Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3027–3040

TETRAHEDRON: *ASYMMETRY*

Stereoselective reductions with macrocyclic NADH models

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Received 19 June 2000; accepted 27 June 2000

Abstract

Macrocyclic NADH models with two (C_2 symmetry) or four (D_2 symmetry) nicotinamide units comprised in a ring have been prepared and found to reduce activated carbonyl compounds in good yields and high enantiomeric excess. The roles of magnesium ions as a cocatalyst and the temperature have also been investigated. The smaller, C_2-symmetric macrocycle gave 96% ee upon reduction of ethyl benzoylformate whereas the best result with the larger D_2-symmetric model was 81% ee for the reduction of methyl benzoylformate.

1. Introduction

Nicotinamide adenine dinucleotide (NAD^+/NADH) is a coenzyme which takes part in many biological oxidation–reduction reactions,[1] such as the conversion of ketones and aldehydes to alcohols and vice versa. These reactions are catalyzed by enzymes commonly named dehydrogenases. The reduction of ketones has been of major interest when mimicking the chemistry of NAD^+/NADH. The enzymatic reduction of a prochiral ketone carried out by NADH proceeds via a selective transfer of one of the two diastereotopic hydrogens in the dihydropyridine ring of NADH. The hydrogen is transferred stereoselectively to the ketone, thus generating a chiral alcohol. Since the first asymmetric reduction using an NADH model reported by Ohno et al. in 1975,[2] there has been a large number of different approaches to NADH mimicking.[3] The general concept is to start with nicotinamide and then modify it, for example, by introducing various chiral auxiliaries in the amide or methyl groups at C-2 and C-4 in the dihydropyridine ring.

The NADH models presented in this report are designed by a supramolecular approach, where the substrate to be reduced is bound into a hydrophobic pocket of the model. The models comprise a chiral cavity as a substrate binding site to keep the substrate in a fixed orientation during the reaction, which should give good control of the stereoselectivity. Several nicotinamide units are incorporated in the macrocyclic framework which creates this hydrophobic pocket.

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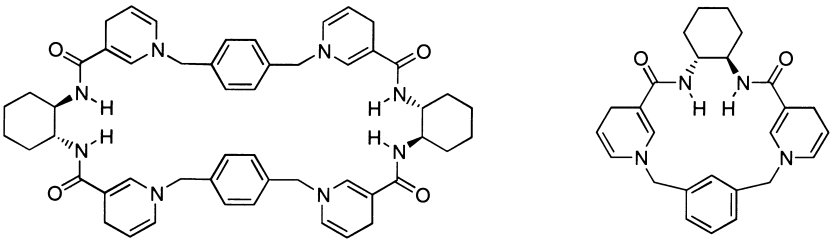
0957-4166/00/$ - see front matter
PII: S0957-4166(00)00248-2

Using nicotinamide as part of a macrocycle gives a versatile host molecule that is soluble in polar solvents when the nicotinamide is oxidized (cationic form) and soluble in apolar solvents when reduced. The nicotinamide units can also function as reducing reagents and possibly as parts of a bioorganic catalyst.

Four nicotinamide units are incorporated in model compound **1H$_4$** and two units in compound **2H$_2$** (Fig. 1), thus creating multicenter redox systems. Metal multicenter systems are often important as redox catalysts, while organic multicenter systems have attracted less attention. In fact, to our knowledge there are only two other groups that have used a multicenter system for NADH mimicking, but their models were not designed for asymmetric reduction of carbonyl substrates.[4,5] Symmetry is important in models of this kind. The symmetry is needed to ensure a uniform environment around the redox centers, which is crucial for the prediction and analysis of the stereochemical outcome of a reduction. In addition, the symmetry and the cyclic structure restrict the number of conformations, which should also simplify the analyses. We have previously reported the synthesis of the C_2-symmetric model **2H$_2$**[6] and a preliminary result of an asymmetric reduction of methyl benzoylformate using the same compound[7] and also a preliminary report on the synthesis of the D_2 model **1H$_4$**.[8] We now present a more thorough study of their syntheses and their ability to reduce prochiral carbonyl substrates stereoselectively.



1H$_4$ **2H$_2$**

Figure 1. The two NADH model compounds, one with D_2 symmetry (**1H$_4$**) and one with C_2 symmetry (**2H$_2$**)

2. Results and discussion

2.1. Synthesis

The building block technique has been used for the synthesis of the NADH models, i.e. the models were constructed from different molecular subunits, namely a chiral subunit, a nicotinamide unit and a spacer. A C_2-symmetric diamine seemed to be a good choice as a chiral subunit and *trans*-1,2-diaminocyclohexane, whose efficiency in asymmetric syntheses and molecular recognition is well-documented,[9] turned out to be best.[6] The relative rigidity of the cyclic diamine reduces the flexibility of the macrocyclic product. The nicotinamide unit is the reactive part as it functions as a hydride acceptor or donor, but it also functions as a wall of the cavity. The spacers make up the center of the cavity and should have hydrophobic properties. The spacers used in our models originated from different bis(bromomethyl)arene building blocks (Fig. 2). The C_2-symmetric NADH models were synthesized in three steps according to Scheme 1 (exemplified by the synthesis of **2H$_2$**).

