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Zinc *meso*-Tetraphenylporphyrin as Shift Reagent for NADH-Models

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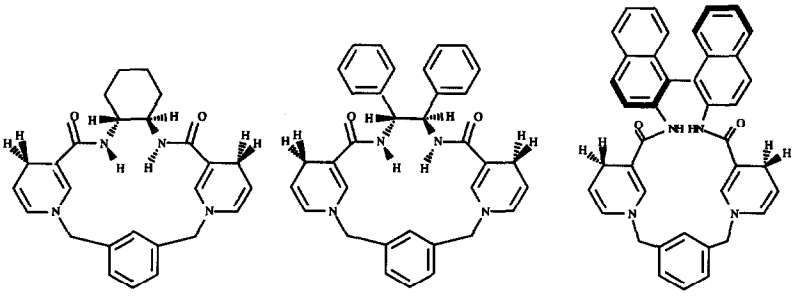
Abstract: By the use of zinc meso-tetraphenylporphyrin as a shift reagent the conformations of two new NADH-models have been determined. Induced ^{1}H-NMR shifts up to five ppm have been observed. The conformations determined in this study are consistent with theoretical calculations and NOE-measurements. It has been proven that zinc, which is a co-catalyst in the reduction of activated carbonyl compounds with NADH-models, coordinates to the oxygen of the amide groups in two of our model compounds.

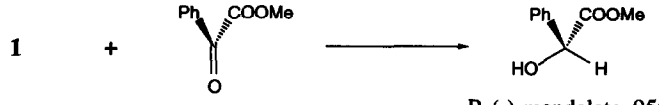
By far the most frequently used biological reduction/oxidation reagent is NAD^+/NADH which is an essential part of several redox enzymes.[1,2] Simple metal ions such as magnesium or zinc are co-enzymes. The modelling of this enzymatic reaction might serve several purposes. An obvious one is a better understanding of the detailed mechanisms of the reactions. Another is to gain proper knowledge to be able to design an organic reagent, based on the NAD^+/NADH-system, for asymmetric reduction of carbonyl compounds. Several model compounds have been prepared and proven to stereoselectively reduce certain activated pro-chiral carbonyl compounds.[1] However, many questions concerning the detailed mechanism of the reaction remain to be answered. Of particular interest is the identification of the reactive hydrogen at C4 in the dihydropyridine ring, the co-ordination site of the co-catalyst, divalent ions of magnesium or zinc, and of course the geometry of the transition state of the reaction.

Since each one of the two hydrogens in the 4-position in the dihydropyridine ring can be the reactive one and since each side of the prochiral substrate can be attacked by the hydride ion it is necessary to enforce steric restrictions on the reagent in order to achieve high stereoselectivity in model systems as is done in the enzymes. The introduction of sterically demanding side chains in the substrate can reduce the number of different possible transition states but positive interactions between substrate and reagent *via* the divalent co-catalysts magnesium and zinc must also be considered. By taking advantage of symmetry (C_2-symmetry) and further restricting the number of possible conformations of the NADH-model compound by locking the dihydropyridine unit into a macrocycle we hope to be able to differentiate between some of the numerous possible reaction paths.



1 2 3

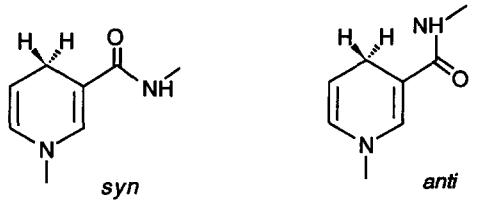
We have recently prepared chiral model compounds built from two dihydropyridine units arranged with C_2-symmetry into the macrocycles, **1-3**. So far, **1** has been shown to function in asymmetric reductions of activated carbonyl compounds.[3] By proper marking of the active hydrogens in the dihydropyridine rings in compound **1** we were able to conclude that the oxidation of the reagent is stereospecific i.e. one specific hydrogen is transferred to the carbonyl compound.



