Complexation of Charged Porphyrins by Charged and Metal-Chelated EDTA-Tethered β-Cyclodextrin Dimers: A Thermodynamic Study on the Influence of Tether Charge and Flexibility on Binding Affinity

Alart Mulder,[a] Jurriaan Huskens,*[a] and David N. Reinhoudt*[a]

Dedicated to Dr. Joop A. Peters on the occasion of his 60th birthday

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Complex formation between charged porphyrin guests and charged or neutral β-cyclodextrin (CD) host dimers linked by ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate (EDTA) tethers was assessed by microcalorimetry. Two EDTA-tethered CD dimers (**1** and **2**) were employed to obtain a series of structurally similar CD dimers with different tether charges and flexibilities through either (partial) protonation or metal chelation (with Ca^{II} or Eu^{III}) of the EDTA tether. Both the tether charge and the flexibility of the CD dimers strongly influenced the binding properties, giving rise to binding constants differing by factors of up to 22. The effect of tether charge was elucidated by the complexation of tetraanionic *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TSPP) by the different forms of CD dimer **1**. Tether charge-dependent complex (de)solvation gave rise to large systematic and counteracting changes in binding enthalpy and entropy, resulting in a binding affinity for the positively charged europium complex of the CD dimer five times larger than that of the negatively charged free ligand CD dimer. No marked differences in binding affinities were observed for the complexation of TSPP by CD dimer **2**. Both restricted tether flexibility by metal chelation and repulsive electrostatic interactions resulted in diminished binding affinity of CD dimer **1** for the relatively large tetracationic *p-tert*-butylbenzyl-functionalized *p*-pyridylporphyrin (TBPyP). The europium complex of CD dimer **1** binds TBPyP less strongly than the corresponding negatively charged uncomplexed forms of the CD dimer, by a factor of up to 22. The tether of CD dimer **2** is too short to allow strong binding of TBPyP.

Introduction

Since the first report of a cyclodextrin dimer by Tabushi in 1979,[1] large numbers of such compounds have been synthesized.[2] Among these, cyclodextrin dimers tethered through metal-chelating linkers are of particular interest as these tethers offer additional functionality. For instance, metallo-cyclodextrin dimers are interesting for catalysis, as they possess two hydrophobic pockets, which can rigidly organize substrate molecules in close proximity to a catalytically active metal center. Such metallo-cyclodextrin dimers have demonstrated impressive reaction rate accelerations and high specificities.[3,4] Metal chelation has also been employed to organize multiple β-cyclodextrin (CD) dimers around a metal ion.[5,6] Additionally, metal-chelating tethers have been utilized to tune binding affinity and selectivity.[7–9]

There are several ways in which metal ligation by the tether can alter the binding properties of a CD dimer. The most impressive results have been obtained when the ligated metal serves as an additional binding site for the guest molecule complexed by the CD dimer. An illustrative example in this respect is the binding of bis(1-adamantylethyl) phosphate by a bipyridine-tethered CD dimer, reported by Breslow and Zhang.[3d] The CD dimer bound the phosphodiester 55 times more strongly in the presence of zinc(II), which was attributed to the coordination of the phosphate anion to the metal cation.

Alternatively, metal ligation has also been used to impose a charge on the CD dimer and thereby to influence its binding affinity for charged guest species through attractive or repulsive electrostatic interactions. Lincoln, Easton, and coworkers demonstrated that coordination of sodium(I) by diazocoronand-tethered CD dimers resulted in a fivefold increase in the binding affinity of the CD dimers for the Brilliant Yellow tetraanion.[7]

Furthermore, ligation of the tether to metal ions has also been used to influence the flexibility of the tether and hence the relative orientation of the two CD cavities, thus altering

[a] Laboratory of Supramolecular Chemistry and Technology, MESA+ Institute for Nanotechnology, University of Twente, P. O. Box 217, 7500 AE Enschede, the Netherlands
Fax: +31-53-4894645
E-mail: smct@ct.utwente.nl

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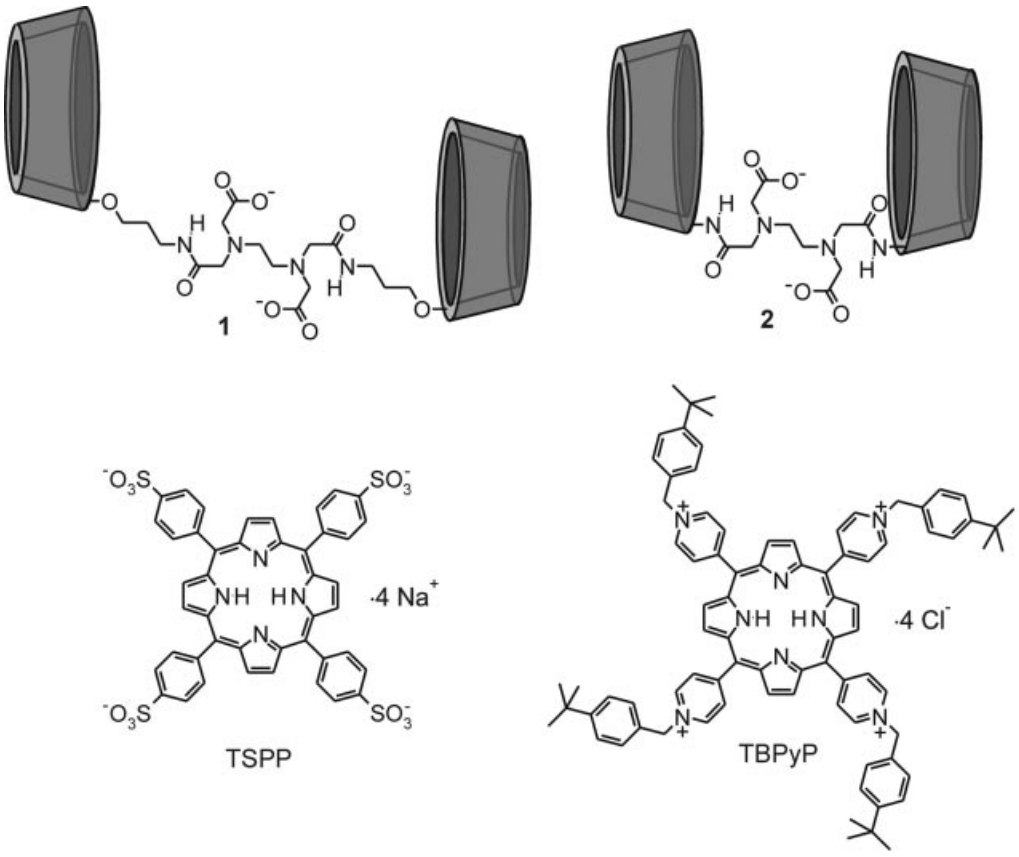


Scheme 1. EDTA-tethered β-cyclodextrin dimers and porphyrin guest molecules used in this study.

