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Di- and Trinuclear Zn^{2+} Complexes of Calix[4]arene Based Ligands as Catalysts of Acyl and Phosphoryl Transfer Reactions

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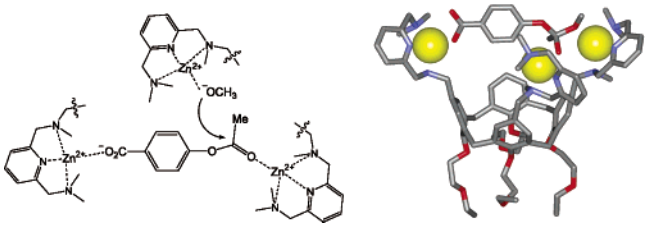

The calix[4]arene scaffold, blocked in the *cone* conformation by proper alkylation of the lower rim hydroxyls, was used as a convenient molecular platform for the design of bi- and trimetallic Zn^{2+} catalysts. The catalytic activity of the Zn^{2+} complexes of calix[4]arenes decorated at the 1,2-, 1,3-, and 1,2,3-positions of the upper rim with 2,6-bis[(dimethylamino)methyl]pyridine units were investigated in the cleavage of ester **6** and of the RNA model compound HPNP. High rate enhancements, up to 4 orders of magnitude, were observed in a number of catalyst−substrate combinations. Interestingly the order of catalytic efficiency among regioisomeric dinuclear complexes in the cleavage of ester **6** is 1,2-vicinal ≫ 1,3-distal, but it is reversed in the reaction of HPNP. The higher efficiency of trinuclear compared to dinuclear complexes provides an indication of the cooperation of three Zn^{2+} ions in the catalytic mechanism.

Many hydrolytic enzymes possess two or three metal ions in their active sites.[1] Common strategies in the mimicry of such enzymes are based on synthetic catalysts consisting of a number of ligated metal ions connected by suitable spacers.[2−4] To this purpose, the use of either rim of calix[4]arenes as convenient platforms for the introduction of metal ion binding sites is well precedented.[2b,4] Particularly successful has been the use of the upper rim of calix[4]arenes blocked in the *cone* conformation by proper alkylation of the lower rim hydroxyls. The dinuclear Zn^{2+} complex of a ditopic receptor (**4**) consisting of two 2,6-bis[(dimethylamino)methyl]pyridine units attached to the 1,3-positions of the calixarene upper rim showed a high degree of catalytic cooperation of the two metal centers in the cleavage of phosphodiester bonds.[2b] More recently, we reported the synthesis of the two regioisomeric ligands **1** and **2**, and an extensive investi-

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(1) (a) Sträter, N.; Lipscomb, W. N.; Klabunde T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2025−2055. (b) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435−2458. (c) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1997**, *36*, 433−450. (d) Williams, R. J. P. *Chem. Commun.* **2003**, 1109−1113. (e) Parkin, G. *Chem. Rev.* **2004**, *104*, 699−767.

(2) For review articles see: (a) Williams, N. H.; Takasaki, B.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485−493. (b) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *29*, 75−86.

(3) For more recent contributions see, for example, the following papers and references therein: (a) Fritsky, I. O.; Ott, R.; Pritzkow, H.; Krämer, R. *Chem. Eur. J.* **2001**, *7*, 1221−1231. (b) Worm, K.; Chu, F.; Matsumoto, K.; Best, M. D.; Lynch, V.; Anslyn, E. V. *Chem. Eur. J.* **2003**, *9*, 741−747. (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *J. Am. Chem. Soc.* **2003**, *125*, 2224−2227. (d) Mancin, F.; Rampazzo, E.; Tecilla, P.; Tonellato, U. *Eur. J. Org. Chem.* **2004**, 281−288. (e) Iranzo, O.; Richard, J. P.; Morrow J. R. *Inorg. Chem.* **2004**, *43*, 1743−1750.

(4) (a) Cacciapaglia, R.; Mandolini, L. Calixarene Based Catalytic Systems. In *Calixarenes in Action*; Mandolini, L., Ungaro, R., Eds.; Imperial Collage Press: London, UK, 2000; pp 241−264. (b) Sansone, F.; Segura, M.; Ungaro, R. Calixarenes in Bioorganic and Biomimetic Chemistry. In *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens J., Eds.; Kluwer: Dordrecht, The Netherlands, 2001; pp 496−512. (c) Ozturk, G.; Akkaya, E. U. *Org. Lett.* **2004**, *6*, 241−243.

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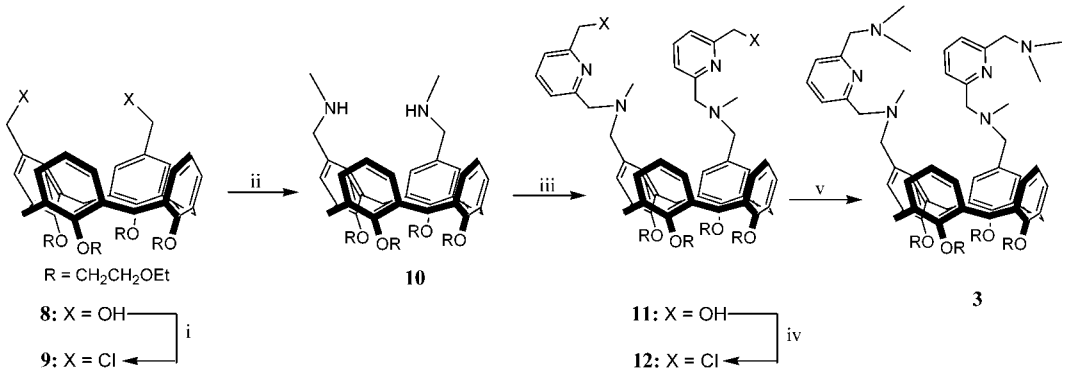
SCHEME 1[a]



[a] Reagents and conditions: (i) $SOCl_2$, CH_2Cl_2; (ii) CH_3NH_2, EtOH; (iii) 2-bromomethyl-6-hydroxymethylpyridine, CH_3CN, K_2CO_3, 42%; (iv) $SOCl_2$, CH_2Cl_2; (v) $(CH_3)_2NH$, EtOH, 76%.

