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An Approach to Features Responsible for the Obtention of High e.e. with a Highly Enantioselective NADH Model

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Key words : NADH models; asymmetric reduction; free rotating ability; entropy

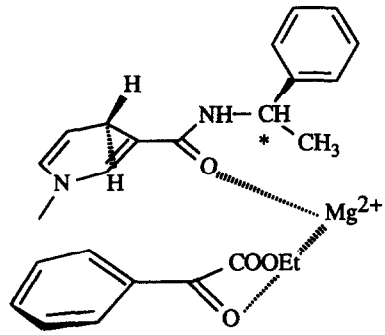
Abstract : A study of the reactivity of several NADH models with free, restricted or suppressed rotating ability of the amide carbonyl group suggests that the main factor responsible for the high e.e. obtained (94 %) with a cyclized reagent is an entropy feature.

The coenzyme NADH is a reagent which, when associated to an enzyme, allows highly chemo and enantioselective reductions (1). The active part of the reagent i.e. the 1,4-dihydronicotinamide moiety is achiral. In asymmetric reductions the stereospecific hydrogen transfer is insured by the enzyme which creates a chiral environment differentiating the two hydrogens of the coenzyme and the two faces of an unsaturated prochiral substrate. The high performances generally obtained in asymmetric reductions in biological systems have, of course, intrigued organic chemists, who have searched to understand the key features responsible of this action and hence developed mimetic systems (2).

Several models have allowed the obtention of high e.e. in the reduction of prochiral substrates (3). Almost all reagents have a chiral auxiliary on the side chain or have a methyl group at the 4-position of the 1,4-dihydropyridine structure with a view to controlling the preferential reactivity of the pro (R) or the pro (S) hydrogen.

Most of the asymmetric reductions are efficient only in the presence of magnesium ions. They are involved in a ternary complex : model/Mg^{2+}/reagent (4).

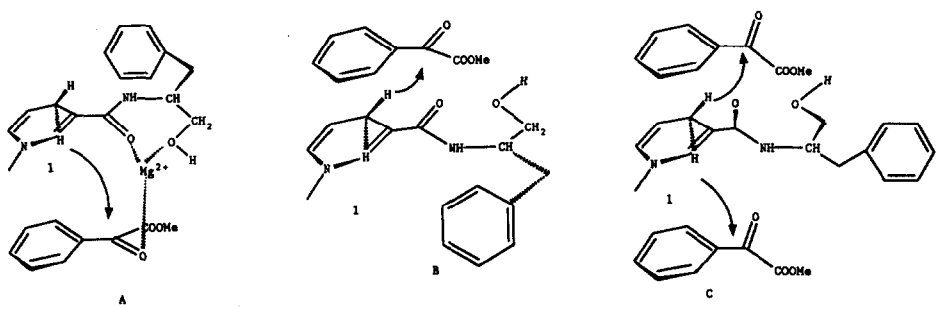
This complex is established on the less hindered face of the dihydropyridine structure. This fact is one of the major points which can explain the high enantioselectivity observed with several models. The other important point is that, in this ternary complex, the polar and non-polar groups of the model and of the reagent are facing each other (scheme 1).



Scheme 1 : Ternary structure proposed for the reduction of ethyl benzoyl formate with a chiral NADH model (4a).

In our laboratory, we have developed models bearing chiral aminoalcohols derivatives on the carbonyl at the 3 position of the 1,4-dihydropyridine (5). It as been shown that the alcoholic oxygen plays an important role in the rigidification of the chiral auxiliary and, as a consequence, in the stereodifferentiation of the two faces of the ring.

Good e.e. were obtained with such models. A study of the ternary complex involved in the reduction shows that with a model such as 1 (scheme 2) the free rotation around the C-3-C=O amide bond can lead to different ternary complexes during the reduction of methyl benzoyl formate.