the binding properties of the CD dimer. This has been demonstrated by the groups of Wu[8] and Liu,[9] who synthesized CD dimers with oligo(ethyleneamine) tethers. Chelation of metal ions by the tethers of these CD dimers resulted in enhanced binding affinities for small organic dye molecules, with maximum binding enhancements of a factor of 5.[9a,9b]

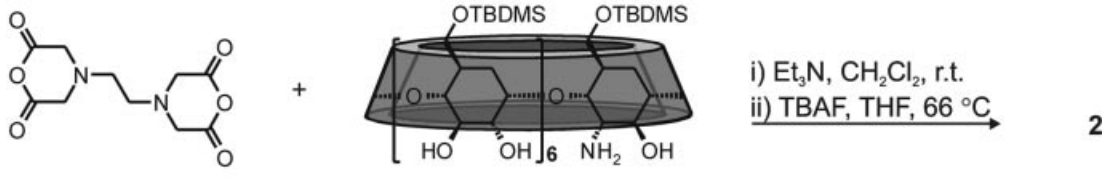
Thus far, the effect of metal chelation by the tether on the binding properties of CD dimers has mainly been discussed in terms of the association constants obtained with the metal-complexed and the free ligand forms of the CD dimers. Here we present a microcalorimetric study on the binding of charged porphyrin guest molecules by two CD dimers tethered through ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate (EDTA). The EDTA tether was used to assess how both tether charge and flexibility influence the binding properties of these CD dimers.

Results and Discussion

Scheme 1 depicts the two EDTA-tethered CD dimers and porphyrin guests used for the complexation studies. The CD dimers differ in the connectivity between the two CD cavities and the EDTA moieties. In CD dimer **1** the CD cavities are connected to the EDTA moiety through flexible propyl spacers, whereas in CD dimer **2** the EDTA moiety is coupled directly to the secondary rims of the CD cavities.

Synthesis of the Dimers

The synthesis of EDTA-tethered CD dimer **1** has been reported previously.[10] For the synthesis of CD dimer **2**, an analogous procedure, outlined in Scheme 2, was followed. Treatment of the commercially available EDTA bis(anhydride)with TBDMS-protected mono-3-amino-3-deoxy-β-



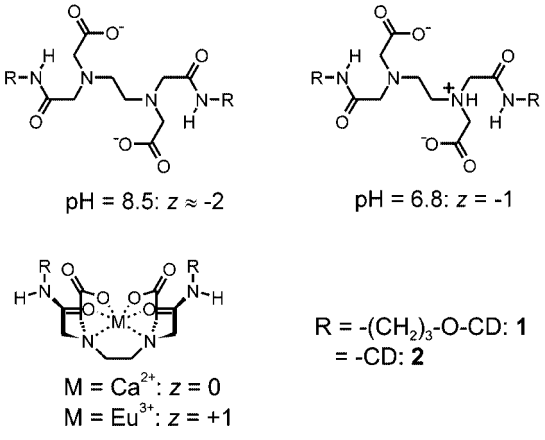
Scheme 2. Synthesis of the EDTA-tethered CD dimer **2**.

cyclodextrin[11] gave the TBDMS-protected precursor of CD dimer **2**, which was purified by silica column chromatography. Subsequent deprotection with tetrabutylammonium fluoride yielded the water-soluble CD dimer **2**. The spectroscopic data obtained for CD dimer **2** were consistent with the data found by Fujita's group, who synthesized this same CD dimer from EDTA bis(anhydride) and unprotected 3-amino-3-deoxy-β-cyclodextrin in DMF.[12]

It should be noted that the CD moieties of CD dimers **1** and **2** are different in the sense that the modified sugar units of the CDs of CD dimer **2** are altrosidic (see Scheme 2). This is inherent in the synthetic pathway towards the TBDMS-protected 3-amino-3-deoxy-β-cyclodextrin,[11] which involves the ring-opening of *manno*-mono-2,3-epoxy-β-cyclodextrin with ammonia, resulting in inversion at the C3 carbon.[13] The presence of the altrose units gives rise to a certain extent of mobility in the CD ring, and as a consequence the CD cavities of CD dimer **2** are relatively flexible in relation to those of CD dimer **1**. CD dimer **1** was synthesized from TBDMS-protected mono(2-*O*-aminopropyl)-β-cyclodextrin,[10] itself obtained by alkylation of TBDMS-protected β-cyclodextrin, the original structure of the CD cavity being left intact.

Charged and Metal-Chelated Dimers

The EDTA tether is a particularly versatile moiety; EDTA is zwitterionic and capable of complexing a variety of metal ions. These characteristic features of EDTA were used to access four different forms of dimers **1** and **2** (see Scheme 3), differing in tether charge (z = –2, –1, 0, and +1) and tether flexibility (free ligand and protonated forms versus metal complexes).



Scheme 3. Charged free ligand, protonated, and metal-chelated forms of the EDTA-tethered CD dimers assessed in the complexation studies.

The ligand protonation constants for CD dimers **1** and **2** were assumed to be comparable to those determined by Peters and co-workers for EDTA-bis(propyl amide) (pK_{a1} = 7.2, pK_{a2} = 3.6, pK_{a3} = 2.0).[14] From these values it can be concluded that especially the free ligand (pH > 8, z = –2) and the monoprotonated species (4.5 < pH < 7, z = –1) are easily accessible. Accordingly, complexation studies were performed at pH 8.5 to assess the binding properties of the free ligands $\mathbf{1}^{2-}$ and $\mathbf{2}^{2-}$ and at physiological pH (6.8), at which the protonated CD dimers $[H\cdot\mathbf{1}]^{1-}$ and $[H\cdot\mathbf{2}]^{1-}$ are assumed to be predominant.

Metal chelation was used to obtain neutral and positively charged CD dimers. Addition of $CaCl_2$ to aqueous solutions of CD dimers **1** and **2** gave the neutral complexes $[Ca\cdot\mathbf{1}]^0$and $[Ca\cdot\mathbf{2}]^0$. Similarly, $EuCl_3$ was used to obtain the positively charged complexes $[Eu\cdot\mathbf{1}]^{1+}$ and $[Eu\cdot\mathbf{2}]^{1+}$. Metal chelation is associated with the organization of the EDTA tether around the metal ion, and consequently the metal-chelated CD dimers have restricted tether flexibility in relation to the uncomplexed CD dimers. This is especially true for CD dimer **2**, in which the CD cavities are directly linked to the EDTA moiety. Restrictions for CD dimer **1** would be expected to be less dramatic, as the propylamine linkers are to some extent able to overcome the rigidity imposed on the EDTA moiety.

Guest Molecules

Porphyrins were chosen as guest molecules for complexation by the CD dimers because they offer multiple equivalent binding sites for the CD components, which are symmetrically positioned at a single molecular platform, giving rise to well defined symmetrical binding modes.[15] For the complexation studies, *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TSPP, Scheme 1) and *p-tert*-butylbenzyl-functionalized *p*-pyridylporphyrin (TBPyP, Scheme 1) were used. These molecules are structurally similar but are differently sized and oppositely charged, enabling a detailed assessment of the influence on the binding properties of the CD dimers of changes both in tether flexibility and in the charge. The smaller tetraanionic TSPP is commercially available, and the larger tetracationic TBPyP was synthesized from the corresponding tetrapyridylporphyrin as reported previously.[16] Both TSPP and TBPyP have been used previously for complexation studies with CD and CD dimers.[15–19]

Complexation Studies

The complexation of the EDTA-tethered CD dimers with the porphyrin guest molecules TSPP and TBPyP was studied by isothermal titration calorimetry (ITC). ITC measurements allow the direct determination of the association constant (K) and the binding enthalpy ($\Delta H°$), and thus provide a complete thermodynamic picture of the interactions under investigation.