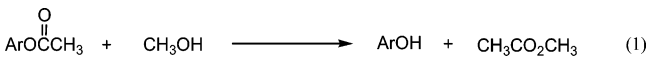
gation of the catalytic activity of their dinuclear Ba^{2+} complexes in the basic ethanolysis of a series of esters endowed with a distal carboxylate anchoring group.[5] We found a high degree of cooperation of the two metal centers in both cases and, most interestingly, a marked superiority in catalysis of the 1,2-vicinal complex (**1**-Ba_2) compared with its 1,3-distal regioisomer (**2**-Ba_2).

In view of our continuing investigations of metal catalysts based on a calix[4]arene scaffold, we report here on a quantitative investigation of the catalytic activity of the upper rim regioisomeric 1,2- and 1,3-bimetallic Zn^{2+} complexes (**3**-Zn_2 and **4**-Zn_2, respectively) and of the analogous trimetallic complex **5**-Zn_3 in the methanolysis of ester **6**, together with a comparison of the catalytic activity of regioisomers **3**-Zn_2 and **4**-Zn_2 in the cleavage of the RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP).

Results and Discussion

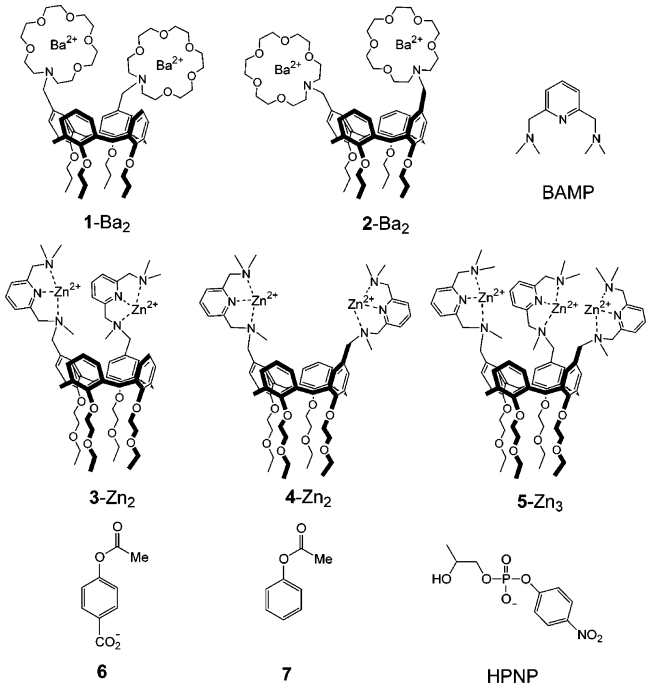
The recently developed[6] synthetic procedure for the preparation of 1,2-diformyl tetraalkoxycalix[4]arenes and corresponding 1,2-dialcohols in synthetically useful amounts has opened an easy way to hitherto elusive upper-rim 1,2-difunctionalized receptors, as illustrated by the procedure adopted for the synthesis of ligand **3** (Scheme 1). Other ligands were available from a previous investigation.[7]

Cleavage of Ester 6. The esterase activity of metal catalysts in the cleavage of esters **6** and **7** was studied in methanol solution (eq 1), buffered at pH 10.4 by means



of a 10 mM 1:1 mixture of *N*,*N*-diisopropyl-*N*-(2-methoxyethyl)amine and its perchlorate salt.[8] Metal catalysts were freshly prepared at 1.00 mM by mixing equivalent amounts of the free ligand and $Zn(ClO_4)_2$. For experimental convenience substrate concentrations in the kinetic runs were 0.10 mM ($k > 1 \times 10^{-3}$ s^{-1}; time-course experiments) or 0.50 mM ($k < 1 \times 10^{-3}$ s^{-1}; initial-rate technique).[9]

CHART 1



A number of control experiments were carried out to evaluate the binding process involving the various species in solution. UV−vis titration of the ligand 2,6-bis-[(dimethylamino)methyl]pyridine (BAMP) with $Zn(ClO_4)_2$ at 268 nm revealed the existence of a fairly strong complexation, $K = 6 \times 10^4$ M^{-1}. Consistently, the absorbance variation observed upon addition of 3 molar equiv of $Zn(ClO_4)_2$ to the tritopic ligand **5** amounted to 95% of the saturation value, thus showing that under the

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(6) Sartori, A.; Casnati, A.; Mandolini, L.; Sansone, F.; Reinhoudt, D. N.; Ungaro, R. *Tetrahedron* **2003**, *59*, 5539−5544.

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(8) The molar autoprotolysis constant of CH_3OH, $K_{ap} = 10^{-16.77}$ (Bosch E.; Bou P.; Allemann H.; Rosés M. *Anal. Chem.* **1996**; *68*, 3651−3657), implies that in this solvent the pH value corresponding to neutrality is 8.39. At pH 10.4 the concentration (activity) of the conjugate base of the solvent is 2 log units higher than under neutral conditions.

(9) Excess catalyst minimizes product inhibition as well as complications arising from multiple binding of carboxylate to di- and trimetallic complexes.

