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NADH Model Systems Functionalized with Zn(II)-Cyclen as Flavin Binding Site—Structure Dependence of the Redox Reaction within Reversible Aggregates

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Abstract: The relative positions and conformations of the prosthetic group FAD and the cofactor NADH have been remarkably conserved within the structurally diverse group of flavin enzymes. To provide a chemical rational for such an obviously optimal relative disposition of the redox partners for efficient reaction we have synthesized NADH models with Zn(II)-cyclen substituents for reversible flavin binding in water. Altogether, four of these model systems with systematically varying spacer length between the recognition site and the redox active dihydronicotinamide were prepared. The binding of these model systems to riboflavin tetraacetate was confirmed by potentiometric pH titration in water and their reaction with flavin was followed by UV−vis spectroscopy in aqueous media under physiological conditions. The measurements reveal a significant rate enhancement of up to 175 times that of an intermolecular reaction. Moreover, a strong dependence of the reaction rate on the spacer length was observed, which clearly shows that within the dynamic reversible assembly only the optimal relative disposition of the redox partners ensures an efficient redox reaction.

Introduction

In living organisms, the flavin prosthetic groups FMN (flavin mononucleotide) and FAD (flavine adenine dinucleotide) act in the cellular redox metabolism as electron-transfer mediators between two-electron reduction and one-electron processes.[1] They accept two electrons from NAD(P)H and transfer one electron to metal centers in proteins containing heme, nonheme iron, or molybdenum sites. This type of redox process is ubiquitous in energy metabolism and has been studied extensively in flavoenzymes[2−6] and chemical model systems.[7−15] However, the electron transfer between reduced pyridine−dinucleotide cofactors and flavins is slow under physiological conditions and has to be catalyzed by enzymes. To mimic the function of these enzymes by chemical models and to enable an efficient electron transfer directly between the redox cofactors, one of them can be functionalized with a recognition site for its counterpart. So far, this has only been done for the flavin part. Flavins have been functionalized with a variety of different recognition moieties including metal chelation sites,[16,17] crown ethers,[18] porphyrins,[19] cyclodextrins,[20] the enzyme papain,[21] and peptides.[22] In this study, we report synthesis and properties of functionalized 1,4-dihydronicotinamides bearing a recognition unit for flavins, which enhances the efficiency of the redox process under physiological conditions.

A comparison of the available X-ray structure analyses of flavin enzymes with both cofactors bound (most of them belong to the glutathione reductase family of enzymes)[23−29] reveal a very similar relative disposition of the redox partners; in all

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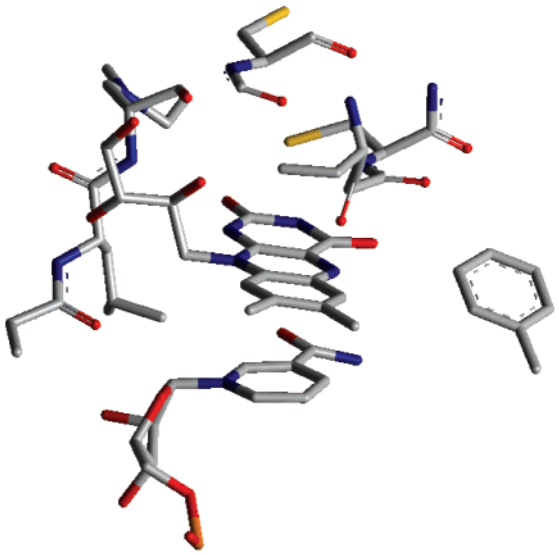

Figure 1. Arrangement of FAD and NADH in glutathione reductase. Water molecules are omitted for clarity. Structure coordinates from Brookhaven protein database, structure no. 1GRB.[25]

cases, the nicotinamide ring is in a nearly parallel arrangement to the flavin centered more or less above the middle ring (see Figure 1 for glutathione reductase). Distances between the planes range from 3 to 4 Å. Marcus theory (if electron transfer mechanism is assumed) and organic reaction mechanisms (if hydride transfer mechanism is assumed) predict an optimal geometrical arrangement as necessary for an efficient process. In this study, we have systematically varied the structure of an NADH and flavin model to demonstrate that changes in the relative orientation of the two cofactors significantly alter reaction rates.

Results and Discussion

Design and Synthesis of the NADH Model Systems. The binding of imides to Lewis-acidic zinc(II) tetraazacyclododecanes has been studied thoroughly by Kimura and coworkers.[30−34] As confirmed by several X-ray structures, the deprotonated imide nitrogen coordinates to the zinc(II) ion, which is complexed by 1,4,7,10-tetraazacyclododecane (cyclen). The binding is probably enhanced by hydrogen bonds between the carbonyl oxygen atoms of the imide and the NH groups of the cyclen. This kinetically labile coordinative bond is stronger than other noncovalent reversible interactions, such as hydrogen bonds, salt bridges, or hydrophobic interactions and, therefore, provides high affinities, even in water at neutral pH. Thus, we chose this binding motif to assemble the imide-containing flavin with a suitable functionalized NADH model system. Moreover, it has been shown that coordination to Zn(II)-cyclen[35] or other receptors[36−41] has a favorable influence on the redox potentials of the flavin facilitating its reduction.

To evaluate the dependence of electron-transfer efficiency on the distance of recognition site and redox-active 1,4-dihydronicotinamide, four NADH model systems with systematic variations of the spacer length were synthesized.[42]

The synthesis is outlined in Scheme 1 and starts with the selective protection of the azamacrocycle to obtain 1,4,7-tris-*tert*-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane **1**.[43] In the following steps, **1** is alkylated to give the nitriles **2** and **14** that were subsequently reduced to the corresponding amines **3** and **6**. Then, the different spacers and/or the redox active 1-benzyl-3-carboxy-pyridinium-bromide[44] were introduced by peptide coupling methods. After deprotection of the Boc-groups and complexation of the azamacrocycle with zinc(II) ions, the pyridinium salts are reduced selectively to the corresponding 1,4-dihydronicotinamides using sodium dithionite. The NADH model systems **5**, **8**, **10**, and **13** are very sensitive to oxygen and therefore have to be handled strictly under an argon atmosphere and prepared freshly prior to use.

Binding Studies by Potentiometric pH Titration. To confirm the binding between the NADH model systems and flavin, potentiometric pH titrations in water were performed. Because of its higher photostability,[45,46] riboflavin tetraacetate was used as the flavin part.

The free ligand 1-benzyl-3-[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl-pyridinium-bromide-tri-hydrobromide**15** shows three pK_a values (log$K$ = 2.1, 8.8, 11.4) for the secondary azamacrocyclic nitrogen atoms that are in the range of comparable compounds found in the literature.[47] Moreover, the Zn^{2+}-ion is tightly bound to the cyclen (log$K$ = 9.8) and the pK_a value of the axial coordinated water molecule (see Figure 2) illustrates the Lewis-acidity of the Zn(II)-complex **16**. As shown in Figure 2 the riboflavin tetraacetate coordinates in its deprotonated form to **16** (log$K$ = 6.3). The same or only slightly different values were obtained for the interaction of riboflavin tetraacetate with the other complexes having longer spacers between the recognition unit and the redox active part.

Proof of the Redox Reaction between the NADH Model Systems and Riboflavin Tetraacetate by UV−Vis Spectroscopy. The redox reaction between the NADH model systems and riboflavin tetraacetate was followed by changes in the UV−vis spectra. The spectra of oxidized and reduced model systems are very similar to the analogous free riboflavin tetraacetate

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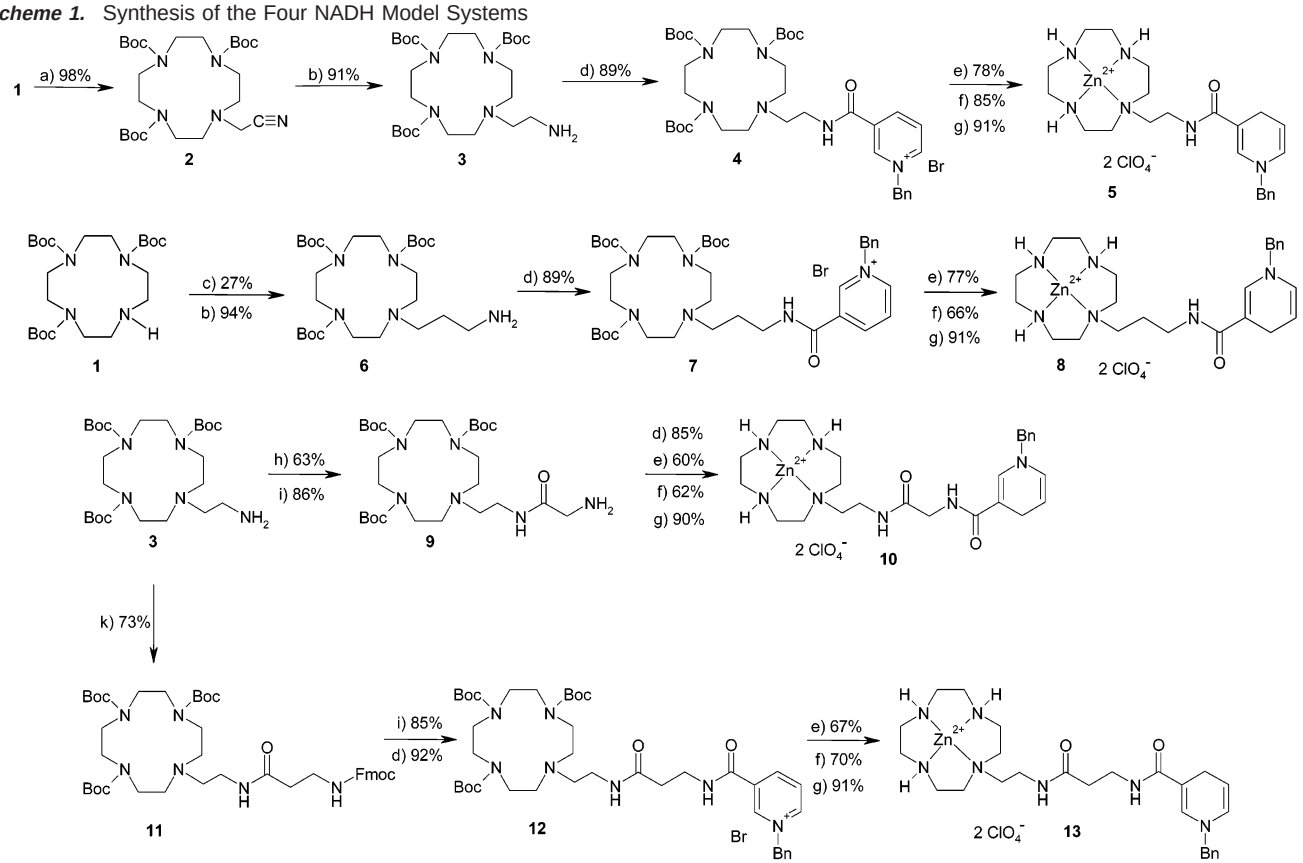
Scheme 1. Synthesis of the Four NADH Model Systems