Figure 1 depicts a typical series of exothermic heat profiles for the titration of the different ligand and metal complex forms of CD dimer **1** with TSPP, and Figure 2 shows two typical titration curves for the titration of TBPyP with CD dimer **1**. The inflection points of the titration curves shown in Figures 1 and 2 indicate that complexation of TSPP and TBPyP with **1** resulted in the formation of com-

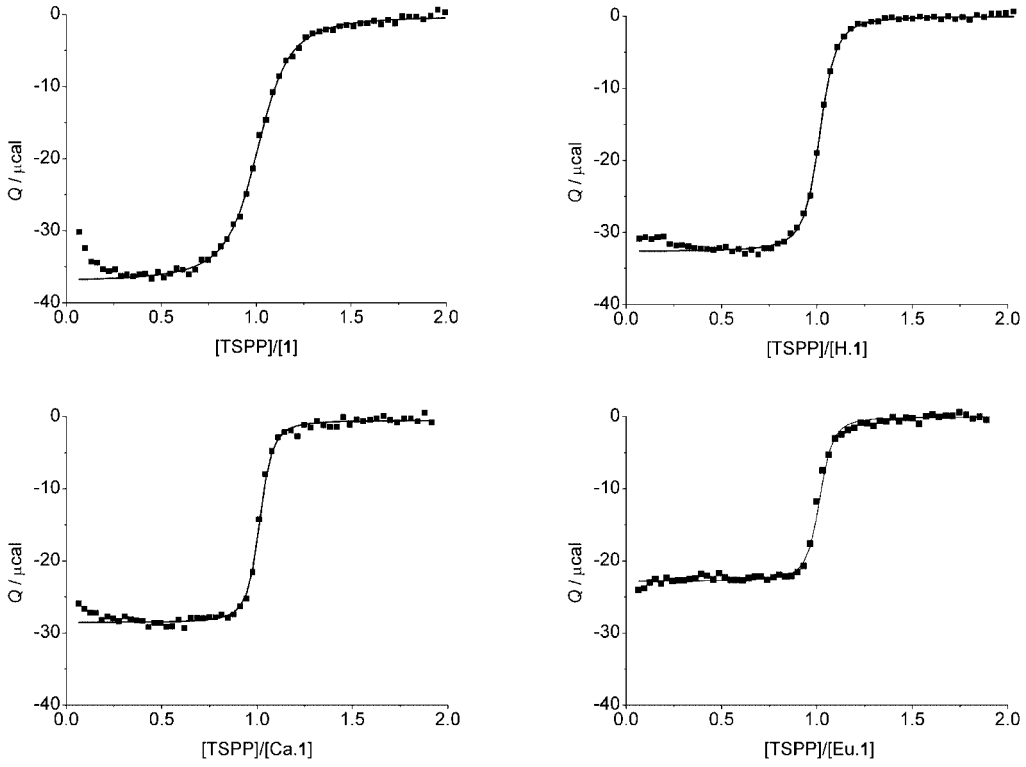


Figure 1. Heat evolved per injection plotted against the molar ratio (markers) and fits (solid lines) for the calorimetric titrations of TSPP (0.5 mM) with 50 μM of $\mathbf{1}^{2-}$ (top left), $[H\cdot\mathbf{1}]^{-}$ (top right), $[Ca\cdot\mathbf{1}]^{0}$ (bottom left), and $[Eu\cdot\mathbf{1}]^{+}$ (bottom right) in water at 25 °C.

plexes with different binding stoichiometries, the smaller TSPP forming a 1:1 complex with **1** and the larger TBPyP being bound by two CD dimers **1**. This is in accordance with previous complexation studies of CD dimers with TSPP[16,17,19] and TBPyP.[16] It is well known that TSPP, because of its small size, can only accommodate two CD cavities (i.e., one CD dimer). Typically, two opposite phenyl rings of the TSPP platform are complexed by CD cavities in a so-called *anti* geometry and complexation of the remaining phenyl rings is sterically not feasible.[20] Complexation studies with TSPP and the different free ligand and metal complexes of CD dimer **2** also gave titration curves with inflection points at a molar ratio of 1, indicative of the formation of 1:1 complexes.

TBPyP has an extended porphyrin platform and thus increased space per binding site in relation to TSPP, since the CD cavities bind to the *tert*-butylbenzyl moieties of TBPyP, so TBPyP is able to accommodate a total of four CDs or two CD dimers with sufficient tether length.[16] The titrations of TBPyP with the free ligand and metal complex forms of CD dimer **1** gave strongly exothermic heat effects indicative of ditopic binding. This contrasts with the titration curves obtained for CD dimer **2**, which gave only small exothermic heat effects and did not give sigmoidal titration curves at sub-millimolar concentrations, indicating that the tether length of this CD dimer is insufficient for efficient ditopic binding of the relatively large TBPyP.

The titration curves obtained for the titration of TSPP with CD dimers **1** and **2** could be fitted well with a 1:1 binding model (solid lines Figure 1), with use of the association constant (K) and the binding enthalpy ($\Delta H°$) as independent fitting parameters. The binding curves obtained for the titration of TBPyP with CD dimer **1** were fitted with a 2:1 (H/G) binding model (solid lines, Figure 2) by use of intrinsic stability constants K_i and binding enthalpies $\Delta H°$ as independent fitting parameters.[21] Table 1 lists the thermodynamic parameters obtained for the complexation of TSPP and TBPyP by the different free ligand and metal complex forms of CD dimers **1** and **2**. The largest differences in binding affinity were observed between $\mathbf{1}^{2-}$ and $[Eu\cdot\mathbf{1}]^{1+}$ for binding TSPP and between $[H\cdot\mathbf{1}]^{1-}$ and $[Eu\cdot\mathbf{1}]^{1+}$ for binding TBPyP, resulting in factors of 5 and 22 difference, respectively.

The Influence of Tether Charge

Comparison of the thermodynamic binding parameters for the binding of a porphyrin by the differently charged forms of a single dimer was used to obtain insight into the effect of tether charge on the binding properties of the EDTA CD dimers. Care was taken in the comparison of the thermodynamic parameters obtained for the calcium-complexed and the monoprotonated forms as these have both different tether charge and different tether flexibility. In this respect, comparison of the two metal-complexed forms on the one hand and the two free ligand forms on the other were considered more useful as these forms were expected to have similar tether flexibilities.

