Synthesis of a Dinuclear Zn^{II}–Calix[4]arene Enzyme Model with Additional General Base Groups − Catalytic Activity in Phosphate Diester Transesterification

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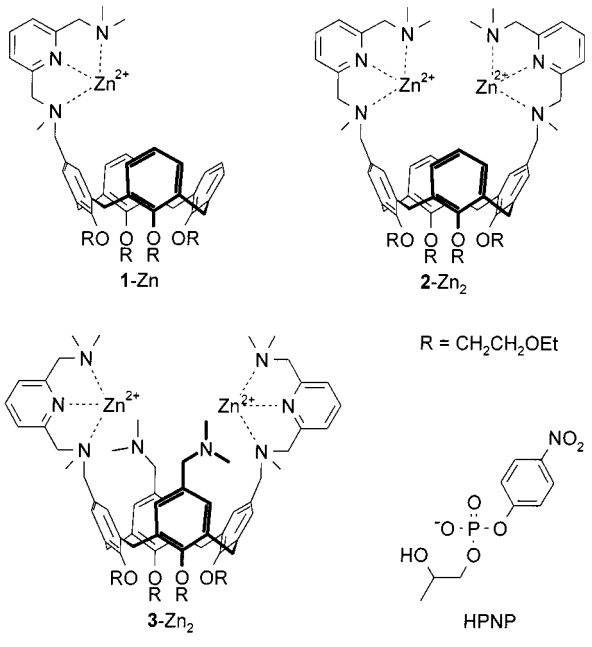
Calix[4]arenes modified at the upper rim with two pairs of functional groups were synthesized and were used as starting materials for the synthesis of an enzyme model **3**-Zn_2 that has two Zn^{II} centers and two dimethylamino groups. Under neutral conditions, this dinuclear metallo-enzyme model is catalytically active in the cleavage of the phosphate diester bond of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP). The kinetics of **3**-Zn_2 were compared with a mononuclear complex **1**-Zn and a dinuclear complex **2**-Zn_2 that lack the dimethylamino groups. A catalysis mechanism is proposed where the Zn^{II} centers of **3**-Zn_2 cooperate in the activation of the phosphoryl group while one of the dimethylamino groups acts as a general base in the deprotonation of the hydroxyl group of HPNP.

Introduction

Many enzymes that cleave phosphate ester bonds use one or two divalent metal centers, like Zn^{II}, for the activation of the substrate. Basic groups, like histidines, are often involved for the generation of an attacking nucleophile.[1] The active site of these phosphodiesterase metallo-enzymes has been mimicked by model compounds that have, connected by a molecular spacer, for instance two metal ion complexes[2−6] or a metal ion complex and a nitrogen base.[6][7] The molecular spacer in the model systems must neither be too flexible nor too rigid since for cooperative catalytic effects a dynamic preorganization of the catalytic groups is required.[4b,5] In this respect, calix[4]arenes[8] are suitable molecular scaffolds. They can be functionalized on two sets of four directionally oriented positions and their structure can adapt by low energy conformational changes. We have previously reported the calix[4]arene Zn^{II} complex **2**-Zn_2 that exhibits at neutral pH a high catalytic activity in the intramolecular transesterification[9] of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP).[4] This dinuclear complex is 50 times more active than its mononuclear analog **1**-Zn due to cooperative catalytic action of the two Zn^{II} centers. Whereas the substrate binding affinity of catalyst **2**-Zn_2 is very high, the rate of the conversion of the substrate within the catalyst-substrate complex is relatively moderate. A possible reason for the latter might be that the catalytic system does not posses a general base that can assist in the deprotonation of the hydroxyl group of the substrate upon nucleophilic attack at phosphorus. The calix[4]arene moiety in the dinuclear complex **2**-Zn_2 possesses two unsubstituted *p*-phenolic positions which can be functionalized for this purpose.[6] Although calix[4]arenes functionalized at two upper rim positions have been used for a long time in the design of molecular receptors, examples of multifunctionalized calix[4]arenes are rare.[8] Here, we report calix[4]arene **3**-Zn_2 that has besides two Zn^{II} centers two basic dimethylamino groups at the upper rim. We have investigated the effect of the basic groups in the vicinity of the Zn^{II} centers in the catalysis of transesterification of HPNP.

The synthesis of a novel class of calix[4]arenes having two pairs of groups at the upper rim that can be differently functionalized is described. These calix[4]arenes were used as the starting materials for the synthesis of the dinucleating ligand **3**. The binding activity of the corresponding Zn^{II} complex **3**-Zn_2 with HPNP and the catalytic turnover is compared with that of calix[4]arenes **1**-Zn and **2**-Zn_2 that lack the additional base groups.



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Results and Discussion

Synthesis

The synthesis of calix[4]arene **2** was described previously.[4] In this synthesis, the aminomethylpyridine ligands were introduced by reacting a bis(*N*-methylaminomethyl)calix[4]arene with 2-bromomethyl-6-hydroxymethylpyridine.[4] For **3**, which is an extension of **2** with two general base groups at the two other aromatic rings, a calix[4]arene starting material is required that has at the upper rim two *N*-methylaminomethyl groups and two other groups that can be further functionalized.[6] The synthesis of upper rim functionalized calix[4]arenes was performed by two different routes, starting from the unsubstituted calix[4]arenes **4**[10] and **8**,[11] respectively, and is shown in Scheme 1.