(a) Bromoacetonitrile, CH_3CN, 50 °C, 16 h; (b) H_2/Raney-Ni, EtOH/NH_3, room temp., 15 bar, 48 h; (c) acrylonitrile, 100 °C, 5 d; (d) 1-benzyl-3-carboxy-pyridinium-bromide, EDC, HOAt, DMF, room temp., 18 h; (e) HBr/glacial acetic acid, room temp., 30 min; (f) (i) basic anion exchange column, (ii) $Zn(ClO_4)_2 \cdot 6\ H_2O$, MeOH, reflux, 1 h; (g) $Na_2S_2O_4$, Na_2CO_3, H_2O, room temp., 1 h; (h) Fmoc-glycine, EDC, HOBt, DMF, 0 °C, 8 h; (i) piperidine, DMF, room temp, 30 min; (k) Fmoc-β-alanine, EDC, HOBt, 0 °C, 4 h.

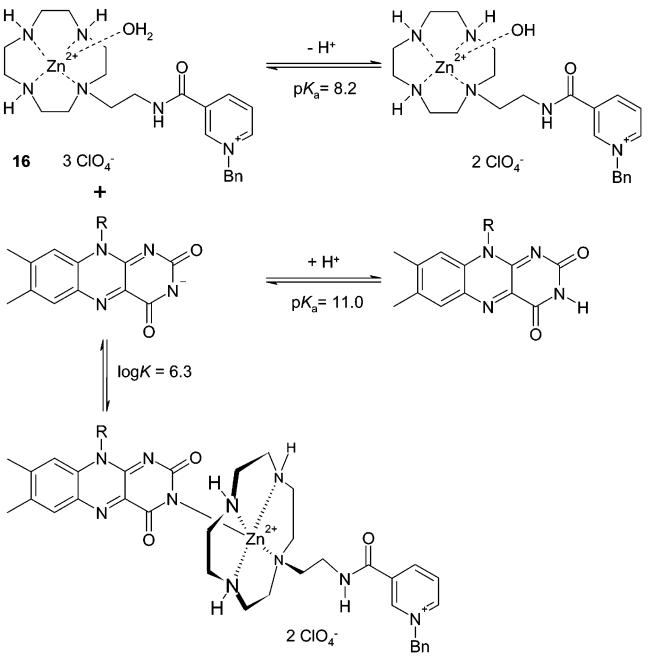


Figure 2. Binding equilibria between **16** and riboflavin tetraacetate. All equilibrium constants were determined by potentiometric titration in water (I = 0.1 mol/l NEt_4^+ ClO_4^-, 25 °C) with NEt_4OH as a base. See the Supporting Information for experimental details. R = ribityl-tetraacetate.

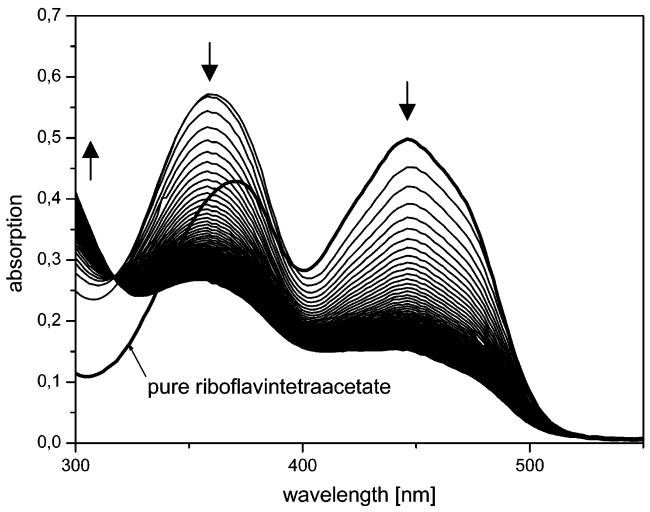


Figure 3. Time course of the absorbance during the reaction of riboflavin tetraacetate with 1 eq NADH model system **13**. Measured in aqueous Tris/HCl buffer pH 9.0 at $c = 5 \times 10^{-5}$ mol/l under argon atmosphere.

species (see Figure 5 of the Supporting Information). The intensity of the long-wave absorption of the flavin at 456 nm decreases significantly during the reaction as shown in Figure 3. This can be attributed to the reduction of the flavin to the corresponding fully reduced species, the flavohydroquinone, that shows only a very weak absorption at this wavelength (see Figure 5 of the Supporting Information). Simultaneously, the intensity of the peak at around 360 nm decreases as well. This is due both to the disappearance of the flavin peak at 384 nm and the oxidation of the 1,4-dihydronicotinamide (λ_{max} = 357 nm) to the corresponding pyridinium species (λ_{max} = 262 nm).

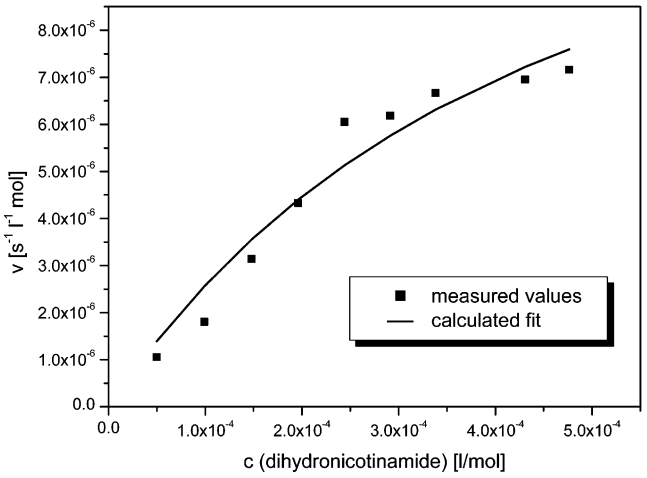


Figure 4. Dependence of the rate of the redox reaction of riboflavin tetraacetate and NADH model system **10** on the concentration of 1,4-dihydronicotinamide as described by eq 1.

Scheme 2. Two Possible Mechanisms for the Redox Reaction between Riboflavin Tetraacetate and the NADH Model Systems

(1) $PyH + Fl \xrightarrow{k_2} Py^+ + FlH_2^-$

(2) $PyH + Fl \overset{K_{ass}}{\rightleftharpoons} PyH\cdots Fl \xrightarrow{k_1} Py^+ + FlH_2^-$ or $Py^+\cdots FlH_2^-$

PyH = NADH model system, Py^+ = oxidized NADH model system, Fl = oxidized flavin, FlH_2 = flavohydroquinone.

At wavelengths shorter than 317 nm, an increase in absorption is observed, which can be assigned to flavin reduction (see Figure 6 of the Supporting Information). At higher concentrations of one reacting species, it is possible to observe an additional absorption band at $\lambda > 500$ nm (see Figure 7 of the Supporting Information). This rather weak long-wavelength band develops during the reaction with an isosbestic point at 516 nm and can be assigned to a charge-transfer complex of flavohydroquinone and the oxidized pyridinium species.[5,8,48]

Altogether, the data confirm the redox reaction between riboflavin tetraacetate and the NADH model systems in water at physiological pH.

Kinetics of the Redox Reaction between Riboflavin Tetraacetate and the NADH Model Systems. Two reasonable models for the kinetic of the reduction of riboflavin tetraacetate by the NADH model systems are shown in Scheme 2. On one hand, the intermolecular redox reaction of the NADH model systems with riboflavin tetraacetate can be described by a second-order rate law (see reaction 1 in Scheme 2). On the other hand if prior association of the flavin and the 1,4-dihydronicotinamide is taken into account, the intramolecular redox reaction should follow first-order rate law kinetics (see reaction 2 in Scheme 2). The second mechanism may describe reality better, but for comparison with the intermolecular redox reaction the first possibility was evaluated, too.

For this comparison, 1-benzyl-1,4-dihydropyridine-3-carboxylic acid diethylamide **17** as an NADH model compound lacking a binding site for flavin was synthesized.[49] The reaction of riboflavin tetraacetate with 1 equiv of **17** was monitored by the decrease in absorbance intensity at 450 nm. The second-order rate constant was calculated from the initial rates by the method of Roseveare[50,51] as $k_2 = 22.8 \pm 1.6$ l·mol^{-1}·s^{-1} at 25 °C in HEPES/KOH buffer pH 7.4. The derived value is in good agreement with comparable systems found in the literature.[52] The rate constants for the reaction of the NADH model systems **5**, **8**, **10**, and **13** with riboflavin tetraacetate were measured under the same conditions, and the results are summarized in Table 1.