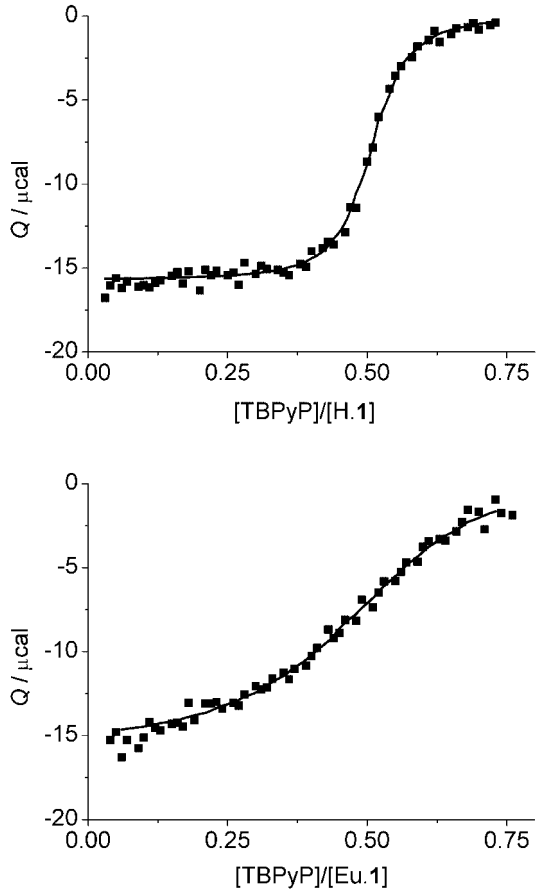


Figure 2. Heat evolved per injection plotted against the molar ratio (markers) and fits (solid lines) for the calorimetric titrations of TBPyP (0.16 mM) with 50 μM $[H\cdot\mathbf{1}]^-$ and $[Eu\cdot\mathbf{1}]^+$(bottom) in water at 25 °C.

The most obvious trends in thermodynamic parameters were observed for the interaction of TSPP with the different forms of CD dimer **1**. Upon going from $\mathbf{1}^{2-}$ to $[Eu\cdot\mathbf{1}]^{1+}$ the association constant for TSPP steadily increased to give a maximum difference of a factor of 5. Actually, the trend in free energy changes are close to the experimental error (see Table 1). The highest association constant was found for the complex involving two oppositely charged species, whereas the complexes formed by two similarly charged species gave relatively low association constants. The increase in association constant was the result of much larger, apparently systematic, and partly counteracting changes in the enthalpies and entropies of binding, as is most clearly seen from a plot of the thermodynamic parameters as a function of the dimer charge (Figure 3, left). Upon going from $\mathbf{1}^{2-}$ to $[Eu\cdot\mathbf{1}]^{1+}$ the enthalpy of binding became less favorable, increasing by 1.6 kcal per added charge unit. These systematic increases in enthalpy were readily visible in the calorimetric titration curves, which showed stronger exothermic heat plateaus for the more negatively charged CD dimers (see Figure 1). The less favorable enthalpies of binding were overridden by systematically increasing entropies. Per added charge unit, the entropy contributions ($T\Delta S$) became 1.9 kcal mol^{-1} more favorable. This can also be seen from the fact that the slope in the enthalpy–entropy compensation plot (Figure 3, right) is smaller than unity (0.84).

These apparently charge-related systematic changes in enthalpy and entropy and the absence of additional effects, possibly related to tether flexibility, between $\mathbf{1}^{2-}$ and $[H\cdot\mathbf{1}]^{1-}$ on the one hand and $[Ca\cdot\mathbf{1}]^0$ and $[Eu\cdot\mathbf{1}]^{1+}$ on the other, imply that electrostatic interactions significantly affect the binding of TSPP by CD dimer **1**, while the difference in tether flexibility has no strong influence. In view of the

Table 1.Thermodynamic parameters of the complexation of TSPP and TBPyP by the different ligand and metal complex forms of **1** and **2** and β-cyclodextrin (β-CD), as determined by ITC at 298 K.

Host	Guest	Stoichiometry (H:G)	K [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]
CD[a]	TSPP	2:1[b]	$(3.1\pm0.4)\times10^4$	–6.1 ± 0.1	–4.3 ± 0.2	1.8 ± 0.3
$\mathbf{1}^{2-}$	TSPP	1:1	$(6.4\pm3.8)\times10^6$	–9.2 ± 0.4	–16.6 ± 0.4	–7.4 ± 0.8
$[H\cdot\mathbf{1}]^{1-}$	TSPP	1:1	$(2.0\pm1.1)\times10^7$	–9.9 ± 0.4	–15.3 ± 0.4	–5.4 ± 0.8
$[Ca\cdot\mathbf{1}]^0$	TSPP	1:1	$(2.0\pm1.3)\times10^7$	–9.9 ± 0.4	–13.7 ± 0.2	–3.8 ± 0.6
$[Eu\cdot\mathbf{1}]^{1+}$	TSPP	1:1	$(3.4\pm1.9)\times10^7$	–10.2 ± 0.3	–11.8 ± 0.6	–1.6 ± 0.9
$\mathbf{2}^{2-}$	TSPP	1:1	$(2.7\pm1.0)\times10^5$	–7.4 ± 0.2	–11.7 ± 0.3	–4.2 ± 0.5
$[Ca\cdot\mathbf{2}]^0$	TSPP	1:1	$(3.1\pm0.9)\times10^5$	–7.4 ± 0.2	–11.4 ± 0.2	–4.0 ± 0.4
$[Eu\cdot\mathbf{2}]^{1+}$	TSPP	1:1	$(2.7\pm1.0)\times10^5$	–7.4 ± 0.3	–10.2 ± 0.6	–2.8 ± 0.9
$\mathbf{1}^{2-}$	TBPyP	2:1[b]	$(4.4\pm1.4)\times10^6$	–9.0 ± 0.2	–11.5 ± 0.2	–2.5 ± 0.4
$[H\cdot\mathbf{1}]^{1-}$	TBPyP	2:1[b]	$(9.1\pm3.8)\times10^6$	–9.5 ± 0.3	–9.6 ± 0.2	–0.1 ± 0.5
$[Ca\cdot\mathbf{1}]^0$	TBPyP	2:1[b]	$(6.3\pm1.7)\times10^6$	–9.2 ± 0.2	–8.7 ± 0.2	0.5 ± 0.4
$[Eu\cdot\mathbf{1}]^{1+}$	TBPyP	2:1[b]	$(4.1\pm1.1)\times10^5$	–7.6 ± 0.2	–10.4 ± 0.5	–2.8 ± 0.7
$\mathbf{2}^{2-}$	TBPyP	–	$< 2\times10^4$	–	–	–

[a] Taken from ref.[17b] [b] Intrinsic binding constants obtained in a 2:1 binding model with the assumption of two sequential, independent binding steps to form the 1:1 and 2:1 complexes (i.e. with equal binding enthalpies for both steps and with the use of $K_1 = 2K_i = 4K_2$ and $K_1 = 4K_i = 8K_2$ for the TSPP and TBPyP complexes, respectively (see text and ref.[21]).