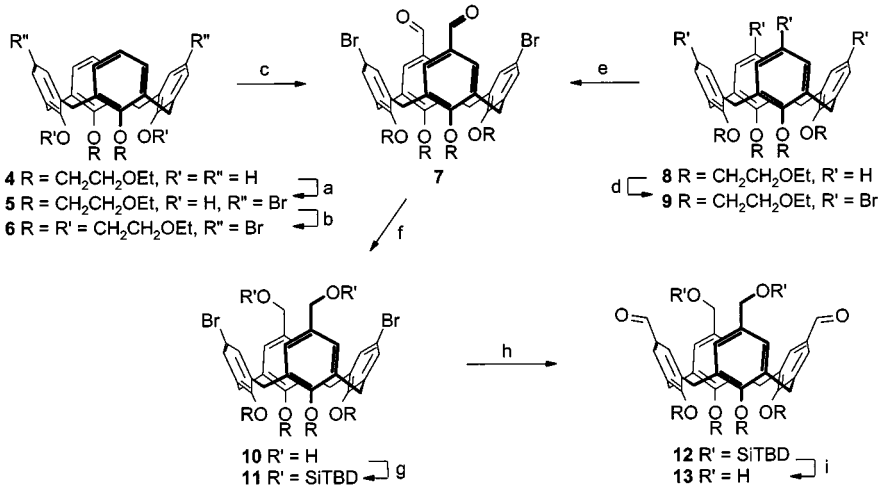
A general method for the selective functionalization of two diametrical upper rim positions comprises electrophilic aromatic substitution at the phenolic *para* positions of di-*O*-alkylated calix[4]arenes.[12] Since phenols are more reactive than anisoles substitution is selective and dihydroxycalix[4]arene **4** was converted in 98% yield to the dibromo derivative **5** by reaction with bromine. Subsequent alkylation of the phenolic groups, using sodium hydride and bromoethyl ethyl ether in DMF, afforded in 86% yield the (tetraethoxyethoxy)calix[4]arene **6**. The remaining *para* positions were then functionalized with formyl groups *via* the Duff reaction,[6][13] by refluxing dibromocalix[4]arene **6** with hexamethylenetetraamine in trifluoroacetic acid. Another method that was used for selective functionalization of the calix[4]arene upper rim was selective bromo−lithium exchange.[14] Tetrabromocalix[4]arene **9**, obtained in 82% yield by reaction of calix[4]arene **8** with *N*-bromosuccinimide,[15] was treated with 2.6 equivalents of *n*BuLi, resulting in selective lithiation of two diametrical positions. Quenching with DMF afforded diformyl derivative **7** in 80% yield. Reduction of the formyl groups to alcohols (**10**) with sodium borohydride proceeded in almost quantitative yield. The hydroxyl groups in **10** were protected with *tert*-butyldimethylsilyl groups (TBDSi, **11**) and silyl ether **11** was lithiated by treatment with *n*BuLi. Formyl groups (**12**) were introduced in 90% yield by quenching with DMF. Subsequently, the protecting TBDSi groups were removed by acidic hydrolysis[16] to afford the multifunctional compound **13**.

The formyl groups were converted into secondary amines by reductive amination with methyl amine under hydrogenation conditions, yielding bisamine **14** in 95% (Scheme 2). Bisamine **14** was reacted with two equivalents of 2-bromomethyl-6-hydroxymethylpyridine,[17] giving in 84% yield the bis(aminomethylpyridine)calix[4]arene **15**. The four hydroxyl groups of compound **15** were transformed into leaving groups by chlorination with thionyl chloride. Since the tetrachloride is not stable, it was not isolated, but immediately reacted with an excess of dimethylamine to afford **3** in 57% yield. Calix[4]arene **3** has at the upper rim two aminomethylpyridine-based metal ion coordinating ligands and two pendant basic dimethylamino groups that are potential catalytic groups.

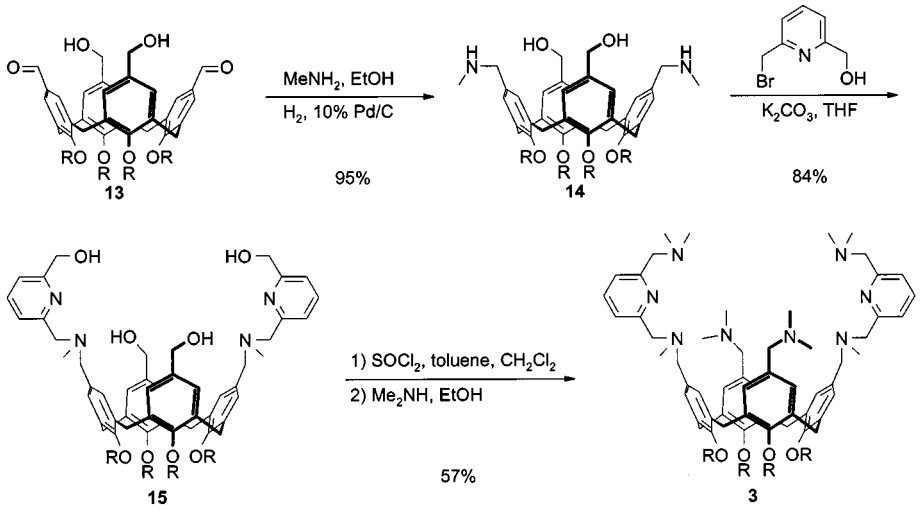
Catalysis

The effect of the presence of the dimethylamino groups in the transesterification of the RNA model substrate[9] HPNP[18] was studied by comparison of the catalytic activity of **3**-Zn_2 with the catalytic activities of the previously[4] investigated Zn^{II} complexes **1**-Zn and **2**-Zn_2. The dinuclear complex **3**-Zn_2 was formed *in situ* by the addition of two equivalents of $Zn(ClO_4)_2$ to the ligand **3**. The kinetic experiments were performed in 50% CH_3CN/20 mM buffer[19] in which **1**-Zn, **2**-Zn_2 and **3**-Zn_2 are well soluble. The pseudo-first-order rate constants and kinetic data are collected in the Table.