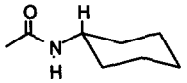
R-(-)-mandelate, 95% ee

If we are able to assign this hydrogen and to determine the conformation of the reagent in solution we have a good chance to discriminate between some of the possible transition states for the reduction which have been suggested in the literature. In the absence of an X-ray structure which eventually will give the structure in the solid state we have to rely on other structural methods. Conclusive evidence is difficult to obtain from one method alone and we are trying to combine data from several methods. So far UV/VIS and ^{1}H-NMR-spectra as well as the CD-spectrum have been used to determine the structure of the reagents in solution. Here, we would like to emphasize one particular method, the use of a zinc porphyrin as a shift reagent to define both the co-ordination site of zinc and to determine the structure of the preferred conformation of the reagents in solution.[4]

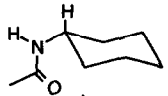
A major problem with the conformation of NADH-models is the orientation of the amide group relative to that of the dihydropyridine ring. In most enzymes the carbonyl group is pointing towards the H_4-hydrogens (here called *syn* conformation) whereas in model compounds the *anti* conformation seems to dominate.[2,5]



The UV/VIS and CD spectra of our model compounds do not give conclusive information on the conformations, nor do theoretical calculations by standard procedures (AM1, PM3) on this type of compound. From ^{1}H-NMR data, that is the coupling constants of the amide hydrogens, it is clear that the amide hydrogens and the adjacent hydrogens on the cyclohexyl ring of **1** are oriented *trans* to each other.

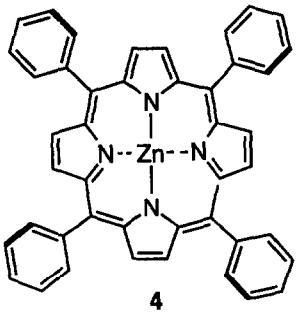


trans



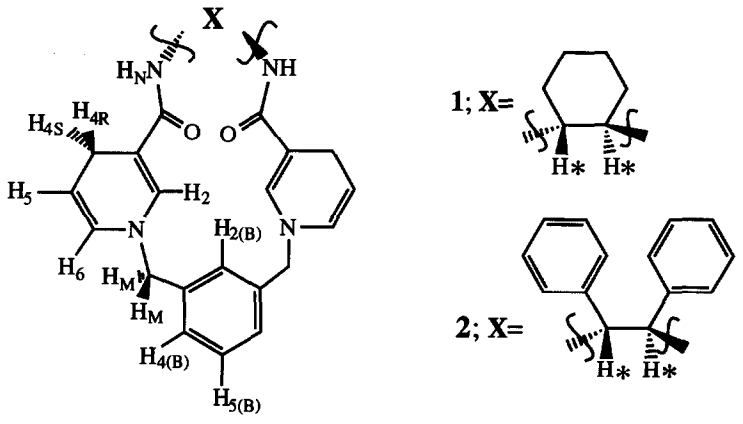
cis

The metal in zinc *meso*-tetraphenylporphyrin **4** is normally tetracoordinated in inert solvents but can increase its coordination number and coordinate to nitrogen or oxygen. The rather planar porphyrin ring sustains a large ring current and the induced chemical shift on nearby hydrogens is considerable in a ^{1}H-NMR experiment. Zinc porphyrin has therefore been used as a shift reagent[4,6] but found little use as a conformational probe, perhaps due to the rather complex calculation of the induced shift which will depend on the angle of rotation of the porphyrin ring. However, since the induced shift is large and the generally weak coordination to nitrogen or oxygen allows for certain conformational freedom in the complex a qualitative interpretation of the induced shift should be appropriate.