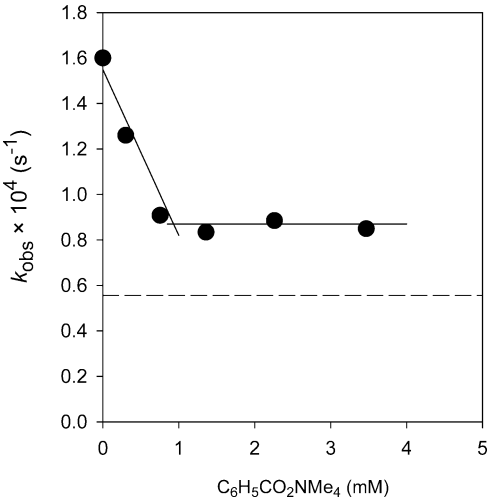
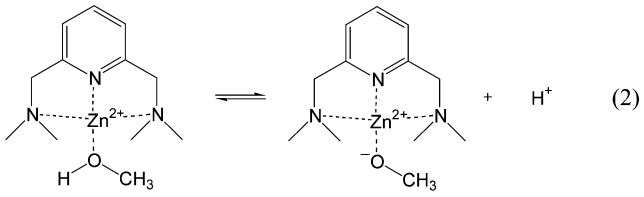


FIGURE 1. Effect of added tetramethylammonium benzoate on k_{obs} for the basic methanolysis of 0.1 mM *p*-nitrophenyl acetate in the presence of 1 mM BAMP-Zn (MeOH, pH 10.4 buffer, 25 °C). The dashed line represents the background rate constant ($k_{bg} = 5.6 \times 10^{-5}$ s^{-1}).

conditions of the kinetic runs binding of Zn^{2+} to the ligand units was nearly complete.

Potentiometric titration[10] with tetramethylammonium methoxide in MeOH showed that the complex of BAMP with $Zn(ClO_4)_2$ behaves as a weak monoprotic acid with $pK_a = 9.5$, which is easily interpreted as the pK_a of a metal-bound solvent molecule[11] (eq 2). This implies that at the pH of the kinetic solutions the Zn^{2+} complexes are mostly in the methoxide-bound form **13**.



(2)

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To provide an estimate of the binding constant between the ligated Zn^{2+} ion and the carboxylate anchoring group in **6**, we carried out a series of experiments in which the BAMP-Zn catalyzed methanolysis of *p*-nitrophenyl acetate (PNPA) is inhibited by increasing amounts of added tetramethylammonium benzoate (Figure 1). Methanolysis of PNPA was chosen as the test reaction despite its moderate sensitivity to the presence of the metal catalyst because the liberated *p*-nitrophenol is easily monitored by UV−vis spectroscopy (400 nm). Addition of a larger than stoichiometric amount of benzoate caused the reaction rate to reach a constant value that is nearly half of the value measured in the absence of added benzoate, but still slightly higher than the background rate observed in the presence of buffer alone.

This finding argues against the simple interpretation that addition of benzoate transforms the catalytically active species **13** into the unreactive complex **14**. We

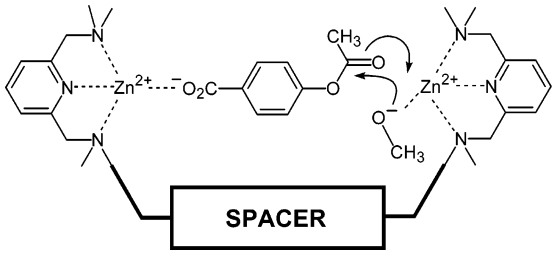
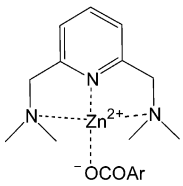


FIGURE 2. Proposed mechanism for the cleavage of ester **6** catalyzed by bimetallic complexes.

rather suggest that inhibition by carboxylate is due to the formation of the pentacoordinate neutral species **15**,[12] in which the Zn^{2+}-bound methoxide is less reactive than that in **13**.[13]



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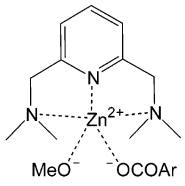

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In any case, independent of the precise nature of the Zn^{2+}-bound carboxylate species, the shape of the kinetic titration plot in Figure 1 indicates that the binding of carboxylate is strong enough ($K \geq 10^4$ M^{-1}) to ensure a complete or nearly complete anchoring of **6** to the metal catalysts.

In conclusion, the results of the above control experiments indicate that under the conditions of the catalytic runs in the presence of the dinuclear catalysts **3**-Zn_2 and **4**-Zn_2, the concentration of the productive complex (Figure 2), in which one of the two Zn^{2+} ions is bound to carboxylate and the other to methoxide, amounts to a significant fraction, no less than $^3/_4$, of the total substrate concentration. Similar considerations apply to the trimetallic catalyst **5**-Zn_3, where only one metal ion is involved in carboxylate binding.[9]

The kinetics of the catalyzed reactions were monitored by HPLC. The chromatograms revealed the presence of unreacted ester and phenol product (eq 1), and the complete absence of undesired byproducts. Pseudo-first-order rate constants for the faster reactions ($k > 1 \times 10^{-3}$ s^{-1}) were obtained from time-course experiments, which showed good adherence to first-order time-dependence in all cases. An initial rate technique was applied to slower reactions ($k < 1 \times 10^{-3}$ s^{-1}). Methanolysis of esters **6** and **7** in the absence of metal catalysts was inconveniently slow. We measured therefore the second-order rate constant (k) for reaction with tetramethylammonium methoxide, and calculated the rate constants for background methanolysis at the pH of the experiments k_{bg}

(10) Neverov, A. A.; McDonald, T.; Gibson, G.; Brown, R. S. *Can. J. Chem.* **2001**, *79*, 1704−1710.

(11) Cacciapaglia, R.; Di Stefano, S.; Fahrenkrug, F.; Lüning, U.; Mandolini L. *J. Phys. Org. Chem.* **2004**, *17*, 350−355.

(12) The X-ray crystal structure of a neutral pentacoordinate species analogous to **15** involving monodentate binding of two carboxylate ions was reported in ref 7b.

(13) The widely accepted bifunctional mechanism for metal-catalyzed hydrolysis (alcoholysis) involves combined nucleophile delivery to and Lewis acid activation of the ester carbonyl (see: Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145−152; for a recent example see ref 11). When applied to complex **15** such a mechanism would require an unlikely hexacoordination for a BAMP complexed Zn center.