At pH 6.8, the rate acceleration induced by 0.48 mM of **3**-Zn_2 (k_{obs} = 2.0 × 10^{-4} s^{-1}) is approximately 7000-fold over the uncatalyzed reaction, whereas **2**-Zn_2 induces under



Scheme 1. Functionalization of the calix[4]arene upper rim. (a) Br_2, $CHCl_3$, 98%; (b) NaH, $EtOCH_2CH_2Br$, DMF, 86%; (c) hexamethylenetetraamine, TFA, 80%; (d) NBS, methyl ethyl ketone, 82%; (e) *n*BuLi, THF, DMF, 80%; (f) $NaBH_4$, EtOH, 98%; (g) TBDSiCl, Et_3N, DMAP, CH_2Cl_2, 88%; (h) *n*BuLi, THF, DMF, 90%; (i) AcOH, H_2O, THF, 82%.



Scheme 2. Synthesis of a dinucleating calix[4]arene ligand (R = CH_2CH_2OEt)

Table 1. Kinetic data for the transesterification of HPNP catalyzed by calix[4]arene Zn^{II} complexes.[a]

Catalyst		k_{obs}[b] (10^{-4} s^{-1})	k_{cat}[c,d] (10^{-4} s^{-1})	K_{ass}[c] (10^{2} M^{-1})	K_m[e] (mM)
1-Zn	pH 7.0[f]	0.12	0.19	7.5	1.3
2-Zn_2	pH 7.0[f]	6.26	7.7	550	0.018
3-Zn_2	pH 6.8[g]	2.0	3.6	19	0.53
none[h]	pH 7.0	2.7×10^{-4}	–	–	–

[a] In 50% CH_3CN/20 mM buffer at 25°C. – [b] At 0.48 mM catalyst and 0.19 mM HPNP. – [c] Determined by curve fitting. – [d] Determined by means of an Eady-Hofstee plot. – [e] Calculated by $K_m = 1/K_{ass}$. – [f] HEPES buffer. – [g] MES buffer. – [h] Measured from a 2.0 mM HPNP solution.

these conditions a 19000-fold rate acceleration ($k_{obs} = 5.2 \times 10^{-4}$ s^{-1}).[4] Consequently, in terms of rate acceleration, the presence of the two dimethylamino groups in **3**-Zn_2 does not directly result in improved catalysis. However, like **2**-Zn_2, the dinuclear complex **3**-Zn_2 cleaves the substrate by cooperative action of the Zn^{II} centers since it is 20 times more active than the mononuclear complex **1**-Zn ($k_{obs} = 1.0 \times 10^{-5}$ s^{-1}). This conclusion is supported by a study in which the rate was measured as a function of the concentration Zn^{II} with respect to ligand **3**. There is a strong increase in rate from 0–2 equivalents Zn^{II} and a plateau is reached at higher Zn^{II} concentrations (Figure 1), indicating the necessity of two Zn^{II} ions in the catalysis.

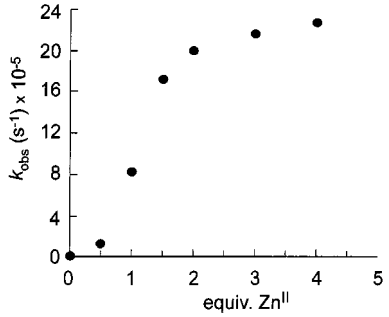


Figure 1. Dependence of k_{obs} for the cleavage of HPNP (0.19 mM) on the amount of $Zn(ClO_4)_2$ with respect to **3** (0.48 mM) in 50% CH_3CN/20 mM MES pH 6.8 at 25°C

Figure 2 shows the pH-rate profiles for **2**-Zn_2 and **3**-Zn_2. The dinuclear complex **2**-Zn_2 exhibits a rate optimum at pH 7.5. The bell shaped pH profile is represented by an increasing and decreasing curve that correspond with acid-base transitions with kinetic pK_a values of 7.1 and 7.8, respectively. These acid-base transitions belong to the deprotonation of Zn^{II} bound water molecules to tightly bound hydroxide ions.[4] The water molecules are weakly bound and allow facile displacement by a substrate molecule (K_{ass}). Consequently, at low pH the rate of substrate cleavage is mainly determined by k_{cat} and not by the substrate binding step (K_{ass}). On the other hand, at high pH the conversion of the substrate within the catalyst-substrate complex (k_{cat}) is more efficient since the conversion requires a general base that can deprotonate the substrate. It is remarkable that the pH optimum of dimethylamino appended complex **3**-Zn_2 is significantly lower than that of **2**-Zn_2, *i.e.* pH 6.8. This shift in the pH optimum can be caused by the presence of the amino groups in **3**-Zn_2 that may act as a general base in the deprotonation of the hydroxyl group of the substrate.[7] The bell shaped profile of **3**-Zn_2 is represented by acid-base transitions with kinetic pK_a values of 6.4 and 7.2. Assuming that the kinetic pK_a values of the water molecules bound to the Zn^{II} centers in **3**-Zn_2 are in the same range as those for **2**-Zn_2, the acid-base transition observed at pH 6.4 may be attributed to the deprotonation of ammonium groups[7c] to basic amino groups. It is also

possible that the pH shift is caused by a lowering of the pK_a of the Zn^{II} bound water molecules due to electrostatic interaction with the neighboring (protonated) amino groups.

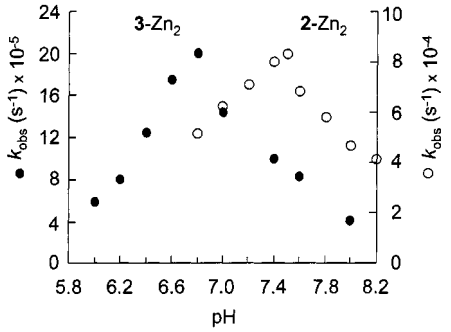


Figure 2. Dependence of k_{obs} on pH for the transesterification of HPNP (0.19 mM) catalyzed by 0.48 mM **2**-Zn_2 and **3**-Zn_2 in 50% CH_3CN/20 mM buffer at 25°C.