4

When mixing a small amount of zinc tetraphenylporphyrin with our three model compounds significant ^{1}H-NMR shift changes occur for the first two only. The steric requirements of the zinc tetraphenylporphyrin and the binaphthyl unit prevent effective coordination in our model compound **3**. Besides, in **3**, the amide nitrogen is a poor electron donor due to conjugation with the naphthyl unit. The induced shifts in the model compounds **1** and **2** are proportional to the amount of zinc porphyrin at relatively low concentrations. At higher concentrations the induced shifts increase and severe band broadening occurs for the signals that are shifted the most. The broadening increases with the shift differences for the various sites. However, the signals for the hydrogens on the C_2-axis of symmetry stay relatively sharp which show that the band broadening must be a dynamic effect. The observed shifts are mean values of the shifts in the free compound, the two sites in the monocomplex and the biscomplex. For protons on the C_2-axis of symmetry the number of different shifts is reduced to three. On further addition of zinc porphyrin (more than twofold excess) the proton signals sharpen again and the induced shift approaches a limiting value. We have interpreted the broadening as a rapid ligand exchange resulting in a dynamic process in which the protons in our model compounds rapidly change between two different sites in the complex and one site the uncomplexed form. Then, when most of the coordination sites in the NADH-models for the zinc porphyrin become occupied the complex regains C_2-symmetry and the exchange process can be neglected and the proton signals again become narrow.



proton		H*	H_N	H_2	H_{4R}	H_{4S}	H_5	H_6	$H_{2(B)}$	$H_{4(B)}$	$H_{5(B)}$	H_M	
shift δ	(1)	3.84	5.24	7.18	2.99	3.22	4.70	5.59	7.35	7.04	7.26	4.41	4.17
(ppm)	(2)	5.49	5.89	a	3.02	3.30	4.76	5.60	7.42	7.06	7.28	4.44	4.21
max. induced Δδ	(1)	5.71	5.32	5.24	2.47	4.55	0.76	0.69	2.11	0.16	0.01	0.96	0.64
(Δppm]													
% of max. Δδ	(1)	100	93	92	43	80	13	12	37	3	0	17	11
	(2) b	100	93	a	57	94	16	14	a	a	a	20	15

a; signals partly overlap

b; calculated from addition of less than one equivalent ZnTPP

Fig.1. Induced shifts in our model compounds 1 and 2 by using ZnTPP.

Fig. 1 shows the chemical shifts for the free compound and the maximum induced shift on addition of six equivalents of zinc porphyrin in a 0.02 M solution of the model compound **1**. The relative shift in percent of the largest maximum induced shift is also shown for **1** and **2**. The induced shifts of model compound **1** as a function of the zinc porphyrin concentration is shown in Fig. 2 for some selected protons. From these data it is possible to calculate the equilibrium constants for the binding of the first (K_1) and second ligand (K_2).

$$1 + 4 \rightleftharpoons 1\text{-}4 \qquad K_1 = \frac{[1\text{-}4]}{[1] \times [4]}$$

$$1\text{-}4 + 4 \rightleftharpoons 4\text{-}1\text{-}4 \qquad K_2 = \frac{[4\text{-}1\text{-}4]}{[1\text{-}4] \times [4]}$$

The best fit between experimental and calculated curves is obtained with the values $K_1 = 1.1 * 10^3\ M^{-1}$ and $K_2 = 5 * 10^1\ M^{-1}$. Thus, there seems to be a weak negative interaction between the two zinc porphyrins.

