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Chiral NADH model systems functionalized with Zn(II)-cyclen as flavin binding site

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Abstract—A series of chiral peptides has been prepared, bearing a 1,4-dihydronicotine amide and a zinc cyclen moiety. The metal complex reversibly binds flavins in aqueous solution, while the dihydronicotine amide serves as a NADH model transferring a hydride to the flavin within the assembly. The reaction rate of the redox reaction was monitored and determined by UV spectroscopy. The reaction rates of the substituted compounds were slower if compared to the non-substituted parent compound **1-H**, but still show a 30–100 fold rate enhancement compared to the compound missing a flavin binding site. It was anticipated to probe the cryptic stereoselectivity of the hydride transfer from dihydropyridine to flavin. Spectroscopic data indicate that the introduction of deuterium labels upon reduction of the pyridinium salts to 1,4-dihydropyridine in D_2O proceeds diastereoselectively, but identical isotope effects on the rate of flavin reduction as with a non-chiral NADH model revealed that the hydride transfer within the assembly proceeds not stereoselective. A more rigid chiral NADH model compound must be prepared to achieve this goal.

1. Introduction

Flavins and nicotine amides are the two most important biological redox cofactors.[1] Nicotine amide nucleotides[2] are the strongest reducing agents found in biology and can transfer electrons to flavins in a thermodynamically allowed process. The transfer of reduction equivalents is catalyzed by enzymes, which bind both cofactors to allow very efficient intramolecular electron transfer. We have recently reported a chemical model system,[3] which mimics this process under physiological conditions.

Enzymatic reactions that involve nicotine amide cosubstrates show stereospecificity in two aspects: the transfer of a hydride to a prochiral substrate is usually highly stereospecific and from the two hydrogen atoms available in the dihydronicotine amide only the pro-*R* or pro-*S* hydrogen atom is transferred. L-Lactate dehydrogenase, as a prominent example, catalyzes the transfer of the pro-*R* hydrogen atom of NADH to pyruvate, whereby only L-lactate, and no D-lactate, is obtained. The arising NAD^+ contains the remaining pro-*S* hydrogen atom. The stereospecific transfer of the pro-*S* hydrogen atom from NADH leads to the same NAD^+ and the cryptic stereospecificity of the process can only be observed by isotope labeling (Scheme 1). All investigated dehydrogenases transfer stereospecifically either the pro-*R* or the pro-*S* hydrogen atom of NADH, with nearly equal distribution.[4] Several explanations for the observations have been proposed. While historical models[5] conclude the evolution of the enzyme families from a common ancestor and random selection of either stereospecificity, because it is a non-adaptive property without selection pressure, this has been criticized by Benner et al.[4] If the specificity is without biological or chemical function, it is a surprisingly conserved property. All lactate dehydrogenases transfer the pro-*R* hydrogen atom of NADH[6] and, therefore, the cryptic stereospecificity is older than the evolutionary separation of life into bacteria, archaee and eucaryonts. The substrate specificity, one of the most important properties of an enzyme, shows a much faster drift. Functional models try to explain the chemical phenomenon with a biological function.[7] The cryptic stereoselectivity is now seen as an adaptive property under selection pressure.[8] The strength as a reducing agent of NADH may be different in its *syn*- and *anti*-conformation. Enzymes, that react with easily reducible substrates may prefer one conformation, while enzymes reacting with substrates that are difficult to reduce use NADH in the other conformation.[9] The high complexity of enzymes makes it difficult to derive conclusive experimental evidence for the phenomenon. Therefore, we were interested to adapt our previously reported chemical model to target stereochemical issues of the hydride transfer from NADH to flavin under

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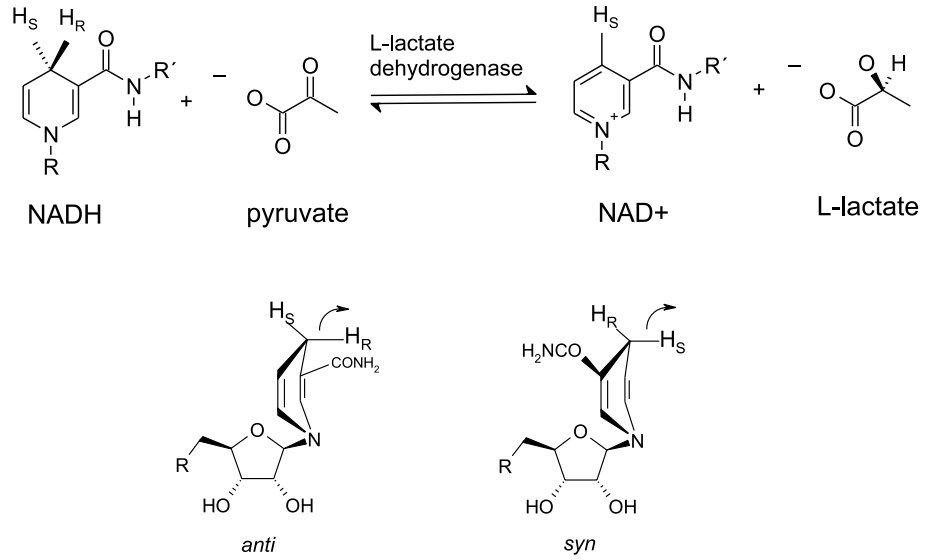


Scheme 1. Stereochemistry of L-lactate dehydrogenase and conformations of NADH in dehydrogenases.

physiological conditions. We report here the synthesis of chiral NADH model compounds, the kinetics of the redox reaction within NADH–flavin aggregates, the diastereoselective reduction of the NAD+ model compounds introducing deuterium atoms and observed deuterium isotope effects in intra-assembly flavin reduction (Scheme 1).

2. Results and discussion

2.1. Synthesis

Figure 1 shows the proposed conformation of the reversible assembly of flavin and the NADH model for the redox reaction. Model compounds with the depicted linker between the zinc cyclen complex and dehydronicotine amide gave the fastest hydride transfer reaction rates in previous studies. Therefore, we chose compound **1** as a starting point and introduced chirality into the linker. Scheme 2 shows the structures of the chiral NADH model compounds prepared and investigated in this study.

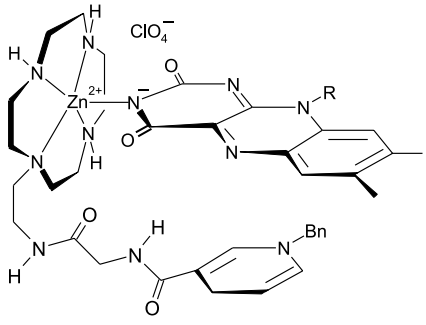
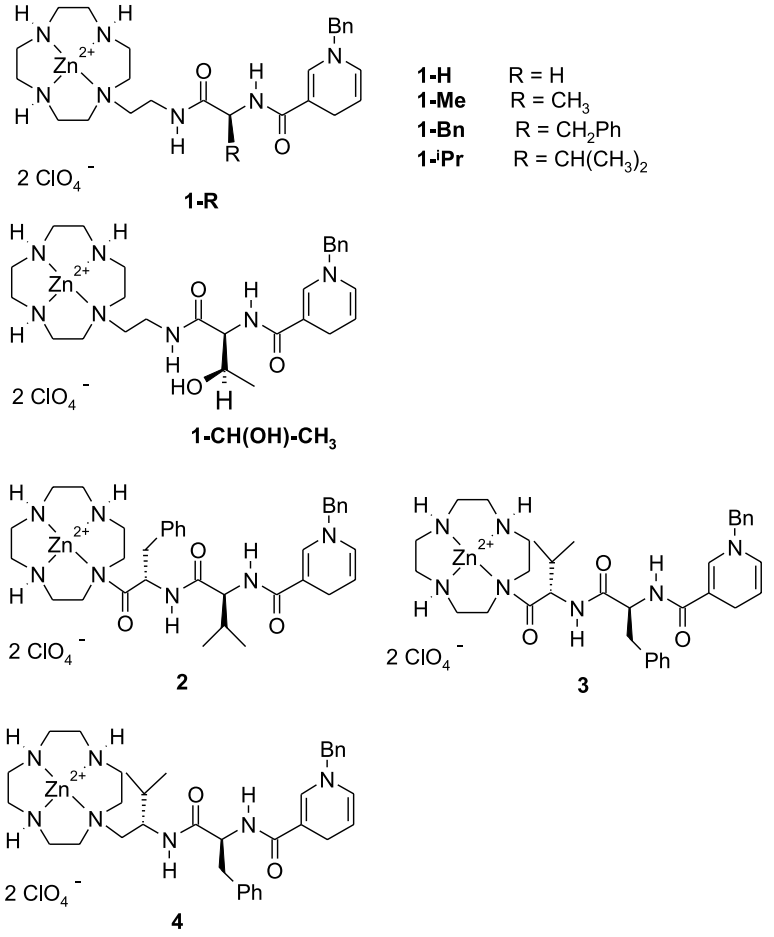


Figure 1. Proposed conformation of the aggregate **1-flavin** for the internal redox reaction (R=ribityl).