Table 1. Second-Order Rate Constants for the Redox Reaction between Riboflavin Tetraacetate and the NADH Model Systems

NADH model compd	k_2 [l·mol^{-1}·s^{-1}]	rate enhancement compared to **17**
17	22.8 ± 1.6	1
5	408 ± 26	18
8	671 ± 37	29
10	3998 ± 321	175
13	646 ± 67	28

[a] Rate constants of a 1:1 mixture of riboflavin tetraacetate and 1,4-dihydronicotinamides ($c = 5 \times 10^{-5}$ mol/l) measured at 25 °C in HEPES/KOH buffer pH 7.4. Detection by spectrophotometry at 450 nm. The given values are the average results of three independent measurements.

For all NADH model systems with the Zn(II)-cyclen recognition unit, a significant rate enhancement was measured in the reaction with riboflavin tetraacetate. Moreover, the rate of reaction shows a strong dependence on the spacer length between the flavin binding site and the redox active dihydronicotinamide part. The maximum rate was with the NADH model system **10**. For compound **13** bearing the longest spacer, the rate is less than that of **10** and is similar to that of **8**. A very high k_2, which reaches nearly 4000 l·mol^{-1}·s^{-1} was obtained for compound **10**. Compared with the intermolecular reaction this corresponds to a rate enhancement of 175-fold. The measured enhancement in reaction rate is to the best of our knowledge the highest efficiency for dihydropyridine reduction of flavins observed in nonenzymatic systems under comparable conditions so far. For a related example, the redox reaction of a flavocyclodextrin and *N*-*n*-hexyl-1,4-dihydronicotinamide a k_2 of 1200 l·mol^{-1}·s^{-1} was measured in water at pH 7.4 and 25 °C.[20]

To confirm these results and to get further mechanistic insights, the reaction between the NADH model systems and riboflavin tetraacetate was studied at varying concentrations of the 1,4-dihydronicotinamide and evaluated according to mechanism 2 (see Scheme 2). Thus, riboflavin tetraacetate ($c = 5 \times 10^{-5}$ mol/l) was mixed with 1 to 10 eq NADH model compound in HEPES/KOH buffer pH 7.4 and the reaction was followed spectrophotometrically at 460 nm and 25 °C. The first-order rate constant k_1 and the binding constant K_{ass} were calculated

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from the initial rates and the concentrations by nonlinear fitting to the following equation[53]

$$\frac{1}{v} = \frac{1}{2 \cdot k_1 \cdot [R]_0 \cdot [D]_0} \cdot \left([R]_0 + [D]_0 + \frac{1}{K_{as}} + \sqrt{\left([R]_0 + [D]_0 + \frac{1}{K_{as}} \right)^2 - 4 \cdot [R]_0 \cdot [D]_0} \right) \quad (1)$$

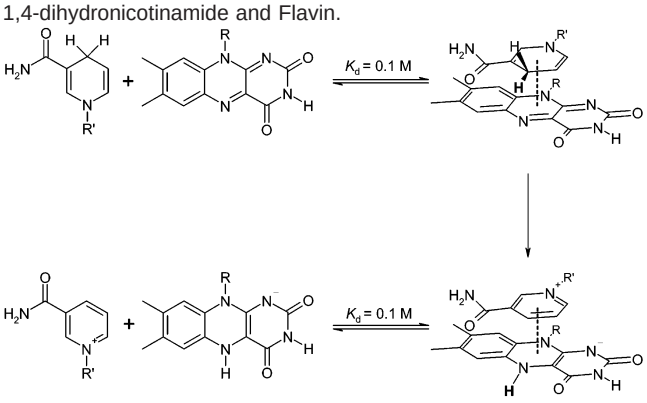
The derived values of k_1 (see Table 2) show the same spacer length dependence as in the corresponding second-order rate constants. The association constants are within the error limits in the same order of magnitude around 10^4 l/mol. These values are 4−5 times higher than the binding constants between riboflavin tetraacetate and the pyridinium species as measured by potentiometric titration: An association constant of $K = 2700$ l/mol was calculated from the titration data at pH 7.5 by the program Hyperquad 2000[54] for the interaction between riboflavin tetraacetate and **16**. This increased affinity of 1,4-dihydronicotinamides to riboflavin tetraacetate as compared to the corresponding pyridinium species can be rationalized by the additional charge-transfer interactions. The strength of these interactions between different flavins and *N*-methyl-1,4-dihydronicotinamide was quantified by Blankenhorn.[7] In these studies, binding constants up to 10 l/mol were measured in aqueous media. The similar values of association strengths of riboflavin tetraacetate and the four NADH model systems lead to the conclusion that the significant variation in redox reaction rate constants must have their origin in the geometry of the complex. During the redox reaction, the hydrogen at the 4-position of the dihydronicotinamide is transferred to the N-5 of the flavin as outlined in Scheme 3.[9] In the assembly between riboflavin tetraacetate and NADH model **10**, it appears that C-4 of dihydronicotinamide and N-5 of the flavin are in a more favorable position to each other than in any of the other models to enable an efficient hydrogen transfer between the two rings. The effect of the linker length in compounds **5, 8, 10**, and **13** on the likely structure of the assembly with flavin was explored with computational methods. The most stable conformer was identified by a systematic conformer analysis (MMFF). The distance between flavin and nicotine amide was constrained to 3.4 Å and the geometry of the structure was optimized by semiempirical methods (PM3). Structures and details of calculations are given in the Supporting Information. A comparison of the assembly structures indicates that in the complex of **10** with **flavin** the optimal arrangement of the redox partners is achieved with little extra strain. Although the simple gas-phase calculations do not allow to derive any quantitative values or give inside into the dynamic behavior of the structures, they support the obvious hypothesis that the structure which keeps flavin and dihydronicotinamide on average in time in the optimal distance and orientation for reaction yields the fastest reaction rates.

Despite the importance of 1,4-dhydronicotinamides in biochemical redox reactions, the mechanism for these reactions continues to be debated. Three possible mechanisms for the transfer of a hydride equivalent from dihydronicotinamide to the substrate are discussed in the literature: (i) transfer of a hydride ion in a single step, (ii) two-step transfer of an electron and a hydrogen atom, or (iii) overall transfer of two electrons and a proton in three separate steps. From a review of the existing literature and studies of the reaction of flavin with various 1,4-dihydronicotinamides, Powell and Bruice[11] came to the conclusion that the reaction proceeds via hydride-transfer in a single step. This observation was confirmed by calculations[55,56] and by studies in phthalate dioxygenase reductase and other systems.[2] By contrast, Miller and Carlson[57] could not rule out a hydrogen transfer followed by an electron transfer in their studies involving the reduction of quinones by NADH. In the reduction of ketones by dihydronicotinamides a hydrogen atom transfer is favored as well.[58] Although Tanaka et al.[59,60] discussed a stepwise mechanism involving electron transfer followed by proton transfer, Bunting[61] suggested in a review a rational merging of all these three possible mechanisms. Thus, the mechanism of the redox reaction between dihydronicotinamides and flavins is not yet fully understood. So we decided to do isotope studies in our system. For this purpose, the reduction of **16** was carried out in D_2O to obtain a 4-monodeuterated NADH model system.[62] This NADH model was then reacted with riboflavin tetraacetate under the same conditions as the nondeuterated compound. A first-order rate constant of $k_1 = 0.031 \pm 0.002\ s^{-1}$ corresponding to a kinetic isotope effect of $k_H/k_D = 1.29 \pm 0.09$ was measured. This indicates that the hydrogen at the 4-position of the dihydronicotinamide is likely to be transferred in the rate-determining step which supports the hydride mechanism in our model system.

Table 2. First-Order Rate and Association Constants of the Redox Reaction between Riboflavin Tetraacetate and the NADH Model Systems as Derived from Eq 1

NADH model compd	k [s^{-1}]	K_{as} [l/mol]
5	0.040 ± 0.003	12000 ± 3400
8	0.053 ± 0.004	14000 ± 4000
10	0.318 ± 0.047	8000 ± 2900
13	0.052 ± 0.008	10200 ± 2000

Scheme 3. Mechanism of the Redox Reaction between 1,4-dihydronicotinamide and Flavin.



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Conclusion

The efficiency of the redox reaction of 1,4-dihydronicotinamide, which is functionalized with a binding site for flavins, and riboflavin tetraacetate under physiological conditions in water is enhanced significantly. The measured rate constant is the highest reported for comparable nonenzymatic model systems so far. Moreover, the distance between the binding site and the redox active part was varied systematically, and this demonstrated a significant dependence of the rate constants on the spacer length. This indicates that the reaction is strongly dependent on the orientation of the dihydropyridine and the isoalloxazine ring to each other.

This observation provides a chemical rational why the geometry of the flavin and NADH binding site in various enzymes including glutathione reductase,[23−26] NADH peroxidase,[27] ferredoxin reductase,[29] and adrenodoxin reductase[28] is highly conserved.

Experimental Section

Materials and Techniques. Compounds **1**,[43] **17**,[49] and 1-benzyl-3-carboxy-pyridinium-bromide[44] were prepared according to known procedures. Melting points were taken on a hot-plate microscope apparatus and are not corrected. The multiplicity of the ^{13}C signals was determined with the DEPT technique and quoted as (+) for CH_3 and CH, (−) for CH_2 and (C_{quart}) for quaternary carbons. UV spectra were recorded on Varian Cary 50 Bio or Zeiss Specord M 500, fluorescence spectra on Varian Cary Eclipse. The fluorescence quantum yields were measured with quinine sulfate in 1 N sulfuric acid as the standard. For details of potentiometric measurements and kinetic measurements, see the Supporting Information.