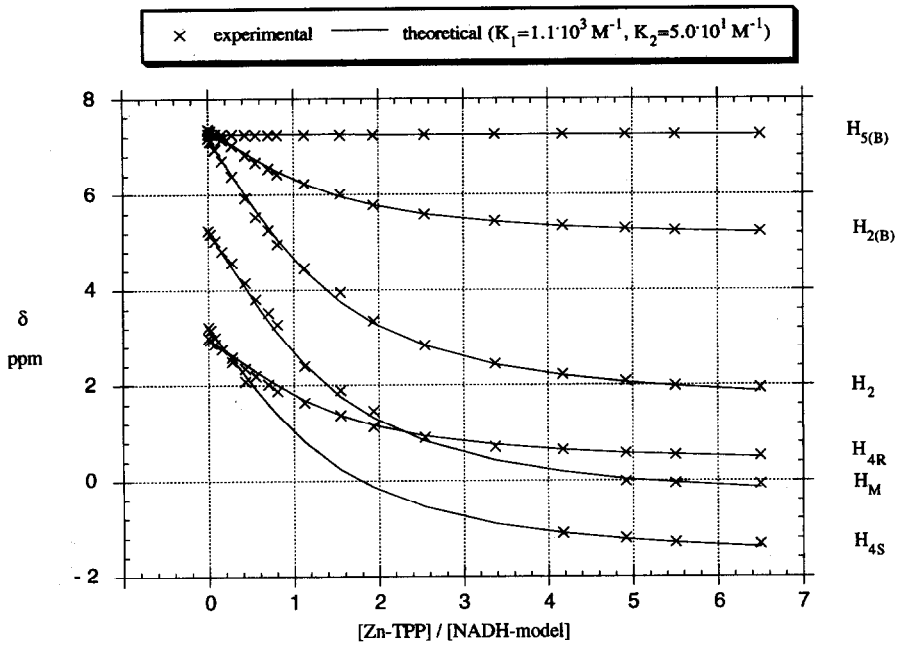


Fig.2. The induced shifts in ppm for some selected protons as a function of the molar ratio of zinc porphyrin and model compound **1**.

Although the complexes are weak they may affect the conformation of the model compounds **1** and **2** in solution. In the absence of conformational changes, the induced shifts of the protons on the C_2-symmetry axis should be proportional to the degree of complexation all the way to the 2:1 complex. Only a minor deviation from this behavior is observed. The two compounds **1** and **2** show similar induced shifts which amounts to more than five ppm for certain protons. We conclude that large conformational changes of our NADH-model compounds **1** and **2** on complexation with zinc tetraphenylporphyrin do not occur.

Our macrocyclic model compounds **1-3** contains amide oxygens and two types of nitrogens which are the potential coordination sites. It is immediately clear from our data that the hydrogens near the dihydropyridine nitrogens are much less affected than those near the amide oxygen and the amide nitrogen. The amide nitrogens are less likely candidates for coordination since they are poor donors. Besides, we have found it unlikely from inspection of models (CPK) that zinc in the porphyrin can bind to the amide nitrogen for steric reasons.

By use of CPK-models of zinc tetraphenylporphyrin and our NADH-models **1** and **2** we can construct two different 2:1 complexes with either *syn* or *anti* orientation of the amide groups, Fig. 3. In these complexes the two porphyrins are essentially coplanar and not interacting. The plane of the amide group is slightly twisted out of conjugation with the dihydropyridine plane to enhance coordination of oxygen to zinc. The structure with the amide groups *anti* is the more compact one.

With the help of these two model complexes the induced shifts for all the protons can be estimated and compared with our experimental results. The most significant differences occur for protons at H_2, H_{4S} and H_{4R} in the dihydropyridine rings. In the *syn* complex the H_2-hydrogens appear close to the center, in the *anti* complex they are forced into the porphyrin rings. In the *syn* complex the H_{4R}- and H_{4S}-hydrogens are forced into the porphyrin rings, in the *anti* complex one of the H_4-hydrogens, H_{4S}, will come close to the oxygen in the amide group on the adjacent dihydropyridine ring and thus relatively close to the porphyrin ring thereby its signal will be shifted upfield much more than that from H_{4R}. The experimental observation that the H_2-proton signals are shifted more than those of the C4-hydrogens is consistent only with the *anti* complex being the stable one. The H_{4S}-hydrogens are the reactive ones towards activated carbonyl compounds.