By introduction of chirality into the spacer between the zinc–cyclen flavin binding site and the nicotine amide, the methylene protons of the dehydronicotine amide become diastereotopic and are, therefore, distinguishable by NMR. In addition, a diastereoselective introduction of deuterium in the reduction of the nicotinium amide may be possible.

The synthesis of compounds **1-Me**, **1-Bn**, **1-^{i}Pr** and **1-CH(OH)–CH$_3$** uses **5** as a common precursor (Scheme 3). The synthesis of **5** has been described previously.[3] The coupling of the Fmoc-protected amino acids **6** to **5** proceeded under standard conditions using HATU and HOAt in high yield. The Fmoc protection group in **7** was removed with TBAF in acetonitrile. These conditions proved to be superior to standard piperidine, but acetonitrile as solvent is essential. Benzylated nicotinic acid was coupled to amines **8** using EDC and HOAt in DMF at room temperature. Isolated product yields range from 75 to 95% depending on the substituent R. The Boc-protecting groups were removed with TFA and the free amine was generated by eluation from a basic ion exchange resin. The stereocenters do not racemize under these conditions as confirmed by control experiments.[10] Finally, the zinc ion is introduced by refluxing compounds **12** with zinc bisperchlorate in ethanol. The choice of the solvent is important to obtain quantitative complexation of the macrocyclic ligand. The zinc complexes are stable salts. For kinetic measurements of the flavin reduction, **13** is reduced to 1,4-dihydronicotinamide **1-R** by treatment with sodium dithionite in aqueous solution. Compounds **1-R** must be handled under strict exclusion of oxygen to avoid rapid reoxidation and decomposition.

The syntheses of compounds **2** and **3** use threefold Boc-protected cyclen **14**[11] as the starting material (Scheme 4). Peptide coupling with Fmoc-protected Phe (**6-Bn**) or Val (**6-^{i}Pr**) proceeds in nearly quantitative yields. Compound **15** was deprotected, again using TBAF in acetonitrile, and the second amino acid was introduced yielding the isomeric compounds **17-Bn-^{i}Pr** and **17-^{i}Pr-Bn** in good yield. The nicotine amide was introduced after deprotection to yield **19**. Removal of all Boc protecting groups and eluation from a basic ion exchange resin[12] set the stage for complexing the macrocyclic ligand. Experiments using methanol as solvent for the complexation reaction were unsuccessful. However,



Scheme 2. Structure of compound **1**-**H** and of chiral model compounds **1**–**4** prepared and investigated in this study.

in acetonitrile solution complexes **22** are obtained in good yields as stable salts. For the reduction to **2** and **3** only a small excess of sodium carbonate is added to avoid hydrolysis of the cyclen acyl bond, which has been observed in other cases in basic aqueous solution. Acetonitrile must be added to the reaction mixture to ensure sufficient solubility of starting materials and products.

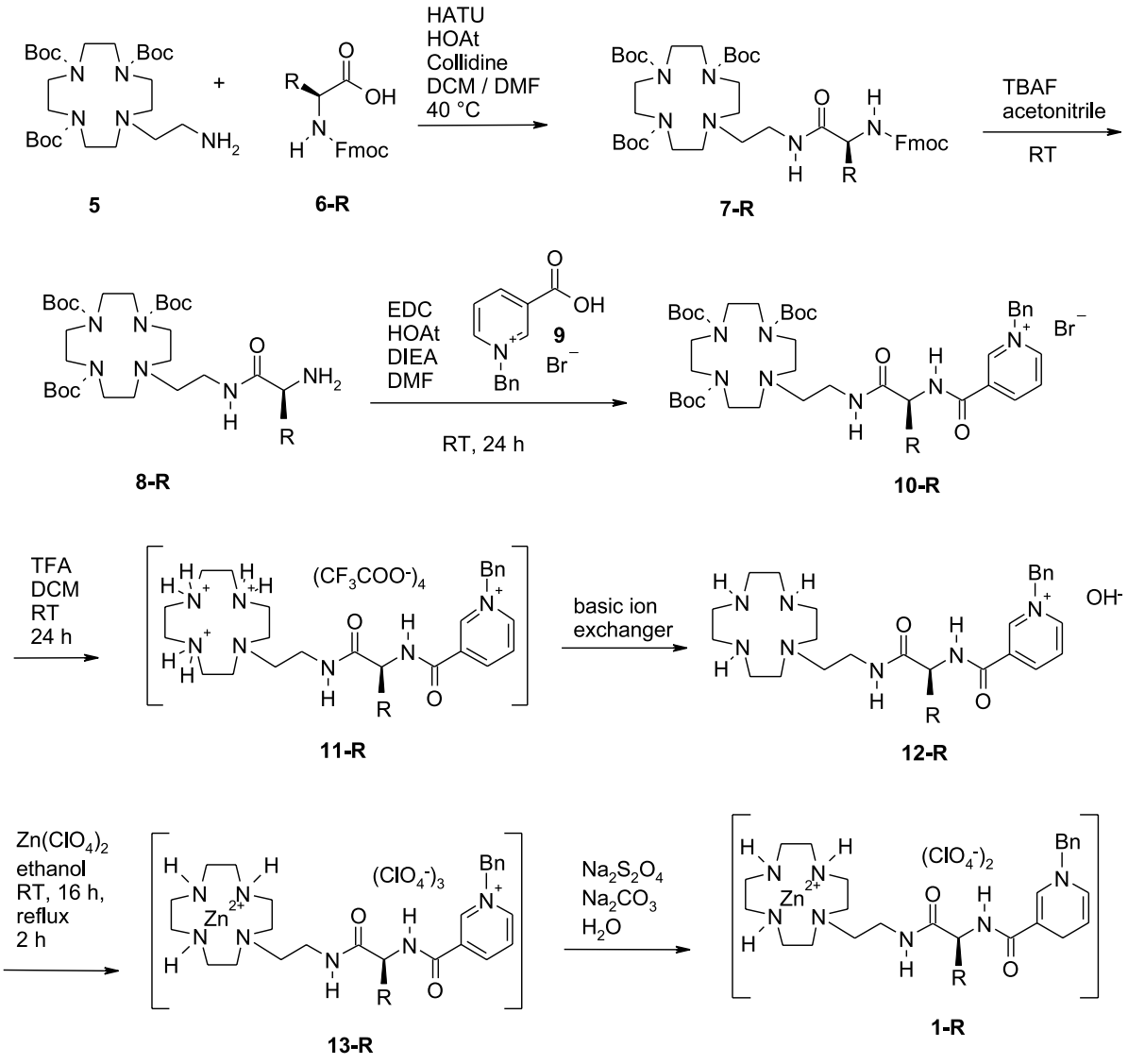
Dipeptide **4** is prepared from **16**-^{i}**Pr** (Scheme 5). Reduction of the amide bond with BH_3–THF proceeds cleanly, but the isolated yield of **23** was only 51%. Peptide coupling with **6**-**Bn**, deprotection and introduction of the nicotine amide follows the previously described procedures. Compound **26** was Boc deprotected and converted into the zinc complex **28**. Reduction using dithionite gave compound **4** in good yield. A round bottom flask under argon atmosphere was charged with the zinc-complex, sodium carbonate, sodium dithionite, degassed water, and degassed acetonitrile. Stirring of this mixture at room temperature for 3 h under strictly exclusion of oxygen afforded a yellow solution. The solvent was evaporated, degassed acetonitrile was added, and the resulting suspension was filtered. The filtrate was evaporated in vacuum to afford the dihydropyridine as a yellow solid, which is highly sensitive to oxygen. (See Supporting information for experimental details). The absorption maxima of the substituted pyridinium salts and 1,4-dihydroniconine amides are similar to the values of the parent compound **1**-**H** (see Table S-1; Supporting information).