Scheme 2

The two complexes A or B (which would give the same mandelate enantiomer), represent limit situations where the C=O of the model is coplanar with the dihydropyridine ring. It is possible to go from one to another through intermediate situations such as C, where the C=O is out of the plane of the ring.

In this case the remote steric blocking group, i.e. the benzyl group, is located in a defined region with respect to the plane of the dihydropyridine ring. Of course A and C are, by far, the most stable conformations of the reagent since the carbonyl of the amide group is conjugated with the dihydropyridine ring and therefore has the stronger electron withdrawing effect on the C_4-H bond. This lowers the reactivity

of the reagent because the disparture of the hydrogen is disfavoured.

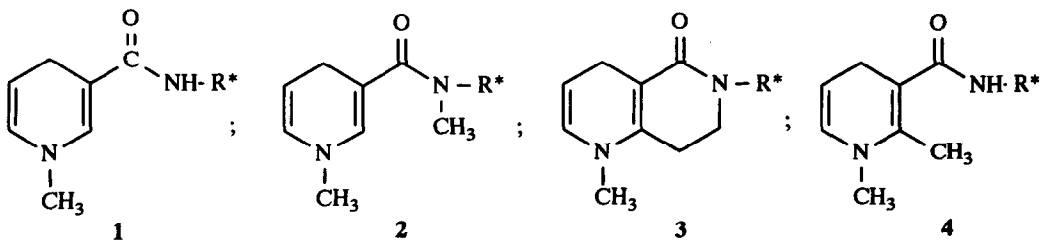
On the other hand in complex C the non conjugation of the carbonyl group with the ring enhances the reactivity of the reagent. But in this case, the stereodifferentiation of the two faces of the dihydropyridine structure is very low compared to situations A or B : the remote steric blocking group is near the mean plane of the dihydropyridine structure and does not hinder preferentially one face or the other. Even if conformations of type C are very much less probable than conformations of type A or B, their high reactivity associated to a very low enantioselectivity can lead to a lowering of the e.e. So, it was of interest to study the behaviour of models with restricted or blocked rotation ability. In model **2** (scheme 3) the steric hindrance caused by the presence of a methyl group on the nitrogen of the amide forces the carbonyl to be out of the plane. In model **3** the cyclized structure suppresses the free rotating ability around the C-3-C=O amide bond. In a preceeding paper we reported the results obtained with models **1,2** and **3** in the asymmetric reduction of methyl benzoylformate (table 1) (6).

Models / Results	1	2	3
e.e.	57	3	85
Major Config.	R	S	R

Reaction time 48 h ; temperature 60°C ; model/Mg^{2+}/substrate : 1/1/1

Table 1

In this paper we will describe in the first part another model **4** bearing a methyl substituent at the 2 position of the dihydropyridine ring which could also force the carbonyl to be out the plane as suggested by examination of molecular models.