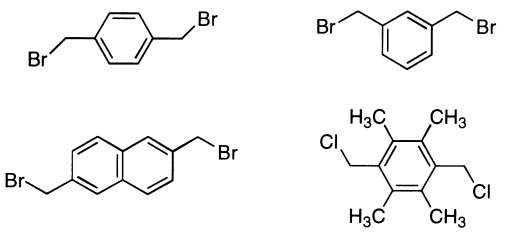
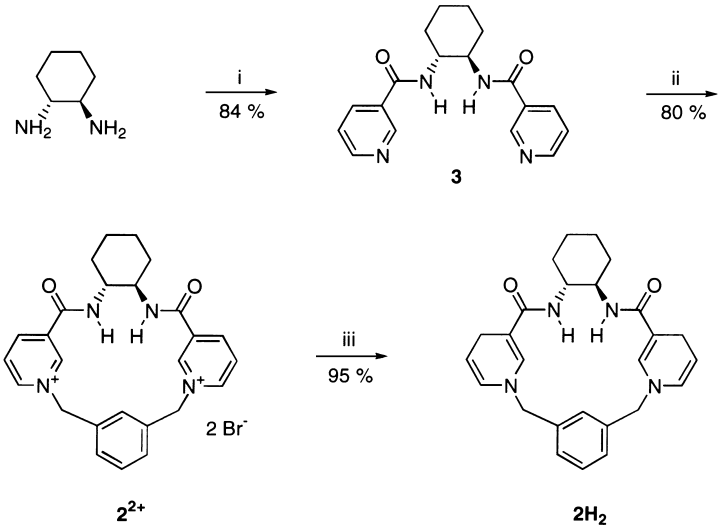


Figure 2. Different building blocks that were incorporated as spacers in macrocyclic NADH models using the same synthetic route



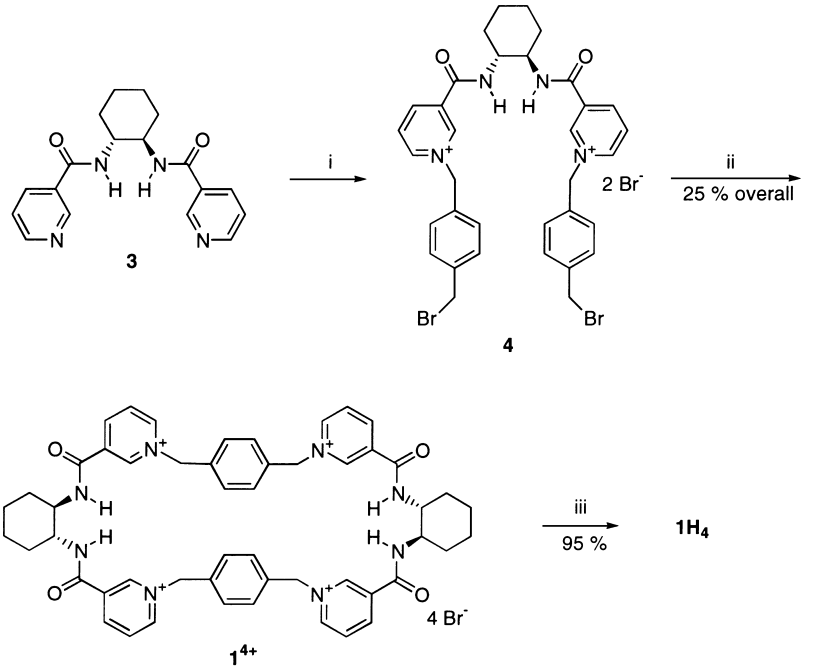
Scheme 1. Reagents and conditions: (i) nicotinoyl chloride hydrochloride, pyridine, rt; (ii) 1,3-bis(bromomethyl)-benzene, DMF, 85°C; (iii) $Na_2S_2O_4/Na_2CO_3$, H_2O, rt

R,*R*-1,2-Diaminocyclohexane was used in the first step as the original building block and chiral source. The resulting cyclic bispyridinium compounds from the second step can be reduced as crude products. The reduced NADH models were then purified by size-exclusion chromatography (Sephadex LH-20) with methanol as an eluent. An alternative way is to purify the bispyridinium compounds prior to reduction. Sodium dithionite is a mild and effective reducing agent with very good regioselectivity. It reduces pyridinium rings exclusively at the *para*-position. This means that in practice no further purification is needed after the reduction. The latter method is more time-consuming but on the other hand it leads to easier analyses and handling of the compounds. Generally, the reduced forms are much more sensitive and labile than are the oxidized forms.

The above-described synthetic route was also used for other spacers such as 1,4-bis-(bromomethyl)benzene, 1,4-bis(chloromethyl)-2,3,5,6-tetramethylbenzene and 1,5-dibromopentane. The first two spacers were linked at the *para*-position of the aromatic ring and resulted in lower yields. The products were also more difficult to purify than $\mathbf{2H_2}$. The *meta*-phenylene spacer (used in $\mathbf{2H_2}$) seemed to be best suited for the incorporation into the macrocyclic framework. The synthesis using 1,5-dibromopentane failed to give a good yield of a defined product, probably because the pentyl chain is too flexible.

Compound **2H$_2$** seemed to be the most promising of the C_2-symmetric NADH models and its host–guest properties and reduction properties were then further studied.

The D_2-symmetric NADH models were synthesized in a similar way. The first step was identical, generating the same building block, **3**, with the same configuration (*R*,*R*). The rest of the synthetic route is shown in Scheme 2, illustrated by the synthesis of **1H$_4$**.



