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Study of the Mechanism of the Hydride Transfer with a Highly Reactive and Stereoselective NADH model

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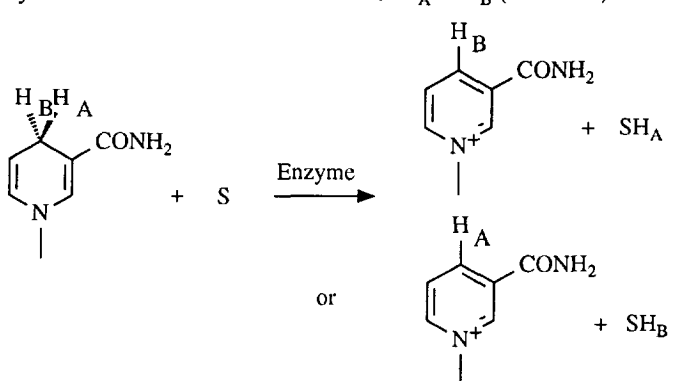
Abstract: The ^{1}H and ^{13}C NMR spectra of the chiral NADH models **3** and **4** were studied both in the presence or absence of Mg^{2+} ions. These two compounds have a chiral carboxamide part derived from (S)-phenylalaninol but contrary to **3**, compound **4** has the ability to free-rotate about the C_3-C=O bond. Careful analysis of the spectral results and comparison with some other studies recently published in the literature allowed the following assumptions: 1-the 1,4-dihydropyridine structure is planar both in the absence or presence of magnesium ions. 2-in the case of the rigid model **3**, the C=O is out of the plane of the dihydropyridine structure. 3-with Mg^{2+} the chiral amino alcohol moiety adopts a quasi-cyclic conformation in the case of **4** but not in the case of **3**. The observed enantioselectivities are explained by the mean of ternary complexes between the model, Mg^{2+} and the substrate .

INTRODUCTION

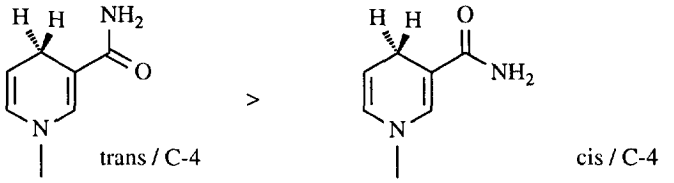
The coenzyme NADH plays an important role in a large number of enzyme catalyzed reductions.[1] Much work has been carried out to elucidate the mechanism of this reaction. In particular, it has been shown early that the hydrogen transfer is stereospecific with respect to both coenzyme and substrates.[2] This specificity is dictated by the enzyme which allows the transfer of either H_A or H_B (Scheme 1).



Scheme 1

X-Ray studies combined with model building suggested that both coenzyme and substrate are fixed in well defined positions inside the binding cleft of the enzyme.[3] As a consequence, one face of the dihydronicotinamide is shielded, whereas the other face is directed toward the substrate. This feature would be responsible for the observed stereospecificity during hydrogen transfer. In this process the role of the amide moiety and its orientation are essential for the stereochemistry. During the last few years large efforts have been made with a view to defining the role of the amide group and to obtain precise information concerning the behaviour of the 1,4-dihydronicotinamide moiety during enantioselective reductions. The main results can be summarized as follows :

- It has been shown from calculations [4] that in most cases, in the absence of an enzyme, the 1,4-dihydronicotinamide prefers a trans structure (with respect to C-4) instead of a cis structure (Scheme 2).



Scheme 2

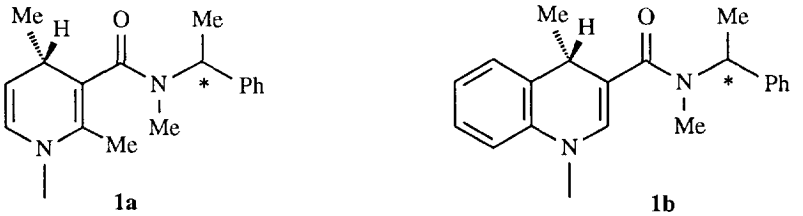
When the coenzyme is placed in an enzyme environment the behaviour is reversed, i.e. the cis conformation is favoured. This factor would be essential for the enantioselectivity of the hydride equivalent.

