Pergamon

Tetrahedron 57 (2001) 8897–8902

TETRAHEDRON

Host–guest properties of NAD^+/NADH models

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Received 4 June 2001; revised 1 August 2001; accepted 23 August 2001

Abstract—The host–guest properties of four macrocyclic NAD^+/NADH models in aqueous solutions have been investigated using ^1H NMR spectroscopy and electrospray ionisation mass spectrometry (ESI-MS). Compounds **1**–**3** have four nicotinamide units and compound **4** has two nicotinamide units linked by a chiral subunit and an aromatic spacer incorporated into the models. All four model compounds formed complexes with the dianion of terephthalate. The larger models **1**–**3** formed stronger complexes than the smaller model **4**. The cavities of the models **1**–**3** are large enough to encapsulate a substrate and it is shown by NMR studies that the dianions of iso- and terephthalate are intercalated between the aromatic spacers of the hosts. ESI-MS revealed that isophthalate formed mostly 1:1 complexes but also to some extent 2:1 complexes with **1** and **2** at higher guest concentrations. In contrast, terephthalate formed only 1:1 complexes with the same model compounds.

1. Introduction

Ion–pair interactions are an important factor which control molecular recognition in many synthetic as well as biological systems. In biological and hence biomimetic systems, molecular recognition of anionic substrates is particularly important and several classes of anion receptors have been designed.[1]

Nicotinamide adenine dinucleotide (NAD^+/NADH) is the most common redox pair in biological systems. The interconversion between a 1,4-dihydropyridine ring and a pyridinium cation is realized by the reversible and stereoselective hydride transfer from NADH to a carbonyl compound and from an alcohol to NAD^+. Our strategy in mimicking NAD^+/NADH has been to use a supramolecular approach, i.e. creating a macrocyclic framework with a hydrophobic cavity wherein the substrate to be reduced can be bound. Of the models presented here, compounds **1** and **2** have D_2 symmetry, compound **3** has C_{2h} symmetry and compound **4** has C_2 symmetry (Fig. 1). Their syntheses are reported elsewhere.[2]

trans-1,2-Diaminocyclohexane was chosen as a chiral subunit and besides introducing chirality it also facilitates the synthesis and it makes the final structure more rigid compared to other chiral building blocks we tried.[3] The aromatic spacers link the nicotinamide units together and make the cavity hydrophobic. The models contain several nicotinamide subunits which is advantageous for creating a water-soluble NAD^+/NADH model able to reduce a substrate. When all of the nicotinamide units are reduced, the model compounds are only soluble in organic solvents such as methylene chloride, but when they are all oxidised the compounds become water-soluble. When only one or two of the nicotinamide units are in their reduced states the models are still water-soluble and with the remaining pyridinium cations it should be possible to bind a substrate containing for example a carboxylate group. Such a complex could provide the prerequisites for a reduction with good stereoselectivity (Fig. 2).

This approach is more suitable for the larger NAD^+/NADH models (**1**–**3**) the cavities of which are large enough to encapsulate a substrate. The cavity of the smaller model (**4**) is too small to encapsulate a substrate, but **4** should still be able to form complexes with anionic substrates. The studies of the four different model compounds presented here have been made with the models in their fully oxidised state.

Compounds **1** and **4** have also been tested as reducing agents in organic solvents and they were found to reduce activated carbonyl compounds in good yields and with high enantiomeric excess.[2]

We have previously reported preliminary results of the host–guest properties of some of the NAD^+/NADH models.[4] We now present a more extensive investigation on the complexation behaviour in aqueous solution of NAD^+/NADH models as hosts and benzoic acid derivatives as guests.

2. Results and discussion

^1H NMR spectroscopy and electrospray ionisation mass

Keywords: NAD^+/NADH models; host–guest complex; terephthalate; isophthalate.