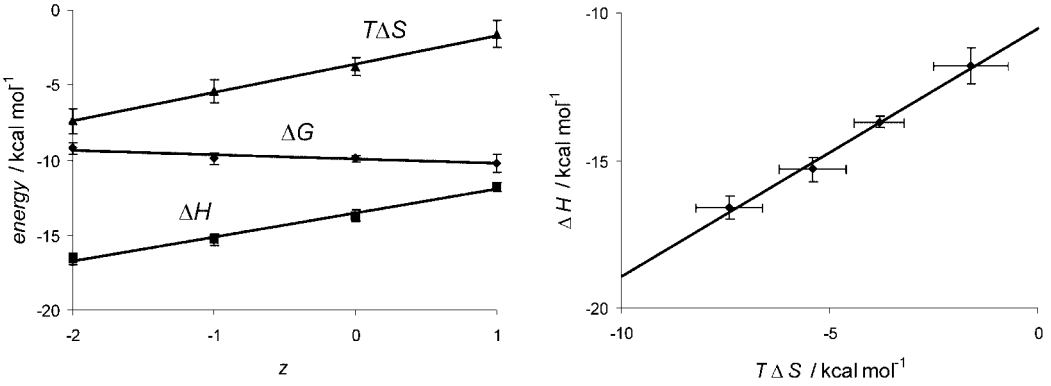


Figure 3. Thermodynamic parameters as a function of charge (left) and enthalpy–entropy compensation plot (right) for the complexation of TSPP by the protonated and metal complex forms of dimer **1**.

small size of TSPP and the flexible propyl spacers of CD dimer **1** this is not surprising. Previous studies with a photoswitchable dithienylethene-tethered CD dimer coupled through propyl linkers also gave only minor differences in binding affinity for TSPP with varying tether flexibility.[17] Influences of electrostatic interactions on binding properties as seen for CD dimer **1** and TSPP are not uncommon. Similarly, electrostatic interactions have been shown to influence the binding properties of diazocoronand-tethered CD dimers,[7] and cationic mono- and diamino-CDs are known to exhibit higher/lower affinities than native CDs towards negatively/positively charged guests.[22–24]

The thermodynamic parameters listed in Table 1 enabled the elucidation of how electrostatic interactions affect the binding of TSPP by CD dimer **1**. The thermodynamic parameters obtained for the interaction between $[Ca\cdot\mathbf{1}]^0$and TSPP are most readily interpreted. $[Ca\cdot\mathbf{1}]^0$ does not bear a charge that could possibly interact with TSPP, and the complexation was therefore attributed solely to the hydrophobic interactions between TSPP and the two CD cavities. The thermodynamic parameters obtained for the binding of TSPP by $[Ca\cdot\mathbf{1}]^0$ were comparable to those obtained for the binding of TSPP by dithienylethene-tethered CD dimers reported previously.[17] Comparison of the thermodynamic parameters obtained for $[Ca\cdot\mathbf{1}]^0$ and $[Eu\cdot\mathbf{1}]^{1+}$ showed that the latter complex formation with TSPP was accompanied by an additional endothermic, entropy-driven process. The enthalpy of binding for complexation of TSPP by $[Eu\cdot\mathbf{1}]^{1+}$ was 1.9 kcal mol^{-1} less favorable than for $[Ca\cdot\mathbf{1}]^0$, while the $T\Delta S°$ contribution was 2.2 kcal mol^{-1} more favorable. Overall this resulted in a favorable contribution of 0.3 kcal mol^{-1} to the free energy of binding.

It is well known that attractive electrostatic interactions between two oppositely charged species are governed by positive entropy values.[25,26] These arise from partial desolvation of the ion groups (ion-pairing). Associated endothermic enthalpies of binding have been ascribed to the energetic cost required to desolvate the charged groups.[27] The differences in thermodynamic parameters for the binding of TSPP by $[Ca\cdot\mathbf{1}]^0$ and $[Eu\cdot\mathbf{1}]^{1+}$ suggest that such attractive electrostatic interactions might also be involved in the interaction of the positively charged CD dimer $[Eu\cdot\mathbf{1}]^{1+}$ and the negatively charged sulfonate groups of TSPP. Similarly, increases in solvation might explain the observed less favorable entropy changes and the corresponding more favorable enthalpies associated with the complexation of TSPP by the negatively charged CD dimers $[H\cdot\mathbf{1}]^{1-}$ and $\mathbf{1}^{2-}$. The close proximity of two oppositely charged molecules requires relatively large amounts of structured water for stabilization. As a result the complexation of TSPP by $\mathbf{1}^{2-}$ is 0.7 kcal mol^{-1} less favorable than the corresponding binding by $[H\cdot\mathbf{1}]^{1-}$. Taken together, the trends in thermodynamic parameters found for the interaction of TSPP with the different forms of CD dimer **1** indicate that the interaction strengths of the complexes are governed by electrostatic interactions.

The (de)solvation effects outlined above for the complexation of TSPP by CD dimer **1** were also observed for the complexation of TSPP by CD dimer **2**, although the effect on the association constants is less strong. All three assessed forms of dimer **2** all bound TSPP with an association constant of 3×10^5 M^{-1}. These association constants are two orders of magnitude lower than those found for the binding of TSPP by dimer **1**. Comparison of the thermodynamic parameters for the complexation of TSPP by the different forms of dimers **1** and **2** showed that the lower association constants observed for the latter dimer were mainly due to a less exothermic enthalpy of binding, indicating a less effective cooperation of the CD cavities in binding the porphyrin guest, which was attributed to the smaller tether of CD dimer **2**. Nevertheless, similarly to CD dimer **1**, the binding of TSPP by $[Eu\cdot\mathbf{2}]^{1+}$ was associated with a less exothermic enthalpy of binding and a compensating, more favorable entropy than seen for the binding of TSPP by $[Ca\cdot\mathbf{2}]^0$, indicating that favorable electrostatic interactions are involved in the binding of TSPP by $[Eu\cdot\mathbf{2}]^{1+}$. The absolute effects, however, were smaller than observed for **1**.

Comparison of the enthalpy and entropy values obtained for the complexation of TBPyP by the metal-chelated dimers $[Ca\cdot\mathbf{1}]^0$ and $[Eu\cdot\mathbf{1}]^{1+}$ indicated that the binding properties of this system were also influenced by electrostatic interactions . Whereas the binding of TSPP by the europium

complexes of dimers **1** and **2** gave more endothermic enthalpy values than the binding of TSPP by the neutral calcium-chelated dimers (Table 1), the binding of TBPyP by $[Eu\cdot\mathbf{1}]^{1+}$ is associated with a more exothermic enthalpy of binding than the binding of TBPyP by $[Ca\cdot\mathbf{1}]^{0}$. The 1.7 kcal mol^{-1} more favorable enthalpy of binding is overridden by a strong decrease of –3.3 kcal mol^{-1} in the $T\Delta S°$ term. These values are in line with the trends in enthalpy and entropy values observed for the binding of TSPP by the negatively charged dimers of **1** in relation to the binding of TSPP by $[Ca\cdot\mathbf{1}]^{0}$ (see Table 1), and are probably due to the relatively large amount of structured water involved in the complexation of TSPP by $[Eu\cdot\mathbf{1}]^{1+}$ in comparison with the corresponding complexation by $[Ca\cdot\mathbf{1}]^{0}$.