Scheme 2. Reagents and conditions: (i) 1,4-bis(bromomethyl)benzene (5 equiv.), DMF, 85°C; (ii) **3**, DMF, 85°C; (iii) $Na_2S_2O_4/Na_2CO_3$, H_2O, rt

The second step was carried out with an excess of the bis(bromomethyl)arene building block and at high concentration, so that undesired side reactions such as polymerization and ring closure were minimized. The crude product was used in the next step because of difficulties in purifying **4**. In the third step, **4** was ring-closed with another unit of **3**. The purification of the resulting **1^{4+}** is worth some comments. Size-exclusion chromatography did not work satisfactorily, due to the complex mixture of products. However, Geuder et al. have used a mixture of methanol and aqueous ammonium bromide to purify macrocyclic polypyridinium ions on silica gel.[10] This system worked well for the purification of **1^{4+}** too, after a slight modification. Size-exclusion chromatography was then used to separate **1^{4+}** from ammonium bromide. However, one of the byproducts formed in the third step was not completely eliminated by this method. We found that dimethylamine present in the DMF reacted with **4** to form a different macrocycle† with similar chromatographic properties as **1^{4+}**. The rather simple solution to this problem was to use amine-free DMF in steps 2 and 3.‡ Finally, **1^{4+}** was reduced to **1H$_4$** using sodium dithionite.

† The macrocycle, with **4** cyclized with dimethylamine instead of **3**, was characterized by ^{1}H NMR spectroscopy and mass spectrometry (electrospray ionization).

‡ It is important to keep the DMF under argon.

Three other compounds were also synthesized according to Scheme 2: two D_2-symmetric models with a *meta*-phenylene spacer ($\mathbf{7}^{4+}$) and a 2,6-naphtylene spacer ($\mathbf{6}^{4+}$), respectively; the third (compound $\mathbf{5}^{4+}$) was an achiral stereoisomer of $\mathbf{1}^{4+}$ that had (*R*,*R*)-configuration at one end and (*S*,*S*)-configuration of the diaminocyclohexane at the other. Host–guest properties of all these four compounds were studied,[11] but only $\mathbf{1H_4}$ was used as a reducing agent. Since the host–guest properties were studied in water with the models in their oxidized states, the final reduction has not been performed for $\mathbf{5}^{4+}$, $\mathbf{6}^{4+}$ and $\mathbf{7}^{4+}$.

The oxidized tetrapyridinium macrocycles are very stable both in the solid state and in solution, even in acidic, aqueous solutions. However, they decompose in basic solutions. Compound $\mathbf{1H_4}$, the reduced form, is much more unstable and must be kept under an inert atmosphere and stored in the dark.

The NMR spectra of $\mathbf{1H_4}$ and $\mathbf{2H_2}$, which show relatively few signals due to the high symmetry, are easy to interpret, but the chemical shifts are concentration- and solvent-dependent. The NMR spectra of the two models are similar. To determine the sizes of the macrocycles we used mass spectrometry, where both FAB and electrospray (ES) can be used to analyze the polypyridinium salts.

2.2. Reductions

The two macrocycles $\mathbf{1H_4}$ and $\mathbf{2H_2}$ were studied as reducing agents. The reductions described in this section were carried out in organic solvents, which means that all of the nicotinamide subunits contained in the models must be in the reduced form to ensure solubility. Generally, reductions using NADH models are carried out in acetonitrile, but since $\mathbf{2H_2}$ and $\mathbf{1H_4}$ are not soluble in acetonitrile the reductions were carried out in methylene chloride and chloroform. Magnesium is the most commonly used cocatalyst.[3] Alcohol dehydrogenases have a zinc ion in their active sites.[12] Magnesium and zinc ions are about equally effective in biomimetic systems, and also in the system presented here. Other metal ions such as nickel, cobalt and cadmium have been used, but they are not as effective.[13] Methyl benzoylformate is often used as a substrate in NADH mimicking reductions, because of the resemblance to pyruvate. α-Keto acids in general are common substrates in enzymatic reductions,[14] where the reduction of pyruvate to lactate catalyzed by lactate dehydrogenase is one of the most well-known reactions. Other activated carbonyl compounds as well as activated imines have also been tested in NADH modeling systems.

The emphasis on this reduction study has been on compound $\mathbf{1H_4}$. A few experiments have been made with $\mathbf{2H_2}$ for comparison. Different substrates have been reduced (Fig. 3) and the influences of magnesium ion concentration and temperature on the stereoselectivity and reaction rate have been studied.

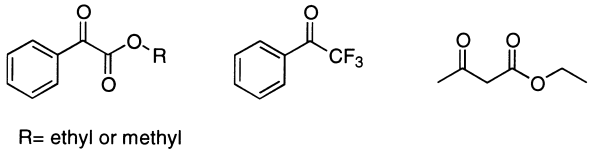


Figure 3. Examples of substrates used in reductions: alkyl benzoylformate, α,α,α-trifluoroacetophenone and ethyl acetoacetate

2.2.1. Reductions with $1H_4$

The influence of magnesium ion concentration. A series of reductions of methyl benzoylformate and α,α,α-trifluoroacetophenone using **$1H_4$** were carried out in methylene chloride with varying concentrations of magnesium. The results are summarized in Table 1.

Table 1
Reductions of methyl benzoylformate and α,α,α-trifluoroacetophenone using **$1H_4$** with varying magnesium concentrations[a]

entry	substrate	ratio[b] $Mg^{2+}/1H_4$	e.e. (%)[c]	yield (%)[d]
1	methyl benzoylformate	0	-	no reaction
2	-"-	0.5	62	22 (3 days)
3	-"-	1.25	54	100 (1 day)
4	-"-	2	33	100 (16 hours)
5	-"-	4	30	100 (4.5 hours)
6	α,α,α-trifluoroacetophenone	0	11	53 (7 days)[e]
7	-"-	2	12	68 (7 days)[e]
8	-"-	4	11	100 (7 days)[e]

[a] The reactions were performed in methylene chloride at room temperature. [b] Magnesium was added in the form of $Mg(ClO_4)_2$. [c] Determined via capillary gas chromatography. The *R*-isomer was in enantiomeric excess for all entries. [d] Determined via capillary gas chromatography; yields are relative to consumed starting material. [e] Determined after quenching the reaction.