- Other studies [5] suggest that in the transition state the $CONH_2$ group is out of the plane of the dihydro pyridine ring. Moreover the transfered hydrogen would be syn with respect to the carbonyl of the amide. This behaviour should favour this transfer, primarily due to electrostatic interactions.

A detailed examination of results obtained from calculations or from vibrational analysis [6] has shown the importance of an other point: the dihydropyridine ring would not be planar but would adopt a quasi-boat conformation. In this case it is the H_4 in an axial position (very close to be syn with the amide carbonyl) which would be transferred.

In a recent paper, Bodor [7] suggested that these hypotheses are somewhat speculative and could be more or less involved in the enantioselectivity of the hydrogen transfer. The stereospecificity of the reduction can also be, in part, explained by enzyme structure.

Efforts have been made to create chiral model compounds mimicking the activity of the coenzyme. Several high performance biomimetic models in asymmetric reductions have been described (Scheme 3).[8]

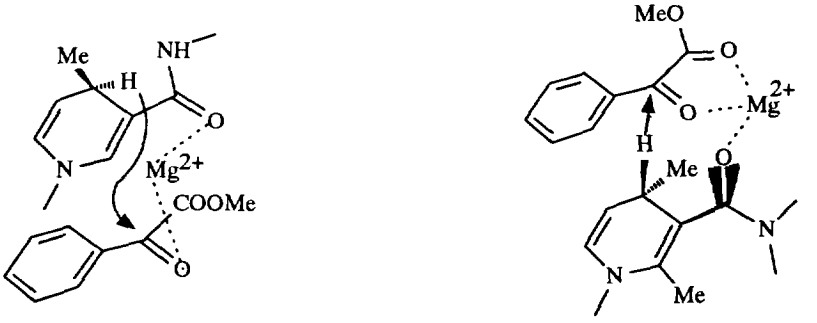


Scheme 3

For example Ohno's model **1a** induces[9] high chirality transfer toward ethyl benzoylformate. The chiral center at C-4 governs the hydride transfer. It was shown that the reduction occurs only in the presence of magnesium ions and Ohno proposed a ternary complex Model/Mg^{2+}/Substrate[10] which would necessarily be involved in the hydrogen transfer. However after reduction the obtained pyridinium salt lost chirality and it was not possible to regenerate reagent **1a**. On the other hand reagent **1b**[11] with a secondary carboxamide moiety, developed by the same group, demonstrated high enantioselectivity combined with reversibility. The secondary amide group prevents the loss of axial chirality in the pyridinium salt and reagent **1b** can be recycled. With this reagent the observed selectivity in the reduction of ethyl benzoylformate is in support with a syn orientation of the transferred hydrogen and of the amide carbonyl group.

Another important family of reactive 4-(R) or 4-(S)-3-*N,N*-(dimethylcarboxamido) -1,2,4-trimethyl-1,4-dihydropyridine derivatives was recently described.[12] In the transition state of the reduction of methyl benzoylformate a strictly organized system was proposed in which the migrating hydride and the amide carbonyl dipole are syn orientated (Scheme 4: B).

Another interesting model was synthesized [13] incorporating a sterically demanding chiral auxiliary at C-3 and a chiral carboxamide at C-5 which insures, by chelation control, the positioning of the substrate. The high enantioselectivity in the hydrogen transfer (i.e. 97 %) is attributed to an out of plane orientation of the carbonyl dipole at C-5 syn to the departing hydride.