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PII: S0040-4020(01)00874-2

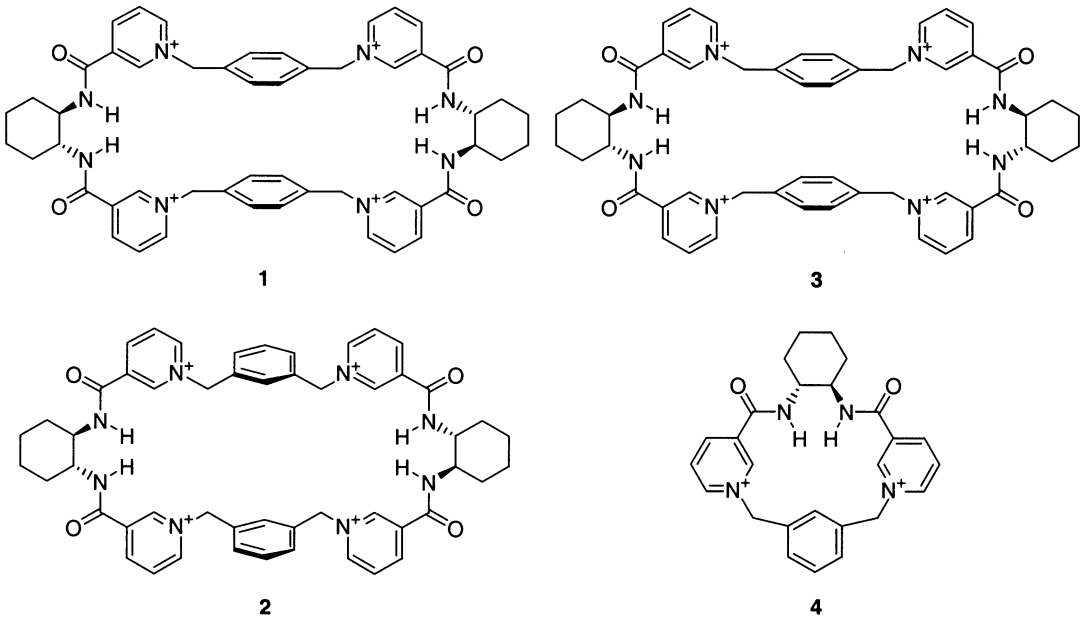

Figure 1. The NAD^+/NADH models used in this study. Compound **3** is an achiral stereoisomer of **1** which has [*R*,*R*]-configuration at one end and [*S*,*S*]-configuration of the diaminocyclohexyl unit at the other.

spectrometry (ESI-MS) have been used to investigate the host–guest properties. Mass spectrometry gives a rough picture of the complexation properties and the stoichiometry of the complexes, while ^{1}H NMR spectroscopy enables a more quantitative analysis. In addition to giving information about the stoichiometry and the structure of the actual complex it also allows for the determination of binding constants.

2.1. Complexation studies using ^{1}H NMR spectroscopy

The NMR studies presented here were made with compounds **1**–**4** as hosts and the dianions of terephthalic and isophthalic acid as guests. The spectra of the host compounds show relatively few signals due to their high symmetry and thus are easy to interpret. The studies were carried out in aqueous media (D_2O) with constant concentration of the host and stepwise additions of the guest. When the guests were added the ^{1}H NMR signals of the hosts and the various dianionic substrates were found to undergo marked shift changes, indicating that complexation occurred. The spectra showed only time-averaged signals of complexed and uncomplexed hosts and guests. The observed chemical shift changes for the different host protons were then plotted as a function of the guest concentration.