In the absence of magnesium ions, **$1H_4$** was able to reduce α,α,α-trifluoroacetophenone, a rather reactive ketone, but not methyl benzoylformate. With 0.5 equivalents of magnesium ions (relative to **$1H_4$**), 22% of the formate was converted within 3 days, whereas all of the formate was converted to the resulting mandelate within 4.5 hours when 4 equivalents were used. On addition to magnesium ions the rate increases significantly more than linearly with the magnesium concentration. In contrast, the presence of metal ions has little effect on the reduction of α,α,α-trifluoroacetophenone. Only a slight increase of the reaction rate was observed. The stereoselectivity of this reduction was poor, only a 12% enantiomeric excess of the *R*-isomer. The reduction without magnesium ions present gave the same enantiomeric excess, in contrast to Ohno's model,[2] where the reduction of the same substrate gave 16% enantiomeric excess in the presence of magnesium ions and a racemic product without magnesium ions. On the other hand, the stereoselectivity of the reduction of methyl benzoylformate was strongly dependent on the amount of metal ions present. The reduction gave *R*-mandelate and the enantiomeric excess dropped from 62% with 0.5 equivalents to 30% with 4 equivalents of magnesium ions.

The comparatively large difference in reactivity between the two substrates when magnesium ions are present needs an explanation. It seems as if the role of the metal ion is not only to act as a Lewis acid, but also to form a ternary complex keeping the reagent and the substrate together. Methyl benzoylformate has three oxygen atoms that can coordinate to a magnesium ion (Fig. 3). The oxygen of the α-keto group and one of the other two oxygens can simultaneously coordinate to a magnesium ion, thus forming a five-membered cyclic complex. In a ternary complex between methyl benzoylformate and **$1H_4$**, where two atoms of the substrate and at least one atom of the reducing agent **$1H_4$** (probably the carboxamide oxygen[15]) are coordinated to magnesium, the conditions for a high enantioselectivity should be present. On the other hand, α,α,α-trifluoroacetophenone has only

one weak donor atom that can coordinate to a metal ion and should not be able to form a similar complex with the same stability and well-defined structure. However, the large catalytic effect of magnesium ions on the reduction of methyl benzoylformate shown in Table 1 cannot be rationalized by the formation of one complex only. The drop in enantiomeric excess from 54 to 33% and the significant increase in reaction rate when the amount of magnesium is changed from 1.25 equivalents to 2 equivalents indicate that there is a fast, less enantioselective, process competing with a slower and more selective reaction at higher magnesium ion concentrations. The fast process could involve magnesium ions bound both to the substrate and the reagent which react in a bimolecular process. The rate of such a reaction involving two magnesium ions should depend on $[Mg^{2+}]^2$. Alternatively, the fast process could be caused by coordination of several metal ions to the model compound **1H$_4$**. The carbonyl oxygen of the nicotinamide units coordinates strongly to magnesium ions.[15] Since **1H$_4$** has four nicotinamide units there is also the possibility that two or more metal ions can be coordinated at the same time, which could cause a change of conformation due to electrostatic repulsion. This new conformation would then be more reactive but less enantioselective.

Temperature dependence. Reductions were also performed where the substrates were added at –30°C to a solution of **1H$_4$** and magnesium perchlorate. The reaction mixture was then allowed to slowly reach room temperature (about 15–20 hours). The results are summarized in Table 2.

Table 2
Reductions carried out at low temperature

entry	substrate	ratio[b] Mg^{2+}/**1H$_4$**	e.e. (%)[c]	reaction temp.
1	methyl benzoylformate	1.25	81	-30°C→r.t.
2	-"-	4	15	-"-
3	α,α,α-trifluoroacetophenone	-	12	-"-
4	-"-	1.25	1	-"-

Footnote b and c, see Table 1.

The results from the limited number of experiments are consistent with the assumption that more than one process might be involved. The dominating mechanism is governed by the metal ion concentration and temperature. A slow enantioselective reaction within a ternary complex between the substrate, a magnesium ion and the reagent dominates at low temperature and low magnesium ion concentrations, whereas a faster, less selective process dominates at higher temperatures and magnesium ion concentrations. This is valid for the reaction with methyl benzoylformate. A noteworthy detail is that the enantiomeric excess is higher at low temperatures when 1.25 equivalents of magnesium is used, whereas the ee is lower at low temperatures when 4 equivalents are used compared to reductions performed at room temperature.

The reaction with α,α,α-trifluoroacetophenone is assumed to be more straightforward, occurring between the substrate and the reagent in a bimolecular process. The coordination of magnesium to the reagent slightly increases the reaction rate for this not very enantioselective reaction.

*Other reductions with **1H$_4$** as the reducing agent*. The results presented in Tables 1 and 2 are from reductions of methyl benzoylformate and α,α,α-trifluoroacetophenone that were carried out

in methylene chloride. Compound **1H$_4$** was also tested for other substrates and the reductions of methyl benzoylformate and α,α,α-trifluoroacetophenone were tested in chloroform.

Compound **1H$_4$** was not completely soluble in methylene chloride and magnesium perchlorate was hardly soluble at all. However, when **1H$_4$** and the metal salt were added together in methylene chloride, a heterogeneous mixture was formed that was reactive. Compound **1H$_4$** seemed to be completely soluble in chloroform but no reaction took place (Table 3, entries 1 and 2).

Table 3
Other reductions using **1H$_4$**[a]

entry	substrate	ratio[b] Mg^{2+}/**1H$_4$**	e.e. (%)[c]	yield (%)[d]
1[e]	α,α,α-trifluoroacetophenone	1.25	-	no reaction
2[e]	methyl benzoylformate	1.25	-	-"-
3	-"- (4 eq.)	4	11	55 (1 day)
4	ethyl benzoylformate	4	21	100 (3 hours)
5	ethyl acetoacetate	4	2	10[f]-25[g] (3 days)
6	acetophenone	4	-	no reaction

Footnote a, b, c and d, see Table 1. [e] Performed in chloroform. [f] Determined via ^{1}H-NMR spectroscopy after work-up. [g] Determined via capillary gas chromatography after quenching the reaction.