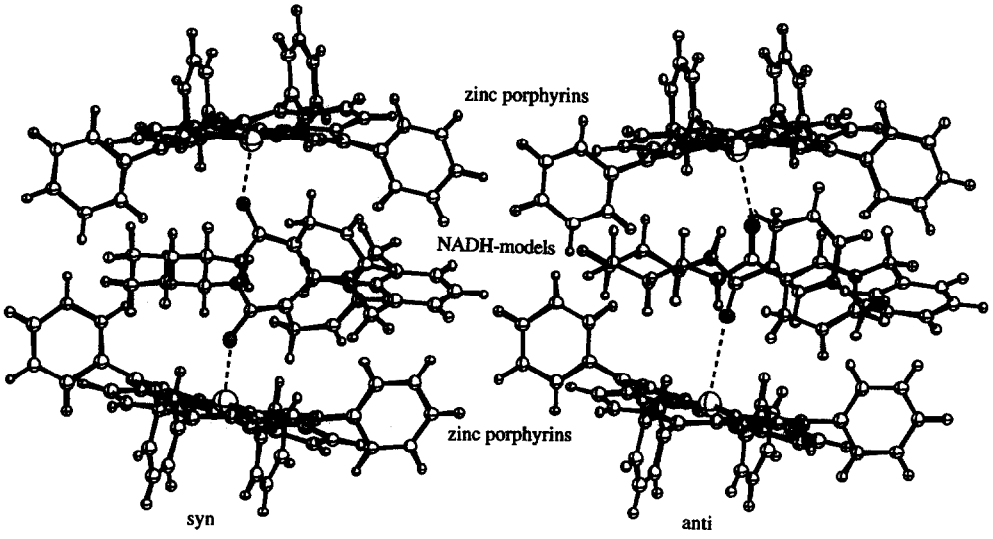


Fig. 3. Proposed structure of the complex between **1** with *syn* and *anti* orientations of the amide groups and two zinc porphyrins, **4**.

Further support for the conclusion that the *anti* orientation of the amide groups is the preferred one has been obtained from ^{1}H-NMR experiments and NOE measurements as well as by quantum chemical calculations of the best ground state conformation by AM1 and PM3 methods. The NOE effect from the NH-hydrogen is largest on the H_{4S} hydrogen and smaller on the H_{4R} hydrogen and negligible on the H_2 hydrogen. The AM1 and PM3 calculations both predict that the *anti* conformation is the preferred one. The *syn* conformation has roughly one kcal mol^{-1} higher energy. No solvent molecules have been included in the calculations and the two amide groups are located close together in an antiparallel way. However, the calculated energy difference is too small to be conclusive although it is fully consistent with the recent, more elaborate, calculations on protonated nicotinamide and 1,4-dihydronicotinamide by Wu and Houk.[3] The macrocycle **3** with the sterically demanding binaphthyl groups does not form a complex with zinc porphyrin under our experimental conditions. This can only be due to steric hindrance which is apparent on inspection of CPK-models.

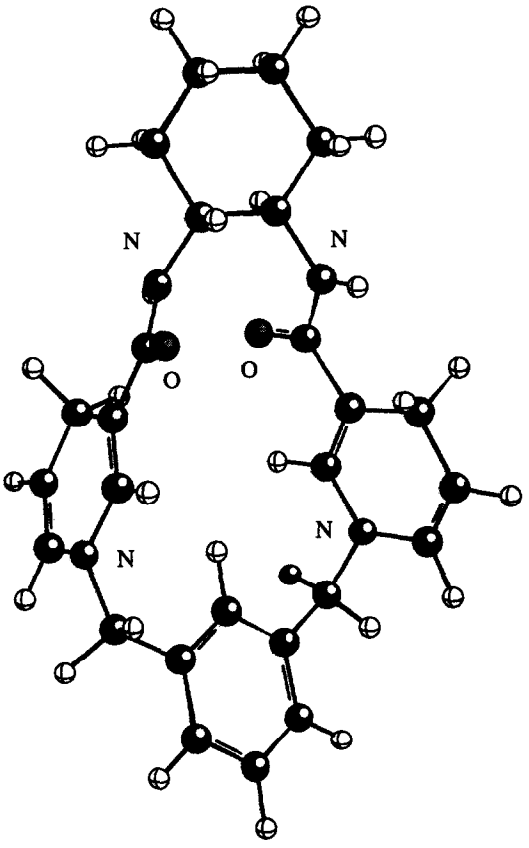


Fig. 4. The conformation of **1** as derived from ^{1}H-NMR-data; spin coupling constants, shift reagents, NOE measurements and AM1 and PM3 calculations.

Knowing the conformation in solution of our model compound **1** with zinc coordinated to the oxygen, the reactive hydrogen, H_{4S}, and the absolute stereochemistry of our reagent, see Fig. 4, and product, we might speculate about the mechanism of the hydride transfer. In several papers the dual function of the co-catalysts magnesium or zinc ions coordinating to both the reagent and the substrate has been suggested. This is a mechanism to create a rather rigid transition state controlled by the metal ion. In our case this is not necessary, maybe not even possible. The reactive hydrogen H_{4S} is closer to the carbonyl group of the other dihydropyridine ring (see Fig. 4).