Synthesis. 10-Cyanomethyl-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (2). 1,4,7-tris-*tert*-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane **1** (2.77 g, 5.9 mmol) was dissolved in 20 mL acetonitrile. Then K_2CO_3 (0.98 g, 7.1 mmol) and bromoacetonitrile (0.45 mL, 0.78 g, 6.5 mmol) were added. The suspension was heated at 50 °C for 16 h. After cooling to room temperature, the K_2CO_3 was filtered off. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica (eluent: EE/PE = 1:1, $R_f = 0.6$) to yield **2** (2.97 g, 5.8 mmol, 98%) as a white solid. mp 71 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1165, 1250, 1366, 1417, 1461, 1685, 2935, 2974; ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.43 (s, 18 H, CH_3), 1.46 (s, 9H, CH_3), 2.82 (bs, 4H, CH_2), 3.35−3.47 (m, 12H, CH_2), 3.82 (bs, 2H, CH_2); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.47 (6C, +), 28.71 (3C, +), 38.88 (−), 46.44 (−), 46.93 (−), 47.33 (2C, −), 49.85 (−), 50.07 (−), 53.93 (−), 54.47 (−), 79.57 (C_{quart}), 79.89 (C_{quart}), 80.19 (C_{quart}), 114.52 (C_{quart}), 155.09 (C_{quart}), 155.84 (C_{quart}), 155.98 (C_{quart}); MS (ESI, CH_3CN) *m*/*z* (%) 512 (100) [MH^+]; Anal. calcd. for $C_{25}H_{45}N_5O_6$, C 58.69, H 8.86, N 13.69; found, C 58.40, H 8.61, N 13.42.

10-(2-Aminoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (3). The nitrile **2** (3.24 g, 6.3 mmol) was dissolved in 50 mL NH_3-saturated ethanol. A 0.5 g portion of Raney-Nickel was added, and the mixture was hydrogenated at room temperature (H_2 pressure: 15 bar) for 48 h. After the catalysator had been filtered off, the solvent was removed in vacuo. The amine **3** (2.93 g, 5.7 mmol, 91%) was obtained as a white solid. mp 63 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1171, 1251, 1366, 1417, 1463, 1689, 2934, 2976, 3443; ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.27 (s, 2H, NH_2), 1.45 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 2.55−2.80 (m, 6H, CH_2), 2.83 (t, 2H, CH_2), 3.20−3.59 (m, 12H, CH_2); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.51 (6C, +), 28.68 (3C, +), 38.21 (−), 48.07 (4 C, −), 50.00 (2 C, −), 54.61 (−), 55.67 (−), 56.65 (−), 79.37 (C_{quart}), 79.59 (2 C, C_{quart}), 155.43 (C_{quart}), 155.78 (C_{quart}), 156.18 (C_{quart}); MS (ESI, CH_2Cl_2) *m*/*z* (%) 516 (100) [MH^+]; Anal. calcd. for $C_{25}H_{49}N_5O_6$, C 58.23, H 9.58, N 13.58; found, C 58.20, H 9.45, N 13.24.

1-Benzyl-3-[2-(4,7,10-tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododec-1-yl)-ethyl-carbamoyl]-pyridinium-bromide (4). A mixture of **3** (0.70 g, 1.36 mmol), HOAt (0.22 g, 1.63 mmol), 1-benzyl-3-carboxy-pyridinium-bromide (0.44 g, 1.50 mmol), EDC (0.29 mL, 0.25 g, 1.63 mmol) and 10 mL DMF was stirred for 18 h at room temperature. The solvent was removed in vacuo and the red precipitate was dissolved in 15 mL CH_2Cl_2. The solution was extracted twice with 5 mL 1 N HCl, dried over $MgSO_4$ and evaporated to dryness to obtain **4** (0.96 g, 1.21 mmol, 89%) as a white solid. mp 68−71 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 706, 1174, 1253, 1368, 1416, 1478, 1686, 2934, 2981, 3425; UV−vis (CH_3CN) λ_{max} (lg ϵ) = 264 nm (3.648); ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.44 (s, 18H, CH_3), 1.45 (s, 9H, CH_3), 3.35 (bs, 4H, CH_2), 3.48 (bs, 10H, CH_2), 3.64 (bs, 4H, CH_2), 3.89 (bs, 2H, CH_2), 5.88 (bs, 2H, CH_2), 7.45 (m, 3H, CH), 7.49 (m, 2H, CH), 8.03 (bs, 1H, CH), 8.91 (bs, 1H, CH), 9.02 (bs, 1H, CH), 9.84 (bs, 1H, CH), 10.22 (bs, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.25 (6C, +), 28.31 (3C, +), 35.16 (−), 45.35 (2C, −), 49.98 (4 C, −), 50.42 (2C, −), 53.85 (−), 65.65 (−), 81.00 (C_{quart}), 81.86 (2C, C_{quart}), 128.18 (+), 129.58 (2C, +), 129.92 (2C, +), 130.49 (+), 131.63 (C_{quart}), 134.62 (C_{quart}), 144.96 (+), 145.03 (+), 145.14 (+), 156.55 (C_{quart}), 156.71 (2C, C_{quart}), 161.90 (C_{quart}); MS (ESI, CH_2Cl_2/MeOH + 1% AcOH) *m*/*z* (%) 356 (10) [$(K^+ + H^+)^{2+}$], 411 (2) [$(K^+$ 3 Boc)$^+$], 511 (2) [$(K^+$ 2 Boc)$^+$], 611 (2) [$(K^+$ Boc)$^+$], 711 (100) [K^+]; HRMS ($C_{38}H_{59}N_6O_7^+$) ber. 711.4445, gef. 711.4433 ± 0.0007.

1-Benzyl-3-[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl-pyridinium-bromide-tri-hydrobromide (15). A mixture of **4** (1.145 g, 1.45 mmol) and 5 mL HBr in glacial acetic acid (33%, 4.1 M) was stirred at room temperature for 30 min. Then 10 mL diethyl ether were added, the precipitate was collected by suction filtration and washed extensively with ether. The crude product was suspended in ethanol, stirred at room temperature for 30 min and collected by suction filtration. The product **15** (0.83 g, 1.13 mmol, 78%) was isolated as a white powder. Decomp. at 125 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1440, 1544, 1628, 1669, 2725, 2980, 3420; UV−vis (H_2O) λ_{max} (lg ϵ) = 263 nm (3.843); ^1H NMR (250 MHz, D_2O, δ [ppm]) 2.75−3.53 (m, 20H, CH_2), 5.80 (s, 2H, CH_2), 7.40 (s, 5H, CH), 8.09 (dd, ^3J = 6.3, 7.9 Hz, 1H, CH), 8.77 (d, ^3J = 7.9 Hz, 1H, CH), 8.96 (d, ^3J = 6.3 Hz, 1H, CH), 9.21 (s, 1H, CH); ^{13}C NMR (62 MHz, D_2O, δ [ppm]) 36.75 (−), 41.62 (2C, −), 42.14 (2C, −), 44.31 (2C, −), 48.02 (2C, −), 51.80 (−), 65.15 (−), 128.73 (+), 129.49 (2C, +), 129.72 (2C, +), 130.24 (+), 132.21 (C_{quart}), 134.02 (C_{quart}), 144.05 (2C, +), 146.64 (+), 163.81 (C_{quart}); MS (ESI, MeOH/H_2O) *m*/*z* (%) 206 (100) [$(K^+ + H^+)^{2+}$], 411 (5) [K^+], 491 (9) [$(K^+ + HBr)^+$]; Anal. calcd. for $C_{23}H_{38}Br_4N_6O \times 2\ H_2O$, C 35.87, H 5.50, N 10.91; found, C 35.75, H 5.54, N 10.92.

1-Benzyl-3-[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl-pyridinium-zinc(II)-tri-perchlorate (16). The hydrobromide **15** (0.71 g, 0.97 mmol) was dissolved 3 mL H_2O and eluated over a basic anion exchange column. The solution was evaporated to dryness and the solid residue was dissolved in 3 mL methanol. Then a solution of $Zn(ClO_4)_2 \times 6\ H_2O$ (0.72 g, 1.94 mmol) in 5 mL methanol was added and the mixture was refluxed for 1 h. After the reaction mixture had been cooled in an ice bath, the precipitate was collected by suction filtration. The crude product was dissolved in acetonitrile, undissoled solids were filtered off and the solvent was removed in vacuo to obtain **16** (0.64 g, 0.82 mmol, 85%) as a orange solid. Decomp. at 230−240 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 549, 1093, 1458, 1552, 1632, 1672, 2938, 3084, 3296, 3363; UV−vis (H_2O) λ_{max} (lg ϵ) = 262 nm (3.689); ^1H NMR (250 MHz, CD_3CN, δ [ppm]) 2.55−3.76 (m, 23H, CH_2, NH), 5.81 (s, 2H, CH_2), 7.48 (m, 5H, CH), 7.81−8.14 (m, 2H, CH, NH), 8.83 (m, 2H, CH), 9.18 (s, 1H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 36.38 (−), 43.47 (2 C, −), 44.76 (2C, −), 45.58 (2C, −), 50.94 (−), 52.27 (2C, −), 65.92 (−), 129.63 (+), 130.48 (2C, +), 130.56 (2C, +), 131.05 (+), 133.53 (C_{quart}), 135.50 (C_{quart}), 145.06 (+), 145.41 (+), 147.20 (+), 163.27 (C_{quart}); MS (ESI, CH_3CN) *m*/*z* (%) 237 (100) [$(M^{3+} - H^+)^{2+}$], 288 (20) [$(M^{3+} + ClO_4^-)^{2+}$], 575 (28) [$(M^{3+} + ClO_4^- - H^+)^+$],

675 (5) [$(M^{3+} + 2\ ClO_4^-)^+$]; Anal. calcd. for $C_{23}H_{35}Cl_3N_6O_{13}Zn \times H_2O$, C 34.82, H 4.70, N 10.59; found, C 35.02, H 4.70, N 10.61.