Since **1H$_4$** has four nicotinamide units capable of transferring a hydride ion, it was of interest to investigate how many of these four hydride ions could be transferred. With 4 equivalents of both magnesium ions and methyl benzoylformate, compound **1H$_4$** converted about 2 equivalents of the substrate (Table 3, entry 3). This means that an average of two out of the four hydrogens were transferred.

The reactivity of **1H$_4$** towards ethyl benzoylformate was also tested (Table 3, entry 4). The effect on the reactivity and selectivity as compared to that towards methyl benzoylformate was only marginal (see entry 5, Table 1). Compound **1H$_4$** was not able to reduce acetophenone (Table 3, entry 6). However, the reduction of ethyl acetoacetate proceeded at a detectable rate (Table 3, entry 5).

*2.2.2. Reductions with **2H$_2$***

Some selected reductions were carried out for the smaller NADH model **2H$_2$**. The results are summarized in Table 4.

Generally, reductions using **2H$_2$** were more stereoselective than reductions using **1H$_4$**. The same enantiomer (*R*-form) of the resulting mandelate was obtained in as high as 92% enantiomeric excess upon reduction of methyl benzoylformate. The reduction of ethyl benzoylformate gave an even higher ee (96%). As in the case of **1H$_4$**, the reduction of α,α,α-trifluoroacetophenone was not as selective as the reduction of esters of benzoylformic acid. The reason is probably the same, namely that the reaction occurred in a bimolecular process in contrast to the reduction of the esters of benzoylformic acid, which was assumed to proceed via a ternary complex between the substrate, a metal ion and the reagent **2H$_2$**. In contrast to **1H$_4$**, the stereoselectivity was not affected by an increase of magnesium ion concentration (entries 2 and 3). A lowering of the temperature did not considerably affect the stereochemical outcome of a reduction either.[7] The reason for these differences could be that a fast, less selective, bimolecular reaction does not take

Table 4
Reductions of different substrates by $\mathbf{2H_2}$[a]

entry	substrate	ratio[b] $Mg^{2+}/\mathbf{2H_2}$	e.e. (%)[c]	yield (%)[d]
1	methyl benzoylformate	1.25	92	90 (16 hours)
2	-"-	2	90	100 (5 hours)
3	-"-	4	89	100 (3 hours)
4	ethyl benzoylformate	-"-	96	97 (16 hours)
5	α,α,α-trifluoroacetophenone	-"-	62	45 (16 hours)
6	methyl ethyl ketone	-"-	-	no reaction
7	benzophenone	-"-	-	-"-
8	acetophenone	-"-	-	-"-

Footnote a, b, c and d, see Table 1.

place in the case of $\mathbf{2H_2}$. Another explanation could be that the macrocyclic framework of $\mathbf{2H_2}$ is more rigid than that of $\mathbf{1H_4}$, which entails that coordination of more than one metal ion to $\mathbf{2H_2}$ does not cause a conformational change leading to a less selective reaction.

2.3. A comparison of the two NADH models

It now seems appropriate to compare the two NADH models $\mathbf{1H_4}$ and $\mathbf{2H_2}$ in more detail. The ^{1}H NMR spectra of the two compounds are very similar. However, the signals for the two diastereotopic hydrogens on the dihydropyridine ring, H-4 and H-4′, in $\mathbf{2H_2}$ are well separated and show different coupling patterns with one hydrogen in an *axial* position and the other in an *equatorial* position. From an analysis of the coupling constants shown in Fig. 4, H-4′ was assigned as the *axial* hydrogen and H-4 as the *equatorial* one. From deuterium marking experiments[7] it was found that H-4′ is the reactive hydrogen, consistent with theoretical calculations.[16] However, the spectrum of $\mathbf{1H_4}$ shows that the two diastereotopic hydrogens have almost exactly the same shift. An attempt to 'freeze out' a conformation wherein the signals for the two hydrogens are well separated failed.

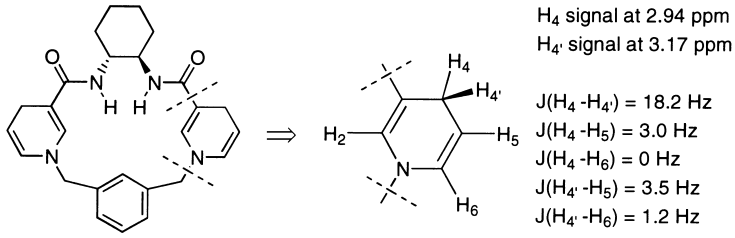


Figure 4. The coupling patterns for the hydrogens at position 4 on the dihydropyridine ring of $\mathbf{2H_2}$

In order to determine the orientation of the carboxamide group (*trans* or *cis* to C-2 of the dihydropyridine ring) zinc tetraphenylporphyrin (ZnTPP) was used as a shift reagent. ZnTPP is particularly attractive as a shift reagent in this case since zinc is a cocatalyst for NADH-mediated reductions. It should therefore be possible to determine the coordination site for the metal ion in the NADH models. The aromatic ring current of the porphyrin ring is large and well-defined and

causes a considerable shift change for protons that are close to the porphyrin ring in a complex. Fukuzumi et al. formed a complex between 1-benzyl-1,4-dihydronicotine amide (BNAH), a simple NADH model, and ZnTPP.[17] Based on the observed shift changes it was suggested that the zinc was coordinated to the carboxamide oxygen, which is consistent with the results of other groups.