In order to investigate the catalytic process in more detail, the rate was measured as a function of the substrate concentration. The resulting saturation kinetics curve was analyzed by means of an Eady-Hofstee plot and was fitted according to the Michaelis−Menten equation (Figure 3). It was previously shown that the high rate accelerations induced by **2**-Zn_2 are primarily due to a very high affinity of **2**-Zn_2 for the substrate HPNP.[4] Comparison of the kinetic parameters (Table) of **3**-Zn_2 with **2**-Zn_2, reveals that for **3**-Zn_2 the substrate binding affinity (K_{ass}) has dropped with almost a factor 30. Apparently, the bulky tertiary dimethylamino groups in **3**-Zn_2 hinder the binding of the substrate to form a catalyst-substrate complex. Also the rate constant k_{cat} is reduced with a factor 2 for **3**-Zn_2, suggesting a less favorable binding orientation for conversion of the substrate and a different catalysis mechanism.

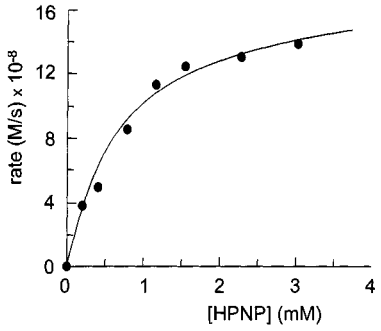


Figure 3. Saturation kinetics curve for the transesterification of HPNP for 0.48 mM of **3**-Zn_2 in 50% CH_3CN/20 mM MES pH 6.8 at 25°C; the experimental data points are fitted according to the Michaelis-Menten equation with K_{ass} and k_{cat} obtained by means of an Eady-Hofstee plot (see Table)

Mechanism of Catalysis

Amino appended complex **3**-Zn_2 exhibits both a lower binding affinity for HPNP and a lower catalytic activity than **2**-Zn_2. Presumably, the bulkiness of the dimethylamino groups hampers a two-point Zn^{II} coordination of HPNP *via* its phosphoryl and hydroxyl group, as was proposed for HPNP binding by **2**-Zn_2 (Figure 4, left).[2] As is indicated by CPK molecular models, coordination of both Zn^{II} centers to the phosphoryl group of HPNP requires less space above the calix[4]arene surface. Such a double Lewis acid activation is widely accepted for dimetal-catalyzed phosphate ester cleavage,[2,3,4b,5] and we propose this binding mode for **3**-Zn_2 (Figure 4, right). For efficient catalysis a general base is then required that can assist in the deprotonation of the substrate hydroxyl group prior to the intramolecular nucleophilic attack at the phosphorus atom. In the catalysis by dinuclear complex **2**-Zn_2 this can be accomplished by buffer ions or by a Zn^{II} bound hydroxide ion. The complex **3**-Zn_2 has two dimethylamino groups and at the optimum pH of 6.8 one of them may act as a general base in the substrate deprotonation.

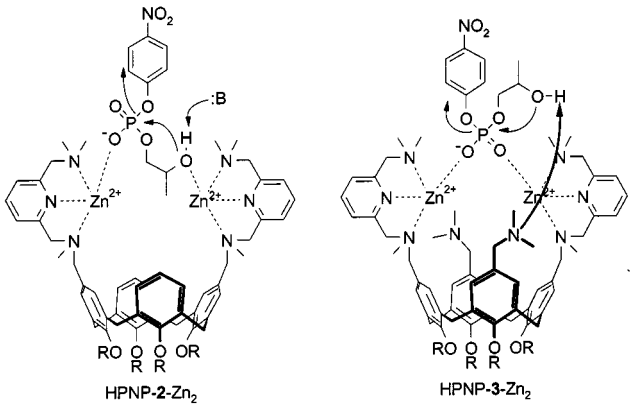


Figure 4. Schematic representations of possible mechanisms for HPNP transesterification catalyzed by **2**-Zn_2 (left) and **3**-Zn_2 (right)

Conclusion

Synthetic methods for the introduction of two pairs of functional groups at the calix[4]arene upper rim have been developed. Diformyl-bis(hydroxymethyl)calix[4]arene **13** is a multifunctional starting material that was used for the synthesis of bifunctional calix[4]arene ligand **3** that has two Zn^{II} binding sites and two basic amino groups. The corresponding dinuclear Zn^{II} complex **3**-Zn_2 mimics dinuclear metallo-enzymes that cleave phosphate diester bonds. It catalyzes efficiently the intramolecular transesterification of the RNA model substrate HPNP by cooperative action of the Zn^{II} ions. The kinetic transition found around pH 6.4 suggests that in the catalysis one of the amino groups acts as a general base in the deprotonation of the substrate hydroxyl group. However, the steric crowding caused by these groups prohibits optimal bifunctional catalysis.

Experimental Section

General Remarks: THF was freshly distilled from Na/benzophenone, CH_2Cl_2 from $CaCl_2$, and $SOCl_2$ from linseed oil.[20] Other solvents and chemicals were of reagent grade and were used as

received from commercial sources. − Column chromatography was performed with silica gel (SiO_2, 0.040−0.063 mm, 230−400 mesh). − Melting points are uncorrected. − ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with Me_4Si as internal standard. − FAB-MS spectra were recorded with *m*NBA as a matrix. − Compounds **4**[10] and **8**,[11] 2-bromomethyl-6-hydroxymethylpyridine,[17] and HPNP[18] were synthesized according to literature procedures. − The pH meter used for adjustment of buffered solutions was calibrated daily. − UV/Vis spectra were measured with a diode array spectrophotometer equipped with a thermostated cuvet holder (7 cuvettes, 1.0 cm path length) and a sample transport accessory.