2.2. Kinetics of NADH–flavin redox reaction

The redox reaction of the NADH model compounds with riboflavin in buffered aqueous solution was monitored spectroscopically using the UV absorption at 450 nm. The experimental set up and the methods to derive the reaction rate constant were the same as described earlier.[3] Table 1 summarizes the determined second order rate constants of the redox reaction. The values of previously tested compounds are given for comparison. With the exception of compound **4** introduction of a substituent into the dipeptide linker of **1**-**H**, significantly reduces the reaction rate. Most likely, substitution makes the conformation of the peptide linker necessary for the arrangement of 1,4-dihydronicotine amide and flavin for hydride transfer less favourable. Due to the limited number of compounds investigated, no predictive relation of molecular structure and chirality, and the reaction rate could be derived.[13]

2.3. Diastereoselective reduction of NADH model and deuterium isotope effects in flavin reduction

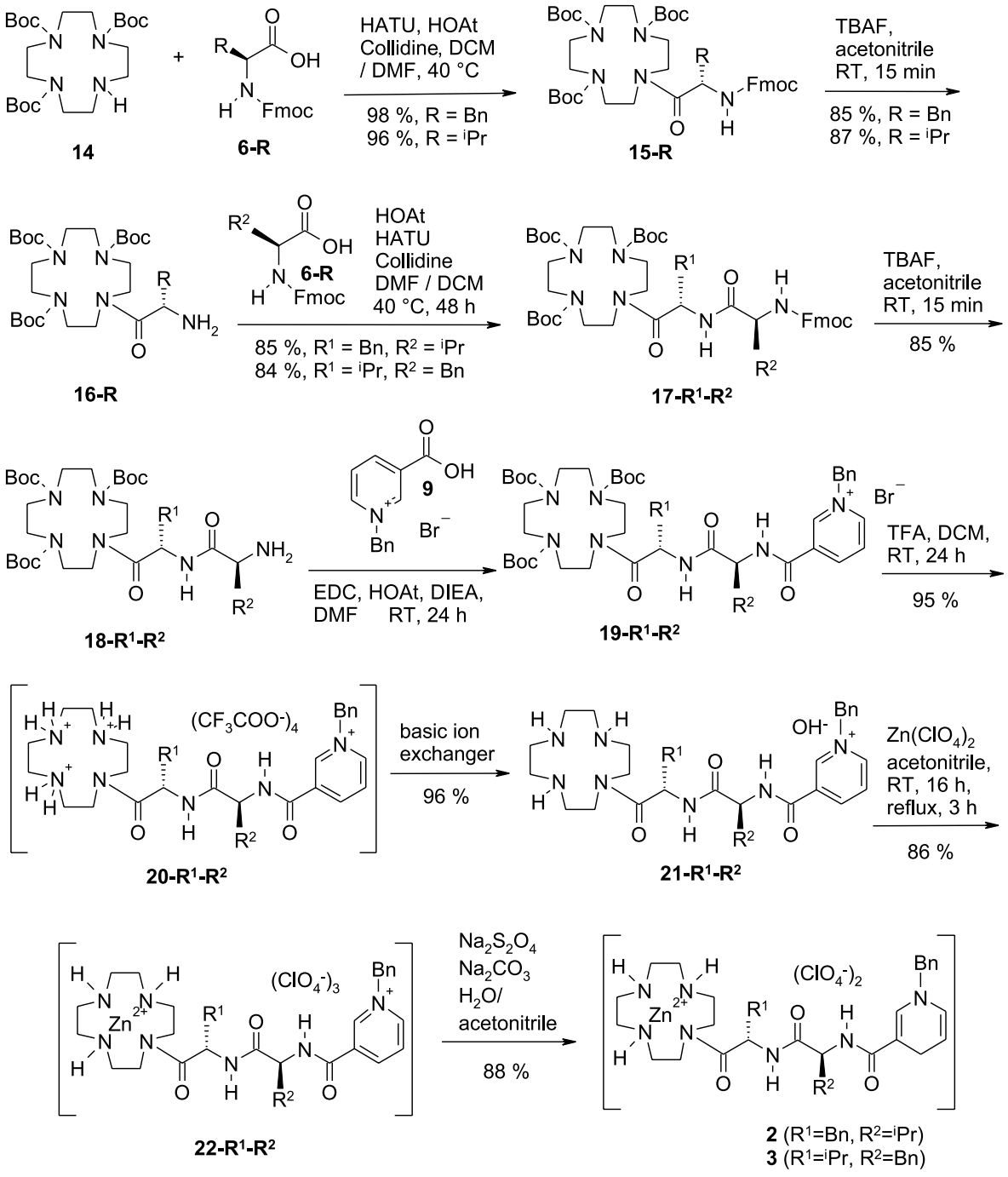
The reduction step of the pyridinium salt to 1,4-dihydronicotine amide in water was exemplarily investigated more



Scheme 3. Synthesis of compounds **1-Me**, **1-Bn**, **1-*i*Pr** and **1-CH(OH)–CH$_3$** from **5**. R = Bn, ^{i}Pr, Me and 1-*R*-1-hydroxyethyl.

closely for **1-CH(OH)–CH$_3$**. Upon reduction, the aromatic resonances of the pyridinium ring between $\delta = 7.8$–9.4 almost disappear.[14] Three new signals appear at $\delta = 4.7$, 5.9 and 7.1, which are assigned to the CH-proton resonances of the dihydropyridine ring (see Supporting information for spectra and assignment). The resonance of the methylene group is in the 1 D proton spectrum in the same region as the resonances of the other 20 methylene protons, but can be identified from HSQC at $\delta = 3.1$–3.3 (see Supporting information for spectrum). In the reduced form, some of the compounds resonance signals broaden or show a double set of signals. This process, which is reversed by oxidation back to the pyridinium salt, indicates the formation of conformers that slowly interconvert on the NMR time scale. Intramolecular coordination of appended hydroxyl- or carbonyl groups onto the Lewis-acidic zinc cyclen complex has been observed in other cases. The reduction reaction in D_2O leads to a compound with nearly identical spectra. The incorporation of deuterium is confirmed by an approximately half integral for the methylene resonance in the proton spectrum and the multiplicity edited HSQC spectrum, which clearly indicates that only one proton is attached to the methylene carbon (see Supporting information for spectrum). To probe the stereochemistry of the reduction reaction variable temperature spectra were recorded to diminish signal broadening or doubling. At 393 K most resonances show coalescence giving a single set of resonances for the compound, which may indicate stereospecific deuteration yielding one diastereomere. A similar diastereoselective non-enzymatic reduction has been described for NAD$^+$.[15] Traces of the pyridinium salt, the air sensitivity, and thermal instability of the compound above 400 K unfortunately prevent a more rigorous structure elucidation.

The reaction rate of deuterated **1-H**, **1-CH(OH)–CH$_3$** and **3** with flavin in aqueous buffer was measured and compared to the rates of the corresponding non-deuterated compounds. All determined isotope effects were identical within their error limits: 1.29 for **1-H**, 1.31 for **1-CH(OH)–CH$_3$** and 1.27 for compound **3**. This shows that either the deuterium incorporation into the pyridinium ring was not diastereoselective, which we cannot finally exclude, or the hydride/deuteride transfer within the assembly is not stereoselective.

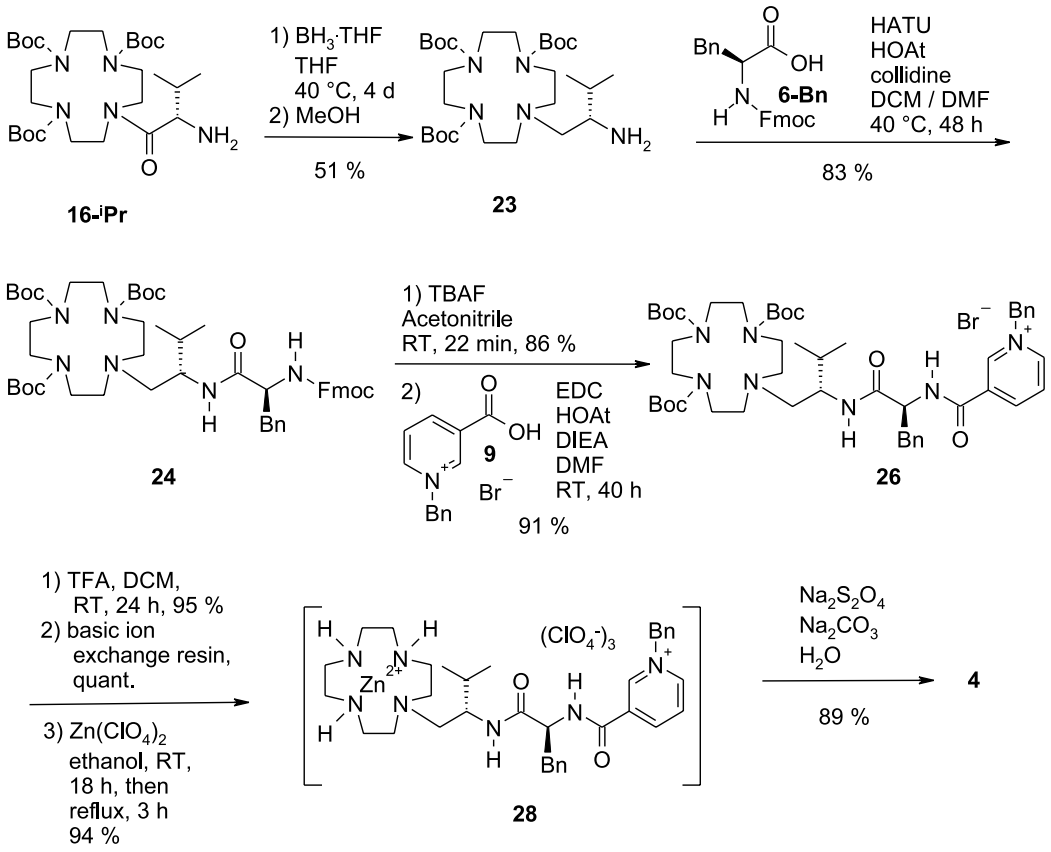


Scheme 4. Synthesis of compounds **2** and **3**. R = Bn or ^{i}Pr.

In any case is the investigated model system not suitable to probe cryptic stereoselectivity of NADH reduction reactions.