When ZnTPP was added to $\mathbf{2H_2}$, dramatic ^{1}H NMR upfield shift changes were observed. The effects were not as large upon addition to $\mathbf{1H_4}$. The shift changes induced by ZnTPP are summarized in Fig. 5, where the shift changes of the different protons are shown as a percentage of the largest shift change.

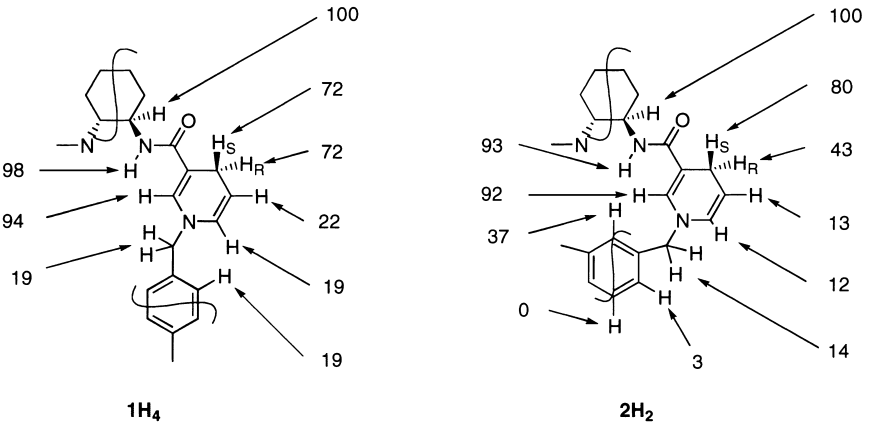


Figure 5. A comparison of the relative induced shift changes for the two NADH models upon addition of ZnTPP (percentage of maximum induced shift)

The relative induced shifts are very similar for the two macrocycles and indicate that the zinc ion is coordinated to the carboxamide oxygen and that the carboxamide group is oriented *cis* to C-2 of the dihydropyridine ring in both cases. All NMR data show that the two compounds have similar conformations. They give the same dominant enantiomers with the same prochiral substrates. However, the smaller macrocycle $\mathbf{2H_2}$ was more enantioselective upon reduction of esters of benzoylformic acid due to the absence of a competing, less selective process. Efforts have been made in order to elucidate the reason for the difference in mechanism between the two model compounds in high magnesium ion concentrations, but so far they have not led to any conclusive evidence for one or the other of the two explanations. The reduction properties of $\mathbf{1H_4}$ have been investigated more thoroughly and the reason is the potential use of $\mathbf{1H_4}$ as a water-soluble reducing agent. The cavity of $\mathbf{1H_4}$ is large enough to encapsulate a substrate whereas the cavity of $\mathbf{2H_2}$ is too small. However, in an apolar solvent such as methylene chloride attractive forces that a hydrophobic cavity can offer will not be strong enough to drive a substrate into the cavity. On the contrary, it is more likely that the substrates react on the outside of the model in apolar solvents, which is probably the case for the reactions presented here. The next step in our research is to use the same or similar macrocyclic structure to design a water-soluble model compound able to stereoselectively reduce substrates encapsulated in a hydrophobic cavity. The results of $\mathbf{1H_4}$ are promising enough to use this compound as the basis of the design for a water-soluble NADH model.

3. Experimental

Materials. The reactions were carried out with oven-dried equipment. Methylene chloride was dried by distillation from calcium hydride under nitrogen. Chloroform was dried by passage through a column of basic alumina (Merck, aluminum oxide 60 active, grade I; 10 g per 14 mL of solvent). Pyridine was distilled prior to use. DMF was purchased from Fluka (absolute, ≥99.5%) and was stored under argon. Commercially available chemicals were used without further purification, if nothing else is mentioned.

Methods. Thin-layer chromatography was performed on silica gel plates (Merck, silica gel 60 F_{254}). Column chromatography was performed using silica gel (Matrex, LC 60 Å/35–70 μm). Size-exclusion chromatography was performed using Sephadex LH-20 in methanol. ^{1}H NMR spectra were recorded at 293 K on a Varian UNITY-400 NMR spectrometer at 400 MHz with DMSO-d_6 or $CDCl_3$ with tetramethylsilane as internal standard as solvents. IR spectra were recorded on an FT-IR instrument, Perkin–Elmer 1600. Mass spectra were recorded on a VG ZabSpec instrument. Positive FAB-MS (fast atom bombardment-mass spectrometry) with thioglycerol/glycerol as the liquid matrix and positive ESI-MS (electrospray ionization-mass spectrometry) were the methods used. Capillary gas chromatography was performed using a Varian 3300 gas chromatograph equipped with a methyl silicone fused silica column (column i.d. 0.32 mm, column length 30 m). Optical rotations were measured on a Perkin–Elmer 241 polarimeter using a 1 dm cell with a total volume of 1 mL. The enantiomeric excess was measured using a Hewlett–Packard 5890 gas chromatograph equipped with a β-cyclodextrin based column (Chrompack, CP-Chirasil-dex CB, column i.d. 0.25 mm, column length 25 m).