5,17-Dibromo-25,27-bis(2-ethoxyethoxy)calix[4]arene (5): To a cooled (0°C) solution of dihydroxycalix[4]arene **4** (4.00 g, 7.03 mmol) in $CHCl_3$ (300 mL) was added dropwise a solution of Br_2 (0.80 mL, 15.5 mmol) in $CHCl_3$ (200 mL) over a period of 1.5 h. The reaction mixture was stirred for an additional 15 min before it was quenched with 5% $NaHSO_3$ solution (400 mL). The layers were separated and the organic layer was washed with 10% $NaHSO_3$ solution (400 mL), water (400 mL) and brine (400 mL), and was dried with $MgSO_4$. The mixture was filtered and the solvent was evaporated under reduced pressure to give the product as a white solid. Recrystallization from CH_2Cl_2/MeOH gave the pure product (5.00 g, 98%). M.p. 253−255°C. − ^1H NMR ($CDCl_3$, 250 MHz): δ = 7.94 (s, 2 H), 7.17 (s, 4 H), 6.87 (d, 4 H, *J* = 7.5 Hz), 6.71 (t, 2 H, *J* = 7.5 Hz), 4.15−4.12 (m, 4 H), 3.89−3.91 (m, 4 H), 3.67 (q, 4 H, *J* = 7.0 Hz), 4.38 and 3.29 (ABq, 8 H, *J* = 13.1 Hz), 1.26 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 152.6, 152.0, 132.8, 130.8, 130.1, 129.2, 125.5, 110.3, 75.6, 69.1, 67.0, 31.0, 15.3. − FAB-MS; *m*/*z*: 724.7 $[M]^+$; calcd. 724.1. − $C_{36}H_{38}Br_2O_6 \cdot 0.8H_2O$ (726.50): calcd. C 58.36, H 5.39; found C 58.08, H 4.99.

5,17-Dibromo-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (6): Calix[4]arene **5** (6.24 g, 8.59 mmol) was added to a suspension of NaH (1.72 g, 60wt% in oil, 43.0 mmol, washed twice with hexane) in DMF (300 mL). After stirring at room temperature for 30 min 2-bromoethyl ethyl ether (4.87 mL, 43.0 mmol) was added. The reaction mixture was stirred for 18 h at 60°C after which it was cooled to room temperature. Water (10 mL) was added and the solvent was removed under reduced pressure. The residue was taken up in EtOAc/saturated NH_4Cl solution (300 mL/300 mL) and the organic layer was washed with saturated NH_4Cl solution (2 × 300 mL), water (300 mL), and brine (300 mL). The solution was dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) and recrystallization (CH_2Cl_2/MeOH) to give the product as white crystals (6.47 g, 86%). M.p. 93−95°C. ^1H NMR ($CDCl_3$, 250 MHz): δ = 6.81 (s, 4 H), 6.59 (s, 6 H), 4.47 and 3.09 (ABq, 8 H, *J* = 13.5 Hz), 4.13−4.06 (m, 8 H), 3.83−3.78 (m, 8 H), 3.52 (q, 8 H, *J* = 7.0 Hz), 1.19 (t, 12 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 156.0, 155.7, 137.4, 134.2, 130.8, 128.4, 122.7, 115.0, 73.4, 69.7, 69.6, 66.4, 30.1, 15.3. − FAB-MS; *m*/*z*: 868.2 $[M]^+$; calcd. 868.2. − $C_{44}H_{54}Br_2O_8$ (870.71): calcd. C 60.70, H 6.25; found C 60.55, H 6.32.

5,11,17,23-Tetrabromo-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (9): To a solution of calix[4]arene **8** (10.00 g, 14.0 mmol) in methylethylketone (500 mL) was added NBS (15.00 g, 112 mmol). After stirring at room temperature for 18 h 10% aqueous $NaHSO_3$ (350 mL) was added and stirring was continued for 1 h. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 250 mL). The combined organic phases were washed with 10% aqueous $NaHSO_3$ (400 mL) and water (400 mL), and dried over $MgSO_4$. The mixture was filtered, concentrated under reduced pressure, and precipitated with CH_2Cl_2/MeOH. Recrystallization from CH_2Cl_2/hexane afforded the pure product (11.81 g, 82%). M.p. 136−137°C. − ^1H NMR ($CDCl_3$, 250 MHz): δ = 6.80 (s, 8 H), 4.09 (t, 8 H, *J* = 5.2 Hz), 3.77 (t, 8 H, *J* = 5.2 Hz), 3.49 (q, 8 H, *J* = 7.0 Hz), 3.07 and 4.45 (ABq, 8 H, *J* = 13.5 Hz), 1.18 (t, 12 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 155.4, 136.5, 131.0, 115.4, 73.5, 69.6, 66.4, 30.6, 15.3. − FAB-MS; *m*/*z*: 1024.9 $[M + H]^+$; calcd. 1025.0. − $C_{44}H_{52}Br_4O_8$ (1028.51) : calcd. C 51.38, H 5.10; found: C 51.33, H 5.01.