R* = — CH(CH2OH)- CH2Ph

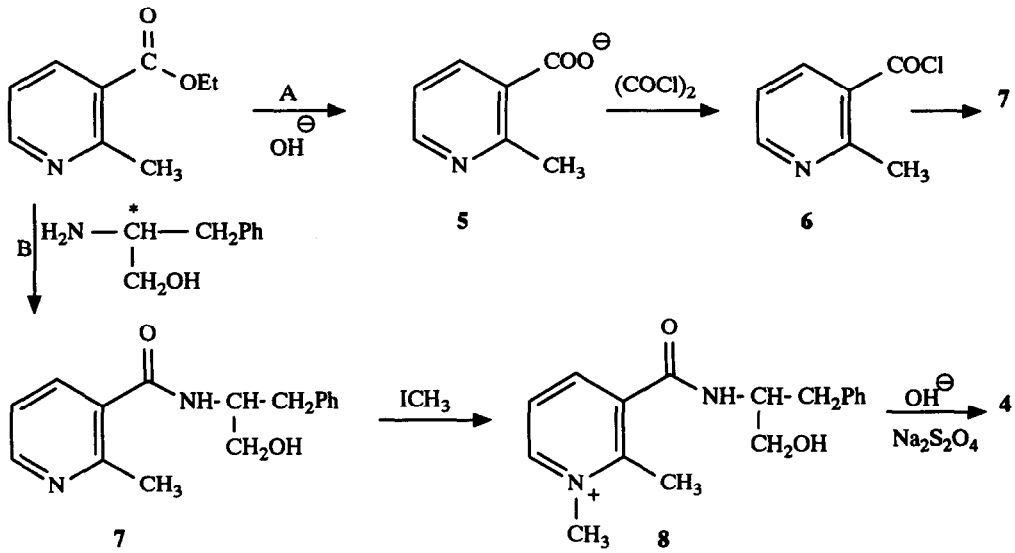
Scheme 3

In a second part, we will approach a rarely discussed point in the case of biomimetic NADH models. In biological systems it is well known that entropy factors play an important role in the kinetic and in the enantioselectivity of reactions performed with enzymes (1). If we compare models **1,2,4** and **3**, the free rotation in the first three probably disrupts good arrangement of the different partners involved in the ternary complex. This behaviour can lead to the occurence of different transition states, some of which can lead to one enantiomer of methyl mandelate and others to the opposite enantiomer.

On the contrary in **3** where the carbonyl is immobilized, the complex must be very easy to establish. This fact can explain the high e.e. obtained with **3** compared to **1**. Moreover as the proximity between the reagent and the model would be easier to insure, it could be assumed that the reactivity of the model would be enhanced. So the reactivity of models **1,2,3** and **4** will be compared in the case of the reduction of methyl benzoyl formate and the results discussed in this second part.

I-Synthesis and behaviour of model 4

Starting from commercially available ethyl 2-methylnicotinic acid two methods were tested (scheme 4).



Scheme 4

By using method A, large amounts of tarry materials were obtained in the reaction **5→6→7** probably due to the reactivity of the methyl group at the 2 position. So, it was easier to perform directly the condensation of the aminoalcohol with the ester at 130°C (method B). The quaternization leading to **8** was nearly quantitative. The reduction with sodium dithionite in classical conditions (room temperature) gave a 20 % yield. It was necessary to work at 50° in a water/dichloromethane mixture. The role of the organic solvent was to extract the dihydropyridine derivative **4** as soon as it was formed with a view to limiting the degradation caused by water.

The ^1H NMR data for **7,8** and **4** are given in table 2.

	1	CH_3 at 2	4	5	6	7	8	9	10	Ph
7	-	2.52	7.50	7.09	8.50	6.17	4.43	3.71 3.83	3.00	7.28
8	4.23	2.45	8.31	7.99	8.99	8.73	4.23	3.51	2.94 2.60	7.26
4	2.88	2.14	2.80	4.57	5.67	5.47	4.20	3.65	2.88	7.24

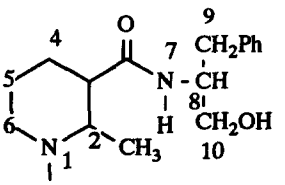


Table 2

A comparison of the NMR data for compounds **7,8** and **4** (series **4**) with the NMR data of the corresponding compounds in series **1,2** and **3** shows that the main features are the following :

- in compounds **7** and **8** the H_4 proton is shielded when compared to the corresponding protons (pyridine precursor or pyridinium salt) in series **1** or **3** (6) (table 3).

H_4 proton	Series 1	Series 3	Series 2	Series 4
pyridine precursor	7.96	8.20	7.45 and 6.94	7.50
pyridinium salt	8.82	8.79	7.38 and 7.76	8.31

Table 3

The chemical shifts are more closely related to those found for the compounds in series **2**. However, in this series the pyridine and the pyridinium derivatives exist under the form of two conformers, contrary to compounds **7** and **8** which exhibit only one conformer. The fact that H_4 is shielded in **7** and **8** is probably a consequence of the anisotropy caused by the out of plane carbonyl group : H_4 is forced to be in the shielding region of this group.