TABLE 1. Basic Methanolysis of Esters 6 and 7 Catalyzed by the Zn^{2+} Complexes of Ligands BAMP and 3−5[a]

	7		6[b]	
catalyst	k_{obs} (s^{-1})	k_{obs}/k_{bg}[c]	k_{obs} (s^{-1})	k_{obs}/k_{bg}[c]
BAMP-Zn	5.3×10^{-6}	5.6	2.1×10^{-5}	38
3-Zn_2	9.7×10^{-6}	10	3.4×10^{-3}	6200
4-Zn_2	4.1×10^{-6}	4.4	1.3×10^{-4}	240
5-Zn_3	1.2×10^{-5}	13	1.3×10^{-2}	24000

[a] Runs carried out in MeOH, pH 10.4 buffer, 25 °C, on 0.10 mM ($k > 1 \times 10^{-3}$ s^{-1}; time-course experiments) or 0.50 mM ($k < 1 \times 10^{-3}$ s^{-1}; initial-rate technique) substrate in the presence of 1.00 mM metal catalyst. [b] Tetramethylammonium salt. [c] Rate constants for background methanolysis (k_{bg}) were calculated from eq 3, with $k = 1.3$ M^{-1} s^{-1} ($k_{bg} = 5.5 \times 10^{-7}$ s^{-1}) for ester **6** and $k = 2.2$ M^{-1} s^{-1} ($k_{bg} = 9.4 \times 10^{-7}$ s^{-1}) for ester **7**.

by means of eq 3, where pOMe = 16.77−10.4.[8]

$$k_{bg} = k \times 10^{-pOMe} \quad (3)$$

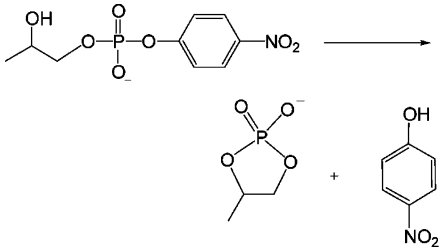
The results of the kinetic measurements are summarized in Table 1. We first note that solutions containing the metal complexes show in all cases enhanced rates compared with those of background methanolysis. In the reaction of **7** only moderate rate enhancements are observed, and differences between the various metal catalysts are small. Thus, nothing in the experimental data suggests that the metals in the bimetallic and trimetallic complexes act in a cooperative fashion. In contrast, rate enhancements experienced by ester **6** are not only much larger, but also strongly dependent on catalyst structure. The markedly different behavior of the two ester substrates illustrates well the important influence of the carboxylate function in **6**. First, as noted in previous instances,[5,14] binding to a metal ion transforms the moderately electron-releasing (rate-retarding) carboxylate substituent into an electron-withdrawing (rate-enhancing) one, with the net result that the inherently less reactive **6** (k^6/k^7 = 1.3/2.2 or 0.59) becomes much more reactive in the presence of BAMP-Zn (k^6_{obs}/k^7_{obs} = $2.1 \times 10^{-5}/5.3 \times 10^{-6}$ or 4.0). Superimposed to this purely electronic influence is the effect due to carboxylate as an anchoring group. The higher efficiency of dinuclear catalyst vs the mononuclear model clearly demonstrates cooperation between metal centers, in accordance with the mechanism depicted in Figure 2, in which one of the metal ions binds to the distal carboxylate and the other delivers a methoxide nucleophile to the ester carbonyl in an intramolecular (intracomplex) reaction.

Catalyst **4**-Zn_2, in which the two metals are in distal positions, shows but a moderate degree of cooperation between metal ions, with a $k_{obs}^{dinuclear}/k_{obs}^{mononuclear}$ ratio of 240/38 or 6.3, whereas the corresponding ratio for the vicinal regioisomer **3**-Zn_2 is as high as 6200/38 or 160. Thus the vicinal dinuclear complex **3**-Zn_2 exhibits a much higher catalytic activity in the cleavage of **6** than its distal regioisomer **4**-Zn_2, as it was already observed with the analogous bis-barium complexes **1**-Ba_2 and **2**-Ba_2. It appears therefore that, in the light of available evidence, the order of catalytic efficiency 1,2-vicinal ≫ 1,3-distal is a general feature of the upper rim dinuclear metallocatalysts for acyl transfer reactions that does not seem to depend in a critical fashion on the nature of the metal ion and of the corresponding ligating units.

Interestingly, the highest catalytic efficiency in the cleavage of **6** is exhibited by the trimetallic complex **5**-Zn_3. In principle, the trimetallic complex could work as a dinuclear complex, with the third metal ion acting as a spectator. In **5**-Zn_3 there are one 1,3-distal and two 1,2-vicinal bimetallic arrangements. Since the efficiency of the 1,3-distal dinuclear complex is very small, the catalytic efficiency of **5**-Zn_3, thought as resulting from the sum of contributions from the above dinuclear arrangements, would be approximately twice as large as that of **3**-Zn_2. The finding that **5**-Zn_3 is about four times as effective as **3**-Zn_2 provides an indication of the cooperation of three Zn^{2+} ions in the catalytic mechanism. Efficient cooperation of three metal ions (Zn, Cu) in the catalytic cleavage of phosphodiesters was reported in a few studies[2b,15] but, to the best of our knowledge, trimetallic catalysis in ester cleavage is unprecedented.

The proposed mechanism depicted in Figure 3 emphasizes the three different functions performed by the metal ions: (i) substrate recognition through binding to carboxylate, (ii) Lewis acid activation of the ester carbonyl, and (iii) nucleophile delivery. The computer drawn model of the complex of **5**-Zn_3 with the tetrahedral intermediate illustrates the geometrical compatibility of the trimetallic catalyst with the proposed mechanism.

Cleavage of HPNP. In previous papers[7] the catalytic activity of a number of calix[4]arene-based Zn^{2+} complexes, including BAMP-Zn, **4**-Zn_2, and **5**-Zn_3, was thoroughly investigated in the intramolecular transesterification of HPNP (eq 4) in CH_3CN-H_2O 1:1 (v/v) at pH around neutrality.