3.1. Synthesis of bispyridine building block **3**

Nicotinoyl chloride hydrochloride (18.3 g, 103 mmol) was added at 0°C to a solution of (1*R*,2*R*)-(–)-1,2-diaminocyclohexane (5.0 g, 44 mmol) in pyridine (125 mL). The solution was stirred under argon at room temperature for 2 days. The pyridine was evaporated and 75 mL of water was added to the light yellow residue. The pH value was adjusted to pH 9 by NaOH (10% aq.) and the solution was cooled to 0°C. The white crystals formed were filtered off by suction and washed with cold water. Yield 12.5 g (88%). Recrystallization from methanol gave white needles, mp: 269.5–270.5°C; $[\alpha]_D^{20} = -131 \pm 1$ (*c* 3.4 mg/mL; pyridine). ^{1}H NMR (DMSO-d_6): δ 1.31 (m, broad, 2H), 1.53 (m, broad, 2H), 1.75 (m, broad, 2H), 1.88 (d, broad, 2H), 3.95 (m, broad, 2H), 7.42 (dd, $J = 5$ and 8 Hz, 2H), 7.99 (dtr, $J = 2$ and 8 Hz, 1H), 8.52 (d, $J = 8$ Hz, 2H), 8.61 (dd, $J = 1.6$ and 5 Hz, 2H), 8.82 (s, 2H).

3.2. Synthesis of macrocyclic bispyridinium compound $\mathbf{2}^{2+}$

A sample of **3** (648 mg, 2 mmol) and 1,3-bis(bromomethyl)benzene (528 mg, 2 mmol) were dissolved in 100 mL of DMF under argon. The solution was heated to 85°C and stirred under argon overnight. The mixture was cooled and poured into 400 mL of diethyl ether. After stirring for 20 min the precipitate formed was filtered off by suction and washed with diethyl ether (3×50 mL). The crude product was purified by flash chromatography (silica gel, methanol:NH_4Br (25% aq.), 4:1) followed by size-exclusion chromatography, giving 940 mg (80% yield) of a white powder. $[\alpha]_D^{20} = -90 \pm 2$ (*c* 3.2 mg/mL; MeOH). FAB-MS: $[M-Br]^+$ 507.140 (calculated 507.140). ^{1}H NMR (DMSO-d_6): δ 1.35 (m, broad, 2H), 1.67 (m, broad, 2H), 1.80 (m, broad, 2H), 1.95 (d,

broad, 2H), 3.92 (m, broad, 2H), 5.94 (s, 4H), 7.01 (s, 1H), 7.56 (tr, $J=8$ Hz, 1H), 7.69 (d, $J=8$ Hz, 2H), 8.40 (dd, $J=6$ and 8 Hz, 2H), 8.90 (d, $J=8$ Hz, 2H), 9.04 (d, $J=8$ Hz, 2H), 9.16 (s, 2H), 9.48 (d, $J=6$ Hz, 2H). IR (KBr): 1662 (C=O), 3425 (N–H, amide).

3.3. Reduction of $\mathbf{2}^{2+}$ to $\mathbf{2H_2}$

Sodium dithionite (2.78 g, 16 mmol) and sodium carbonate (1.69 g, 16 mmol) were dissolved in 20 mL of water. An aqueous solution of $\mathbf{2}^{2+}$ (940 mg, 1.6 mmol) was added dropwise and the resulting solution, which turned light red and became viscous within minutes, was stirred at room temperature under argon for 3 hours. Cold water (20 mL) was added and the yellow-red precipitate was filtered off by suction and washed with cold water (3×20 mL). Yield 650 mg (95%) of $\mathbf{2H_2}$. FAB-MS: $[M]^+$ 430.237 (calculated 430.237). ^{1}H NMR ($CDCl_3$): δ 1.34 (s, broad, 4H), 1.78 (s, broad, 2H), 1.92 (m, broad, 2H), 2.99 (dd, $J=3.6$ and 17 Hz, 2H), 3.22 (dm, $J=17$ Hz, 2H), 3.84 (m, broad, 2H), 4.17 (d, $J=16$ Hz, 2H), 4.41 (d, $J=16$ Hz, 2H), 4.70 (m, 2H), 5.24 (d, $J=10$ Hz, 2H), 5.59 (dd, $J=1.2$ and 8 Hz, 2H), 7.04 (d, $J=8$ Hz, 2H) 7.18 (s, 1H) 7.26 (tr, $J=8$ Hz, 1H).

3.4. Synthesis of 4

A sample of **3** (648 mg, 2 mmol) and 1,4-bis(bromomethyl)benzene (2.64 g, 10 mmol) were dissolved in 4 mL of DMF under argon. The solution was heated to 85°C and stirred under argon for 3 hours. The mixture was cooled and poured into 100 mL of chloroform, whereupon 500 mL of diethyl ether was added. The mixture was stirred for 20 min and a white precipitate was filtered off by suction and washed with diethyl ether (3×50 mL) to give the crude product **4**, which was used without purification in the next step.

3.5. Synthesis of macrocyclic tetrapyridinium compound $\mathbf{1}^{4+}$

A sample of **3** (639 mg, 1.97 mmol) and **4** (1.68 mg, 197 mmol) were dissolved in 100 mL of DMF under argon. The solution was heated to 85°C and stirred under argon for 3 hours. The mixture was cooled and poured into 500 mL of diethyl ether. After stirring for 20 min the precipitate was filtered off by suction and washed with diethyl ether (3×50 mL) to give the crude product. Flash chromatography (silica gel, methanol:NH_4Br (25% aq.), 4:1) followed by size-exclusion chromatography gave 587 mg (25% yield) of white crystals. $[\alpha]_D^{20}=-73\pm2$ (c 3.06 mg/mL; MeOH). FAB-MS: $[M-Br^-]^+$ 1093.197 (calculated 1093.198). ^{1}H NMR (DMSO-d_6): δ 1.33 (m, broad, 4H), 1.59 (m, broad, 4H), 1.80 (m, broad, 4H), 1.92 (d, broad, 4H), 3.91 (m, broad, 4H), 5.81 (d, $J=14$ Hz, 4H), 5.90 (d, $J=14$ Hz, 4H), 7.55 (s, 8H), 8.21 (dd, $J=6$ and 8 Hz, 4H), 8.58 (d, $J=8$ Hz, 4H), 9.00 (d, $J=6$ Hz, 4H), 9.26 (d, $J=6$ Hz, 4H), 9.33 (s, 4H). IR (KBr): 1650 (C=O), 3425 (N–H, amide).