The differences in the thermodynamic parameters found for complexation of TBPyP with $[H\cdot\mathbf{1}]^{1-}$ and $\mathbf{1}^{2-}$ are not readily interpretable. It is not likely that $[H\cdot\mathbf{1}]^{1-}$ and $\mathbf{1}^{2-}$ have strongly different tether flexibilities. Furthermore, in view of the trends in enthalpy and entropy observed for the complexation of TSPP by dimers **1** and **2**, and also for the binding of TBPyP by dimers $[Eu\cdot\mathbf{1}]^{1+}$ and $[Ca\cdot\mathbf{1}]^{0}$, a less exothermic enthalpy of binding would be expected, overridden by a more favorable entropy of binding. This is in sharp contrast with the thermodynamic values found for the complex of $\mathbf{1}^{2-}$ with TBPyP, which indicated that this formation is more exothermic and entropically less favorable than the complexation of TBPyP by $[H\cdot\mathbf{1}]^{1-}$. It might be that strong attractive electrostatic interactions (ion-pairing) are involved in the complexation of TBPyP by the negatively charged dimers $[H\cdot\mathbf{1}]^{1-}$ and $\mathbf{1}^{2-}$. The pyridinium ions of TBPyP are situated close to the binding sites of the CD cavities, and are easily accessible, so interactions between the carboxylates of the EDTA tether and the positively charged pyridinium moieties of TBPyP are not unlikely. Strong ion-pairing is typically associated with exothermic heat effects, and in this respect the more exothermic enthalpy found for the binding of TBPyP by $\mathbf{1}^{2-}$ could be attributable to the possible formation of an additional ion-pair. Such strong electrostatic interactions do not necessarily have to result in a much stronger energy of binding overall, as ion-pairing can interfere with ideal complexation of the *tert*-butylbenzyl moieties by the CD cavities, as has been observed for the binding of anionic guests by mono-6-amino-6-deoxy-β-cyclodextrin.[22a] However, it is not reasonable to assume that such interference would result in a less favorable energy of binding overall. Furthermore, the formation of ion-pairs conflicts with the relatively large unfavorable entropy values found for the complexation of TBPyP by the negatively charged dimers. The fact that these complexations probably involve an interplay between hydrophobic and electrostatic interactions makes it difficult to draw conclusions on the extents of the relative contributions of the two interactions and on the outcome of the combination of the two.

Taken together, these results show that association of charged porphyrins and oppositely charged CD dimers is entropically more favorable and therefore stronger than the corresponding binding of the porphyrins by the neutral forms of the CD dimer. This is attributed to improved desolvation of the formed complex, which is associated with a more favorable entropy of binding partly compensated by an unfavorable enthalpy contribution. In contrast, the interaction between charged porphyrins and similarly charged CD dimers is entropically less favorable, which is ascribed to an increase in structured water upon complex formation, giving rise to lower association constants.

The Influence of Tether Flexibility

To examinethe effect of tether flexibility on the binding properties of the EDTA-tethered CD dimers, the thermodynamic binding parameters of the metal-complexed CD dimers were compared to those of the corresponding free ligand CD dimers. As these CD dimers differ both in tether flexibility and in charge, direct comparison of the thermodynamic parameters in terms of tether flexibility is troublesome and care should be taken in the interpretation of these parameters. As discussed above, no tether flexibility effects were observed for the interaction of TSPP and CD dimer **1**. The reason for this is probably that metal complexation has only a moderate effect on the tether flexibility and this is therefore not expected to give rise to large differences in binding properties, especially when dealing with small guests such as TSPP.

However, subtle differences in tether flexibility become more crucial for larger guests such as TBPyP. Comparison of the thermodynamic parameters obtained for the complexation of TBPyP by $[Ca\cdot\mathbf{1}]^{0}$ with those obtained for the negatively charged CD dimers $[H\cdot\mathbf{1}]^{1-}$ and $\mathbf{1}^{2-}$ indicated that restriction of tether length and flexibility by metal complexation hampers the binding abilities of the metal-chelated CD dimers. Given the attractive electrostatic interactions that are potentially involved in the binding of TBPyP by $[H\cdot\mathbf{1}]^{1-}$, a less favorable enthalpy and a more favorable entropy of binding would be expected than in the binding by $[Ca\cdot\mathbf{1}]^{0}$. However, more exothermic enthalpy values were found, accompanied by relatively less favorable entropies of binding, resulting in larger association constants overall. These thermodynamic parameters, and in particular the more favorable enthalpy values, imply that $[H\cdot\mathbf{1}]^{1-}$ employs its CD cavities more effectively than the metal-chelated dimers $[Eu\cdot\mathbf{1}]^{1+}$ and $[Ca\cdot\mathbf{1}]^{0}$ in binding TBPyP. The negative entropy values can be explained in terms of enthalpy–entropy compensation,[28] which is often observed for CDs and CD dimers.[22,29] Apparently, the increased tether flexibility allows better cooperation of the CD cavities in complexation of TBPyP. The higher association constant (by a factor of 22) observed for the complexation of TBPyP with $[H\cdot\mathbf{1}]^{1-}$ in relation to $[Eu\cdot\mathbf{1}]^{1+}$ can therefore probably be assigned both to more favorable electrostatic interaction and to more effective binding by the CD cavities, while the stronger binding (by a factor 5) of TSPP by $[Eu\cdot\mathbf{1}]^{1+}$ relative to $\mathbf{1}^{2-}$ stems solely from electrostatic interactions.

Conclusions

Tethering of two CDs by an EDTA moiety gives access to tunable CD dimers with binding properties that can be altered by metal chelation and protonation. The binding affinity of these CD dimers towards charged guest molecules is strongly dependent on the charge and rigidity imposed on the EDTA tether. Electrostatic interactions between charged guest molecules and charged CD dimers give rise to systematic changes in the thermodynamic parameters of complexation. ITC experiments revealed that the association of charged porphyrins and oppositely charged CD dimers is entropically more favorable than the corresponding binding of the porphyrins by the neutral forms of the CD dimer. This is attributed to improved desolvation of the formed complex, which is associated with an unfavorable enthalpy contribution that is overridden by a more favorable entropy of binding. In contrast, the interaction between charged porphyrins and similarly charged CD dimers is less strong because of less favorable entropies of binding, which is ascribed to an increase in structured water upon complex formation. Association constants determined for the differently charged CD dimers were found to differ by a factor of up to 5 in the case of the complexation of TSPP by CD dimer **1**, which was solely attributed to changes of tether charge, and not of tether flexibility.