(4)

The very high catalytic efficiency of **4**-Zn_2 compared with that of mononuclear counterparts demonstrates that both metal centers in **4**-Zn_2 are involved in the binding of the altered substrate in the transition state. A likely mode of catalysis is a bifunctional mechanism in which one of the Zn^{2+} ions binds and activates the phosphate group, whereas the other has a role in the activation of the neighboring β-hydroxyl nucleophile. The proposed

(14) (a) Cacciapaglia, R.; Di Stefano, S.; Kelderman, E.; Mandolini, L. *Angew. Chem., Int. Ed.* **1999**, *38*, 348−351. (b) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *J. Org. Chem.* **2001**, *66*, 5926−5928. (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *J. Org. Chem.* **2002**, *67*, 521−525.

(15) (a) Komiyama, M.; Kina, S.; Matsumura, K.; Sumaoka, J.; Tobey, S.; Lynch, V. M.; Anslyn, E. *J. Am. Chem. Soc.* **2002**, *124*, 13731−13736. (b) Scarso, A.; Scheffer, U.; Göbel, M.; Broxterman, Q. B.; Kaptein, B.; Formaggio, F.; Toniolo, C.; Scrimin, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5144−5149. (c) Bencini, A.; Berni, E.; Bianchi, A.; Giorgi, C.; Valtancoli, B.; Chand D. K.; Schneider H.-J. *Dalton Trans.* **2003**, 793−800. (d) Kovbasyuk, L.; Pritzkow, H.; Krämer, R.; Fritsky, I. O. *Chem. Commun.* **2004**, 880−881. (e) Takebayashi, S.; Ikeda, M.; Takeuchi, M.; Shinkai, S. *Chem. Commun.* **2004**, 420−421.

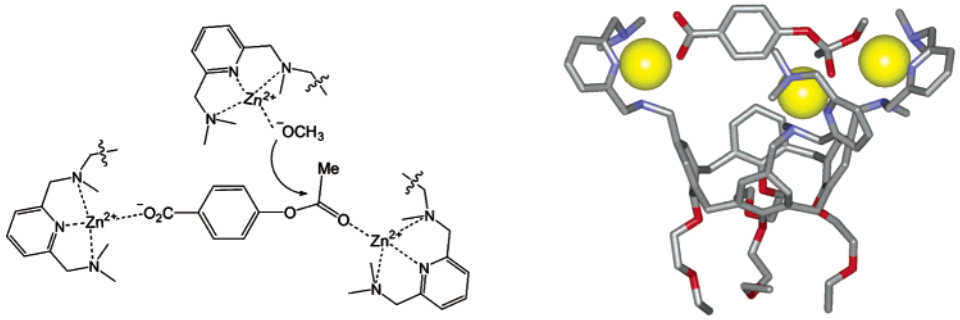


FIGURE 3. Proposed mechanism of trimetallic catalysis in the methanolysis of **6** in the presence of **5**-Zn_3 (left) and the computer-generated model of the tetrahedral intermediate/catalyst complex (right).

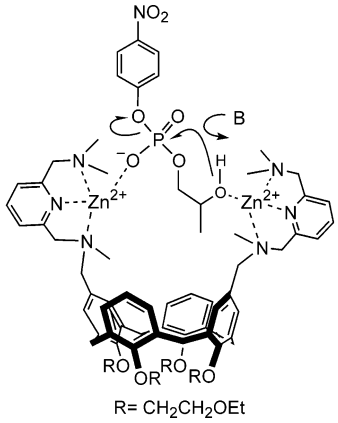


FIGURE 4. Bifunctional catalytic mechanism of **4**-Zn_2 in HPNP transesterification.

mechanism is depicted in Figure 4.[16] Evidence for the cooperative involvement of the three Zn^{2+} centers in **5**-Zn_3 was also obtained.

In light of the results obtained in the methanolysis of ester **6**, it seemed of interest to investigate the catalytic activity in the cleavage of HPNP of the 1,2-vicinal complex **3**-Zn_2, which was not available at the times of previous investigations.[7] In the context of the present study, the relevant questions are whether and to what an extent the two metal centers in **3**-Zn_2 work together in a cooperative fashion, and how the catalytic activity of **3**-Zn_2 compares to that of the 1,3-distal regioisomer **4**-Zn_2.

The spectrophotometrically determined catalytic rate constants listed in Table 2, obtained under conditions very close to those of the previous investigations, clearly reveal a high degree of synergism between metal centers in **3**-Zn_2, with a 61-fold rate enhancement of dinuclear over mononuclear catalyst. However, in contrast to what was observed in the catalytic cleavage of ester **6**, the 1,2-vicinal dinuclear complex **3**-Zn_2 is significantly less effective a catalyst than its 1,3-distal regioisomer **4**-Zn_2.

Concluding Remarks

Examples of dinuclear complexes whose catalytic activity is not much larger, or even lower, than that of analogous mononuclear complexes are not rare,[3b,d,4c,17] and show that two metal ions at a short distance do not necessarily make a good catalyst. It is remarkable, therefore, that the calix[4]arene-based di- and trinuclear Zn^{2+} complexes described in this work show high levels of cooperativity between metal ions both in the methanolysis of **6** and in the intramolecular transesterification of HPNP. Interestingly, and quite unpredictably, among dinuclear complexes the order of catalytic efficiency 1,2-vicinal > 1,3-distal is observed in the reaction of ester **6**, but the reverse order 1,3-distal > 1,2-vicinal holds for the transesterification of HPNP. Clearly, our catalysts possess degrees of conformational freedom arising from rotations around the C−C and C−N bonds connecting the ligand units to the calix[4]arene skeleton. If, on one hand, such a high conformational mobility makes it difficult to provide a rationale for the observed catalytic orders even a posteriori, on the other hand it ensures a large adaptability to transition states of different nature and geometry.

In conclusion, the data reported in this work, when combined with analogous data from previous investigations,[2b,5] show that calix[4]arenes are suitable building blocks for the design of di- and trinuclear metallocatalysts of acyl and phosphoryl transfer reactions. The calix[4]-arenes scaffold provides quite a reasonable compromise between proper preorganization of catalytic groups and adaptability resulting from conformational flexibility.