The plots fitted nicely to the theoretical curves for 1:1 complexes[5] (exemplified in Fig. 3) with one exception (Table 1, entry 5). In the experiment with host **3** and the

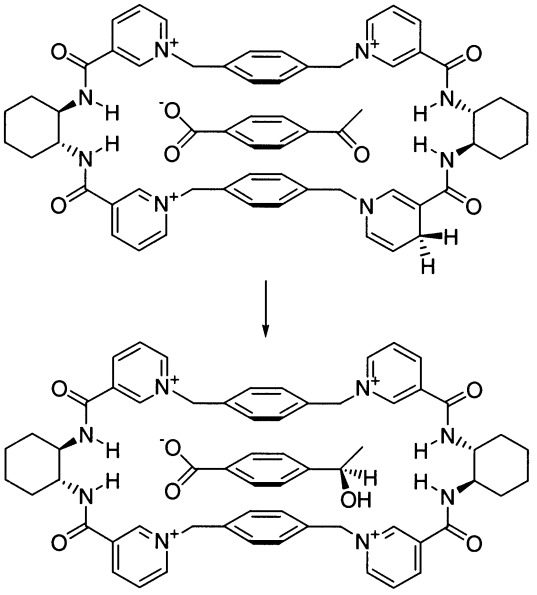

Figure 2. Hypothetical representation of stereoselective reduction within a host–guest complex.

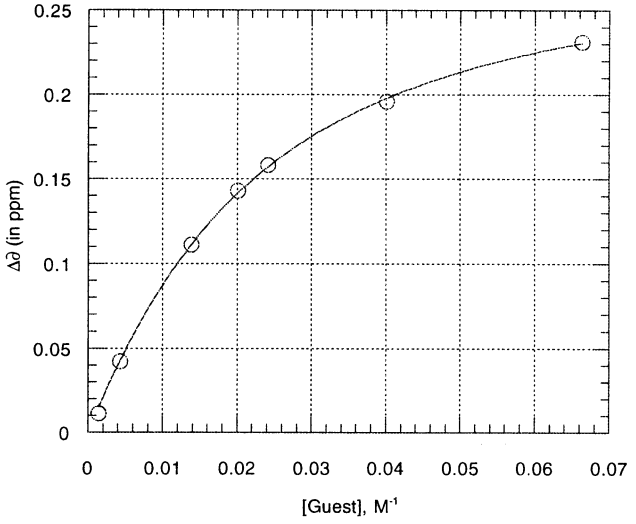

Figure 3. NMR titration of the dianion of terephthalate with model compound **1**. Experimental (symbols) and calculated (line) chemical shifts for the aromatic protons of **1**.

Table 1. Association constants K_a and chemical shifts $\Delta\delta_{max}$ for complexes with compounds **1**–**4** as hosts and the dianions of tere- and isophthalate as guests in D_2O measured by ^1H NMR

Entry	Host[a]	Guest		H-1[b]	H-2[b]	H-3[b]	Ar–H[c]
1	**1**	Terephthalate	K_a	59±2		46±2	
			$\Delta\delta_{max}$ (host)	−0.30		−0.21	
			$\Delta\delta_{max}$ (guest)				−0.58±0.04
2	**2**	Terephthalate	K_a	40±1	63±2	50±2	
			$\Delta\delta_{max}$ (host)	−0.20	−0.24	−0.21	
			$\Delta\delta_{max}$ (guest)				−0.70±0.1
3	**1**	Isophthalate	K_a	60±3		65±6	
			$\Delta\delta_{max}$ (host)	−0.33		−0.21	
			$\Delta\delta_{max}$ (guest)				−0.57±0.01
4	**2**	Isophthalate	K_a	56±2	68±3	65±3	
			$\Delta\delta_{max}$ (host)	−0.20	−0.26	−0.20	
			$\Delta\delta_{max}$ (guest)				−0.58±0.03
5	**3**	Terephthalate	K_a	Not determined[d]			
			$\Delta\delta_{max}$ (host)	Not determined[d]			
			$\Delta\delta_{max}$ (guest)	Not determined[d]			
6	**4**	Terephthalate	K_a	33±1.5	35±2	35±2	
			$\Delta\delta_{max}$ (host)	−0.22	−0.13	−0.14	
			$\Delta\delta_{max}$ (guest)				−0.22±0.005

Seven to nine ^1H NMR measurements were made for each entry in D_2O at 20 °C. K_a: association constant in M^{-1}. $\Delta\delta_{max}$: calculated maximum upfield chemical shift induced by full complexation.