3. Conclusion

We have prepared a series of chiral NADH model compounds to probe stereochemical effects on the rate of the redox reaction between 1,4-dehydronicotine amide and flavin within a reversible aggregate in buffered water. Chiral α-amino acids were introduced into the linker tethering a zinc cyclen complex, which serves as the flavin-binding site, and nicotine amide. The redox reaction was followed by UV spectroscopy and measurements revealed a decrease in reaction rate in substituted compounds in comparison to the non-substituted parent compound **1-H**. We explain this by substituent effects on the conformation of the linker, which force the flavin binding site and 1,4-dehydropyridine in less favorable relative orientation for the redox reaction with flavin. Reduction of the pyridinium salts to 1,4-dihydronicotine amides in D_2O leads to deuterium incorporation. For compound **1-CH(OH)–CH$_3$** NMR measurements indicate the formation of only one diastereomere, similar to the non-enzymatic reduction of NAD^+. However, identical deuterium isotope effects on the redox reaction



Scheme 5. Synthesis of compound **4**.

with flavin show that the hydride transfer within the assembly is not stereospecific. The pro-*R* and pro-*S* hydrogen atoms of the 1,4-dehydropyridine are randomly transferred to coordinated flavin, which reveals that the conformational flexibility of the peptide linker between zinc cyclen and 1,4-dehydropyridine does not sufficiently confine the reactive conformation. A more rigid linker structure within the NADH model restricting the rotation of the 1,4-dehydropyridine is necessary to obtain a NADH model compound, which will selectively transfer either its pro-*R* or pro-*S* hydrogen atom to a bound substrate.

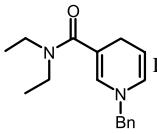
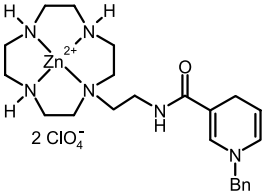
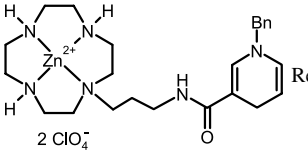
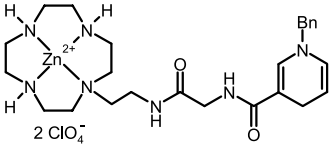
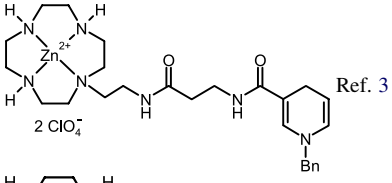
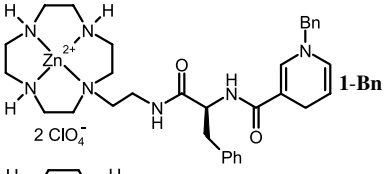
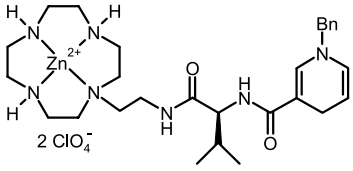
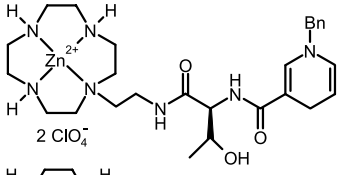
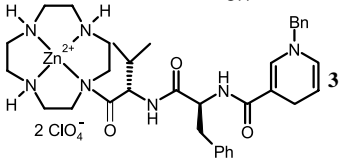
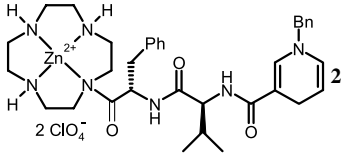
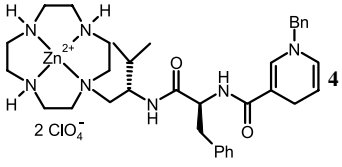
4. Experimental

4.1. General procedure 1 (GP 1) for the synthesis of compounds 7-Bn, 7-^{i}Pr, 7-Me, 7-CH(OH)–CH$_3$, 15-Bn, 15-^{i}Pr, 17-Bn-^{i}Pr, 17-^{i}Pr-Bn, 24

A round bottom flask was charged with the amine (1.0 equiv), Fmoc-protected amino acid (1.1 equiv), coupling reagents HOAt and HATU (each 1.2 equiv) and collidine (9.0 equiv). The compounds were dissolved in a 1:1-mixture of dry DMF and dry DCM. A minimum amount of solvent was used. The yellow solution was stirred at 40 °C for 2 days and then diluted with 100 ml of DCM. The reaction conversion was monitored by TLC. The mixture was extracted with 50 ml of aqueous HCl (c=1 mol/l), the organic layer was dried over $NaSO_4$ and concentrated under reduced pressure. CC with ethyl acetate–petroleum ether (EE/PE) afforded the fully protected compounds as colourless solids.

4.1.1. 10-{2-[2-(9*H*-Fluoren-9-yl-methoxycarbonylamino)-2-benzyl-acetylamino]-ethyl}-1,4,7,10-tetraazacyclododecan-1,4,7-tricarbonicacid-tri-*tert*-butylester (7-Bn). The synthesis follows GP 1 using **5** (1.00 g, 1.94 mmol), **6-Bn** (0.83 g, 2.13 mmol), HOAt (0.32 g, 2.35 mmol), HATU (0.89 g, 2.35 mmol) and collidine (2.12 g, 2.3 ml, 17.5 mmol). CC with PE/EE (40:60) to PE/EE (20:80) afforded **7-Bn** in a yield of 1.66 g (1.88 mmol, 97%); R_f=0.10 (EE/PE=1:1); mp: 103–105 °C. ^{1}H NMR (600 MHz, CD_2Cl_2): δ=1.48 (s, 18H, Boc-CH$_3$), 1.49 (s, 9H, Boc-CH$_3$), 2.56–2.65 (m, 6H, CH$_2$), 3.09–3.11 (m, 2H, Phe-CH$_2$), 3.24–3.51 (m, 14H, CH$_2$), 4.23 (dd, ^3J=6.9 Hz, 1H, Fmoc-CH), 4.30–4.33 (m, 1H, Fmoc-CH$_2$), 4.44 (dd, ^3J=6.9 Hz, ^2J=10.5 Hz, 1H, Fmoc-CH$_2$), 4.47 (m, 1H, C*H), 5.66 (d, ^3J=7.0 Hz, 1H, NH), 6.67 (m, 1H, NH), 7.22–7.23 (m, 2H, arom. CH), 7.25–7.28 (m, 1H, arom. CH), 7.30–7.36 (m, 4H, arom. CH), 7.44 (dd, ^3J=7.5 Hz, 2H, arom. Fmoc-CH), 7.59–7.62 (m, 2H, arom. Fmoc-CH), 7.82 (d, ^3J=7.5 Hz, 2H, arom. Fmoc-CH); ^{13}C NMR (150.1 MHz, CD_2Cl_2): δ=28.7 (+, Boc-CH$_3$), 28.8 (+, Boc-CH$_3$), 36.5 (−), 39.2 (−, Phe), 47.6 (+, Fmoc-CH), 48.2 (−), 48.7 (−), 50.1 (−), 52.4 (−), 54.9 (−), 56.3 (+, C*H), 67.2 (−, Fmoc-CH$_2$), 79.5 (C$_{quat}$, Boc), 79.8 (C$_{quat}$, Boc), 120.3 (+, 2 arom. Fmoc-C), 125.4 (+, 1 arom. Fmoc-C), 125.5 (+, 1 arom. Fmoc-C), 127.2 (+, 1 arom. C), 127.4, (+, 2 arom. C), 128.1 (+, 2 arom. Fmoc-C), 128.8 (+, 2 arom. C), 129.9 (+, 2 arom. C), 137.2

Table 1. Reaction rate constants of the redox reaction of the respective NADH-model compound with 1 equiv of riboflavin tetraacetate in aqueous solution (HEPES/KOH pH 7.4); $c = 4.51 \cdot 10^{-5}$ mol/l. UV detection at 447 nm. Rate constants are derived from a minimum of two independent measurements

Compound	k_2 [l mol^{-1} s^{-1}]	Relative rates
 Ref. 3	22	1
 Ref. 3	408 ± 26	18
 Ref. 3	671 ± 37	29
 **1-H**	3998 ± 321	175
 Ref. 3	646 ± 67	28
 **1-Bn**	643 ± 132	28
 **1-^{i}Pr**	706 ± 141	31
 **1-CH(OH)CH$_3$**	725 ± 65	32
 **3**	783 ± 151	34
 **2**	806 ± 155	37
 **4**	2352 ± 228	103

(arom. C_{quat}), 141.7 (arom. C_{quat}), 144.4 (arom. C_{quat}), 144.4 (arom. C_{quat}), 155.8 (C_{quat}, urethane-C), 156.1 (C_{quat}, urethane-C), 156.3 (C_{quat}, urethane-C), 171.1 (C_{quat}, amide-C); UV–vis (CH_3CN): λ (log ε)=205 nm (4.781), 265 nm (4.246), 289 nm (3.674), 300 nm (3.747); MS (ESI, CH_2Cl_2/MeOH): m/z=885.6 [MH^+] (100%), 907.7 [$M+Na^+$] (10%); MS-HR (FAB, CH_2Cl_2): [MH^+] (Calcd)=885.5116, [MH^+] (found)=885.5116±0.58 ppm; IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3064, 2970, 2932, 1683, 1534, 1462, 1250, 1161, 742; MF: $C_{49}H_{68}N_6O_9$; MW=885.12.