TABLE 2. Transesterification of HPNP Catalyzed by Calix[4]arene Zn^{2+} Complexes[a]

catalyst	k_{obs} (s^{-1})	k_{obs}/k_{bg}[b]	$k_{obs}^{dinuclear}/k_{obs}^{mononuclear}$
BAMP-Zn	3.4×10^{-6}	180	
3-Zn_2	2.0×10^{-4}	11 000	61
4-Zn_2	1.3×10^{-3}	68 000	380

[a] Spectrophotometric rate measurements in 50% CH_3CN/H_2O, 20 mM HEPES, pH 7.0, T = 25.0 °C. In all runs [cat.]=1.00 mM, [HPNP] = 0.2 mM. [b] $k_{bg} = 1.9 \times 10^{-8}$ s^{-1} (from initial-rate measurements).

Experimental Section

Instruments and General Methods. NMR spectra were recorded on either a 300- or 400-MHz spectrometer. Chemical shifts are reported as δ values in ppm from tetramethylsilane added as an internal standard. Mass spectra by electrospray ionization (ESI) and chemical ionization (CI) methods were recoded on a Micromass ZMD and on a Finnigan Mat SSQ710 spectrometer, respectively. Spectrophotometric measurements (kinetic runs involving pNPA, HPNP, and reaction of 0.1 mM **6** or **7** with 5 mM tetramethylammonium methoxide) were

(16) A kinetic equivalent mechanism is one in which a Zn^{2+}-bound hydroxide acts as a general base. Another possibility is that both Zn^{2+} ions are involved in the binding of the phosphate moiety, whereas base catalysis is provided by OH^- bound to one of the Zn^{2+} centers in a pentacoordinate geometry.

carried out on either a double beam or on a diode array spectrophotometer. HPLC analyses (methanolysis of esters **6** and **7** carried out in the presence of metal complexes) were performed on a liquid chromatograph fitted with a UV−vis detector operating at 220 nm. Kinetic runs monitored by HPLC were carried out in the presence of 1,4-dimethoxybenzene as an internal standard. Samples were analyzed on a Supelcosil LC-18 DB column (25 cm × 4.6 mm i.d., particle size 5 μm). At convenient time intervals 0.1 mL of the reaction mixture was quenched with 0.1 mL of a 10 mM aqueous solution of HBr, and subjected to HPLC analysis with CH_3CN/MeOH/H_2O−0.03% trifluoroacetic acid 10/45/45 (v/v) as eluent, at a flow rate of 0.6 mL/min.

Error limits of rate constants are in the order of ±5% (time-course kinetics) and ±10% (initial-rate technique). Nonlinear least-squares calculations of kinetic data were carried out with the program SigmaPlot 2002 for Windows, Version 8.0 (SPSS, Inc.).

Potentiometric titrations in methanol solution were carried out as reported in the literature,[10] by automatic titration under an argon atmosphere.

Materials. THF was dried by distillation from sodium benzophenoneketyl. 1,4-Dimethoxybenzene, phenyl acetate, benzoic acid, and $Zn(ClO)_2{\cdot}6H_2O$ were commercial samples used without further purification. Acid **6**·H^+,[14] HPNP, and nitrogen ligands BAMP, **4**, and **5** were available from previous investigations.[7]

Substrate **6** was generated in situ by addition of the parent phenolic compound to the buffered reaction medium. The stock solution of tetramethylammonium benzoate that was used in the kinetic titration of BAMP-Zn was freshly prepared from the parent carboxylic acid by addition of a stoichiometric amount of tetramethylammonium methoxide. A solution of the latter in methanol was prepared and handled as previously described.[18]

Other materials, apparatus, and techniques for the transesterification of HPNP in 50% (v/v) CH_3CN-H_2O were as reported previously.[7]

***N,N*-Diisopropyl-*N*-(2-methoxyethyl)amine.** *N,N*-Diisopropylethanolamine (14.5 g, 0.10 mol) was added at room temperature to a stirred mixture of 6 g of 60% NaH dispersion in mineral oil in 300 mL of THF. When addition of the reagent was complete, the reaction mixture was heated at 40 °C for 30 min. After the mixture was cooled, a solution of methyl iodide (12 mL, 0.14 mol) in THF (40 mL) was added dropwise. The reaction mixture was allowed to stand for 16 h at room temperature. The solvent and excess methyl iodide were removed under vacuum. Fractional distillation afforded the product (9.2 g, 58 mmol, 58% yield) as a volatile colorless oil (bp 170.5−171.5 °C, 760 mmHg), pK_a 10.4 in MeOH at 25 °C: ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.34 (s, 3H), 3.33 (t, J = 7.5 Hz, 2H), 2.99 (hept, J = 6.5 Hz, 2H), 2.61 (t, J = 7.5 Hz, 2H), 1.00 (d, J = 6.5 Hz, 12H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 67.5, 59.1, 56.7, 47.7, 18.9, 17.4.

***N,N*-Diisopropyl-*N*-(2-methoxyethyl)ammonium Perchlorate Salt.** This salt was prepared according to a literature procedure[19] (56% yield of a white solid (needles) after recrystallyzation from 2-propanol). ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.82 (hept, J = 6.5 Hz, 2H), 3.75 (t, J = 5.5 Hz, 2H), 3.42 (s, 3H), 3.29 (t, J = 5.5 Hz, 2H), 1.45 (br s, 12H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 67.5, 59.1, 56.7, 47.7, 18.9, 17.4. Anal. Calcd for $C_9H_{22}ClNO_5$ (259.73): C, 41.62; H, 8.54; N, 5.39. Found: C, 41.71; H, 9.04; N, 5.41.

Warning! Care was taken when handling *N,N*-diisopropyl-*N*-(2-methoxyethyl)ammonium perchlorate because it is potentially explosive.[20] No accident occurred in the course of the present work.