1-Benzyl-1,4-dihydropyridine-3-carboxylic acid [2-(1,4,7,10-tetraazacyclododec-1-yl)-ethyl]-amide-zinc(II)-di-perchlorate (5). Compound **16** (34 mg, 44 μmol) is dissolved in 5 mL degassed H_2O and Na_2CO_3 (19 mg, 0.18 mmol) and $Na_2S_2O_4$ (18 mg, 88 μmol) were added. The solution was stirred at room temperature for 1 h. Then the reaction mixture was evaporated to dryness and to the residue was added 1 mL degassed acetonitrile. The suspension was filtered and the solvent was removed under reduced pressure to obtain **5** (27 mg, 40 μmol, 91%) as a yellow, very air-sensitive solid. UV−vis (H_2O) λ_{max} (lg ϵ) = 356 nm (3.781); fluorescence (H_2O, λ_{ex} = 360 nm) λ_{max} = 472 nm, Φ = 0.050; ^{1}H NMR (250 MHz, CD_3CN, δ [ppm]) 2.46−2.98 (m, 18H, CH_2), 3.04 (m, 2H, CH_2), 3.20−3.43 (m, 5H, CH_2, NH), 4.32 (s, 2H, CH_2), 4.75 (dt, ^3J = 8.0, 3.6 Hz, 1H, CH), 5.88 (ddt, ^3J = 8.0 Hz, ^4J = 1.6, 1.7 Hz, 1H, CH), 6.31 (bs, 1H, NH), 7.03 (m, 1H, CH), 7.29 (m, 5H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 22.85 (−), 36.00 (−), 43.49 (2C, −), 44.69 (2C, −), 45.55 (2C, −), 51.53 (2C, −), 53.88 (−), 57.59 (−), 103.90 (+), 128.44 (2C, +), 128.73 (+), 129.81 (2C, +), 130.33 (2C, +), 139.31 (C_{quart}), 140.21 (C_{quart}), 169.98 (C_{quart}).

10-(2-Cyanoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (14). 1,4,7-Tris-*tert*-butyloxycarbonyl-1,4,7,10-tetraazacyclododecane **1** (1.08 g, 2.27 mmol) was dissloved in acrylonitrile (50.0 mL, 40.5 g, 0.76 mol) and refluxed for 5 days. The reaction mixture was evaporated to dryness and to the residue were added 50 mL dichloromethane. The polmeric material was filtered off, the solvent was removed in vacuo and the crude product was purified by column chromatography on silica (eluent: EE, R_f = 0.7). The product **14** (0.32 g, 0.62 mmol, 27%) was isolated as a white powder and starting material **1** (0.58 g, 1.23 mmol) was recovered. mp 55−56 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 773, 1171, 1251, 1366, 1417, 1462, 1686, 2248, 2977; ^{1}H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.45 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 2.49 (t, ^3J = 6.9 Hz, 2H, CH_2), 2.72 (m, 4 H, CH_2), 3.00(t, ^3J = 6.9 Hz, 2H, CH_2), 3.32−3.51 (m, 12H, CH_2); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.49 (6C, +), 28.68 (3C, +), 47.61 (3C, −), 48.00 (3C, −), 50.14 (2C, −), 53.35 (−), 54.35 (−), 79.49 (C_{quart}), 79.87 (2 C, C_{quart}), 119.11 (C_{quart}), 155.34 (C_{quart}), 155.84 (C_{quart}), 156.16 (C_{quart}); MS (ESI, CH_3CN) *m*/*z* (%) 526 (100) [MH^+]; Anal. calcd. for $C_{26}H_{47}N_5O_6$, C 59.41, H 9.01, N 13.32; found, C 59.20, H 9.15, N 13.04.

10-(3-Aminopropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (6). Compound **6** was synthesized from the above nitrile (0.43 g, 0.82 mmol) by a method similar to that of **3**. The amine **6** (0.41 g, 0.77 mmol, 94%) was obtained as a white solid. mp 52−56 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1172, 1251, 1366, 1416, 1463, 1690, 2933, 2976, 3438; ^{1}H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.45 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 1.67 (m, 2H, CH_2), 2.05 (bs, 2H, NH_2), 2.57−2.75 (m, 8H, CH_2), 3.29−3.61 (m, 12H, CH_2); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 27.44 (−), 28.53 (6C, +), 28.70 (3C, +), 40.14 (−), 47.56 (−), 47.94 (3C, −), 49.98 (3C, −), 53.76 (−), 54.82 (−), 79.34 (C_{quart}), 79.57 (2C, C_{quart}), 155.44 (C_{quart}), 155.77 (C_{quart}), 156.19 (C_{quart}); MS (ESI, MeOH + 1% AcOH) *m*/*z* (%) 265 (10) [$(M + 2H^+)^{2+}$], 530 (100) [MH^+], 1059 (1) [$(2M + H^+)^+$], 1081 (1) [$(2\ M + Na^+)^+$]; Anal. calcd. for $C_{26}H_{51}N_5O_6 \times H_2O$, C 57.01, H 9.75, N 12.79; found, C 57.09, H 9.46, N 12.38.

1-Benzyl-3-[3-(4,7,10-tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododec-1-yl)-propylcarbamoyl]-pyridinium-bromide (7). Compound **7** was synthesized from amine **6** (0.38 g, 0.71 mmol) by a method similar to that of **4**. The product **7** (0.51 g, 0.63 mmol, 89%) was isolated as a white powder. mp 83 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1162, 1251, 1367, 1414, 1468, 1694, 2932, 2975, 3433; UV−vis (CH_3CN) λ_{max} (lg ϵ) = 257 nm (3.729); ^{1}H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.44 (bs, 27H, CH_3), 2.25 (bs, 2H, CH_2), 3.29−3.84 (m, 20H, CH_2), 6.06 (bs, 2H, CH_2), 7.34 (m, 3H, CH), 7.49 (m, 2H, CH), 8.03 (m, 1H, CH), 8.91 (m, 1H, CH), 9.22 (m, 1H, CH), 9.68 (bs, 1H, CH), 10.21 (bs, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 23.91 (−), 28.44 (6C, +), 28.51 (3C, +), 37.03 (−), 45.56 (2C, −), 48.52 (2C, −), 50.10 (2C, −), 50.50 (2C, −-), 52.21 (−), 65.06 (−), 80.98 (C_{quart}), 81.59 (2C, C_{quart}), 128.06 (+), 129.70 (2C, +), 129.88 (2C, +), 130.10 (+), 132.49 (C_{quart}), 134.73 (C_{quart}), 144.78 (+), 145.06 (+), 145.52 (+), 156.53 (2C, C_{quart}), 156.64 (C_{quart}), 161.56 (C_{quart}); MS (ESI, MeOH) *m*/*z* (%) 363 (100) [$(K^+ + H^+)^{2+}$], 725 (80) [K^+]; HRMS ($C_{39}H_{61}N_6O_7^+$) calcd, 725.4602; found, 725.4622 ± 0.0037.

1-Benzyl-3-[3-(1,4,7,10-tetraazacyclododec-1-yl)-propylcarbamoyl]-pyridinium-bromid-tri-hydrobromide. The compound was synthesized from **7** (0.46 g, 0.57 mmol) by a method similar to that of **15** and was obtained as a white powder (0.33 g, 0.44 mmol, 77%). Decomp at 169 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1456, 1551, 1669, 2955, 3433; UV−vis (H_2O) λ_{max} (lg ϵ) 263 nm (3.759); ^{1}H NMR (250 MHz, D_2O, δ [ppm]) 1.83 (tt, ^3J = 6.7, 8.1 Hz, 2H, CH_2), 2.85 (t, ^3J = 8.0 Hz, 2H, CH_2), 3.03−3.24 (m, 16H, CH_2), 3.33 (t, ^3J = 6.7 Hz, 2H, CH_2), 5.75 (s, 2H, CH_2), 7.36 (m, 5H, CH), 8.02 (dd, ^3J = 6.3, 8.2 Hz, 1H, CH), 8.72 (d, ^3J = 8.3 Hz, 1H, CH), 8.89 (d, ^3J = 6.2 Hz, 1H, CH), 9.19 (s, 1H, CH); ^{13}C NMR (62 MHz, D_2O, δ [ppm]) 23.53 (−), 37.94 (−), 42.33 (2C, −), 42.61 (2C, −), 43.94 (2C, −), 49.14 (2C, −), 51.57 (−), 65.11 (−), 128.60 (+), 129.41 (2C, +), 129.67 (2C, +), 130.17 (+), 132.20 (C_{quart}), 134.44 (C_{quart}), 144.11 (2C, +), 146.34 (+), 163.81 (C_{quart}); MS (ESI, H_2O) *m*/*z* (%) 213 (100) [$(K^{4+}\ 2\ H^+)^{2+}$], 425 (20) [$(K^{4+}\ 3\ H^+)^+$], 507 (9) [$(K^{4+}\ 2\ H^+ + Br^-)^+$]; Anal. calcd. for $C_{24}H_{40}Br_4N_6O \times 4\ H_2O$, C 35.14, H 5.90, N 10.25; found, C 35.03, H 5.63, N 10.39.