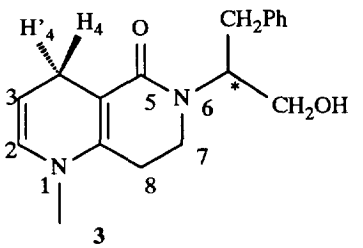


A: Ohno **B: Vekemans**

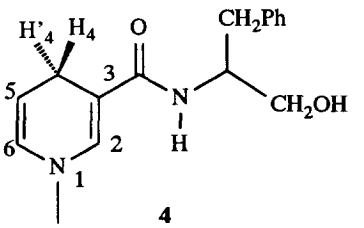
Scheme 4

If we compare the ternary complexes proposed by Ohno[10] or by Vekemans[12] (Scheme 4, A and B), some comments can be made: 1)in the two cases the 1,4-dihydropyridine ring is supposed to be planar. 2)in Ohno's ternary complex, magnesium ions are complexed with the carbonyl dipole through the intervention of p-orbitals. On the other hand, in the Vekeman's scheme, it seemed that complexation of magnesium ions occurs through the intervention of n-orbitals. In the first situation Mg^{2+} is not in the plane of the involved carbonyl groups, in the second it is in the same plane.

In our laboratory, a few years ago, we developed a new generation of efficient chiral NADH models combining high reactivity, high enantioselectivity and recycling properties (Scheme 5).[14]



3



4

Scheme 5

The rigidified reagent **3** allows a 90 % e.e (94 % at 0°C) to be obtained in a very fast reaction during the reduction of methyl benzoylformate. These results are spectacular when compared with the behaviour of the non-rigidified reagent **4** in the same reduction (medium enantioselectivity and reactivity). The main feature of reagent **3** is that, due to the cyclized structure, the amide part lost its free rotating ability compared to the situation with reagent **4**.

The purpose of this paper is to discuss the results obtained with **3** and **4**, by examining the following points : 1- NMR studies of **3** and **4** in the absence of magnesium. 2- NMR studies of the same reagents in the presence of magnesium. 3- attempts to propose a structure for the transition state with a view to explain the large difference in behaviour of the two reagents.

RESULTS

I- Geometry of the chiral auxiliary.

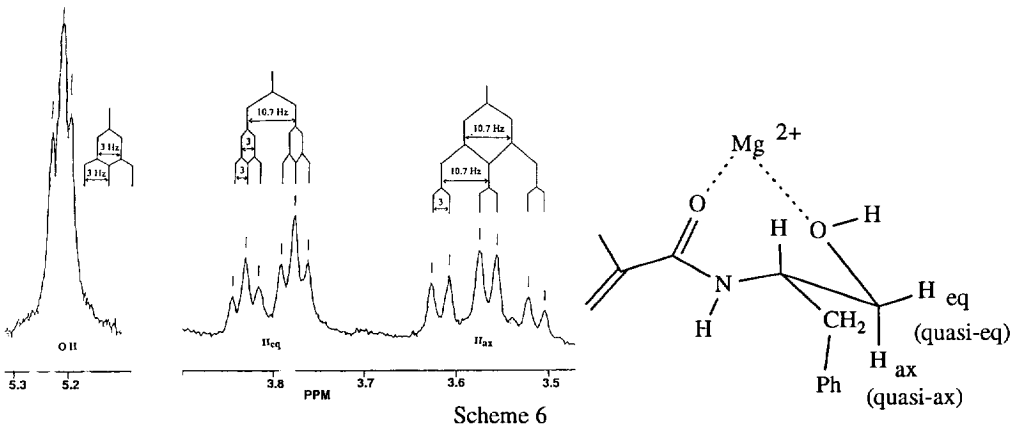
The data for compounds **3** and **4** are reported in Table 1.

Table 1: ^1H NMR chemical shifts of protons of chiral CH, CH_2OH and CH_2 at 7 with or without Mg^{2+}

	Without Mg^{2+}				With 1 eq Mg^{2+}			
	CH*	CH_2OH	OH	CH_2 at 7	CH*	CH_2OH	OH	CH_2 at 7
3	4.48 m	3.58 d	3.48 m	3.22 m 3.10 m	4.63 m	3.61 d	5.32 m	3.28 m 3.13 m
4	4.08 m	3.47 br d	3.55 m	–	4.28 m	H_{eq} 3.81 dt H_{ax} 3.58 dt	5.21 t	–

Spectra were recorded at 360 MHz in CD_3CN as a solvent at 20 °C. m: multiplet; br : broad; d : doublet; t: triplet; dt: doublet of triplets.