5,17-Dibromo-25,26,27,28-tetrakis(2-ethoxyethoxy)-11,23-diformyl-calix[4]arene (7). − Method A: To a solution of dibromocalix[4]arene **6** (500 mg, 0.580 mmol) in TFA (30 mL) was added hexamethylenetetraamine (810 mg, 5.74 mmol). The reaction mixture was refluxed for 18 h, cooled to room temperature and poured out in ice water (150 mL). The aqueous suspension was extracted with CH_2Cl_2 (3 × 50 mL) after which the combined organic phases were washed with saturated Na_2CO_3 solution (75 mL) and water (75 mL). The mixture was dried with $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) to give the product as a colorless oil (900 mg, 80%). − **Method B:** To a solution of tetrabromo-calix[4]arene **9** (10.00 g, 9.72 mmol) in THF (350 mL), cooled to −78°C, was added *n*BuLi (10.1 mL, 2.5 M, 25.3 mmol). After stirring for 15 min at −78°C DMF (15.0 mL, 194 mmol) was added. The reaction mixture was stirred 5 min at −78°C and 5 min at room temperature, and was subsequently quenched with 0.1 N HCl (25 mL). The solvent was removed under reduced pressure, the residue was taken up in CH_2Cl_2 (500 mL) and was washed with 0.1 N HCl (2 × 400 mL) and water (400 mL). The solution was dried with $MgSO_4$, filtered, and concentrated in *vacuo* to give the crude product, which was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) to give the product as a colorless oil (7.21 g, 80%). − ^1H NMR ($CDCl_3$, 250 MHz): δ = 9.69 (s, 2 H), 7.26 (s, 4 H), 6.72 (s, 4 H), 4.55 and 3.22 (ABq, 8 H, *J* = 13.7 Hz), 4.26 (t, 4 H, *J* = 5.1 Hz), 4.07 (t, 4 H, *J* = 5.0 Hz), 3.81 (t, 4 H, *J* = 5.1 Hz), 3.77 (t, 4 H, *J* = 5.0 Hz), 3.53 (q, 4 H, *J* = 7.0 Hz), 3.48 (q, 4 H, *J* = 7.0 Hz), 1.20 (t, 6 H, *J* = 7.0 Hz), 1.15 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 191.5, 162.2, 155.1, 136.2, 136.0, 131.4, 131.1, 130.2, 115.6, 73.7 (2×), 69.7, 69.5, 66.4, 66.3, 30.7, 15.2. − FAB-MS; *m*/*z*: 925.2 $[M + H]^+$; calcd. 925.2; 947.1 $[M + Na]^+$; calcd. 947.1.

5,17-Dibromo-25,26,27,28-tetrakis(2-ethoxyethoxy)-11,23-bis-(hydroxymethyl)calix[4]arene (10): To a solution of calix[4]arene **7** (690 mg, 0.734 mmol) in EtOH/THF (50 mL/5 mL) was added $NaBH_4$ (167 mg, 4.40 mmol). The reaction mixture was stirred at room temperature for 3 h and quenched with acetic acid until hydrogen formation stopped (pH 5). The solvents were evaporated under reduced pressure, the residue was taken up in EtOAc (100 mL) and was washed with saturated Na_2CO_3 solution (100 mL), water (100 mL) and brine (100 mL). The solution was dried over $MgSO_4$, filtered and concentrated in *vacuo* to give a colorless oil, which was pure enough for further modification (670 mg, 98%). − ^1H NMR ($CDCl_3$, 250 MHz): δ = 7.00 (s, 4 H), 6.45 (s, 4 H), 4.48 and 3.09 (ABq, 8 H, *J* = 13.5 Hz), 4.24 (s, 4 H), 4.21 (t, 4 H, *J* = 5.6 Hz), 3.99 (t, 4 H, *J* = 5.1 Hz), 3.81 (t, 4 H, *J* = 5.6 Hz), 3.77 (t, 4 H, *J* = 5.1 Hz), 3.54 (q, 4 H, *J* = 7.0 Hz), 3.52 (q, 4 H, *J* = 7.0 Hz), 2.45 (br s, 2 H), 1.21 (t, 6 H, *J* = 7.0 Hz), 1.17 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 156.3, 155.1, 137.8, 135.2, 133.5, 131.0, 126.6, 114.8, 73.7, 73.0, 69.7, 69.6, 66.5, 66.3, 64.4, 30.8 (2×). − FAB-MS; *m*/*z*: 929.4 $[M]^-$, calcd. 928.2; 930.1 $[M + H]^+$, calcd. 929.2; 953.0 $[M + Na]^+$, calcd. 951.2.

5,17-Dibromo-11,23-bis[(*tert*-butyldimethyl)silyloxymethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (11): To a solution of calix[4]arene **10** (5.05 g, 5.42 mmol) in CH_2Cl_2 (300 mL) was added triethylamine (4.50 mL, 32.5 mmol), 4-(dimethylamino)-pyridine (663 mg, 5.43 mmol), and *tert*-butyldimethylsilyl chloride (4.91 g, 32.6 mmol). After refluxing for 48 h the solution was washed subsequently with 0.1 N HCl (2 × 300 mL), water (300 mL), saturated Na_2CO_3 solution (300 mL), and water (300 mL). The solution was dried with $MgSO_4$, filtered, and concentrated in *vacuo* to give the crude product, which was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) to give the product as a colorless oil (5.56 g, 88%). − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 6.70 (s, 4 H), 6.56 (s, 4 H), 4.47 (s, 4 H), 4.39 and 3.02 (ABq, 8 H, *J* = 13.4 Hz), 4.08 (t, 4 H, *J* = 5.8 Hz), 3.96 (t, 4 H, *J* = 5.2 Hz), 3.77−3.70 (m, 8 H), 3.46 (q, 4 H, *J* = 7.0 Hz), 3.43 (q, 4 H, *J* = 7.0 Hz), 1.13 (t, 6 H, *J* = 7.0 Hz), 1.10 (t, 6 H, *J* = 7.0 Hz), 0.88 (s, 18 H), 0.00 (s, 12 H). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 155.7, 154.8, 136.5, 135.5, 134.6, 130.6, 126.6, 115.3, 73.6, 73.0, 69.6, 66.4, 66.3, 64.9, 30.9, 26.0, 25.7, 18.4, 15.3 (2×). − FAB-MS; *m*/*z*: 1157.2 $[M + H]^+$; calcd. 1157.4.