1-Benzyl-3-[3-(1,4,7,10-tetraazacyclododec-1-yl)-propylcarbamoyl]-pyridinium-zinc(II)-tri-perchlorate. The zinc(II) complex was synthesized from the above compound (0.31 g, 0.41 mmol) by a method similar to that of **16** and was isolated as an orange solid (0.21 g, 0.27 mmol, 66%). mp 127−133 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 627, 1091, 1552, 1668, 2933, 3437; UV−vis (H_2O) λ_{max} (lg ϵ) = 263 nm (3.774), 375 (2.658), 442 (2.163); ^{1}H NMR (250 MHz, CD_3CN, δ [ppm]) 1.85 (m, 2H, CH_2), 2.70−3.44 (m, 23H, CH_2, NH), 5.81 (s, 2H, CH_2), 7.49 (m, 5H, CH), 7.76 (bs, 1H, NH), 8.11 (dd, ^3J = 6.3, 7.9 Hz, 1 H, CH), 8.82 (m, 2 H, CH), 9.16 (s, 1 H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 23.17 (−), 38.76 (−), 43.39 (2C, −), 44.71 (2C, −), 45.22 (2C, −), 50.47 (2C, −), 51.40 (−), 65.85 (−), 129.53 (+), 130.50 (2C, +), 130.55 (2C, +), 131.03 (+), 133.54 (C_{quart}), 136.03 (C_{quart}), 145.04 (+), 145.30 (+), 147.11 (+), 162.64 (C_{quart}); MS (ESI, CH_3CN) *m*/*z* (%) 274 (96) [$(K^{3+} + CH_3COO^-)^{2+}$], 294 (100) [$(K^{3+} + ClO_4^-)^{2+}$], 587 (14) [$(K^{3+}\ H^+ + ClO_4^-)^+$], 647 (14) [$(K^{3+} + ClO_4^- + CH_3COO^-)^+$], 687 (10) [$(K^{3+} + 2\ ClO_4^-)^+$]; Anal. calcd. for $C_{24}H_{37}Cl_3N_6O_{13}Zn \times H_2O$, C 35.71, H 4.87, N 10.41; found, C 36.10, H 5.08, N 10.41.

1-Benzyl-1,4-dihydropyridine-3-carboxylic acid [3-(1,4,7,10-tetraazacyclododec-1-yl)-propyl]-amide-zinc(II)-di-perchlorate (8). The 1,4-dihydronicotinamide **8** was synthesized from the above compound (35 mg, 44 μmol) by a method similar to that of **5** and obtained as a yellow solid (28 mg, 40 μmol, 91%). UV−vis (H_2O) λ_{max} (lg ϵ) = 357 nm (3.641); fluorescence (H_2O, λ_{ex} = 360 nm) λ_{max} = 462 nm; ^{1}H NMR (400 MHz, CD_3CN, δ [ppm]) 1.79 (m, 2H, CH_2), 2.65−3.28 (m, 25H, CH_2, NH), 4.29 (s, 2H, CH_2), 4.72 (dt, ^3J = 8.0, 3.5 Hz, 1H, CH), 5.85 (dd, ^3J = 8.0 Hz, ^4J = 1.6 Hz, 1H, CH), 6.21 (s, 1H, NH), 7.09 (m, 1 H, CH), 7.34 (m, 5 H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 22.89 (−), 25.06 (−), 37.96 (−), 43.31 (2C, −), 44.62 (2C, −), 45.51 (2C, −), 51.01 (2C, −), 52.13 (−), 57.53 (−), 103.62 (+), 128.31 (2C, +), 128.59 (+), 129.71 (2C, +), 130.17 (+), 130.49 (+), 139.36 (C_{quart}), 140.03 (C_{quart}), 169.65 (C_{quart}).

10-{2-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-acetylamino]-ethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester. The amine **3** (1.0 g, 1.9 mmol) was dissolved in 20 mL CH_2Cl_2 and the solution was cooled in an ice-bath. Then HOBt (0.32 g, 2.4 mmol), EDC (0.42 mL, 0.37 g, 2.4 mmol) and Fmoc-Glycin (0.63 g, 2.1 mmol) were added. After the solution had been stirred in an ice-bath for 8 h, it was extracted with 5 mL 2 N NaOH, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography on silica (eluent: EE, R_f = 0.6). The product (0.92 g, 1.2 mmol, 63%) was obtained as a white powder.

mp 82 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 739, 760, 1159, 1250, 1366, 1417, 1462, 1540, 1685, 2933, 2977, 3329; UV−vis (CH_3CN) λ_{max} (lg ϵ) = 265 (4.092), 289 (3.511), 300 (3.596); ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.46 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 2.61−3.51 (m, 20H, CH_2), 3.91 (d, 2H, CH_2), 4.22 (t, ^3J = 7.1 Hz, 1H, CH), 4.38 (d, ^3J = 7.1 Hz, 2H, CH_2), 5.73 (bs, 1H, NH), 7.01 (bs, 1H, NH), 7.27−7.39 (m, 4H, CH), 7.60 (d, ^3J = 7.3 Hz, 2H, CH), 7.75 (d, ^3J = 7.3 Hz, 2H, CH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.57 (9C, +), 36.81 (−), 44.20 (−), 47.14 (+), 47.63 (2C, −), 49.68 (4C, −), 52.50 (−), 54.67 (2C, −), 67.08 (−), 79.87 (C_{quart}), 80.06 (2C, C_{quart}), 119.96 (2C, +), 125.18 (2C, +), 127.06 (2C, +), 127.69 (2C, +), 141.29 (2C, C_{quart}), 143.89 (2C, C_{quart}), 155.63 (C_{quart}), 156.63 (3 C, C_{quart}), 168.77 (C_{quart}); MS (ESI, CH_2Cl_2 / MeOH + 1% AcOH) *m/z* (%) 495 (100) [(MH^+ 3 Boc)$^+$], 595 (27) [(MH^+ 2 Boc)$^+$], 695 (10) [(MH^+ Boc)$^+$], 795 (8) [MH^+]; Anal. calcd. for $C_{42}H_{62}N_6O_9 \times H_2O$, C 62.05, H 7.93, N 10.34; found, C 61.97, H 7.96, N 10.05.

10-[2-(2-Amino-acetylamino)-ethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (9). The above compound (0.85 g, 1.06 mmol) was dissolved in 3.2 mL DMF, 0.8 mL piperidine were added and the solution was stirred at room temperature for 30 min. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica (eluent: CH_2Cl_2/MeOH = 10:1, R_f = 0.7). The amine **9** (0.52 g, 0.91 mmol, 86%) was isolated as a white solid. mp 79 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 774, 1173, 1251, 1366, 1418, 1464, 1558, 1682, 2977, 3460; ^1H NMR (400 MHz, DMSO-d_6, δ [ppm]) 1.37 (s, 18H, CH_3), 1.40 (s, 9H, CH_3), 2.55−2.70 (m, 6H, CH_2, NH_2), 3.18−3.49 (m, 18H, CH_2), 7.89 (t, 1H, NH); ^{13}C NMR (62 Hz, DMSO-d_6, δ [ppm]) 28.02 (6C, +), 28.28 (3C, +), 33.47 (−), 43.77 (−), 46.31 (−), 46.74 (−), 47.13 (2C, −), 48.84 (2C, −), 49.42 (−), 53.67 (−), 54.32 (−), 78.17 (C_{quart}), 78.48 (2C, C_{quart}), 154.45 (C_{quart}), 154.75 (C_{quart}), 155.02 (C_{quart}), 171.24 (C_{quart}); MS (ESI, MeOH + 1% AcOH) *m/z* (%) 573 (100) [MH^+], 1146 (4) [($2M + H^+$)$^+$], 1167 (3) [($2M + Na^+$)$^+$]; HRMS ($C_{27}H_{53}N_6O_7^+$) calcd, 573.3976; found, 573.3970 ± 0.55 ppm.

1-Benzyl-3-({[2-(4,7,10-tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-methyl}-carbamoyl)-pyridinium-bromide. The compound was synthesized from amine **9** (0.44 g, 0.76 mmol) by a method similar to that of **4** and obtained as a white solid (0.55 g, 0.65 mmol, 85%). mp 59 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1162, 1252, 1368, 1414, 1478, 1545, 1686, 2933, 2976, 3428; UV−vis (MeOH) λ_{max} (lg ϵ) = 262 nm (3.884); ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.45 (s, 9H, CH_3), 1.47 (s, 9H, CH_3), 3.14−3.76 (m, 20H, CH_2), 4.19 (bs, 2H, CH_2), 6.03 (s, 2H, CH_2), 7.37 (m, 3H, CH), 7.64 (m, 2H, CH), 8.47 (m, 1H, CH), 8.97 (m, 2H, CH), 9.92 (m, 1H, CH), 10.39 (bs, 1H, NH), 10.48 (bs, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.42 (6C, +), 28.46 (3C, +), 33.86 (−), 43.69 (−), 45.01 (2C, −), 49.09 (2C, −), 49.79 (2C, −), 50.31 (2C, −), 54.07 (−), 65.14 (−), 81.70 (C_{quart}), 81.85 (2C, C_{quart}), 127.73 (+), 129.71 (2C, +), 129.98 (2C, +), 130.12 (+), 132.35 (C_{quart}), 134.47 (C_{quart}), 144.83 (+), 144.92 (+), 146.20 (+), 156.54 (3C, C_{quart}), 161.81 (C_{quart}), 169.88 (C_{quart}); MS (ESI, MeOH + 1% AcOH) *m/z* (%) 378 (15) [(K^+ 3 Boc − Benzyl + H)$^+$], 468 (7) [(K^+ 3 Boc)$^+$], 568 (12) [(K^+ 2 Boc)$^+$], 668 (30) [(K^+ Boc)$^+$], 768 (100) [K^+]; HRMS ($C_{40}H_{62}N_7O_8^+$) calcd, 768.4660; found, 768.4660 ± 0.0013.