See electronic Supporting information for the synthesis and characterisation of compounds **7-^{i}Pr**, **7-Me**, **7-CH(OH)–CH$_3$**, **15-Bn**, **15-^{i}Pr**, **17-Bn-^{i}Pr**, **17-^{i}Pr-Bn**, **24**.

4.2. General procedure 2 (GP 2) for the synthesis of compounds 8-^{i}Pr, 8-Bn, 8-Me, 8-CH–(OH)CH$_3$, 16-^{i}Pr, 18-Bn-^{i}Pr, 18-^{i}Pr-Bn, 25

In a round bottom flask the Fmoc-protected product from GP 1 is dissolved in a solution of tetrabutylammonium fluoride–trihydrate (TBAF) in acetonitrile (c=0.05 mol/l, 2.0 equiv of TBAF) and stirred at room temperature for 17 min. The reaction conversion was monitored by TLC. Then 150 ml of DCM were added to stop the reaction. The mixture was extracted twice with 75 ml of water. The combined aqueous layers were extracted with 75 ml of DCM, the combined organic layers were dried over $NaSO_4$ and concentrated under reduced pressure. CC with EE/PE or methylene chloride–methanol (DCM/MeOH) afforded the Fmoc-deprotected compounds as colourless solids.

4.2.1. 10-[2-(2-Amino-3-methyl-butyrylamino)-ethyl]-1,4,7,10-tetraazacyclododecan-1,4,7-tri-carbonicacid-tri-*tert*-butylester (8-^{i}Pr). The synthesis follows GP 2 using 1.36 g (1.62 mmol) of **7-^{i}Pr** and 1.03 g (3.24 mmol) of TBAF. CC with EE/PE (70:30) to CH_2Cl_2/MeOH (95:5) gave **8-^{i}Pr** (0.87 g, 1.41 mmol, 87%); R_f=0.42 (DCM/MeOH=9:1); mp: 81–84 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.75 (d, ^3J=6.9 Hz, 3H, Val-CH_3), 0.91 (d, ^3J=6.9 Hz, 3H, Val-CH_3), 1.38 (s, 18H, Boc-CH_3), 1.40 (s, 9H, Boc-CH_3), 1.63 (bs, 2H, NH_2), 2.19 (dhept, ^3J=4.2, 6.9 Hz, 1H, Val-CH), 2.60–2.65 (m, 6H, 3CH_2), 3.12 (d, ^3J=4.2 Hz, 1H, C*H), 3.24–3.46 (m, 14H, 7CH_2), 7.39 (m, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=16.2 (+, Val-CH_3), 19.67 (+, Val-CH_3), 28.5 (+, Boc-CH_3), 28.6 (+, Boc-CH_3), 30.8 (+, Val-CH), 35.2 (−, 1C), 48.0 (−, 4C), 49.8 (−, 2C), 51.9 (−, 1C), 54.2 (−, 1C), 55.3 (−, 1C), 60.2 (+, C*H), 79.3 (C_{quat}, Boc), 79.6 (C_{quat}, Boc), 155.4 (C_{quat}, urethane-C), 155.8 (C_{quat}, urethane-C), 156.1 (C_{quat}, urethane-C), 174.7 (C_{quat}, amide-C); MS (ESI, MeOH): m/z=615.6 [MH^+] (100%), 1251.9 [$2M+Na^+$] (0.7%), 1345.8 [$2M+H^+$+CH_3COOH] (1.0%); IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3391, 2974, 2931, 1695; MF: $C_{30}H_{58}N_6O_7$; MW=614.83.

See electronic Supporting information for the synthesis and characterisation of compounds **8-Bn**, **8-Me**, **8-CH(OH)–CH$_3$**, **16-Bn**, **16-^{i}Pr**, **18-Bn-^{i}Pr**, **18-^{i}Pr-Bn**, **25**.

4.3. General procedure 3 (GP 3) for the synthesis of compounds 10-Bn, 10-^{i}Pr, 10-Me, 10-CH(OH)–CH$_3$, 19-Bn-^{i}Pr, 19-^{i}Pr-Bn, 26

A round bottom flask was charged with the amine (1.0 equiv), the nicotinic acid derivative (1.1 equiv), coupling reagents HOAt and EDC (each 1.2 equiv) and *N*-ethyldiiso-propylamine (1.2 equiv). The mixture was dissolved in the minimum amount of dry DMF. The yellow solution was stirred at room temperature for 24 h and the reaction conversion was monitored by TLC. The mixture was evaporated and dried in vacuum. The resulting oil was dissolved in 50 ml of DCM and extracted three times with 10 ml of aqueous HBr (c=1 mol/l) to remove excess coupling reagents and amine. The combined organic layers were dried over $NaSO_4$ and concentrated under reduced pressure. CC (DCM/MeOH) afforded the fully Boc-protected compounds as reddish solids.

4.3.1. 1-Benzyl-3-{2-phenyl-1-[2-(4,7,10-tris-*tert*-butoxy-carbonyl-1,4,7,10-tertaaza-cyclododec-1-yl)-ethylcarbamoyl]-ethylcarbamoyl}-pyridinium-bromide (10-Bn). The synthesis follows GP 3 using **8-Bn** (1.10 g, 1.66 mmol), **9** (0.54 g, 1.83 mmol), HOAt (0.27 g, 2.01 mmol), EDC (0.31 g, 0.36 ml, 2.01 mmol) and *N*-ethyldiisopropylamine (0.26 g, 0.34 ml, 2.01 mmol). CC with CH_2Cl_2/MeOH (98:2) to CH_2Cl_2/MeOH (87:13). After concentrating the solution under reduced pressure and drying in vacuum the obtained solid was diluted in as little dry DCM as possible and stored at −18 °C over night. Any separated silica gel was removed by filtration and the obtained solution was dried in vacuum. This afforded **10-Bn** in a yield of 1.34 g (1.43 mmol, 86%); R_f=0.16 (CH_2Cl_2/MeOH=9:1); mp: 146–149 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.42 (s, 18H, Boc-CH_3), 1.45 (s, 9H, Boc-CH_3), 2.67–2.74 (m, 6H, CH_2), 3.26–3.48 (m, 16H, CH_2), 4.87–4.92 (m, 1H, C*H), 6.05 (bs, 2H, Bn–CH_2), 7.09–7.12 (m, 1H, arom. CH), 7.15–7.18 (m, 2H, arom. CH), 7.34–7.36 (m, 2H, arom. CH), 7.41–7.45 (m, 3H, arom. CH), 7.60–7.62 (m, 2H, arom. CH), 7.67 (bs, 1H, NH), 7.98–8.02 (m, 1H, py-CH), 8.95 (d, ^3J=8.2 Hz, 1H, py-CH), 9.09 (d, ^3J=5.2 Hz, 1H, py-CH), 9.51 (m, 1H, NH), 10.22 (s, 1H, py-CH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=28.5 (+, Boc-CH_3, 6C), 28.7 (+, Boc-CH_3, 3C), 35.4, (−, 1C), 38.2 (−, Phe), 47.9 (−, 4C), 49.8 (−, 2C), 50.5 (−, 1C), 53.9 (−, 1C), 55.0 (−, 1C), 57.4 (+, C*H), 65.0 (−, Bn), 79.4 (C_{quat}, Boc, 1C), 79.6 (C_{quat}, Boc, 2C), 126.7 (+, 1 arom. C), 127.9 (+, 1 py-C), 128.4 (+, 2 arom. C), 129.4 (+, 2 arom. C), 129.7 (+, 2 arom. C), 129.9 (+, 2 arom. C), 130.5 (+, 1 arom. C), 131.9 (arom. C_{quat}), 134.4 (arom. C_{quat}), 137.3 (arom. C_{quat}), 144.7 (+, 1 py-C), 145.1 (+, 1 py-C), 145.3 (+,1 py-C), 155.5 (C_{quat}, urethane-C), 155.8 (C_{quat}, urethane-C), 156.1 (C_{quat}, urethane-C), 160.7 (C_{quat}, amide-C), 171.0 (C_{quat}, amide-C); UV–vis (MeOH): λ (log ε)=264 nm (3.755), 204 nm (4.577); MS (ESI, H_2O/MeOH/AcN): m/z=429.9 [$(M^+ + H^+)^{2+}$] (20%), 858.6 [M^+] (100%); IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3063, 2977, 2930, 2856, 1679, 1545, 1460, 1416, 1366, 1250, 1162, 749, 702; $[\alpha]_D^{20}$ (MeOH)=−2±1°; MF: $C_{47}H_{68}N_7O_8Br$; MW=939.00.