[a] Host concentrations: 11±0.5 mM, except for entry 6 where the host concentration was 17 mM.

[b] Host compounds **1** and **3**: H-1 corresponds to the aromatic protons of the spacers. Host compounds **2** and **4**: H-1 corresponds to the aromatic protons in positions 4–6 and H-2 corresponds to the aromatic protons in position 2 of the spacers. H-3 corresponds to the methylene protons linking the spacers and the pyridinium rings in all four host compounds.

[c] Corresponds to the aromatic protons of the guest.

[d] The K_a and $\Delta\delta_{max}$ values were not determined in entry 5, since the experimental data did not fit the theoretical 1:1 complex model.

terephthalate dianion a precipitate was formed at higher guest concentrations (about 7–8 times the concentration of the host). The precipitate was dissolved in DBr/D_2O and the composition of this complex was determined by ^1H NMR to be a 2:1 guest–host complex. The reason why the terephthalate dianion formed a precipitate with **3** and not with **1** and **2** can be rationalized by the difference in structure. CPK model studies show that compounds **1** and **2** have helix-like structures due to the chiral diaminocyclohexyl subunits, whereas the achiral compound **3** has a nonhelical, rectangular structure. This open structure seems to have the ability to more easily form 2:1 complexes and at higher concentrations the complexes precipitate due to extensive π-stacking. So far, we have not been able to obtain a X-ray structure of this complex.

The association constants (K_a) are listed in Table 1. As expected, the K_a value for the smaller C_2 symmetric model **4** is slightly lower than for the other models, since **4** has fewer positive charges and thus fewer ion-pair binding sites. In addition, the cavity of **4** is too small to encapsulate the substrate. Hosts **1** and **2** display a similar behaviour in the complexation of terephthalate and isophthalate; the K_a values are about the same for all four complexes (Table 1, entries 1–4). The magnitude of the K_a values are comparable to the values obtained by Hossain and Schneider.[6] Their complexes resemble the ones mentioned above, consisting of open-chained diammonium salts with aromatic spacers between the two cations, and the dianions of terephthalate and isophthalate. Consequently, in the complexes between **1** and **2** and the iso- and terephthalate dianions the electrostatic forces contribute the most to the stability of the complexes and the contribution from the hydrophobic cavity which enables the encapsulation of a substrate is relatively small. The host–guest formation is an equilibrium process and should give the same stability constant (K_a) regardless of which proton of the host that is observed. However, different protons of the hosts give different K_a values. The K_a value can differ as much as from 40 to 63 M^{-1} within the same complex (Table 1, entry 2). Given the relatively small chemical shift variations in the titration experiments, errors in the order of 10–20 % might be more realistic than the errors given by the iteration process. Also, due to the rather flexible structure of **1** and **2** the complexation process can give rise to a conformational change, which could be responsible for the difference in K_a values within the same compound. This process can be compared to the effect that is responsible for the difference in chemical shifts between DMSO-d_6 and D_2O that is observed for the hosts **1** and **2** (Fig. 4).

These shift changes indicate that the model compounds have different conformations in DMSO and water and can in part be explained by assuming that the bromide ions are more tightly bound to the pyridinium cations in DMSO due to the poor anion solvation ability of DMSO. A spectrum with **2** and NaBr was recorded to investigate if the salt could influence the conformation, but the effect was negligible.