- in compound **4** the chemical shifts of the different protons are similar to those observed for the corresponding protons in compounds **1,2** or **3** (6).

The reduction of methyl benzoylformate with **4** under classical conditions leads to the obtention of methyl mandelate with a 55 % chemical yield and a 3 % e.e. (major enantiomer S). This result can be compared with the behaviour of coumpound **2** under the same conditions (chem. yield 60 %, e.e. : 3 % major enantiomer S).

We can suppose that in compound **4**, as in compound **2**, the out of the plane amide carbonyl group forces, in the ternary complex, the hindered group,(the benzyl substituent) on the chiral atom, to be located in a position very closely related to the mean plane of the dihydropyridine structure (as in complex C in

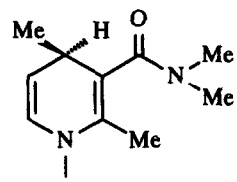
scheme 2). So the stereodifferentiation of the two faces of the dihydropyridine is bad and the departure of either the pro (R) or pro (S) hydrogen atom from C_4 is nearly equally favoured leading to a quasi racemic methyl mandelate.

This discussion will be completed in the second part by using reactivity considerations.

II - Reactivity of models 1,2,3 and 4 towards methyl benzoylformate

The conjugation or the non conjugation of the amide carbonyl group with the plane of the 1,4-dihydropyridine ring plays an important role in the reactivity of NADH models.

For example with reagent **9** described by Vekemans et al. (9) it was etablished that the presence of methyl groups at the 2 and 4 positions of the 1,4-dihydropyridine ring and the nitrogen atom of the amide group forces the C=O substituent to be out of the plane. As a consequence the reagent has a high reactivity (reduction of the substrate in 1 hour at -25°C) and a high enantioselectivity (e.e. 98 %). But the reduction was performed at low temperature because at room temperature the reagent is too unstable and the e.e. falls largely (from 98 % to 66 %).



Vekemans' model : **9**

It must be noticed that contrary to our models the chirality of the Vekeman's reagent is located at the 4 position. So, after reduction of a substrate, the regeneration of chirality at this position is a difficult problem.

First of all, we performed the reduction of methyl benzoylformate at various temperatures with model **3** (table 4).

Température °C	Chem. Yield (%)	e.e. (%)
60	95	85
20	95	90
0	95	94
-30	95	94

model **3**/Mg^{2+}/substrate : 1/1/1.

table 4

Of course, as expected, lowering the temperature affords a better e.e.. As can be seen a high chemical reactivity for the reagent is maintained since the yield is nearly quantitative. The main point is that our model **2** remains stable at room temperature for a period of several days. In fact, it appears that the cyclized model **2** is one of the most high performing NADH models in asymmetric synthesis (3c).(Table 5)

In a secondly time, we have studied the reaction rates of the reduction of methyl benzoyl formate with models **1,2,3** and **4**. The reductions were performed at room temperature with a ratio 1/1/1 (model/Mg^{2+}/substrate) and the conversion was monitored by g.l.c. (table 5).

Model (temp.) / Time (min)	1 r. t.	2 r. t.	3 r. t.	4 r. t.	3 at 0°C	3 at 0°C
5		100 %	98 %	46 %		
10				48 %	95 %	71 %
20	56 %			49 %		
40	97 %				100 %	
60				48 %		92 %

Table 5: % conversion during the reduction of methyl benzoylformate with models **1**, **2**, **3** and **4**

Models **2**,**3** and **4** are very much more reactive than model **1**. For model **2**, as mentioned already the main reason is probably, the non conjugation in the ternary complex between the carbonyl amide and the dihydropyridine ring.

The behaviour is probably the same with model **4** in which the electrondonating effect of the methyl group at the 2 position reinforces the departing ability of H_4. This non conjugation seems to be confirmed by the fact that the reagent is less stable than **1** (this could explained the lower chemical yield).