The differentiation between the two sides of the substrate is done by the different steric requirements for the phenyl group and the methyl ester group in the substrate. The best fit is shown in Fig.5. As a result the dihydropyridine ring and the carbonyl group in the substrate chose an antiparallel approach. Although it is possible to derive at a rather well defined transition state further studies of new model compounds and reactive carbonyl compounds as substrate is indeed necessary before the different mechanistic pathways can be properly evaluated.

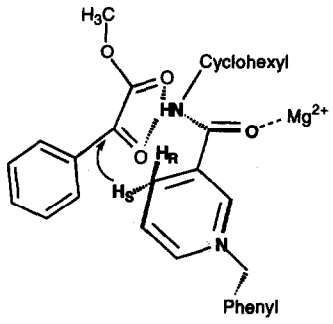


Fig 5. A model of the antiparallel transition state for the asymmetric reduction of methyl phenyl pyruvate to R-mandelate by the R,R-isomer of **1**. Only a part of the reagent is shown for clarity.

As always there are limits to the method and it is possible that the observed conformation in solution is not the reactive one. However, the cyclic nature of our models reduces the number of possible conformations and thus the risk of misinterpretation of the stereochemical conditions for the reaction. Low temperature ^{1}H-NMR studies (in CD_2Cl_2,-80 °C) of our model compound **1** did not reveal any dynamic process with a significant barrier but such processes leading to new reactive conformations cannot be rigorously excluded. According to the ab initio calculations by Wu and Houk as well as the AM1 calculations by Almarsson and Bruice the barrier to rotation around the single bond between the dihydropyridine ring and the carbonyl group is small (5-10 kcal mol^{-1}).[2,5]

Conclusion.

We have found that zinc porphyrin is an excellent shift reagent for two of our three NADH-model compounds. The induced shifts are large and the initially observed signal broadening is supressed when all coordination sites become occupied.

By this method we have been able to confirm that zinc can bind to the amide oxygen[4] and how H_R and H_S, the hydrogens involved in the reduction of carbonyl groups, are oriented in our NADH-models. Although more experimental data must be collected before the detailed knowledge of the structure of the complex allows us to select between different possible transition states for the hydride transfer step in the reduction of an activated carbonyl compound.

Experimental

General information: All solvents were distilled prior to use. Dry N,N-dimethylformamide (DMF) was distilled from calcium hydride. All commercially available reagents were used without further purification. Zinc(II) *meso*-tetraphenylporphyrin (ZnTPP) was made according to the literature.[7]

^{1}H-NMR spectra were recorded on a Varian 400 MHz NMR spectrometer. Mass spectra of the NADH-models were recorded on a VG Auto-Spec mass spectrometer with FAB technique. Melting points were measured using a Mettler FP90 melting point apparatus.

Preparation of the C_2-symmetric N,N'-bis(3-carbonylpyridines).[8]

Standard procedure:
To a cooled solution of the appropriate diamine (88 mmol) in pyridine (250 ml) nicotinoylchloride hydrochloride (185 mmol) was added. After the required reaction conditions (see below) the slightly yellow suspension was evaporated. Water was added and titrated with 10% NaOH (aq) to pH=8 and stirred for two hours. The precipitate was collected by filtration, washed with 10% NaOH (aq) and water.

[R,R]-1,2-N,N'-Bis(aminocarbonylpyridine)-cyclohexane
Reaction conditions; stirred at room temperature for 24 h. Recrystallized in methanol (needles), 77% yield, mp; 268-269°C, ^{1}H-NMR ($CDCl_3$); 1.42(m,4H), 1.84(d,2H), 2.21(d,2H), 4.05(m,2H), 7.25(m,2H), 7.30(br.d,2H), 8.01(d,2H), 8.64(q,2H), 8.97(d,2H). Abs.mass; M= 324.169 m/e (calc. 324.159 m/e). $[\alpha]_D^{20}$ -130°±1° (c 0.3 in pyridine).