Some conclusions can be drawn from the data in Table 1 about the structure of the observed complexes. As mentioned, compound **4** is not able to encapsulate a substrate, therefore the dianion of terephthalate has to be located on the outside of **4** in entry 6. For the larger

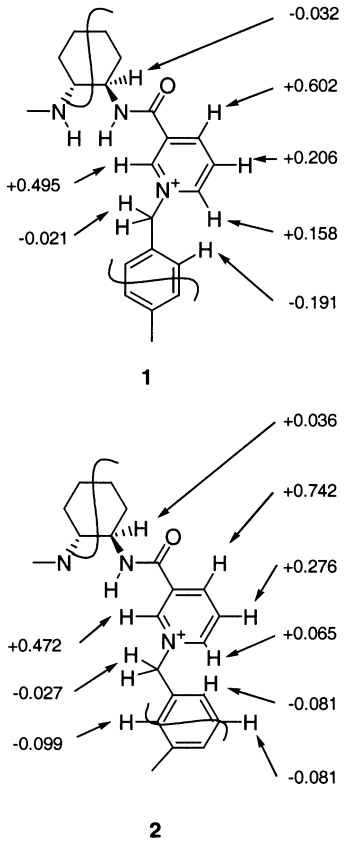

Figure 4. ^{1}H NMR shift changes for compounds **1** and **2** as δ_{DMSO}-δ_{water} in ppm.

NAD^+/NADH models the substrates can be located either on the outside or on the inside of the cavity. In the 2:1 complex between the dianion of terephthalate and the achiral model **3** only one guest molecule can be encapsulated and the other has to be stacked on the outside. For the complexes in entries 1–4 involving models **1** and **2** the values of $\Delta\delta_{max}$, the maximum upfield shift induced by full complexation obtained from curve fitting, indicate that the substrates are intercalated between the aromatic spacers of the host compounds as shown in Fig. 5.

The $\Delta\delta_{max}$ values are about twice as large for the guests as for the hosts, which is explained by the fact that the aromatic protons of the guests are affected by the anisotropy of two aromatic rings while the aromatic protons of the hosts are only affected by one aromatic ring. Also, the $\Delta\delta_{max}$ values for the guests in entries 1–4 are much higher than for the guest in entry 6, where the guest is not intercalated. As mentioned, the contributions from hydrophobic interactions to the stability of the complexes is relatively small and yet the guests seem to be intercalated between the aromatic spacers of the hosts. The reason could be found in the 3-D structure of the hosts, which should have helix-like structures. The most straightforward way for the dianionic guest molecule to bind to two positive charges of the host simultaneously is to bind inside the cavity due to the twisted structure of the host.

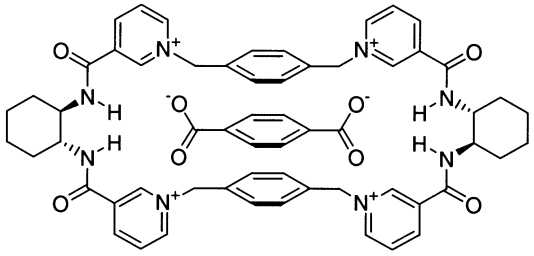

Figure 5. Proposed complex between **1** and terephthalate, where the guest is intercalated between the aromatic spacers of the host.

A more bowshaped guest is required to get a good fit on the outside of the host. This could also be the reason why isophthalate forms a small amount of 2:1 complexes with hosts **1** and **2** at high guest concentrations (detected by ESI-MS, vide infra) whereas terephthalate does not.

2.2. Complexation studies using ESI-MS

Mass spectrometry has become more and more important for studies of molecular recognition processes due to the development of efficient 'soft' ionisation methods along with high speed handling and small sample requirements. Fast atom bombardment (FAB)[7] and electrospray ionisation (ESI)[8] are the two most widely used ionisation methods for studying host–guest complexes. The ionic complexes observed using ESI-MS are formed in solution outside the vacuum region, i.e. the detected complexes correspond to solution-phase behaviour,[8d,9] which makes ESI-MS more suitable than FAB-MS in this case.