1-Benzyl-3-({[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-methyl}-carbamoyl)-pyridinium-bromide-tri-hydrobromide. The deprotection of the above compound (0.56 g, 0.65 mmol) was done by a method similar to that of **15**. The product (0.31 g, 0.39 mmol, 60%) was isolated as a white powder. Decomp. at 200 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1456, 1548, 1669, 2964, 3429; UV−vis (H_2O) λ_{max} (lg ϵ) = 263 nm (3.832); ^1H NMR (250 MHz, D_2O, δ [ppm]) 2.64 (t, ^3J = 5.7 Hz, 2H, CH_2), 2.81−2.92 (m, 8H, CH_2), 3.09 (m, 8H, CH_2), 3.27 (t, ^3J = 5.7 Hz, 2H, CH_2), 3.99 (s, 2H, CH_2), 5.78 (s, 2H, CH_2), 7.36 (m, 5H, CH), 8.06 (dd, ^3J = 6.2, 8.1 Hz, 1H, CH), 8.80 (d, ^3J = 8.2 Hz, 1H, CH), 8.93 (d, ^3J = 6.2 Hz, 1H, CH), 9.27 (s, 1H, CH); ^{13}C NMR (62 MHz, D_2O, δ [ppm]) 36.26 (−), 41.76 (2C, −), 42.16 (2C, −), 43,54 (−), 44.12 (2C, −), 48.46 (2C, −), 52.76 (−), 65.16 (−), 128.59 (+), 129.39 (2 C, +), 129.61 (2C, +), 130.12 (+), 132.15 (C_{quart}), 133.67 (C_{quart}), 144.20 (+), 144.43 (+), 146.61 (+), 164.27 (C_{quart}), 171.43 (C_{quart}); MS (ESI, H_2O) *m/z* (%) 234 (100) [(K^++ H^+)$^{2+}$], 468 (1) [K^+], 550 (2) [(K^++ HBr)$^+$]; Anal. calcd. for $C_{25}H_{41}Br_4N_7O_2 \times 2\ H_2O$, C 36.30, H 5.48, N 11.85; found, C 36.12, H 5.66, N 11.76.

1-Benzyl-3-({[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-methyl}-carbamoyl)-pyridinium-zinc(II)-tri-perchlorate. The zinc(II) complex was synthesized from the above compound (0.29 g, 0.37 mmol) by a method similar to that of **16** and obtained as a an orange solid (0.19 g, 0.23 mmol, 62%). Decomp. at 117 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 627, 1090, 1110, 1548, 1668, 2932, 3406; UV−vis (H_2O) λ_{max} (lg ϵ) = 264 nm (3.745); ^1H NMR (250 MHz, CD_3CN, δ [ppm]) 2.69−3.50 (m, 23H, CH_2, NH), 4.10 (s, 2H, CH_2), 5.83 (s, 2H, CH_2), 7.50 (m, 5H, CH), 8.15 (dd, ^3J = 6.2, 8.0 Hz, 1H, CH), 8.86 (m, 2H, CH), 9.22 (s, 1H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 36.56 (−), 43.47 (2C, −), 44.46 (−), 44.80 (2C, −), 45.51 (2C, −), 51.92 (2C, −), 54.32 (−), 65.99 (−), 129.75 (+), 130.52 (2C, +), 130.60 (2C, +), 131.10 (+), 133.52 (C_{quart}), 135.21 (C_{quart}), 145.20 (+), 145.51 (+), 147.37 (+), 163.27 (C_{quart}), 172.33 (C_{quart}); MS (ESI, CH_3CN) *m/z* (%) 265 (100) [(K^{3+} H^+)$^{2+}$], 316 (10) [(K^{3+}+ ClO_4^-)$^{2+}$], 440 (10) [(K^{3+} H^+ $PhCH_2^+$)$^+$], 630 (7) [(K^{3+} H^++ ClO_4^-)$^+$], 732 (2) [(K^{3+}+ 2 ClO_4^-)$^+$]; Anal. calcd. for $C_{25}H_{38}Cl_3N_7O_{14}Zn \times 2\ H_2O$, C 34.58, H 4.87, N 11.29; found, C 34.59, H 4.97, N 11.25.

1-Benzyl-1,4-dihydropyridine-3-carboxylic acid {[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-methyl}-amide-zinc(II)-diperchlorate (10). 10 was synthesized from the above compound (37 mg, 44 μmol) by a method similar to that of **5** and was isolated as a yellow solid (29 mg, 40 μmol, 90%). UV−vis (H_2O) λ_{max} (lg ϵ) = 359 nm (3.480); fluorescence (H_2O, λ_{ex} = 360 nm) λ_{max} = 463 nm, Φ = 0.061; ^1H NMR (250 MHz, CD_3CN, δ [ppm]) 2.65−3.38 (m, 27H, CH_2, NH), 4.30 (s, 2H, CH_2), 4.72 (m, 1H, CH), 5.84 (m, 1H, CH), 6.35 (bs, 1H, NH), 6.54 (m, 1H, NH), 7.04 (d, ^4J = 1.5 Hz, 1H, CH), 7.32 (m, 5H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 22.88 (−), 35.94 (−), 43.58 (2C, −), 44.68 (2C, −), 45.66 (2C, −), 51.75 (2C, −), 53.38 (−), 57.56 (−), 65.86 (−), 103.87 (+), 128.40 (2C, +), 128.69 (+), 129.81 (2C, +), 130.42 (2C, +), 139.40 (C_{quart}), 140.16 (C_{quart}), 169.81 (C_{quart}), 172.08 (C_{quart}).

10-{2-[3-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-ethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (11). The amine **3** (1.08 g, 2.10 mmol) was dissolved in 20 mL CH_2Cl_2 and the solution was cooled in an ice-bath. Then HOBt (0.34 g, 2.54 mmol), EDC (0.45 mL, 0.39 g, 2.54 mmol) and Fmoc-β-Alanin (0.72 g, 2.31 mmol) were added. After the solution had been stirred in an ice-bath for 4 h, it was extracted with 5 mL 2 N NaOH, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography on silica (eluent: EE, R_f = 0.5). The product **11** (1.25 g, 1.54 mmol, 73%)was obtained as a white powder. mp 94 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 742, 760, 1158, 1250, 1366, 1416, 1462, 1540, 1690, 2933, 2976, 3067; UV−vis (MeOH) λ_{max} (lg ϵ) = 265 (3.990), 289 (3.394), 300 (3.486); ^1H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.45 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 2.46 (m, 2H, CH_2), 2.63 (m, 6H, CH_2), 3.33−3.52 (m, 16H, CH_2), 4.20 (t, ^3J = 7.2 Hz, 1H, CH), 4.33 (d, ^3J = 7.1 Hz, 2H, CH_2), 5.85 (bs, 1H, NH), 6.73 (bs, 1H, NH), 7.27−7.42 (m, 4H, CH), 7.60 (m, 2H, CH), 7.74 (m, 2H, CH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.53 (6C, +), 28.60 (3C, +), 35.51 (−), 36.70 (−), 37.24 (−), 47.27 (+), 49.40 (−), 50.35 (6C, −), 52.55 (+), 54.77 (2C, −), 66.71 (−), 79.86 (3C, C_{quart}), 119.93 (2C, +), 125.19 (2C, +), 127.03 (2C, +), 127.64 (2C, +), 141.27 (2C, C_{quart}), 144.04 (2C, C_{quart}), 155.61 (C_{quart}), 156.52 (2C, C_{quart}), 171.16 (C_{quart}), 171.81 (C_{quart}); MS (ESI, CH_2Cl_2/MeOH + 1% AcOH) *m/z* (%) 509 (100) [(MH^+ 3 Boc)$^+$], 609 (75) [(MH^+ 2 Boc)$^+$], 709 (40) [(MH^+ Boc)$^+$], 809 (55) [MH^+], 831 (10) [MNa^+], 1640 (3) [($2M$ + Na^+)$^+$]; Anal. calcd. for $C_{43}H_{64}N_6O_9 \times H_2O$, C 62.45, H 8.04, N 10.16; found, C 62.67, H 8.00, N 9.85.

10-[2-(3-Amino-propionylamino)-ethyl]-1,4,7,10-tetraazycyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester. The amine was prepared from **11** (1.13 g, 1.40 mmol) by a method similar to that of **9** and was obtained as a white solid (0.68 g, 1.19 mmol, 85%). mp 78 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 774, 1122, 1252, 1366, 1418, 1466, 1560, 1686, 2977, 3454; ^{1}H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.45 (s, 18H, CH_3), 1.47 (s, 9H, CH_3), 1.67 (bs, 2H, NH_2), 2.33 (t, ^3J = 6.0 Hz, 2H, CH_2), 2.67 (m, 6H, CH_2), 3.00 (t, ^3J = 6.0 Hz, 2H, CH_2), 3.33−3.53 (m, 14H, CH_2), 7.20 (bs, 1 H, NH); ^{13}C NMR (62 MHz, DMSO-d_6, δ [ppm]) 28.00 (6C, +), 28.26 (3C, +), 33.57 (−), 37.98 (−), 38.65 (−), 46.29 (−), 46.78 (−), 47.10 (2C, −), 48.90 (2C, −), 49.46 (−), 53.72 (−), 54.35 (−), 78.15 (C_{quart}), 78.47 (2C, C_{quart}), 154.46 (C_{quart}), 154.75 (C_{quart}), 155.02 (C_{quart}), 171.17 (C_{quart}); MS (ESI, MeOH + 1% AcOH) *m*/*z* (%) 587 (100) [MH^+], 1173 (1) [$(2M + H^+)^+$], 1195 (1) [$(2M + Na^+)^+$]; Anal. calcd. for $C_{28}H_{54}N_6O_7 \times H_2O$, C 55.61, H 9.33, N 13.90; found, C 56.10, H 9.28, N 13.64.