11,23-Bis[(*tert*-butyldimethyl)silyloxymethyl]-25,26,27,28-tetrakis-(2-ethoxyethoxy)-5,17-diformylcalix[4]arene (12): To a solution of calix[4]arene **11** (1.00 g, 0.863 mmol) in THF (30 mL), cooled to −78°C, was added *n*BuLi (1.40 mL, 1.6 M, 2.24 mmol). After stirring at −78°C for 15 min DMF (1.30 mL, 16.9 mmol) was added. The reaction mixture was stirred 5 min at −78°C and 5 min at room temperature, and was subsequently quenched with 0.1 N HCl (10 mL). The solvent was removed under reduced pressure, the residue was taken up in CH_2Cl_2 (50 mL) and was washed with 0.1 N HCl (2 × 40 mL) and water (40 mL). The solution was dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) to give the product as a colorless oil (821 mg, 90%). − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 9.45 (s, 2 H), 7.01 (s, 4 H), 6.65 (s, 4 H), 4.51 and 3.18 (ABq, 8 H, *J* = 13.5 Hz), 4.43 (s, 4 H), 4.15 (t, 4 H, *J* = 5.1 Hz), 4.08 (t, 4 H, *J* = 5.4 Hz), 3.80−3.74 (m, 8 H), 3.54 (q, 8 H, *J* = 7.0 Hz), 1.15 (t, 6 H, *J* = 7.0 Hz), 1.14 (t, 6 H, *J* = 7.0 Hz), 0.87 (s, 18 H), 0.00 (s, 12 H). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 191.4, 161.8, 155.4, 136.0, 135.5, 134.2, 131.2, 129.9, 126.4, 73.7, 73.2, 73.0, 69.7, 69.6, 66.3 (2×), 64.8, 64.5, 30.9, 26.0 (2×), 18.4 (2×), 15.3, 15.2. − FAB-MS; *m*/*z*: 1156.5 $[M]^+$; calcd. 1156.6.

25,26,27,28-Tetrakis(2-ethoxyethoxy)-5,17-diformyl-11,23-bis-(hydroxymethyl)calix[4]arene (13): Calix[4]arene **12** (370 mg, 0.350 mmol) was dissolved in THF (5 mL) to which acetic acid (15 mL) and water (5 mL) were added. After stirring for 18 h at room temperature the reaction mixture was concentrated in *vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated Na_2CO_3 solution (2 × 40 mL) and water (40 mL), and dried over Na_2SO_4. The mixture was filtered and concentrated in *vacuo*. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 95/5) to give the product as a colorless oil (240 mg, 82%). − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 9.67 (s, 2 H), 7.36 (s, 4 H), 6.46 (s, 4 H), 4.57 and 3.25 (ABq, 8 H, *J* = 13.6 Hz), 4.34 (t, 4 H, *J* = 5.2 Hz), 4.20 (s, 4 H), 4.03 (t, 4 H, *J* = 4.9 Hz), 3.86 (t, 4 H, *J* = 5.2 Hz), 3.80 (t, 4 H, *J* = 4.9 Hz), 3.56 (q, 4 H, *J* = 7.0 Hz), 3.49 (q, 4 H, *J* = 7.0 Hz), 3.21 (br. s, 2 H), 1.22 (t, 6 H, *J* = 7.0 Hz), 1.15 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 191.9, 162.9, 155.0, 136.7, 135.4, 133.4, 131.0, 130.3, 126.7, 73.7, 73.4, 69.8, 69.6, 66.4, 66.2, 64.1, 30.9, 15.2. − FAB-MS; *m*/*z*: 827.5 $[M - H]^+$; calcd. 827.4, 851.4 $[M + Na]^+$; calcd. 851.4.

25,26,27,28-Tetrakis(2-ethoxyethoxy)-5,17-bis(hydroxymethyl)-11,23-bis(methylaminomethyl)calix[4]arene (14): Calix[4]arene **13** (1.10 g, 1.33 mmol) was dissolved in a 33% methylamine solution in EtOH (40 mL) after which 10% Pd/C (100 mg) was added. The reaction mixture was stirred for 16 h in a hydrogen atmosphere, filtered over Celite and evaporated till dryness. The residue was taken up in CH_2Cl_2/saturated Na_2CO_3 solution containing 5% EDTA (30 mL/60 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with K_2CO_3, filtered and concentrated in *vacuo* to give the product as a colorless oil (1.09 g, 95%), which was pure enough for further modification. − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 6.84 (s, 4 H), 6.40 (s, 4 H), 4.48 and 3.11 (ABq, 8 H, *J* = 13.1 Hz), 4.22 (t, 4 H, *J* = 6.1 Hz), 4.13 (s, 4 H), 3.99 (t, 4 H, *J* = 5.0 Hz), 3.89 (t, 4 H, *J* = 6.1 Hz), 3.80 (t, 4 H, *J* = 5.0 Hz), 3.60−3.48 (m, 8 H), 3.51 (s, 4 H), 2.29 (s, 6 H), 1.22 (t, 6 H, *J* = 7.0 Hz), 1.18 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 156.3, 154.6, 135.7, 135.4, 133.7, 133.2, 128.6, 126.2, 77.4, 73.7, 72.7, 69.7, 69.6, 66.4, 66.2, 64.0, 55.5, 35.6, 30.8, 15.4, 15.3. − FAB-MS; *m*/*z*: 859.5 $[M + H]^+$; calcd. 859.5.