3.6. Reduction of $\mathbf{1}^{4+}$ to $\mathbf{1H_4}$

Sodium dithionite (1.74 g, 10 mmol) and sodium carbonate (1.06 g, 10 mmol) were dissolved in 10 mL of water. An aqueous solution of $\mathbf{1}^{4+}$ (585 mg, 0.5 mmol) was added dropwise and the solution, which turned yellow and became turbid within minutes, was stirred at room temperature under argon for 6 hours. Methanol (40–50 mL) was added and the solvents were

removed under reduced pressure. The product was dissolved in chloroform, filtered to remove inorganic salts and concentrated. Yield 420 mg (98%) of **1H$_4$** as a red solid. ^{1}H NMR ($CDCl_3$): δ 1.25 (m, broad, 4H), 1.35 (m, broad, 4H), 1.74 (m, broad, 4H), 2.10 (d, broad, $J=13$ Hz, 4H), 3.05 (m, broad, 8H), 3.70 (m, 4H), 4.15 (d, $J=16$ Hz, 4H), 4.23 (d, $J=16$ Hz, 4H), 4.65 (m, 4H), 5.65 (dd, $J=1.2$ and 8 Hz, 4H), 5.90 (d, $J=7$ Hz, 4H), 7.05 (s, 4H), 7.20 (s, 8H).

3.7. Synthesis of macrocyclic tetrapyridinium compound 5^{4+}

Prepared as described for $\mathbf{1}^{4+}$. In the last step, the *S*,*S*-form of **3** was used. Yield 14% of a white powder. FAB-MS: $[M-3Br^-]^+$ 935.359 (calculated 935.361). ^{1}H NMR (DMSO-d_6): δ 1.35 (m, broad, 4H), 1.59 (m, broad, 4H), 1.80 (m, broad, 4H), 1.94 (d, broad, 4H), 3.96 (m, 4H), 5.84 (d, $J=14$ Hz, 4H), 5.91 (d, $J=14$ Hz, 4H), 7.51 (s, 8H), 8.25 (dd, $J=6$ and 8 Hz, 4H), 8.76 (d, $J=8$ Hz, 4H), 9.07 (d, $J=6$ Hz, 4H), 9.31 (d, $J=6$ Hz, 4H), 9.44 (s, 4H). IR (KBr): 1650 (C=O), 3412 (N–H, amide).

3.8. Synthesis of macrocyclic tetrapyridinium compound 6^{4+}

Prepared from 2,6-bis(bromomethyl)naphthalene as described for $\mathbf{1}^{4+}$. Yield 12% of a white powder. ESI-MS: $[M-2Br^-]^{2+}$ 557.3 (calculated 557.2). ^{1}H NMR (DMSO-d_6): δ 1.32 (m, broad, 4H), 1.58 (m, broad, 4H), 1.78 (m, broad, 4H), 1.93 (m, broad, 4H), 3.95 (m, broad, 4H), 5.97 (d, $J=14$ Hz, 4H), 6.04 (d, $J=14$ Hz, 4H), 7.47 (d, $J=8$ Hz, 4H), 7.77 (d, $J=8$ Hz, 4H), 7.92 (s, 4H), 8.20 (dd, $J=6$ and 8 Hz, 4H) 8.76 (d, $J=8$ Hz, 4H), 9.06 (d, $J=7$ Hz, 4H), 9.31 (d, $J=6$ Hz, 4H), 9.45 (s, 4H). IR (KBr): 1668 (C=O), 3418 (N–H, amide).

3.9. Synthesis of macrocyclic tetrapyridinium compound 7^{4+}

Prepared from 1,3-bis(bromomethyl)benzene as described for $\mathbf{1}^{4+}$. Yield 14% of a white powder. $[\alpha]_D^{20}=-40\pm2$ (*c* 2.75 mg/mL; MeOH) MS: $[M-Br^-]^+$ 1093.219 (calculated 1093.198). ^{1}H NMR (DMSO-d_6): δ 1.36 (m, broad, 4H), 1.64 (m, broad, 4H), 1.81 (m, broad, 4H), 1.95 (d, $J=12$ Hz, broad, 4H), 3.99 (m, 4H), 5.87 (d, $J=14$ Hz, 4H), 5.92 (d, $J=14$ Hz, 4H), 7.38 (tr, $J=8$ Hz, 2H), 7.49 (d, $J=8$ Hz, 4H), 7.89 (s, 2H), 8.27 (tr, $J=6$ and 8 Hz, 4H), 8.79 (d, $J=8$ Hz, 4H), 9.02 (d, $J=7$ Hz, 4H), 9.27 (d, $J=6$ Hz, 4H), 9.47 (s, 4H). IR (KBr): 1650 (C=O), 3425 (N–H, amide).

3.10. General procedure for reductions using models 2H$_2$ and 1H$_4$

A 10 mL round-bottomed flask was charged with magnesium perchlorate and the model compound (80–100 μmol), sealed with a rubber septum, and flushed with argon. Freshly distilled methylene chloride (2.5–3 mL) was added via syringe and the mixture was stirred for 1 to 2 hours, whereupon the substrate was added via syringe. The flask was kept in the dark and the reaction mixture was stirred under argon. The reduction was monitored by capillary gas chromatography. The reaction was quenched by adding 7–8 mL of water. The aqueous phase was extracted with methylene chloride (3×10 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated. The optical rotation was measured to determine the enantiomer in excess and the enantiomeric excess was measured using capillary GC.

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