*: examination of coupling constants has shown that H_7 at 3.10 ppm (3.13 with Mg^{2+}) is axial and that H_7 at 3.22 ppm (3.38 with Mg^{2+}) is equatorial. J H_{7ax}-H_{7eq}= 12.5 Hz. J H_{7ax}-H_{8ax}=9.7 Hz; J H_{7ax}-H_{8eq} = 5.4 Hz;



Scheme 6

The choice of an aminoalcohol as a chiral auxiliary was governed by the asumption that the alcohol function would be involved in the complexation with magnesium ions. As a consequence, the chiral auxiliary would be rigidified through the complexation of Mg^{2+} both with the amide carbonyl and with the alcohol hydroxy. In compound **4** the behaviour of the CH_2 group of CH_2OH seems to support this hypothesis as can be shown from the evolution of the signals of this group without or with Mg^{2+}. In the absence of Mg^{2+} ions the two hydrogen of the CH_2OH group have the same chemical shift (see Table 1). So it can be assumed that the structure of the aminoalcohol moiety is not well defined. On the other hand, in the presence of Mg^{2+} ions, the rigidification of the chiral part causes a differentiation of these two hydrogen atoms (scheme 6) and the observed coupling constants can be closely related to a quasi six-membered ring for the chiral auxiliary where the sterically demanding group i.e. the $-CH_2-Ph$ substituent is normally in an equatorial position. It must be noticed that if this group would have been in an axial position the stereodifferentiation of the two faces of the 1,4-dihydropyridine ring would have been better.

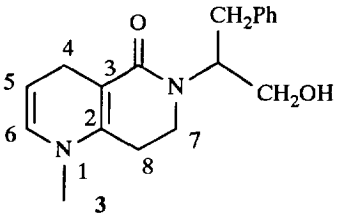
In the case of the rigidified model **3** the behaviour is quite different. In the presence of Mg^{2+} ions, signals were not observed to be compatible with the above mentionned six membered ring for the chiral auxiliary. In particular the two protons of the CH_2OH group appear as a doublet (J = 4.2 Hz : coupling with the hydrogen at the chiral carbon, no other coupling observed). So the geometry of the chiral auxiliary does not seem to be not so well defined as with **4**. A n.O.e. experiment was performed with model **3**. In the absence of Mg^{2+} ions, no effect was observed. On the other hand, in the presence of Mg^{2+}, irradiation of the proton H_7 at 3.28 ppm gave a 1.5 % enhancement of the multiplet at 7.25 ppm (aromatic protons of the phenyl ring). Irradiation of the other H_7 proton (δ = 3.13 ppm) gave no modification of the signals of the phenyl ring. The first information suggests that the phenyl group is near the CH_2 at the 7 position. Despite the non evidence in NMR spectroscopy of a defined structure for the chiral auxiliary, it is probable that interactions of Mg^{2+} with this auxiliary allow the stericlly demanding group to be able to insure a stereodifferentition of the two faces of the 1,4-dihydropyridine ring.

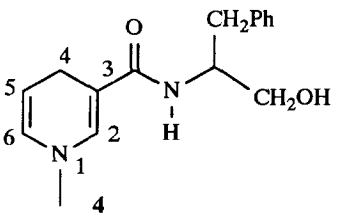
II - Geometry of the 1,4-dihydropyridine ring.

With a view to facilitate the comparison between the two reagents **3** and **4**, the same numeration was adopted for ring atoms in the two cases (see Table 2).