Restriction of tether flexibility by metal chelation can have a more pronounced influence on the binding properties of the CD dimer. Suitable design of the CD dimer with respect to size–fit compatibility with the guest molecule may result in significant differences in binding, as was shown by the complexation of the relatively large guest porphyrin TBPyP by CD dimer **1**. Metal chelation of the tether resulted in a restricted tether flexibility and consequently in less favorable enthalpies of binding, which was ascribed to less efficient cooperation of the two CD cavities. For the interaction of TBPyP with CD dimer **1**, metal chelation of the EDTA tether resulted in a binding affinity that was reduced by a factor of 22. Such significant differences in binding can be sufficient to achieve substantial release of guest molecules from the CD dimer upon metal chelation.[17]

Calorimetry was shown to be a powerful tool for the elucidation of the complexation thermodynamics, as it allows interpretation of the influence of metal chelation and charges upon the binding properties of the EDTA-tethered CD dimers. Attribution of the separate contributions of electrostatic and hydrophobic interactions is readily achieved for interactions in which one of the two is systematically changed. Comparison of interactions for which both contributions are altered are more difficult to analyze and require further, preferably structural, investigation.

Experimental Section

Materials and Methods: All chemicals were used as received, unless stated otherwise. Solvents were purified by standard laboratory methods.[30] 3-Amino-3-deoxy-heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin[11] was prepared by literature procedures. Chromatographic separations were performed on silica gel 60 (Merck, 0.040–0.063 mm, 230–240 mesh). MALDI-TOF mass spectra were recorded with a PerSpective Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer with dihydroxybenzoic acid as a matrix. NMR spectra were recorded at 25 °C with a Varian Inova 300 spectrometer. ^{1}H NMR chemical shifts (300 MHz) are given relative to residual HDO (δ = 4.65 ppm). ^{13}C NMR chemical shifts (75 MHz) are given relative to CH_3OD (δ = 49.3 ppm, used as an external standard for samples measured in D_2O).

EDTA-Tethered CD Dimer2: A solution of EDTA-bisanhydride (12 mg, 0.05 mmol) in CH_2Cl_2 was added dropwise to a solution of TBDMS-protected 3-amino-3-deoxy-β-cyclodextrin **4** (200 mg, 0.10 mmol) and Et_3N (20 μL, 0.15 mmol) in CH_2Cl_2 at room temperature. The reaction mixture was stirred overnight, after which the solution was diluted with CH_2Cl_2 and washed with HCl (0.1 M) and brine. The organic layer was dried over Na_2SO_4, and the product was purified over silica gel with ETOAc/EtOH/H_2O 12:2:1 as the eluent, and used as such in the deprotection step. The TBDMS-protected precursor of **2** (125 mg, 0.03 mmol) was dissolved in THF at room temperature, and a solution of tetrabutylammonium fluoride (TBAF) in THF (1 M, 1 mL, 1.0 mmol) was added. The reaction mixture was heated at reflux overnight. The solvent was evaporated in vacuo, and the residue was diluted with water and washed twice with diethyl ether. The aqueous layer was freeze-dried to give CD dimer **2** as a white solid (76 mg, overall 64% yield). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 3.20 (br., 4 H), 3.29 (br., 4 H), 3.43–3.91 (m, 78 H), 4.00 (br., 2 H), 4.07 (br., 2 H), 4.25 (br., 2 H), 4.88 (d, J = 5.5 Hz, 2 H), 4.92–5.01 ppm (m, 12 H). ^{13}C NMR (75 MHz, D_2O, 25 °C): δ = 51.2, 54.0, 56.6, 59.7–60.5, 72.2–73.8, 81.0–81.8, 101.2–104.0, 167.1, 167.6 ppm. MALDI-TOF-MS: *m/z* calcd. for $[M + Na]^+$ 2545.8; found 2546.1.

Preparation of the Protonated Ligand and Metal Complex Forms of 1 and 2: The metal complexes of the EDTA-tethered CD dimers **1** and **2** were prepared by addition of aliquots of concentrated solutions of $CaCl_2$ or $EuCl_3$ in doubly distilled water (Millipore) to solutions of **1** and **2**. The addition of the metal salts to solutions of the EDTA-tethered CD dimers was followed by monitoring of the pH; strong decreases in pH indicated the formation of the metal complexes. A slight excess of CD dimers **1** and **2** relative to the metal salts was maintained (1:0.95) to prevent the formation of metal hydroxides. After addition of the metal salts the solutions were adjusted to pH 7 with NaOH. The free ligands of **1** and **2** were prepared by adjusting the pH of solutions of **1** and **2** to pH 8.5, with NaOH.

Calorimetric Titrations: Calorimetric titrations were performed at 25 °C with a Microcal VP-ITC titration microcalorimeter. Sample solutions were prepared as described above. For the titrations performed with CD dimer solutions of pH 8.5, the pH values of the guest solutions were adjusted to the pH of the host solution with NaOH. Titrations were performed by addition of aliquots (5 or 10 μL) of a guest solution to a host solution. For the titrations of TSPP with CD dimer **1**, the titrant contained 0.01 to 0.1 mM of TSPP, while the cell solutions contained 10 to 100 μM of CD dimer **1**. Titrations of TSPP with CD dimer **2** were performed with 0.1 to 1 mM of TSPP as the titrant and 0.01 to 0.1 mM solutions of CD dimer **2** in the cell. For the titrations of TBPyP with CD dimer **1**, the titrant contained 0.05 to 0.5 mM of TBPyP, while the cell solutions contained 10 to 100 μM of CD dimer **1**. All calorimetric titrations were corrected for dilution heats by subtraction of the calorimetric dilution experiments from the calorimetric titration experiments. The titrations were analyzed by use of a least-squares curve fitting procedure. The sets of thermodynamic parameters

given in Table 1 are based on at least three independent titrations performed at three different concentrations.