See electronic Supporting information for the synthesis and

characterisation of compounds **10-^{i}Pr**, **10-Me**, **10-CH(OH)–CH$_3$**, **19-Bn-^{i}Pr**, **19-^{i}Pr-Bn**, **26**.

4.4. General procedure 4 (GP 4) for the synthesis of compounds 11-Bn, 11-^{i}Pr, 20-Bn-^{i}Pr, 20-^{i}Pr-Bn, 27

In a round-bottomed flask the Boc-protected product from GP 3 (1 equiv) was dissolved in DCM and treated with trifluoroacetic acid (TFA) (42 equiv). The yellow solution was stirred at room temperature for 24 h and was then evaporated and dried in vacuum. This afforded the fully deprotected compounds as yellow solids in sufficient purity for use in subsequent steps.

4.4.1. 1-Benzyl-3-{2-phenyl-1-[2-(1,4,7,10-tertaaza-cyclododec-1-yl)-ethylcarbamoyl]-ethyl-carbamoyl}-pyridinium-trifluoroacetate-trihydro-trifluoroacetate (11-Bn).

The synthesis follows GP 4 using 1.28 g (1.36 mmol) of **10-Bn** dissolved in 60 ml CH_2Cl_2 and 6.53 g (4.4 ml, 57.30 mmol) of TFA. This gave 1.24 g of **11-Bn** (1.22 mmol, 90%); mp: 92–94 °C. ^{1}H NMR (400 MHz, CD_3CN): δ=2.61–3.18 (m, 19H), 3.10 (dd, ^2J=13.8 Hz, ^3J=11.9 Hz, 1H, Phe-CH_2), 3.44–3.51 (m, 1H, CH_2), 3.56 (dd, ^2J=13.8 Hz, ^3J=3.4 Hz, 1H, Phe-CH_2), 4.88 (ddd, ^3J=3.4, 8.6, 11.9 Hz, 1H, C*H), 5.86 (s, 2H, Bn–CH_2), 7.11–7.21 (m, 3H, arom. CH), 7.38–7.40 (m, 2H, arom. CH), 7.45–7.49 (m, 3H, arom. CH), 7.54–7.57 (m, 2H, arom. CH), 8.01 (dd, ^3J=6.2, 8.1 Hz, 1H, py-CH), 8.23 (t, ^3J=6.0 Hz, 1H, NH), 8.82–8.83 (m, ^3J=6.2 Hz, 1H, py-CH), 8.96 (dt, ^3J=8.1 Hz, ^4J=1.3 Hz, 1H, py-CH), 9.47 (d, ^3J=8.6 Hz, 1H, NH), 9.82 (m, 1H, py-CH); ^{13}C NMR (100.6 MHz, CD_3CN): δ=37.6 (−, Phe), 39.1 (−, 1C, CH_2–NH), 43.1 (−, 4C, cyclen), 45.3 (−, 2C, cyclen), 50.2 (−, 1C, cyclen), 50.7 (−, 1C, cyclen), 55.7 (−, 1C, CH_2–N), 57.3 (+, C*H), 65.5 (−, Bn), 127.6 (+, 1 arom. C), 129.2 (+, 1 py-C), 129.3 (+, 2 arom. C), 130.4 (+, 2 arom. C), 130.5 (+, 4 arom. C), 131.0 (+, 1 arom. C), 133.9 (arom. C_{quat}, 1C), 135.2 (arom. C_{quat}, 1C), 139.3 (arom. C_{quat}, 1C), 145.8 (+, 1 py-C), 146.1 (+, 1 py-C), 146.9 (+, 1 py-C), 163.2 (C_{quat}, amide-C), 173.4 (C_{quat}, amide-C); UV–vis (MeOH): λ (log ε)=264 nm (3.889), 205 nm (4.626); MS (ESI, CH_3CN): m/z=391.9 [(M^++H^++KCl+CF_3COOH+HCl)$^{2+}$] (68%), 392.9 [(M^++H^++KCl+HBr+2HCl)$^{2+}$] (75%), 419.4 (100%), 558.4 [M^+] (20%), 694.3 [M^++CF_3COONa] (4%), 746.3 [M^++KCl+CF_3COOH] (6%), 748.3 [M^++KCl+HBr+HCl] (8%); IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3432, 3070, 2970, 2856, 2362, 1676, 1545, 1497, 1458, 1200, 1135, 706; MF: [$C_{32}H_{47}N_7O_2$]$^{4+}$(CF_3COO^-)$_4$/$C_{40}H_{47}N_7O_{10}F_{12}$; MW=1013.83; $[\alpha]_D^{20}$ (MeOH)=−13±1°.

See electronic Supporting information for the synthesis and characterisation of compounds **11-^{i}Pr**, **20-Bn-^{i}Pr**, **20-^{i}Pr-Bn**, **27**.

4.5. General procedure 5 (GP 5) for the synthesis of compounds 12-Me, 12-CH(OH)CH$_3$, 21-Bn-^{i}Pr, 21-^{i}Pr-Bn

The TFA-salt (1 equiv) from *GP 4* was dissolved in water and passed over a strongly basic ion-exchanger column (loading: 0.9 mmol/ml, 6 equiv). The obtained solution was lyophilized to afford the corresponding amine as a pale yellow solid.

4.5.1. 1-Benzyl-3-{1*S*-1-[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-ethylcarbamoyl}-pyridinium-hydroxide (12-Me).

The synthesis follows GP 5 using 245 mg (0.30 mmol) of **11-Me** and 2.0 ml of the basic ion-exchanger (1.80 mmol). This afforded **12-Me** in a yield of 145 mg (0.29 mmol, 97%); mp: 85–87 °C. UV–vis (CH_3CN): λ_{max} [nm] (log ε)=322 (3.829); MS (ESI, MeOH/CH_3CN+0.1% TFA): m/z (%)=196.4 (30) [(K^++H^+–Bn)$^{2+}$], 216.9 (45), 241.5 (100) [(K^++H^+)$^{2+}$], 392.1 (64) [(K^+−Bn)$^+$], 482.3 (93) [K^+], 596.4 (24) [K^++CF_3CO_2H)]; IR (KBr): $\tilde{\nu}$ [cm^{-1}]=704, 735, 1179, 1212, 1279, 1348, 1413, 1456, 1540, 1665, 2830, 2934, 3424; $[\alpha]_D^{20}$ (CH_3CN)=−68±7°; MF: $C_{26}H_{41}N_7O_3$; MW=499.66.

See electronic Supporting information for the synthesis and characterisation of compounds **12-CH**(OH)-**CH$_3$**, **21-Bn-^{i}Pr**, **21-*i*Pr-Bn**.

4.6. General procedure 6 (GP 6) for the synthesis of compounds 22-Bn-^{i}Pr, 22-^{i}Pr-Bn

In a round bottom flask $Zn(ClO_4)_2\cdot 6H_2O$ was dissolved in acetonitrile and a suspension of the amine in acetonitrile was slowly added. The resulting reddish solution was stirred at room temperature for 16 h and then heated to reflux for 4 h. After cooling to room temperature the solvent was evaporated to afford the crude product as orange oil. Ethanol (2 ml) was added and the resulting pale red precipitate was treated with ultrasound for 15 min. After filtration, the solid was washed with 2 ml of ethanol and dissolved in 1.5 ml of acetonitrile. Any resulting precipitate was removed by centrifugation. Drying of the solution in vacuum afforded the zinc-complex as a hygroscopic orange solid.

4.6.1. 1-Benzyl-3-{1-[1-benzyl-2-oxo-2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethyl-carbamoyl]-2-methyl-propylcarbamoyl}-pyridinium-zinc-(II)-tri-perchlorate (22-Bn-^{i}Pr).

The synthesis follows GP 6 using 0.22 g (0.60 mmol, 3 equiv) $Zn(ClO_4)_2\cdot 6H_2O$ dissolved in 5 ml acetonitrile and a suspension of **21-Bn-^{i}Pr** (0.13 g, 0.20 mmol) in 7 ml acetonitrile. This gave **22-Bn-*i*Pr** in a yield of 0.17 g (0.18 mmol, 88%); mp: 230 °C (dis.).