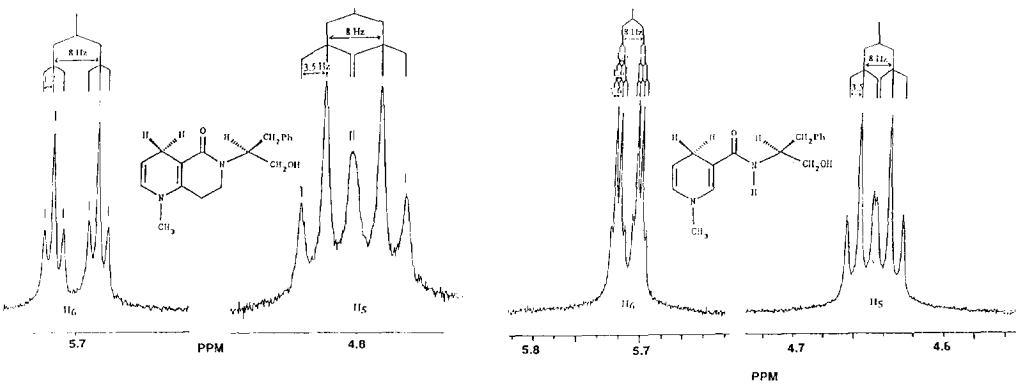
In the absence of Mg^{2+} ions, in the case of compound **3**, H_5 and H_6 appear as a doublet of triplet with the coupling constants given on Scheme 7. In the case of compound **4**, H_5 appears also as a doublet of triplet but H_6 appears as a doublet of quadruplet (coupling with H_2). In the presence of Mg^{2+} ions, the ^1H spectrum is very similar (see Table 2). These data suggest that the 1,4-dihydropyridine ring is planar because otherwise the coupling constants would have been different.[15]

Table 2: ^1H NMR chemical shifts protons in positions 4, 5 or 6.





	Without Mg^{2+}			With 1 eq Mg^{2+}		
	H_6	H_5	H_4	H_6	H_5	H_4
3	5.70dt	4.63dt	3.48 m	5.72dt	4.73dt	2.93 brd 2.89 brd
4	5.71dq	4.64dt	2.65-3.0	5.77dq	4.81dt	2.70-3.10



Scheme 7

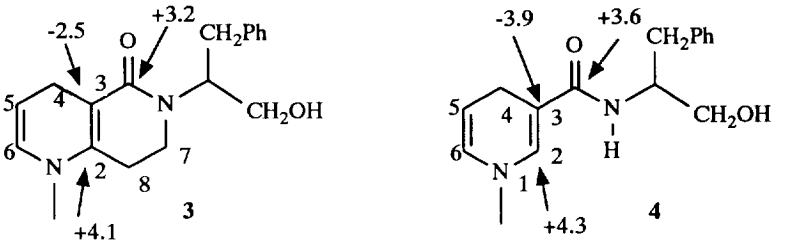
This behaviour is confirmed by measurements of C-H coupling constants in ^{13}C NMR spectroscopy. The main features are the following (either in the presence or in the absence of Mg^{2+} ions). For compound **3**: J C_5-H_4 = J C_5-$H_{4'}$ = 6.7 Hz. For compound **4**: J C_5-H_4 = J C_5-$H_{4'}$= 3.6 Hz. These values indicate similar dihedral angles between H_4 and $H_{4'}$ with C_5 and C_6 corresponding to a planar structure for the dihydropyridine moiety.

III - Conformation of C=O amide.

In the absence of Mg^{2+} ions, the signals for H_4 and H'_4 of the compound **4** appear as a broad peak where the two atoms have almost identical chemical shifts. On the other hand H_4 and H'_4 in compound **3** show a single peak. In particular the coupling constants with H_5 and H_6 are not measurable. A simulation of the NMR spectra (PANIC) confirms this observation.

In the presence of Mg^{2+} ions, a COSY experiment performed on compound **4** clearly shows that H_4 and H'_4 are distinct. In the case of compound **3**, in the presence of Mg^{2+}, the two protons H_4 and H'_4 are also distinct.

The ^{13}C NMR data for compounds **3** and **4** in the absence or the presence of Mg^{2+} ions are given in table 3 (Scheme 8 remembers the numbering).