25,26,27,28-Tetrakis(2-ethoxyethoxy)-5,17-bis(hydroxymethyl)-11,23-bis[((6-hydroxymethyl)pyridin-2-yl-methyl)-(methylamino)-methyl]calix[4]arene (15): A mixture of calix[4]arene **14** (510 mg, 0.469 mmol), K_2CO_3 (130 mg, 0.938 mmol), and 2-(bromomethyl)-6-hydroxymethyl)pyridine[17] (190 mg, 0.938 mmol) was stirred in THF (40 mL) for 2 days. The solvent was removed under reduced pressure, the residue was taken up in EtOAc/saturated Na_2CO_3 solution (40 mL/40 mL) and the aqueous phase was extracted with EtOAc (3 × 40 mL). The combined organic phases were dried over K_2CO_3, filtered and concentrated in *vacuo*. The residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N, 90/9/1) to give **15** as a colorless oil (434 mg, 84%). − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 7.65 (t, 2 H, *J* = 7.6 Hz), 7.44 (d, 2 H, *J* = 7.6 Hz), 7.20 (d, 2 H, *J* = 7.6 Hz), 7.02 (s, 4 H), 6.21 (s, 4 H), 4.66 (s, 4 H), 4.47 and 3.10 (ABq, 8 H, *J* = 13.1 Hz), 4.26 (t, 4 H, *J* = 6.3 Hz), 3.94−3.89 (m, 8 H), 3.79 (t, 4 H, *J* = 4.9 Hz), 3.71 (s, 4 H), 3.59 (s, 8 H), 3.55 (q, 4 H, *J* = 7.0 Hz), 3.53 (q, 4 H, *J* = 7.0 Hz), 2.26 (s, 6 H), 1.24 (t, 6 H, *J* = 7.0 Hz), 1.18 (t, 6 H, *J* = 7.0 Hz). − ^{13}C NMR ($CDCl_3$, 250 MHz): δ = 159.3, 158.8, 156.8, 154.1, 137.5, 136.1, 135.1, 133.2, 131.0, 130.1, 126.0, 121.2, 119.4, 73.8, 72.6, 69.7, 69.6, 66.5, 66.2, 64.7, 63.5, 61.8, 61.2, 42.3, 30.7, 15.4, 15.3. − FAB-MS; *m*/*z*: 1100.0 $[M]^-$; calcd. 1100.6, 1102.1, $[M + H]^+$; calcd. 1101.6).

25,26,27,28-Tetrakis(2-ethoxyethoxy)-5,17-bis(dimethylamino-methyl)-11,23-bis[((6-dimethylaminomethyl)pyridin-2-ylmethyl)-(methylamino)methyl]calix[4]arene (3): To a solution of calix[4]arene **15** (270 mg, 0.245 mmol) in CH_2Cl_2/toluene (25 mL/5 mL) was added $SOCl_2$ (0.72 mL, 9.87 mmol), and the solution was stirred for 5 h. The solvent was removed under reduced pressure to give the product as a hydrochloride, which was not characterized but directly used for further modification. A freshly prepared solution of the crude hydrochloride in CH_2Cl_2 (20 mL) was added dropwise to a cooled 33% dimethylamine solution in EtOH (20 mL). The reaction mixture was stirred for 18 h at room temperature and subsequently concentrated to dryness. The residue was taken up in CH_2Cl_2/saturated Na_2CO_3 solution (20 mL/20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried with K_2CO_3, filtered and concentrated in *vacuo*. The crude product was purified by column chromatography (basic Al_2O_3, CH_2Cl_2/MeOH, 98/2) to give **3** as a colorless oil (169 mg, 57%). − ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 7.64 (t, 2 H, *J* = 7.5 Hz), 7.35 (d, 2 H, *J* = 7.5 Hz), 7.26 (d, 2 H, *J* = 7.5 Hz), 6.77 (s, 4 H), 6.52 (s, 4 H), 4.46 and 3.13 (ABq, 8 H, *J* = 13.1 Hz), 4.15 (t, 4 H, *J* = 6.0 Hz), 4.07 (t, 4 H, *J* = 5.6 Hz), 3.91−3.83 (m, 8 H), 3.59−3.49 (m, 16 H), 3.24 (s, 4 H), 2.94 (s, 4 H), 2.28 (s, 12 H), 2.05 (s, 6 H), 1.96 (s, 12 H), 1.25−1.17 (m, 12 H). − ^{13}C NMR

($CDCl_3$, 250 MHz): δ = 159.3, 158.2, 155.5, 154.9, 136.7, 134.8, 134.2, 132.1, 131.7, 129.0, 121.0, 73.3, 72.9, 69.6, 66.3, 66.2, 65.9, 63.9, 63.2, 61.8, 45.7, 44.8, 42.0, 30.7, 15.3. − FAB-MS; *m*/*z*: 1209.9 $[M + H]^+$; calcd. 1209.8.

Kinetics: Solutions for spectrophotometric titrations and kinetic measurements were made by adding CH_3CN (spectrophotometric grade) up to 50% (v/v) to a 20 mM aqueous buffer solution adjusted with NaOH to the desired pH. Buffers (MES, pH 6.0−7.0; HEPES, pH 7.0−8.2; EPPS pH 8.2−8.6) were obtained from commercial sources and used without further purification in deionized distilled water. HPNP was prepared according to the literature.[18] Stock solutions were freshly prepared before performing the kinetic measurements. In a typical experiment, the ligand **3** (20 μL, 50 mM in EtOH) and $Zn(ClO_4)_2 \cdot 6\,H_2O$ (40 μL, 50 mM in water) were added to a cuvet containing 2 mL of 50% CH_3CN/20 mM buffer solution (v/v) and thermostated at 25°C. After a couple of minutes equilibration time, HPNP (4 μL, 100 mM in water) was injected and the increase in UV absorption at λ = 400 nm due to the release of *p*-nitrophenolate was recorded every 30 seconds. Final concentrations were 0.98 mM for complex **1**-Zn and 0.48 mM for complexes **2**-Zn_2, and **3**-Zn_2, 0.19 mM in HPNP, and 10 mM in buffer. All solutions remained clear during the time of the kinetic measurements. In the absence of ligand precipitation of polymeric Zn^{II} hydroxide took place. The observed pseudo-first-order rate constants k_{obs} (s^{-1}) were calculated with the extinction coefficient of *p*-nitrophenolate at λ = 400 nm by an initial slope method (< 4% conversion). The kinetic runs were carried out in at least triplicate and the obtained average rate constants were obtained with an uncertainty of less than 5%. The pseudo-first-order rate constants for uncatalyzed reactions (k_{uncat}, s^{-1}) were measured from a 2.0 mM HPNP solution by following the increase in absorbance at 400 nm due to the release of *p*-nitrophenolate for one week.

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