5,11-Bis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (9). Dialcohol calix[4]arene **8** (280 mg, 0.36 mmol) was dissolved in CH_2Cl_2 and $SOCl_2$ (0.53 mL, 7.5 mmol) was added. The solution was stirred for 2 h at room temperature and then the solvent was evaporated under vacuum to give **9** as a colorless oil in quantitative yield (290 mg, 0.36 mmol). The crude was pure enough to be used in the subsequent reaction. An analytically pure sample was obtained by dissolving the crude prodouct in 10 mL of CH_2Cl_2 and by washing with 10 mL of $NaHCO_3$ saturated solution and water. The organic phase was dried over Na_2SO_4 and filtered and the solvent was evaporated under reduced pressure. ^{1}H NMR (300 MHz; $CDCl_3$) δ 6.62−6.56 (m, 10H), 4.49 (d, J = 13.5 Hz, 4H), 4.29 (s, 4H), 4.11 (t, J = 5.6 Hz, 4H), 4.10 (t, J = 5.6 Hz, 4H), 3.82 (t, J = 5.6 Hz, 8H), 3.54 (q, J = 7.0 Hz, 8H), 3.14 (d, J = 13.5 Hz, 4H), 1.20 (t, J = 7.0 Hz, 12H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 156.6, 156.3, 135.5, 135.2, 135.1, 134.7, 131.0, 128.6, 128.4, 128.2, 128.1, 122.0, 73.2, 73.1, 69.6, 66.3, 46.6, 30.8, 29.6, 15.2. MS (ES) *m/z* (%) 831.6 $[M + Na]^+$ (100). Anal. Calcd for $C_{46}H_{58}Cl_2O_8$ (809.87): C, 68.22; H, 7.22. Found: C, 68.31; H, 7.33.

5,11-Bis[(*N*-methyl)aminomethyl]-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (10). A 33% methylamine solution in EtOH (10 mL) was added to a round-bottomed flask containing the bischloromethyl calixarene **9** (260 mg, 0.32 mmol). The solution was stirred ca. 48 h at room temperature, until completion was confirmed by TLC (Al_2O_3, CH_2Cl_2/MeOH 9/1). The solvent was evaporated under vacuum; the crude was dissolved in CH_2Cl_2 (30 mL) and washed with a 10% $NaHCO_3$ solution (30 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated under vacuum to give **10** (252 mg, 0.32 mmol) as colorless oil. Yield 97%. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.18 (d, J = 1.6 Hz, 2H), 6.86 (dd, J = 7.4 Hz, J = 1.2 Hz, 2H), 6.73 (d, J = 1.6 Hz, 2H), 6.68 (d, J = 7.4 Hz, 2H), 6.58 (t, J = 7.4 Hz, 2H), 4.51 (d, J = 13.1 Hz, 1H), 4.50 (d, J = 13.1 Hz, 2H), 4.47 (d, J = 13.1 Hz, 1H), 4.16−4.07 (m, 8H), 3.94 (d, J = 13.4 Hz, 2H), 3.82 (t, J = 5.5 Hz, 8H), 3.62 (d, J = 13.4 Hz, 2H), 3.53 (q, J = 7.0 Hz, 4H), 3.51 (q, J = 7.0 Hz, 4H), 3.24 (d, J = 13.1 Hz, 1H), 3.14 (d, J = 13.1 Hz, 3H), 2.28 (s, 6H), 1.19 (t, J = 7.0 Hz, 12H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 156.7, 155.9, 135.8, 135.6, 134.9, 134.3, 130.4, 129.6, 128.5, 128.3, 124.4, 122.6, 73.5, 73.2, 69.6, 69.5, 66.3, 51.6, 30.8, 30.7, 30.5, 15.2. MS (CI) *m/z* (%) 799.4 $[M + H]^+$ (75), 767.5 $[M - NHCH_3]^+$ (100). Anal. Calcd for $C_{48}H_{66}N_2O_8$ (799.06): C, 72.15; H, 8.33; N, 3.51. Found: C, 72.32; H, 8.41; N, 3.44.

5,11-Bis[*N*-((6-hydroxymethyl)pyridin-2-ylmethyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (11). K_2CO_3 (88 mg, 0.64 mmol) and 2-bromomethyl-6-hydroxymethylpyridine (130 mg, 0.64 mmol) were added to a solution of diamine calix[4]arene **10** (250 mg, 0.315 mmol) in dry CH_3CN (10 mL). The solution was stirred under N_2 atmosphere for 2 days. The solvent was removed under reduced pressure and the residue dissolved again in CH_2Cl_2 (70 mL). The organic layer was washed with a Na_2CO_3 saturated solution (70 mL) and the aqueous phase extracted with CH_2Cl_2 (70 mL). The combined organic layers were evaporated under vacuum and the product was obtained as a colorless oil (138 mg, 0.132 mmol) after purification by column chromatography (SiO_2; CH_2Cl_2/MeOH, 9/1). Yield 42%. ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.62 (t, J = 7.4 Hz, 2H), 7.19 (d, J = 7.4 Hz, 2H), 7.13 (d, J = 7.4 Hz, 2H), 6.68 (d, J = 1.6 Hz, 2H), 6.63 (br s, 4H), 6.47 (d, J = 7.4 Hz, 2H), 6.34 (t, J = 7.4 Hz, 2H), 4.72 (s, 4H), 4.49 (d, J = 12.9 Hz, 1H), 4.48 (d, J = 12.9 Hz, 2H), 4.46 (d, J = 13.3 Hz, 1H), 4.12 (t, J = 5.8 Hz, 4H), 4.10 (t, J = 5.8 Hz, 4H), 3.86 (t, J = 5.8 Hz, 4H), 3.85 (t,