Scheme 8

Table 3: ^{13}C NMR chemical shifts with or without Mg^{2+}

	Compound **3**			Compound **4**		
	Without Mg^{2+}	With Mg^{2+}	Δδ	Without Mg^{2+}	With Mg^{2+}	Δδ
C_2	148.2	152.3	+4.1	139.7	144.0	+4.3
C_3	97.9	95.4	-2.5	99.9	96.0	-3.9
C_4	22.9	22.4	-0.5	22.6	21.9	-0.7
C_5	103.1	105.3	+2.2	102.4	104.8	+2.4
C_6	126.9	127.4	+0.5	126.9	127.4	+0.5
C=O	168.7	171.9	+3.2	168.7	172.3	+3.6
CH*	59.1	58.4	-0.7	53.9	53.6	-0.3
CH_2OH	63.6	64.4	+0.8	64.8	67.6	+2.8
C_7	41.5	41.0	-0.5	---	---	---
C_8	25.2	24.9	-0.3	---	---	---

Δδ = δ with Mg^{2+} - δ without Mg^{2+}. The more significative values are depicted in scheme 8

It should be emphasized that the signals of C_7 and CH* of compound **3** in the presence of Mg^{2+} are almost faded. These two features are probably a consequence of a high complexation of Mg^{2+} with atoms in the neighborhood of these two carbons i.e, a high complexation with C=O and N in compound **3**. This lead to suppose that the nitrogen atom of the amide function in **3** would be near to be pyramidalized.

This behaviour is different to that observed in compound **4**: the complexation is not as high (no extinction of signals) and the carbon atoms corresponding to the methylene of CH_2OH and C=O are the more affected. However, a comparison of $\Delta\delta$ on C_2, C_3 and C=O in the two compounds shows that there is a lower degree of conjugation between the enamine system of the ring and the carbonyl in compound **3** compared to compound **4**.

DISCUSSION

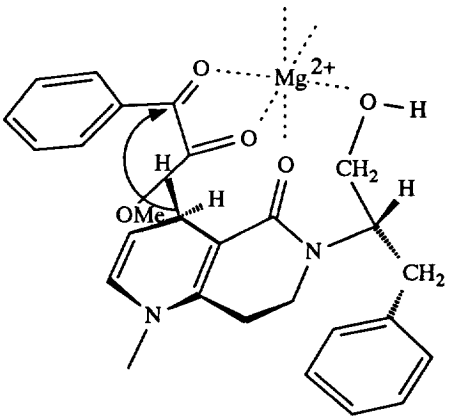
From the above results it can be deduced that :

- The 1,4-dihydropyridine is planar for **3** and **4** both in the absence and the presence of Mg^{2+} ions.
- In the case of model **4** the chiral auxiliary, in the presence of Mg^{2+}, adopts a six membered cyclized conformation with a rigid structure which could reinforce the role of the sterically demanding group. However this group adopts a quasi equatorial position which is not the most favourable for the stereodifferentiation of the two faces of the 1,4-dihydropyridine ring.

The behaviour of model **3** is more complicated. Despite the lack of evidence for the above mentioned cyclized structure, it appears that the sterically demanding group is able to ensure a stereodifferentiation of the two faces since, this group being near the protons borne by C_7 as shown by nOe, the interaction between Mg^{2+} ions and the alcohol function may exist, but it is too weak to be deduced from ^1H NMR data. Moreover it can be observed that the interactions of Mg^{2+} ions are different for models **3** and **4** :

- In **3**, Mg^{2+} ions seem to be strongly linked with the C=O and N atom (probably more pyramidalized than in 4) of the carbamoyl function.
- In **4**, Mg^{2+} is linked with the C=O and OH functions, but with a lower intensity than in **3**.
- The conjugation of the C=O amide with the 1,4-dihydropyridine is less important in **3** compared with **4**, suggesting an out of plane orientation of the C=O in **3** in the presence of Mg^{2+}. Moreover due to the rigid structure of this model, the C=O would be, in this case syn orientated.