Compounds **1** and **2** have been investigated with ESI-MS together with different benzoic acid derivatives. All of the ESI mass spectra were recorded with H_2O as the mobile phase. The ESI mass spectrum of free host (**1**) displays singly, doubly, three- and fourfold charged species of the form $[\mathbf{1}+3Br^-]^+$, $[\mathbf{1}+2Br^-]^{2+}$, $[\mathbf{1}+Br^-]^{3+}$ and $[\mathbf{1}]^{4+}$ (Fig. 6, top spectrum).[†]

The first guest to be tested was the dianion of terephthalic acid, which seemed to have the right features to match the host **1**, i.e. the structural complementarity of the structures enables both ion-pair interactions and π–π interactions. Indeed, complexation occurred and only traces of uncomplexed **1** could be seen in the mass spectrum (Table 2, entry 1).

The rest of the peaks were derived from 1:1 complexes between **1** and the terephthalate dianion (Fig. 6, bottom spectrum). When the dianion of isophthalic acid was added as the guest to the host **1**, again almost all of the host molecules were complexed. In contrast to terephthalate, isophthalate formed a considerable amount of 2:1 guest–host complex (Table 2, entry 3). Host **2** displayed a similar behaviour. With the above mentioned guests, **2** formed only 1:1 complexes with the terephthalate dianion and formed mostly 1:1 complexes and to some extent 2:1 complexes with the isophthalate dianion (Table 2, entries 2 and 4). It should be noted that in the ESI-MS experiments, up to 15 equiv. of the guests were added as a comparison to

[†] Bromide ions was used as counterions in all of the experiments presented in this report.

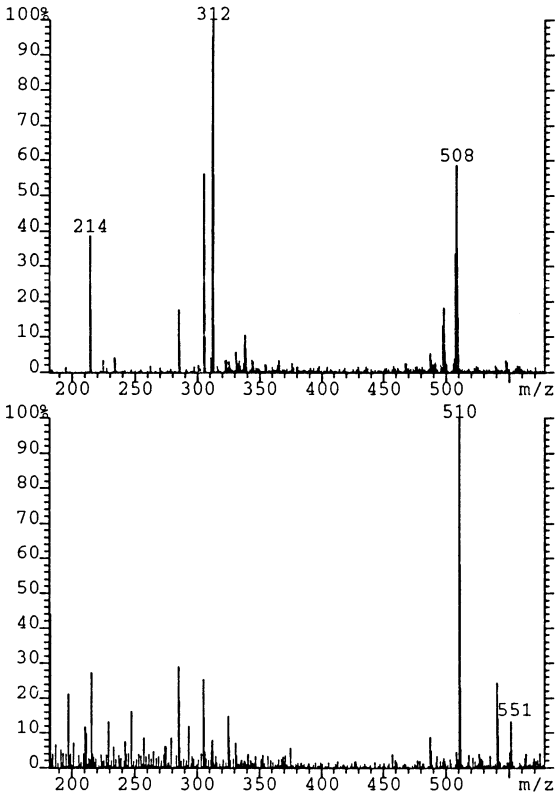

Figure 6. *Top spectrum.* An aqueous solution of model compound **1**: $[\mathbf{1}]^{4+}$ *m/z* 214, $[\mathbf{1}+Br^-]^{3+}$ *m/z* 312 and $[\mathbf{1}+2Br^-]^{2+}$ *m/z* 508. *Bottom spectrum.* The same solution after addition of the dianion of terephthalate (TP): $[\mathbf{1}+TP]^{2+}$ *m/z* 510 and $[\mathbf{1}+Br^-+TP+H^+]^{2+}$ *m/z* 551.

each complex gave rise to several peaks due to different charges. A large amount of free host was observed which indicates that, as expected, the affinity for singly carboxylate functioned substrates is lower than substrates with two carboxylate groups. Also, the amount of 2:1 complexes were much higher and even 3:1 complexes were present.