^{1}H NMR (400 MHz, CD_3CN): δ=0.95 (d, ^3J=6.7 Hz, 3H, Val-CH_3), 0.98 (d, ^3J=6.7 Hz, 3H, Val-CH_3), 2.05–2.13 (m, ^3J=6.7 Hz, 1H, CH), 2.31–2.36 (m, 1H, CH_2), 2.48–2.61 (m, 1H, CH_2), 2.68–2.93 (m, 9H, CH_2), 3.00–3.13 (m, 5H, CH_2), 3.28–3.35 (m, 1H, CH_2), 3.48–3.54 (m, 1H, CH_2), 3.67–3.71 (m, 1H, NH), 3.83–3.86 (m, 1H, NH), 3.95–3.97 (m, 1H, NH), 4.53–4.56 (m, 1H, Val-C*H), 4.71–4.75 (m, 1H, Phe-C*H), 5.80 (d, ^2J=14.8 Hz, 1H, Bn–CH_2), 5.84 (d, ^2J=14.8 Hz, 1H, Bn–CH_2), 7.22–7.31 (m, 6H, arom. CH), 7.44–7.52 (m, 4H, arom. CH), 7.82 (d, ^3J=8.3 Hz, 1H, Val-NH), 8.06 (dd, ^3J=6.1, 8.0 Hz, 1H, py-CH), 8.05–8.08 (m, 1H, Phe-NH), 8.76 (d, ^3J=6.1 Hz, 1H, py-CH), 8.93 (d, ^3J=8.0 Hz, 1H, py-CH), 9.66 (bs, 1H, py-CH); ^{13}C NMR (100.6 MHz, CD_3CN): δ=18.5 (+, Val-CH_3), 19.3 (+, Val-CH_3), 32.8 (+, Val-CH), 37.3 (−, Phe), 42.8 (−, 1C), 45.2 (−, 1C), 45.5 (−, 1C), 46.1–46.4 (−, 3C), 46.7 (−, 1C), 48.9 (−, 1C), 55.1 (+, Phe-C*H), 59.4 (+, Val-C*H), 65.8 (−, 1C, Bn), 128.3 (+, 1 arom. C), 129.2 (+, 1 py-C), 129.7 (+, 2 arom. C), 130.3 (+, 2

arom. C), 130.4 (+, 1 arom. C), 130.5 (+, 1 arom. C), 130.8 (+, 1 arom. C), 133.8 (arom. C_{quat}), 135.6 (arom. C_{quat}), 136.4 (arom. C_{quat}), 145.9 (+, py-C), 146.1 (+, py-C), 146.7 (+, py-C), 162.3 (C_{quat}, amide-C), 173.0 (C_{quat}, amide-C), 175.3 (C_{quat}, amide-C); UV–vis (CH_3CN): λ (log ε)=264 (3.813), 385 nm (2.609); MS (ESI,CH_3CN): m/z=338.7 [$(M^{3+}-H^+)^{2+}$] (90%), 356.6 [$(M^{3+}+Cl^-)^{2+}$] (100%), 368.6 [$(M^{3+}+CH_3COO^-)^{2+}$] (35%), 388.6 [$(M^{3+}+ClO_4^-)^{2+}$] (50%), 586.3 [$(M^{3+}-H^+-Bn^+)^+$] (20%), 712.4 [$(M^{3+}-H^++Cl^-)^+$] (10%), 776.4 [$(M^{3+}-H^++ClO_4^-)^+$] (6%), 876.4 [$(M^{3+}+2ClO_4^-)^+$] (4%); IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3386, 3078, 2966, 2928, 1642, 1539, 1497, 1455, 1367, 1096, 746, 703; $[\alpha]_D^{20}$ (CH_3CN)=+5±1°; MF: $[C_{35}H_{48}N_7O_3Zn]^{3+}(ClO_4^-)_3$/$C_{35}H_{48}N_7O_{15}Cl_3Zn$; MW=978.54.

See electronic Supporting information for the synthesis and characterisation of compound **22-^{i}Pr-Bn**.

4.7. General procedure 7 (GP 7) for the synthesis of compounds 1-Bn, 1-Me, 1-^{i}Pr, 1-CH(OH)–CH_3, 2, 3, 4

A round bottom flask under argon atmosphere was charged with the zinc-complex (obtained from GP 6, GP 8 or GP 9), sodium carbonate, sodium dithionite, 4 ml of degassed water and 2 ml of degassed acetonitrile. Stirring of this mixture at room temperature for 3 h under strictly exclusion of oxygen afforded a yellow solution. The solvent was evaporated, 2 ml of degassed acetonitrile were added and the resulting suspension was filtered and the filtrate evaporate in vacuum to afford the dihydropyridine as a yellow solid, which is highly sensitive to oxygen.

4.7.1. 1-Benzyl-1,4-dihydropyridin-3-carbonicacid-{[2-phenyl-1-[2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethylcarbamoyl]-ethyl}-amide-zinc(II)-di-perchlorate (1-Bn). The synthesis follows GP 7 using **13-Bn** (32 mg, 35 μmol), sodium carbonate (15 mg, 139 μmol, 4 equiv) and sodium dithionite (15 mg, 87 μmol, 2.5 equiv). This afforded **1-Bn** in a yield of 26 mg (31 μmol, 90%). ^{1}H NMR (300 MHz, CD_3CN): δ= 2.50–3.67 (m, 27H, CH_2), 4.30 (s, 2H, Bn–CH_2), 4.60–4.67 (m, 1H, C*H), 4.74 (dt, ^3J=8.1, 3.3 Hz, 1H, CH), 5.86 (dd, ^3J=8.1 Hz, ^4J=1.3 Hz, 1H, CH), 6.08 (d, ^3J=7.0 Hz, 1H, NH), 7.06 (d, ^4J=1.3 Hz, 1H, CH), 7.18–7.49 (m, 11H, arom. CH+NH); ^{13}C NMR (75.5 MHz, CD_3CN): δ=22.9 (−), 36.6 (−, 1C), 38.5 (−, 1C), 43.3 (−, 2C), 44.9 (−, 2C), 45.4 (−, 2C), 52.8 (−, 2C), 55.2 (−, 1C), 56.8 (+, C*H), 57.6 (−, 1C), 103.6 (+), 127.8 (+, 1arom. C), 128.5 (+, 2 arom. C), 128.8 (+), 129.4 (+, 2 arom. C), 129.9 (+, 2 arom. C), 130.3 (+), 130.3 (+, 2 arom. C), 130.4 (+, 1 arom. C), 137.6 (C_{quat}), 139.5 (C_{quat}), 139.8 (C_{quat}), 169.2 (C_{quat}, amide-C), 175.2 (C_{quat}, amide-C); UV–vis (H_2O): λ_{max} (log ε)=361 nm (3.794); MF: $[C_{32}H_{45}N_7O_2Zn]^{2+}(ClO_4^-)_2$/$C_{32}H_{45}N_7O_{10}Cl_2Zn$; MW=824.04.

See electronic Supporting information for the synthesis and characterisation of compounds **1-Me**, **1-^{i}Pr**, **1-CH(OH)–CH_3**, **2**, **3**, **4**.

4.8. General procedure 8 (GP 8) for the synthesis of compounds 13-Bn, 13-^{i}Pr, 28

The TFA-salt (1 equiv) was dissolved in water and passed over a strongly basic ion-exchanger column (loading: 0.9 mmol/ml, 6 equiv). The obtained solution was lyophilized to afford the corresponding amine as a pale yellow solid. In a round bottom flask $Zn(ClO_4)_2\cdot 6H_2O$ was dissolved in ethanol and a solution of the amine in 5 ml of ethanol was slowly added. A white precipitate formed immediately. The suspension was stirred at room temperature for 16 h and then heated to reflux for 2 h. The white precipitate became an orange oil which separated from the solution. The solution was removed and the oil was dried in vacuum. Ethanol (1 ml) was added and the resulting pale red precipitate was treated with ultrasound for 15 min. After filtration, the solid was washed with 2 ml of ethanol and was dissolved in 1.5 ml of acetonitrile. Any resulting precipitate was removed by centrifugation. Evaporation of the solution in vacuum afforded the zinc-complex as a hygroscopic orange solid.

4.8.1. 1-Benzyl-3-{2-phenyl-1-[2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethylcarbamoyl]-ethyl-carbamoyl}-pyridinium-zinc-(II)-tri-perchlorate (13-Bn). The synthesis follows GP 8 using 101 mg (0.1 mmol) of **11-Bn** and 74 mg (0.2 mmol, 2 equiv) $Zn(ClO_4)_2\cdot 6H_2O$ dissolved in 3 ml ethanol. This afforded **13-Bn** in a yield of 92 mg (0.1 mmol, 100%); mp: 142–145 °C. ^{1}H NMR (300 MHz, CD_3CN): δ=2.70–3.17 (m, 19H, CH_2), 3.32–3.55 (m, 6H, CH_2+3 NH), 4.80–4.87 (m, 1H, C*H), 5.80 (s, 2H, Bn–CH_2), 7.21–7.30 (m, 5H, arom. CH), 7.45–7.49 (m, 5H, arom. CH), 7.99 (d, ^3J=6.6 Hz, 1H, Phe-NH), 8.10 (dd, ^3J=6.1, 8.0 Hz, 1H, py-CH), 8.21 (m, 1H, NH), 8.73–8.76 (m, ^3J=8.1 Hz, 1H, py-CH), 8.82–8.84 (m, ^3J=6.1 Hz, 1H, py-CH), 9.09 (m, 1H, py-CH); ^{13}C NMR (75.5 MHz, CD_3CN): δ=37.6 (−, 1C, Phe), 39.5 (−, 1C), 43.3 (−, 2C), 44.8 (−, 1C), 45.1 (−, 1C), 45.3 (−, 1C), 45.4 (−, 1C), 52.7 (−, 1C), 52.9 (−, 1C), 55.2 (−, 1C), 57.2 (+, C*H), 66.0 (−, 1C), 128.0 (+, 1 arom. C), 129.7 (+, 2 arom. C), 129.7 (+, 1 py-C), 130.3 (+, 2 arom. C), 130.6 (+, 2 arom. C), 130.6 (+, 2 arom. C), 131.1 (+, 1 arom. C), 133.5 (arom. C_{quat}), 135.0 (arom. C_{quat}), 137.8 (arom. C_{quat}), 145.3 (+, py-C), 145.4 (+, py-C), 147.4 (+, py-C), 163.0 (C_{quat}, amide-C), 177.1 (C_{quat}, amide-C); UV–vis (CH_3CN): λ (log ε)=265 nm (3.863); MS (pos. ESI, CH_3CN): m/z= 310.7 [$(M^{3+}-H^+)^{2+}$] (100%), 530.2 [$(M^{3+}-H^+-Bn^+)^+$] (18%), 720.3 [$(M^{3+}-H^++ClO_4^-)^+$] (12%), 820.2 [$(M^{3+}+2ClO_4^-)^+$] (1%); MS (neg. ESI, CH_3CN): m/z=918.1 [$(M^{3+}-H^++3ClO_4^-)^-$] (100%), 1018.1 [$(M^{3+}+4ClO_4^-)^-$] (30%); IR (KBr): $\tilde{\nu}$ [cm^{-1}]=3426, 3297, 3082, 2963, 2933, 1658, 1627, 1539, 1495, 1458, 1092, 748, 703; $[\alpha]_D^{20}$ (CH_3CN)=−24±2°; MF: $[C_{32}H_{44}N_7O_2Zn]^{3+}(ClO_4^-)_3$/$C_{32}H_{44}N_7O_{14}Cl_3Zn$; MW=922.48.