In the case of compound **3** the reactivity is the same as with **2** and **4** (half time reaction at room temperature : 5 minutes). This high rate of reduction is, to our point of view, a consequence of the ease with which the ternary complex is established with this cyclized model.

Despite the fact that the reactivity could be lowered by conjugation of the carbonyl with the ring, it appears that the entropy factor associated to the rigidified structure plays a more important role in the enhancement of the reactivity. It must be remembered that in the transition state of the reaction, the three partners must be in proper situation, with a view to insuring the hydrogen transfer from the model to the substrate.

So with models such as **1** the free rotation around the C3-C=O bond could perturb the establishment of the ternary complex. It is well known that in biological systems the efficiency of enzymes in chemical reactions is related to an increase of the "order" of the reactants and a consequent loss of entropy. Entropy is one of the most important factors in enzyme catalysis (8). There is, in the transition state of an enzymatic reaction, no loss of translation and/or rotational entropy.

We can assume that the situation is comparable with reagent **2** where the rigidified structure leads to a loss of the rotational entropy during the etablishment of the ternary complex compared to the behaviour with compound **1**.

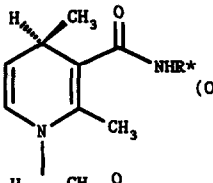
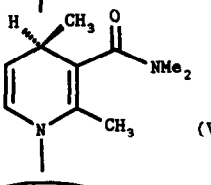
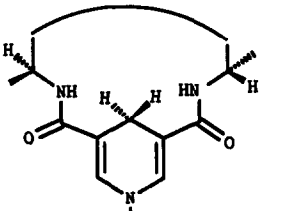
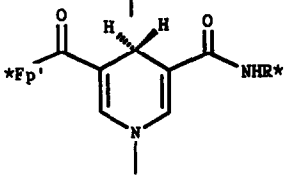
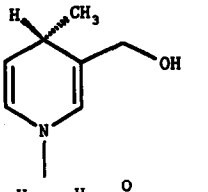
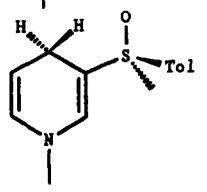
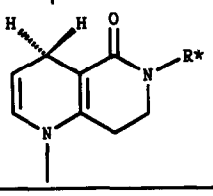
Reagent (principal author)	% e.e (*) of Mandelate (configuration)	Time
 (Ohno)	98 (R)	2 days (r.t.)
 (Vekemans)	92 (R)	1 hour (-25°C)
 (Kellog)	90 (S)	4 days (r.t.)
 (Davies)	98 (R)	90 minutes (r.t.)
 (Meyers)	83 (S)	5 days (r.t.)
 (Iwata)	96 (R)	7 days (r.t.)
 (Combret)	94 (R)	15 mn (0 °C)

Table 5 : asymmetric reduction of alkyl benzoylformate by NADH Mimics.

EXPERIMENTAL

The infra red spectra were recorded on a Beckman IR 4250 spectrometer. The ^1H NMR spectra were recorded on a 60 MHz Varian EM 360 L spectrometer, on a 200 MHz or on 400 MHz Brucker AM 400 spectrometer. Microanalyses were obtained from a Carlo Erba 1106 apparatus. Enantiomeric excesses were determined by HPLC, after separation of the enantiomers by using a Waters apparatus and a L.K.B. Enantiopac as a chiral column (9) or after derivatization of the chiral alcohols by Mosher's method and analysis by GPC (10). Solvents were degassed after bubbling with dry argon for, at least, a quarter of an hour before use. Acetonitrile was distilled on calcium hydride prior to use.

2-Methyl-3-[N-(S)-2-benzyl-1-hydroxyethyl)]carbonylaminopyridine : **7**

In a flask 3.30 g (0.02 mol) of ethyl 2-methylnicotinate and 3.2 g (0.02 mol) of (S) phenylalaninol were heated at 110°C for 5 days under argon. The obtained oil was purified by column chromatography (eluent CH_2Cl_2/EtOH : 90/10). Yield 42 %, m.p.=160°C.