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[1] I. Tabushi, Y. Kuroda, K. Shimokawa, *J. Am. Chem. Soc.* **1979**, *101*, 1614–1619.
[2] T. Lecourt, J.-M. Mallet, P. Sinay, *Eur. J. Org. Chem.* **2003**, 4553–4560, and references therein.
[3] a) M.-M. Luo, R.-G. Xie, P.-F. Xia, L. Tao, D.-Q. Yuan, H.-M. Zhao, *J. Phys. Org. Chem.* **2001**, *14*, 515–520; b) J. Yan, R. Breslow, *Tetrahedron Lett.* **2000**, *41*, 2059–2062; c) X. Ren, J. Liu, G. Luo, Y. Zhang, Y. Luo, G. Yan, J. Shen, *Bioconjugate Chem.* **2000**, *11*, 682–687; d) B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1997**, *119*, 1676–1681.
[4] a) J. Yang, B. Gabriele, S. Belvedere, Y. Huang, R. Breslow, *J. Org. Chem.* **2002**, *67*, 5057–5067; b) R. Breslow, J. Yang, J. Yan, *Tetrahedron* **2002**, *58*, 653–659; c) J. Yang, R. Breslow, *Angew. Chem. Int. Ed.* **2000**, *39*, 2692–2695.
[5] a) H. F. M. Nelissen, A. F. J. Schut, F. Venema, M. C. Feiters, R. J. M. Nolte, *Chem. Commun.* **2000**, 577–578; b) H. F. M. Nelissen, M. Kercher, L. De Cola, M. C. Feiters, R. J. M. Nolte, *Chem. Eur. J.* **2002**, *8*, 5407–5414.
[6] Y. Liu, Y. Chen, L. Li, H.-Y. Zhang, S.-X. Liu, X.-D. Guan, *J. Org. Chem.* **2001**, *66*, 8518–8527.
[7] L. C. West, O. Wyness, B. L. May, P. Clements, S. F. Lincoln, C. J. Easton, *Org. Biomol. Chem.* **2003**, *1*, 887–894.
[8] Y. Liu, C.-T. Wu, G.-P. Xue, J. Li, *J. Inclusion Phenom. Macrocyclic Chem.***2000**, *36*, 95–100.
[9] a) Y. Liu, C. C. You, T. Wada, Y. Inoue, *Tetrahedron Lett.* **2000**, *41*, 6869–6873; b) Y. Liu, C.-C. You, B. Li, *Chem. Eur. J.* **2001**, *7*, 1281–1288; c) Y. Liu, L. Li, H.-Y. Zhang, Y. Song, *J. Org. Chem.* **2003**, *68*, 527–536.
[10] J. J. Michels, J. Huskens, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2002**, *124*, 2056–2064.
[11] E. van Dienst, B. H. M. Snellink, I. von Piekartz, M. H. B. Grote Gansey, F. Venema, M. C. Feiters, R. J. M. Nolte, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem.* **1995**, *60*, 6537–6545.
[12] J.-M. Yan, M. Atsumi, D.-Q. Yuan, K. Fujita, *Helv. Chim. Acta* **2002**, *85*, 1496–1504.
[13] a) R. Breslow, A. W. Czarnik, M. Lauer, R. Leppkes, J. Winkler, S. Zimmerman, *J. Am. Chem. Soc.* **1986**, *108*, 1969–1979; b) D.-Q. Yuan, K. Ohta, K. Fujita, *Chem. Commun.* **1996**, 821–822.
[14] C. Platas-Iglesias, D. M. Corsi, L. van der Elst, R. N. Muller, D. Imbert, J.-C. G. Bünzli, É. Tóth, T. Maschmeyer, J. A. Peters, *J. Chem. Soc., Dalton Trans.* **2003**, 727–737.
[15] K. Kano, R. Nishiyabu, T. Asada, Y. Kuroda, *J. Am. Chem. Soc.* **2002**, *124*, 9937–9944.
[16] J. J. Michels, R. Fiammengo, P. Timmerman, J. Huskens, D. N. Reinhoudt, *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *41*, 163–172.
[17] a) A. Mulder, A. Juković, L. N. Lucas, J. van Esch, B. L. Feringa, J. Huskens, D. N. Reinhoudt, *Chem. Commun.***2002**, 2734–2735; b) A. Mulder, A. Juković, F. W. B. van Leeuwen, H. Kooijman, A. E. Spek, J. Huskens, D. N. Reinhoudt, *Chem. Eur. J.* **2004**, *10*, 1114–1123; c) A. Mulder, A. Juković, J. Huskens, D. N. Reinhoudt, *Org. Biomol. Chem.* **2004**, *2*, 1748–1755.
[18] a) X.-P. Wang, J.-H. Pan, S.-M. Shuang, *Spectrochimica Acta A* **2001**, *57*, 2755–2762; b) T. Carofiglio, R. Fornasier, V. Lucchini, C. Rosso, U. Tonelatto, *Tetrahedron Lett.* **1996**, *37*, 8019–8022; c) J. M. Ribo, J. A. Farrera, M. L. Valero, A. Virgili, *Tetrahedron* **1995**, *51*, 3705–3712.
[19] a) F. Venema, A. E. Rowan, R. J. M. Nolte, *J. Am. Chem. Soc.* **1996**, *118*, 257–258; b) F. Venema, H. F. M. Nelissen, P. Berthault, N. Birlirakis, A. E. Rowan, M. C. Feiters, R. J. M. Nolte, *Chem. Eur. J.* **1998**, *4*, 2237–2250.
[20] a) J. S. Manka, D. S. Lawrence, *J. Am. Chem. Soc.* **1990**, *112*, 2440–2442; b) D. L. Dick, T. V. S. Rao, D. Sukumaran, D. S. Lawrence, *J. Am. Chem. Soc.* **1992**, *114*, 2664–2669.
[21] CPK modeling of the complexes of TBPyP and the different forms of dimer **1** indicated that in all cases the tether length is insufficient to bind two opposite *tert*-butylphenyl moieties, but sufficient to bind two adjacent *tert*-butylphenyl moieties. These implications dictate that $K_1 = 4K_i = 8K_2$.
[22] a) M. V. Rekharsky, Y. Inoue, *J. Am. Chem. Soc.* **2002**, *124*, 813–826; b) M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1917.
[23] S. E. Brown, J. H. Coates, P. A. Duckworth, S. F. Lincoln, C. J. Easton, B. L. May, *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 1035–1040.
[24] a) T. Kitae, H. Takashima, K. Kano, *J.Inclusion Phenom. Macrocyclic Chem.* **1999**, *33*, 345–359; b) T. Kitae, T. Nakayama, K. Kano, *J. Chem. Soc., Perkin Trans. 2* **1998**, 207–212; c) K. Kano, *J. Phys. Org. Chem.* **1997**, *10*, 286–291.
[25] a) D. Foguel, J. L. Silva, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8244–8247; b) R. Mahtab, H. H. Harden, C. J. Murphy, *J. Am. Chem. Soc.* **2000**, *122*, 14–17; c) T. W. Lynch, S. G. Sligar, *J. Biol. Chem.* **2000**, *275*, 30561–30565.
[26] a) M. Stodeman, N. Dhar, *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 899–903; b) R. Prohens, M. C. Rotger, M. N. Pina, P. M. Deya, J. Morey, P. Ballester, A. Costa, *Tetrahedron Lett.* **2001**, *42*, 4933–4936; c) R. Meissner, X. Garcias, S. Mecozzi, J. Rebek, Jr., *J. Am. Chem. Soc.* **1997**, *119*, 77–85; d) D. J. Cram, H. J. Choi, J. A. Bryant, C. B. Knobler, *J. Am. Chem. Soc.* **1992**, *114*, 7748–7765.
[27] a) S. Tobey, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 14807–14815; b) F. Corbellini, R. Fiammengo, P. Timmerman, M. Crego-Calama, K. Versluis, A. J. R. Heck, I. Luyten, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2002**, *124*, 6569–6575; c) L. Sebo, B. Schweizer, F. Diederich, *Helv. Chim. Acta* **2000**, *83*, 80–92.
[28] J. E. Leffler, *J. Org. Chem.* **1955**, *20*, 1202–1231.
[29] a) B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1993**, *115*, 9353–9354; b) Y. Liu, Y. Chen, B. Li, T. Wada, Y. Inoue, *Chem. Eur. J.* **2001**, *7*, 2528–2535.
[30] D. D. Perrin, W. F. L. Armarego, *Purification of Laboratory Chemicals*, 3rd ed., Pergamon, Oxford, **1989**.

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