See electronic Supporting information for the synthesis and characterisation of compounds **13-^{i}Pr**, **28**.

4.9. General procedure 9 (GP 9) for the synthesis of compounds 13-Me, 13-CH(OH)–CH_3

In a round bottom flask $Zn(ClO_4)_2\cdot 6H_2O$ was dissolved in ethanol and a solution of the amine in 5 ml of ethanol was added slowly. A white precipitate formed immediately. The suspension was stirred at room temperature for 16 h and then heated to reflux for 2 h. The white precipitate became an orange oil which separated from the solution. The

solution was removed and the oil was dried in vacuum. Ethanol (1 ml) was added and the resulting pale red precipitate was treated with ultrasound for 15 min. After filtration, the solid was washed with 2 ml of ethanol and was dissolved in 1.5 ml of acetonitrile. Any resulting precipitate was removed by centrifugation. Evaporation of the solution in vacuum afforded the zinc-complex as a hygroscopic orange solid.

4.9.1. 1-Benzyl-3-{1*S*-1-[2-(1,4,7,10-tetraazacyclododec1-yl)-ethylcarbamoyl]-ethylcarbamoyl}-pyridinium-zink-(II)-tri-perchlorate (13-Me). The synthesis follows GP 9 using 134 mg (0.36 mmol, 2 equiv) $Zn(ClO_4)_2 \cdot 6H_2O$ dissolved in 3 ml ethanol and 90 mg (0.18 mmol) **12-Me**. This afforded **13-Me** in a yield of 135 mg (0.16 mmol, 91%), mp: 105–108 °C. ^{1}H NMR (400 MHz, CD_3CN): $\delta = 1.56$ (d, $^3J = 7.2$ Hz, 3H, CH_3), 2.66–3.11 (m, 18H, CH_2), 3.35 (m, 1H, NH), 3.41–3.57 (m, 4H, $2CH_2$, 2 NH), 4.57 (dq, $^3J = 1.5$, 7.2 Hz, 1H, CH), 5.84 (s, 2H, CH_2), 7.48–7.54 (m, 5H, CH), 8.10–8.11 (m, 1H, NH), 8.16 (dd, $^3J = 6.3$, 6.6 Hz, 1H, CH), 8.38 (bs, 1H, NH), 8.85–8.87 (m, 1H, CH), 8.91–8.93 (m, 1H, CH), 9.27 (s, 1H, CH); ^{13}C NMR (100.6 MHz, CD_3CN): $\delta = 17.2$ (+), 40.1 (−), 43.4 (−), 43.4 (−), 45.0 (2C, −), 45.3 (2C, −), 52.4 (+), 53.3 (2C, −), 56.0 (−), 66.0 (−), 129.7 (+), 130.5 (2C, +), 130.6 (2C, +), 131.1 (+), 133.6 (C_{quat}), 134.9 (C_{quat}), 145.6 (+), 145.8 (+), 147.5 (+), 163.4 (C_{quat}), 180.0 (C_{quat}); UV–vis (CH_3CN): λ_{max} [nm] ($\log \varepsilon$) = 264 (3.713); MS (ESI, CH_3CN): m/z (%) = 272.5 (100) $[(L^+ - H^+ + Zn^{2+})^{2+}]$, 454.1 (21), 644.3 (5) $[(L^+ - H^+ + Zn^{2+} + ClO_4^-)^+]$, 746.3 (1) $[(L^+ + Zn^{2+} + 2ClO_4^-)^+]$; IR (KBr): $\bar{\nu}$ [cm^{-1}] = 626, 706, 749, 1091, 1454, 1497, 1542, 1669, 2938, 3079, 3294, 3407; $[\alpha]_D^{20}$ (CH_3CN) = +13 ± 1°; MF: $(C_{26}H_{40}N_7O_2Zn)^{3+}$ $(ClO_4^-)_3$, respectively $C_{26}H_{40}N_7O_{14}Cl_3Zn$; MW = 846.39.

See electronic Supporting information for the synthesis and characterisation of compound **13-CH(OH)-CH$_3$**.

4.10. Electronic supporting information

The electronic Supporting information contains experimental procedures and characterization of new compounds, copies of proton and/or carbon NMR spectra of new compounds, a table of the UV absorption maxima of compounds **1–4**, copies of multiplicity edited HSQC and variable temperature spectra of deuterated **1CH(OH)–CH$_3$**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.03.081

References and notes

1. (a) How important they are, is illustrated best by the consequences of non-sufficient supply in human nutrition. Until the end of the 19th century the disease Pellagra (from Italian 'pelle agra' for rough skin) was widespread. Since 1918 it was recognized that the disease resulted from insufficient supply of riboflavine and nicotinic acid. From 1938 on nicotinic acid and amide was added to cereal products in the USA, which drastically reduced the number of deaths from Pellagra. (b) Offermanns, H.; Kleemann, A.; Tanner, H.; Beschke, H.; Friedrich, H. In *Kirk-Othmer Encyclopedia of Chemical Technology*, *Vol. 24*; Wiley: New York, 1984. (c) Gopalan, C.; Rao, K. S. J. *Vitam. Horm.* **1975**, *33*, 505.
2. For a recent review on the mechanism of NADH to NAD+ interconversion, see: Gebicki, J.; Marcinek, A.; Zielonka, J. *J. Acc. Chem. Res.* **2004**, *37*, 379–386.
3. Reichenbach-Klinke, R.; Kruppa, M.; Koenig, B. *J. Am. Chem. Soc.* **2002**, *124*, 12999–13007.
4. Benner, S. A.; Glasfeld, A.; Piccirilli, J. A. *Top. Stereochem.* **1989**, *19*, 127–207.
5. Glasfeld, A.; Leanz, F. G.; Benner, S. A. *J. Biol. Chem.* **1990**, *265*, 11692–11699.
6. You, K.-S. *CRC Crit. Rev. Biochem.* **1985**, *17*, 313.
7. Benner, S. A.; Nambiar, K. P.; Chambers, G. K. *J. Am. Chem. Soc.* **1985**, *107*, 5513–5517.
8. It is assumed that equilibrium constants of an enzymatic reaction approach their optimal values in evolution: Ellington, A. D.; Benner, S. A. *J. Theor. Biol.* **1987**, *127*, 491.
9. Benner, S. A. *Experientia* **1982**, *38*, 633.
10. Solutions of Boc-protected L-valine with equimolar amounts of cyclen, basic ion-exchange resin or 10 equivalents of KOH in water and methanol were prepared. Their optical rotation was monitored over 24 h. While solutions with 10 equivalents of KOH showed rapid racemization, the optical rotation does not change of solutions with cyclen or basic ion-exchange resin.
11. Brandes, S.; Gros, C.; Denat, F.; Pullumbi, P.; Guilard, R. *Bull. Soc. Chim. Fr.* **1996**, *133*, 65–73.
12. The possible risk of racemization under the basic conditions of the ion-exchanger was excluded by a series of control experiments (see Ref. 10). Under the experimental conditions, no loss of stereochemical information could be detected.
13. Force field and semi-empirical calculations of minimum energy conformations have been performed, but results from gas phase cannot reliably predict conformations in aqueous solution, which would be needed to predict and correlate reaction rates.
14. A small amount of reoxidation is difficult to avoid due to the high air sensitivity of the 1,4-dihydronicotine amide.
15. Pullman, M. E.; San Pietro, A. *J. Biol. Chem.* **1954**, *206*, 129.