As mentioned, the behaviour of models **3** and **4** is very different : rigidified model **3** has both high reactivity and enantioselectivity compared with model **4**. At this point, one can consider that these two models would react with different pathways. In the case of model **3**, the main point is that the C=O carbonyl is out of plane of the 1,4-dihydropyridine. In a first approach, we can consider that there is little conjugation between the C=O and the ring system and as a consequence the departure of the hydrogen atom is not disfavoured, the reactivity being high. The out of plane orientation of the C=O amide group may be responsible of the strong interaction of Mg^{2+} with this group. It is then reasonable to consider that the C=O amide would be on the superior part of the ring, the sterically demanding benzyl group being near the C_7 position. We have performed some calculations on structure **3**, using molecular mechanics force field or MNDO and PM3 methods.[16] These calculations clearly show that, both in the presence or the absence of Mg^{2+} ions, the carbonyl group is about 15 ° out of the plane of the dihydropyridine ring. If we adopt similar hypotheses than those proposed by Vekemans [12] for the construction of the ternary complex we can propose the following scheme for the transition state:



Scheme 9

By analogy with the proposed behaviour of the coenzyme itself during the reduction of a prochiral substrate the out of plane orientation of the C=O amide largely favor the enantioselective transfer of the syn orientated hydride equivalent. As a consequence, it is normal that in the case of model **3**, the e.e. is high. Moreover, this situation is kinetically favourable and the rigidified structure (i.e. the absence of the rotating ability about the C_3-C=O amide bond) facilitates the establishment of the ternary complex. These factors lead to an explanation of the observed higher reactivity.

In the case of model **4**, the hydroxy function seems to play an important role. So it is reasonable to involve this group in the hexacoordinated bipyramidal system of Mg^{2+}. Molecular models show that it is easy to construct the ternary complex represented. The position of the C=O ester of the substrate can be rationalized through a dipolar interaction with the C=O amide of the model. In this case the sterically demanding group adopts an equatorial position which is not the most favourable for a good stereodifferentiation of the two faces of the 1,4-dihydropyridine ring. So this behaviour can explain the medium observed e.e. On the other hand the medium observed reactivity can be explained by the conjugation of the C=O amide group with the dihydropyridine ring which does not favour the departure of the hydride equivalent at the 4-position. Another factor can be suggested : the free rotating ability about the C-3-C=O amide bond. This does not favour the construction of the necessary involved ternary complex since the three partners must be in their correct positions.

In the case of model **3** the out-plane and syn oriented position of the C=O amide group seems to play an important role along with the position of the sterically demanding group near the C_7 atom. The out of plane location of the C=O amide group may be responsible of the strong interaction of Mg^{2+} with this group.

EXPERIMENTAL

The ^1H NMR spectra were recorded on 200 MHz, 360 MHz, 400 MHz and 500 MHz Brucker spectrometers. The solvent was deuterated acetonitrile d_3 purchased from Spectrométrie Spin et Techniques and it was used without further purification. Anhydrous magnesium perchlorate was purchased from Merck corp Ltd (art n° 5879). Compounds **3** and **4** were synthesized according to previously published procedures.[14]

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15. Skog K. and Wennerström O., *Tetrahedron*, **1992**, *33*, 1751. With a well established non planar 1,4-dihydronicotinamide the following coupling constants were observed for H_{4ax}, H_{4eq}, H_5 and H_6 (in DMSO d_6). J-H_{4eq}-H_5 = 4 Hz ; J-H_{4eq}-H_{4ax} = 18 Hz; J-H_{4ax}-H_5 = 3 Hz ; J-H_{4ax}- H_{4eq} = 18 Hz; J-H_{4ax}-H_6 = 1 Hz.

16. Molecular mechanics force field calculations were performed with PC-Model on a PC-486 computer. MNDO calculations were performed using MOPAC-6.0 on a PC-486 computer. The PM3 Hamiltonian was used in the case of calculations involving Mg^{2+} ions.

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