3. Conclusions

Compounds **1** and **2** are the most interesting of the NAD^+/NADH models presented here considering our purposes, namely to use the models in further studies as asymmetric catalysts in reductions of carbonyl compounds by inclusion of the substrate in a chiral cavity. The NMR studies showed that the cavities of **1** and **2** are very similar, both in size and binding properties. The largest binding contribution originates from ion-pair interactions although some structural recognition provided by the cavities is observed. It was also shown that the substrates were encapsulated in the cavities of the hosts, which was of major importance to ensure a fixed orientation of a substrate and hence to give good stereocontrol upon reduction.

ESI-MS revealed a difference in binding between terephthalate and isophthalate. Isophthalate formed not only 1:1 but also 2:1 complexes with hosts **1** and **2**, which is not desirable for our purposes. In a 2:1 complex, one substrate molecule may be located inside the cavity and the other on the outside of the NAD^+/NADH model. Two different binding modes make the analyses more difficult and lower the possibilities for highly selective reductions.

Table 2. Distribution of different observed complexes with model compounds **1** and **2** as hosts and benzoic acid derivatives as guests using ESI-MS

Entry	Host[a]	Guest[b]	0:1 (free host)[c] (%)	1:1 complex[c] (%)	2:1 complex[c] (%)	3:1 complex[c]
1	**1**	Terephthalate	<10	100	–	–
2	**2**	Terephthalate	<5	100	–	–
3	**1**	Isophthalate	<10	100	26	–
4	**2**	Isophthalate	<10	100	10	–
5	**1**	Benzoic acid	94	100	60	32
6	**2**	Benzoic acid	87	100	75	26

[a] Host concentration: 1±0.1 mM.
[b] Fifteen molar equivalents were added.
[c] The values are given as a percentage of the largest peak.

the NMR experiments where only around seven equivalents were added in total. The presence of 2:1 complexes only seems to be observed at higher concentrations of the guests. Neither of the two hosts formed complexes with the dianion of phthalic acid. The fact that the two hosts **1** and **2** are able to form complexes with terephthalate and isophthalate but not with phthalate indicates that the observed complexes are not the result of anomalous aggregate ions but are structure-specific ions.

Benzoic acid was also tested as a guest and the results with the two different hosts were similar (Table 2, entries 5 and 6). The spectra contained a larger number of peaks than the spectra with the other guests, which made the analyses more difficult than in the other cases. The reason for this was that a higher number of different complexes were present and

The achiral compound **3** was of interest because it has a more open structure which turned out to lead to the formation of 2:1 complexes even at comparatively low substrate (guest) concentration.

4. Experimental

4.1. General

^1H NMR spectra were recorded at 293 K on a Varian UNITY-400 NMR spectrometer at 400 MHz with D_2O as solvent and chemical shifts were measured relative to the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid. Mass spectra were recorded on a VG ZabSpec instrument. Positive ESI-MS (electrospray ionisation-mass

spectrometry) was the used method with H_2O as the mobile phase. Syntheses of NAD^+/NADH models **1**–**4** are described elsewhere.[2] The disodium salt of isophthalic acid was prepared according to a literature procedure,[10] using NaOH instead of LiOH. Commercially available chemicals were used without further purification, unless stated otherwise.

4.2. NMR measurements and determination of association constants

In a typical experiment, a total of 7–9 additions of the dianionic guest as a solid were made to a 11 ± 0.5 mM solution of a NAD^+/NADH model host in D_2O. After each addition increment, a spectrum was recorded and the changes in chemical shifts of the host signals were then plotted against the guest concentration. The values of the association constants K_a and the calculated maximum upfield chemical shifts induced by full complexation $\Delta\delta_{max}$ were obtained by non-linear regression fitting to the theoretical curve for the 1:1 complex model described elsewhere.[5]

4.3. Complexation studies using mass spectrometry

Fifteen molar equivalents of a guest was added to a 1 ± 0.1 mM solution of a NAD^+/NADH model host in H_2O. The solution was stirred vigorously and was allowed to settle prior to injection.

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