Analysis $C_{16}H_{18}N_2O_2$: calcd : C, 71.09 ; H, 6.71 ; N, 10.36. Found : C, 70.7 ; H, 6.5 ; N, 10.1. I.R. υ (C=0): 1640 cm^{-1}. ^1H NMR ($CDCl_3$): 8.50 (dd,1H) ; 7.50 (dd, 1H); 7.28 (m, 5H); 7.09 (dd, 1H); 6.17 (d, 1H, NH) ; 4.43 (m, 1H) ; 3.83 (dd, 1H) ; 3.71 (dd, 1H) ; 3.00 (m, 2H) ; 2.52 (s,3H).

1,2-dimethyl-3-[N-(S)-2-benzyl-1-hydroxyethyl]carbonylaminopyridinium iodide : **8**

Compound **7** (1.35 g, 0.005 mol) and 5 ml of methyl iodide were heated under reflux of acetonitrile (5 ml) for 12 hours.

After concentration to one third of the initial volume, 10 ml of anhydrous diethyl ether were added. A gummy product was filtered and triturated with ether. A yellow solid was obtained and dried. Yield 95 % ; m.p. = 204°C.

Analysis $C_{17}H_{21}IN_2O_2$: calcd : C, 49.53 ; H, 5.13 ; N, 6.79. Found : C, 49.6 ; H, 4.9 ; N, 6.7. I.R. (υ (C=0) : 1650 cm^{-1}). ^1H N.M.R (DMSO- d_6) : 8.99 (d, 1H) ; 8.73 (d, 1H, NH) ; 8.31 (d, 1H) ; 7.99 (t, 1H) ; 7.26 (m, 5H) ; 4.96 (t, 1H, OH) ; 4.23 (m, 4H) ; 3.51 (t, 2H) ; 2.94 (dd, 1H) ; 2.60 (dd, 1H) ; 2.45 (s, 3H).

1,2-dimethyl-3-[N-(S)-2-benzyl-1-hydroxyethyl]carbonylamino-1,4-dihydropyridine : **4**

In a 25 ml flask, 0.412 g (0.001 mol) of the pyridinium salt **8** were dissolved at 50°C in 4 ml of degassed water to which were then added 4 ml of dichloromethane. The mixture was placed under argon in the dark and a solution of 0.53 g (0.005 mol) of sodium carbonate and 1.69 g of (0.008 mol) of sodium dithionite in 4 ml of degassed water was added. The mixture was vigourously stirred for 20 minutes at 50°C and the dihydropyridine **4** was extracted with 3X10 ml of dichloromethane. The organic phase was washed with 3X10 ml of water, then dried and evaporated to dryness.

The crude derivative **4** was stored under vacuum in the dark. Yield 49 %. ^1H N.M.R ($CDCl_3$) : 7.24 (m, 5H) ; 5.67 (d, 1H) ; 5.47 (d, 1H, NH) ; 4.57 (t, 1H) ; 4.20 (m, 1H) ; 3.91 (m, 1H, OH) ; 3.65 (m, 2H) ; 2.88 (m, 7H) ; 2.80 (m, 2H); 2.14 (s, 3H).

Reduction of methyl benzoylformate

In a flask flushed with dry argon, the appropriate NADH model (1 mmol), methyl benzoylformate (0.9 mmol) and magnesium perchlorate (1 mmol) were dissolved in acetonitrile (5 ml). The reaction mixture was stirred in the dark, under argon (temperatures and reaction times are mentioned in the text). Water, (0.5 ml) was then added and the product extracted with ether. The organic phase was dried and the solvent evaporated. The crude methyl mandelate was purified by chromatography (column : silica, eluent : ether/hexane : 1/3).