[R,R]-1,2-N,N'-Bis(aminocarbonylpyridine)-1,2-diphenylethane
Reaction conditions; refluxed for 16 h. Recrystallized in methanol (needles), 80% yield. mp; 287-288°C, ^{1}H-NMR ($CDCl_3$); 5.62(d,2H), 7.26(s,10H), 7.33(q,2H), 7.60(br.s,2H), 8.04(d,2H), 8.69(d,2H), 8.97(s,2H). Abs.mass; M= 422.196 m/e (calc. 422.174 m/e).$[\alpha]_D^{20}$ -124°±1° (c 0.2 in pyridine).

2,2'-N,N'-Bis(aminocarbonylpyridine)-[R]-1,1'-binaphthyl
Reaction conditions; refluxed for 5 h. Purified by column chromatography (SiO-gel, eluent; EtOAc:EtOH, gradient 9:1 to 1:1), TLC(EtOAc:EtOH, 1:1); R_f=0.7, 58% yield. ^{1}H-NMR ($CDCl_3$); 7.21(q,2H), 7.25(d,2H), 7.36(tr,2H), 7.51(tr,2H), 7.66(m,2H), 7.91(s,2H), 7.99(d,2H), 8.11(d,2H), 8.37(d,2H), 8.57(q,2H). Abs.mass; M= 494.178 m/e (calc. 494.174 m/e). $[\alpha]_D^{20}$+52°±1° (c 0.2 in pyridine).

Preparation of the C_2-symmetric (R,R)-1,4-Dihydronicotinamides (1-3).[8]

Standard procedure;
To a dry DMF solution of the appropriate N,N'-Bis-(3-carbonylpyridine) (1 mmol / 50 ml solvent) 1,3-bisbromomethylbenzene was added. The mixture was stirred under nitrogen at 80-90°C over night (>12 h). The mixture was cooled and ether (200 ml / 1 mmol) was added. The precipitate was collected by filtration and washed with ether. This crude cyclic bispyridinium salt was then reduced without prior work-up.
To an aqueous solution (10ml) of sodium dithionite (5 mmol) and sodium carbonate (5 mmol), an aqueous solution (10 ml) (for the bispyridinium salt of **2** methanol must be added) of the bispyridinium salt was added dropwise during 1 h at room temperature under nitrogen. The solution became turbid after a few minutes. After an additional hour, 25 ml of water was added. The yellow precipitate was collected by filtration. The crude products **1-3** were purified by gel chromatography (Sephadex®, eluent; MeOH). Yield **1** 74%, **2** 16%, **3** 8%. ^{1}H-NMR ($CDCl_3$); **1**; 1.34(s,4H), 1.80(s,4H), 1.92(d,2H), 2.99(q,2H), 3.22(d,2H), 3.84(br.s,2H), 4.17(d,2H), 4.41(d,2H), 4.70(m,2H), 5.24(d,2H), 5.59(q,2H), 7.04(d,2H), 7.18(s,2H), 7.26(tr,1H), 7.35(s,1H) **2**; 3.03(q,2H), 3.30(d,2H), 4.21(d,2H), 4.44(d,2H), 4.76(m,2H), 5.49(q,2H), 5.60(d,2H), 5.89(q,2H), 7.07(d,2H), 7.21(s,10H), 7.26(tr,1H), 7.42(s,1H) **3**; 2.57(d,2H), 2.68(d,2H), 4.19(d,2H), 4.43(d,2H), 4.60(m,2H), 5.61(d2,H), 6.91(d,2H), 6.97(s,2H), 7.04(d,2H), 7.20(tr,2H), 7.25(s,1H hidden under $CHCl_3$) 7.26(tr,1H), 7.40(tr,2H), 7.91(d,2H), 8.03(d,2H), 8.86(d,2H). Abs. mass; **1**; M= 430.237 m/e (calc. 430.237 m/e), **2**; M+1=529.260 m/e (calc. 529.261 m/e), **3**; M= 600.248 m/e (calc. 600.253 m/e) .

Complexation studies. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian 400 MHz spectrometer with TMS as an internal standard. Small portions of ZnTPP were added to a 0.02 M solution of the different NADH-model compounds. The induced shifts for the hydrogens in the NADH-models were measured after each addition.

Acknowledgements
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