1-Benzyl-3-{2-[2-(4,7,10-tris-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-ethylcarbamoyl}-pyridiniumbromide (12). 12 was synthesized from the above amine (0.62 g, 1.06 mmol) by a method similar to that of **4** and isolated as a white powder (0.84 g, 0.97 mmol, 92%). mp 67 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1162, 1253, 1368, 1416, 1479, 1550, 1675, 2976, 3433; UV−vis (CH_3CN) λ_{max} (lg ϵ) = 255 nm (3.761); ^{1}H NMR (250 MHz, $CDCl_3$, δ [ppm]) 1.43 (bs, 27H, CH_3), 2.61 (bs, 2H, CH_2), 3.20−3.78 (m, 22H, CH_2), 6.09 (s, 2H, CH_2), 7.35 (m, 3H, CH), 7.64 (m, 2H, CH), 8.51 (m, 1H, CH), 9.02 (d, 1H, CH), 9.09 (d, 1H, CH), 9.59 (m, 1H, CH), 10.31 (bs, 1H, NH), 11.16 (bs, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$, δ [ppm]) 28.42 (6C, +), 28.48 (3C, +), 34.53 (−), 36.06 (−), 37.07 (−), 45.29 (2C, −), 49.13 (2C, −), 49.99 (2C, −), 50.31 (2C, −), 54.81 (−), 65.01 (−), 80.97 (C_{quart}), 81.68 (2C, C_{quart}), 127.86 (+), 129.72 (2 C, +), 129.87 (2C, +), 130.14 (+), 132.42 (C_{quart}), 134.79 (C_{quart}), 144.98 (2C, +), 145.36 (+), 156.52 (C_{quart}), 156.56 (2 C, C_{quart}), 161.39 (C_{quart}), 173.17 (C_{quart}); MS (ESI, MeOH) *m*/*z* (%) 241 (4) [$(K^+ + H^+ 3\ Boc)^{2+}$], 291 (4) [$(K^+ + H^+ 2\ Boc)^{2+}$], 341 (7) [$(K^+ + H^+ Boc)^{2+}$], 391 (12) [$(K^+ + H^+)^{2+}$], 782 (100) [K^+]; HRMS ($C_{41}H_{64}N_7O_8^+$) calcd, 782.4816; found, 782.4784 ± 0.0034.

1-Benzyl-3-{2-[2-(1,4,7,10-tetraazacyclododec-1-yl)ethylcarbamoyl]-ethylcarbamoyl}-pyridinium-bromid-trihydrobromide. The compound was synthesized from **12** (0.76 g, 0.89 mmol) by a method similar to that of **15** and obtained as a white solid (0.48 g, 0.60 mmol, 67%). Decomp. at 116 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 1456, 1550, 1669, 2927, 3428; UV−vis (H_2O) λ_{max} (lg ϵ) = 264 nm (3.647); ^{1}H NMR (250 MHz, D_2O, δ [ppm]) 2.47 (t, ^3J = 6.9 Hz, 2H, CH_2), 2.60 (t, ^3J = 6.1 Hz, 2H, CH_2), 2.75−2.88 (m, 8H, CH_2), 3.06 (m, 8H, CH_2), 3.20 (t, ^3J = 6.1 Hz, 2H, CH_2), 3.55 (t, ^3J = 6.9 Hz, 2H, CH_2), 5.77 (s, 2H, CH_2), 7.38 (m, 5H, CH), 8.06 (dd, ^3J = 6.2, 8.2 Hz, 1H, CH), 8.71 (d, ^3J = 8.2 Hz, 1H, CH), 8.93 (dt, ^3J = 6.2 Hz, ^4J = 1.3 Hz, 1H, CH), 9.17 (t, ^4J = 1.3 Hz, 1H, CH); ^{13}C NMR (62 MHz, D_2O, δ [ppm]) 35.36 (−), 35.95 (−), 36.86 (−), 41.75 (2C, −), 42.08 (2C, −), 44.15 (2C, −), 48.25 (2C, −), 52.25 (−), 65.13 (−), 128.65 (+), 129.38 (2C, +), 129.67 (2C, +), 130.17 (+), 132.19 (C_{quart}), 134.42 (C_{quart}), 144.03 (2C, +), 146.42 (+), 163.70 (C_{quart}), 174.26 (C_{quart}); MS (ESI, MeOH/H_2O) *m*/*z* (%) 644 (20) [$(K^{4+}\ 3\ H^+ + 2\ Br^-)^-$], 724 (50) [$(K^{4+} - 2\ H^+ + 3\ Br^-)^-$], 804 (100) [$(K^{4+} - H^+ + 4\ Br^-)^-$]; Anal. calcd. for $C_{26}H_{43}Br_4N_7O_2 \times 3\ H_2O$, C 36.34, H 5.75, N 11.41; found, C 36.32, H 5.45, N 11.36.

1-Benzyl-3-{2-[2-(1,4,7,10-tetraazacyclododec-1-yl)ethylcarbamoyl]-ethylcarbamoyl}-pyridinium-zinc(II)-tri-perchlorate. The zinc-(II) complex was synthesized from the above compound (0.43 g, 0.54 mmol) by a method similar to that of **16** and isolated as an orange solid (0.32 g, 0.38 mmol, 70%). mp 124−129 °C; IR (KBr) $\bar{\nu}$ (cm^{-1}) 627, 1091, 1550, 1663, 2930, 3374; UV−vis (H_2O) λ_{max} (lg ϵ) = 263 nm (3.731), 372 (2.505), 459 (2.279); ^{1}H NMR (250 MHz, CD_3CN), δ [ppm]) 2.64−3.76 (m, 27H, CH_2, NH), 5.82 (s, 2H, CH_2), 7.34 (m, 1H, NH), 7.50 (m, 5H, CH), 7.89 (m, 1H, NH), 8.13 (dd, ^3J = 6.1, 8.1 Hz, 1H, CH), 8.82 (m, 2H, CH), 9.13 (bs, 1H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 37.13 (−), 37.18 (−), 39.75 (−), 43.19 (2C, −), 44.96 (2C, −), 45.29 (2C, −), 53.04 (2C, −), 55.35 (−), 65.87 (−), 129.61 (+), 130.54 (2C, +), 130.57 (2C, +), 131.06 (+), 133.52 (C_{quart}), 135.73 (C_{quart}), 145.05 (+), 145.29 (+), 147.09 (+), 162.98 (C_{quart}), 178.63 (C_{quart}); MS (ESI, CH_3CN/MeOH) *m*/*z* (%) 272 (100) [$(K^{3+} - H^+)^{2+}$], 644 (5) [$(K^{3+}\ H^+ + ClO_4^-)^+$], 746 (2) [$(K^{3+} + 2\ ClO_4^-)^+$]; Anal. calcd. for $C_{26}H_{40}Cl_3N_7O_{14}Zn$, C 36.90, H 4.76, N 11.58; found, C 36.59, H 4.92, N 11.51.

1-Benzyl-1,4-dihydropyridine-3-carboxylic acid {2-[2-(1,4,7,10-tetraazacyclododec-1-yl)-ethylcarbamoyl]-ethyl}-amide-zinc(II)-diperchlorate (13). 13 was synthesized from the above compound (37 mg, 44 μmol) by a method similar to that of **5** and was obtained as a yellow solid (30 mg, 40 μmol, 91%). UV−vis (H_2O) λ_{max} (lg ϵ) = 357 nm (3.547); fluorescence (H_2O, λ_{ex} = 360 nm) λ_{max} = 461 nm; ^{1}H NMR (250 MHz, CD_3CN, δ [ppm]) 2.31−3.48 (m, 29H, CH_2, NH), 4.29 (s, 2H, CH_2), 4.71 (dt, ^3J = 8.1, 3.4 Hz, 1H, CH), 5.84 (dd, ^3J = 8.1 Hz, ^4J = 1.6 Hz, 1H, CH), 6.28 (t, ^3J = 5.8 Hz, 1H, NH), 7.04 (d, ^4J = 1.5 Hz, 1H, CH), 7.25 (m, 1H, NH), 7.34 (m, 5H, CH); ^{13}C NMR (62 MHz, CD_3CN, δ [ppm]) 22.90 (−), 35.44 (−), 36.28 (−), 37.33 (−), 43.43 (2C, −), 44.58 (2C, −), 45.56 (2C, −), 51.30 (2C, −), 53.12 (−), 57.56 (−), 103.50 (+), 128.35 (2C, +), 128.65 (+), 129.77 (2C, +), 130.29 (+), 139.46 (C_{quart}), 139.83 (2C, +, C_{quart}), 169.26 (C_{quart}), 173.59 (C_{quart}).

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Supporting Information Available: Details of potentiometric titrations and kinetic measurements; UV−vis-spectra of **5**, **16**, riboflavin tetraacetate and reduced riboflavin tetraacetate; long-wavelength spectra during the reaction of riboflavin tetraacetate and **5**; synthesis of compounds **18**−**21**; Calculated structures of compounds **5**, **8**, **10**, and **13** with coordinated flavin. This material is available free of charge via the Internet at http://pubs.acs.org.

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