The e.e. was determined by HPLC : sample 1.5 mg in 50 ml of solvent (phosphate buffer pH 7 and propanol 98/2 ; λ = 210 nm ; temperature 15°C ; retention times : 15 mn for the (S) and 18 mn for the (R). The same results were obtained by GPC analysis of the diastereoisomers after derivatization by Mosher's method. Sample 1 µl., capillary column DB1 (l=50 m), oven temperature : 170°C. FID detector and injector temperatures : 250°C, vector gas : He, pressure 1 bar ; retention times : 36 min. for the (R,S) and 38.8 min. for the (S,S).

Kinetics of the reductions

At various intervals 50 µl samples of the reaction mixture were collected with a syringe and transferred in a test tube. Dichloromethane (0.5 ml) was added and the organic phase was washed with 1 ml of water.

After drying, 1 µl of the organic phase was analyzed by gas phase chromatography in the following conditions : apparatus Varian 3400, capillary column CP Sil 19 30X0.25X0.25 (m x mm x µm) ; injector "Split" : 250°C ; detector FID : 250°C ; vector gas : H_2 (pressure 1 bar), oven temperature 100°C. Retention times : near 19 min.

REFERENCES AND NOTES

1) Dugas H. : *Bioorganic chemistry. A chemical Approach to Enzyme Action*; Second Edition; Springer-Verlag 1988; pp. 187-189 and 489-513.

2) a) Ohnishi Y., Kagami M. and Ohno A., *J. Am. Chem. Soc*, **1975**, *97*, 4766-4768. b) Ohnishi Y., Numakumai T. and Ohno A., *Tetrahedron Lett.*, **1975**, 3813-3814.

3) For a review see :a) Ohno A., Ushida S. Mechanistic Models for Asymmetric Reductions. In *Lecture Notes in Bio-Organic Chemistry*; Springer-Verlag 1986. b) Baba N., Oda J. and Inouye Y. *Asymmetric Synthesis* Vol 2, Academic Press 1983; pp. 90. c) Burgess A. V., Davies S. G., Skerly R. T. *Tetrahedron : Asymmetry*, **1991**, *2*, 299-328 and references cited therein.

4) a) Ohno A., Kimura T., Yamamoto H., Kim S. G., Oka S. and Ohnishi Y., *Bull. Chem. Soc. Japan*, **1977**, *50*, 1535-1538. b) Ohno A., Yamamoto H., Okamoto T., Oka S. and Ohnishi Y., *Bull. Chem. Soc. Japan*, **1977**, *50*, 2385-2386. c) Hughes M. and Prince R.H., *J. Inorg. Nucl. Chem.*, **1978**, *40*, 703-712.

5) Binay P., Dupas G., Bourguignon J. and Quéguiner G. *Tetrahedron Lett.*, **1988**, *29*, 931-932.

6) Combret Y., Torché J.J., Plé N., Duflos J., Dupas G., Bourguignon J. and Quéguiner G., *Tetrahedron*, **1991**, *47*, 9369-9382. Prior to these results it was proposed that in the ternary complex the ring nitrogen could be chelated with magnesium ions. So, in this type of complex the carbonyl dipole of the amide part is pointed towards the nitrogen of the dihydropyridine. With the cyclized model **3** this situation, is of course, impossible and however the e.e. is good.

7) De Kok P. M. T., Bastiaansen L. A. M., Van Lier P. M., Vekemans J. A. J. M. and Buck H. M., *J. Org. Chem.*, **1989**, *54*, 1313-1320.

8) Ferscht A. *Enzyme Structure and Mechanism*, Freeman, 1977, pp. 44-48.

9) Levacher V., Benoit R., Duflos J., Dupas G., Bourguignon J. and Quéguiner G., *Tetrahedron*, **1991**, *47*, 429-440.

10) a) Dale J., Dull D. L. and Mosher H. S., *J. Org. Chem.*, **1969**, *34*, 2543-2549. b) Dale J. and Mosher H. S., *J. Am. Chem. Soc.*, **1973**, *95* 512-519.