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J = 5.8 Hz, 4H), 3.55 (q, J = 7.0 Hz, 4H), 3.53 (q, J = 7.0 Hz, 4H), 3.35 (d, J = 14.0 Hz, 2H), 3.28 (d, J = 14.0 Hz, 2H), 3.25 (d, J = 14.4 Hz, 2H), 3.12 (d, J = 14.4 Hz, 2H), 3.12 (d, J = 12.9 Hz, 3H), 3.07 (d, J = 13.3 Hz, 1H), 1.97 (s, 6H), 1.21 (t, J = 7.0 Hz, 6H), 1.20 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 159.1, 159.0, 156.5, 155.8, 137.6, 135.4, 135.2, 135.0, 132.1, 129.5, 129.2, 128.5, 128.4, 122.5, 121.8, 119.2, 73.6, 73.5, 70.0, 66.8, 64.8, 62.2, 61.8, 42.8, 32.6, 31.2, 15.7. MS (CI) *m/z* (%) 1041.6 $[M + H]^+$ (100). Anal. Calcd for $C_{62}H_{80}N_4O_{10}$ (1041.34): C, 71.51; H, 7.74; N, 5.38. Found: C, 71.64; H, 7.84; N, 5.46.

5,11-Bis[*N*-((6-chloromethyl)pyridin-2-ylmethyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (12). Thionyl chloride (0.14 mL, 1.9 mmol) was added to a solution of calix[4]arene **11** (100 mg, 0.096 mmol) in CH_2Cl_2 (15 mL) and the mixture was stirred for 3 h. The solvent was removed under reduced pressure to give the bis-(2-chloromethylpyridine) **12** (102 mg, 0.096 mmol) in quantitative yield, and pure enough for further reactions. An analytical sample of **12** was dissolved in CH_2Cl_2, washed with a saturated $NaHCO_3$ solution, and dried over Na_2SO_4. Dichloromethane was removed in vacuo to give compound **12**. ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.67 (t, J = 7.4 Hz, 2H), 7.33 (d, J = 7.4 Hz, 2H), 7.25 (d, J = 7.4 Hz, 2H), 6.67 (s, 2H), 6.65 (s, 2H), 6.62 (d, J = 7.4 Hz, 2H), 6.47 (d, J = 7.4 Hz, 2H), 6.39 (t, J = 7.4 Hz, 2H), 4.65 (s, 4H), 4.47 (d, J = 13.3 Hz, 3H), 4.45 (d, J = 13.3 Hz, 1H), 4.12 (t, J = 5.8 Hz, 4H), 4.10 (t, J = 5.5 Hz, 4H), 3.85 (t, J = 5.5 Hz, 4H), 3.84 (t, J = 5.8 Hz, 4H), 3.54 (q, J = 7.0 Hz, 4H), 3.53 (q, J = 7.0 Hz, 4H), 3.39 (s, 4H), 3.27 (d, J = 12.9 Hz, 2H), 3.24 (d, J = 12.9 Hz, 2H), 3.14 (d, J = 12.9 Hz, 1H), 3.13 (d, J = 12.9 Hz, 2H), 3.08 (d, J = 13.3 Hz, 1H), 2.03 (s, 6H), 1.20 (t, J = 7.0 Hz, 12H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 160.2, 156.5, 156.0, 155.8, 137.7, 135.3, 135.1, 135.0, 132.2, 129.2, 129.0, 128.5, 128.4, 122.8, 122.5, 121.2, 73.6, 70.1, 66.7, 62.9, 62.0, 47.2, 42.9, 31.2, 15.7. MS (CI) *m/z* (%) 1077.7 $[M + H]^+$ (100). Anal. Calcd for $C_{62}H_{78}N_4O_8Cl_2$ (1079.23): C, 69.06; H, 7.29; N, 5.20. Found: C, 69.21; H, 7.16; N, 5.29.

5,11-Bis[*N*-((6-*N*,*N*-dimethylaminomethyl)pyridin-2-ylmethyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (3). A 33% dimethylamine solution in EtOH (10 mL) was added to a round-bottomed flask containing the calix[4]arene **12** (98 mg, 0.09 mmol). The solution was stirred overnight and then evaporated. The product was purified by column chromatography (neutral Al_2O_3, CH_2Cl_2/MeOH 99/1) to give **3** as a pale yellow oil (75 mg, 0.068 mmol). Yield 76%. ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.61 (t, J = 7.4 Hz, 2H), 7.25 (d, J = 7.4 Hz, 2H), 7.19 (d, J = 7.4 Hz, 2H), 6.66 (s, 4H), 6.60 (d, J = 7.4 Hz, 2H), 6.47 (d, J = 7.4 Hz, 2H), 6.38 (t, J = 7.4 Hz, 2H), 4.46 (d, J = 12.9 Hz, 3H), 4.45 (d, J = 13.3 Hz, 1H), 4.09 (t, J = 5.8 Hz, 4H), 4.08 (t, J = 5.4 Hz, 4H), 3.84 (t, J = 5.4 Hz, 4H), 3.83 (t, J = 5.8 Hz, 4H), 3.56 (s, 4H), 3.53 (q, J = 7.0 Hz, 8H), 3.43 (s, 4H), 3.25 (d, J = 12.9 Hz, 2H), 3.19 (d, J = 12.9 Hz, 2H), 3.13 (d, J = 13.3 Hz, 1H), 3.12 (d, J = 12.9 Hz, 2H), 3.07 (d, J = 12.9 Hz, 1H), 2.27 (s, 12H), 2.02 (s, 6H), 1.194 (t, J = 7.0 Hz, 6H), 1.192 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 159.8, 158.5, 156.6, 155.7, 137.0, 135.4, 135.0, 132.7, 129.2, 129.0, 128.6, 128.5, 122.5, 121.6, 121.4, 73.5, 70.1, 66.7, 66.3, 63.5, 62.1, 46.1, 42.8, 31.2, 30.1, 15.7. MS (CI) *m/z* (%) 1095.6 $[M + H]^+$ (100). Anal. Calcd for $C_{66}H_{90}N_6O_8$ (1095.48): C, 72.36; H, 8.28; N, 7.67. Found: C, 72.48; H, 8.19; N, 7.78.

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