Pergamon

Tetrahedron Letters 42 (2001) 7837–7840

TETRAHEDRON LETTERS

Synthesis and characterization of imidazole-substituted calix[4]arenes as simple enzyme-mimics with acyltransferase activity

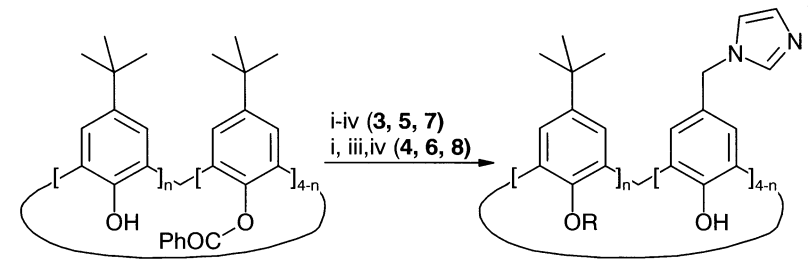
Günter Dospil and Jürgen Schatz*

Division of Organic Chemistry I, University of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany

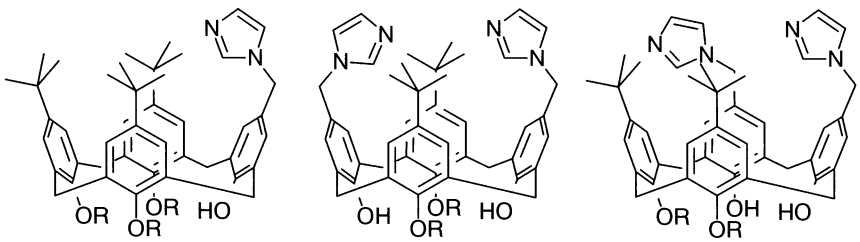
Received 16 August 2001; accepted 10 September 2001

Abstract—Calix[4]arenes which bear imidazole groups at different positions at the wide rim act as catalysts for enzyme-like release of *p*-nitrophenol from *p*-nitrophenyl esters in buffered solution.

To mimic the action and efficiency of natural enzymes was and still is a major challenge for supramolecular chemistry. Such enzyme mimics usually consist of a hydrophobic cavity and suitable catalytic substituents preorganized to enable catalysis of an organic transformation.[1–3] Cyclodextrins[4,5] or cyclophanes[6] bearing thiazolium[7] or imidazole moieties[8] as well as metal complexes as reactive sites have found widespread application as artificial enzymes. Both bond-breaking such as hydrolytic reactions[8–10] as well as bond-forming



2 **3–8**



3 (R = H; 70%)
4 (R = Bz; 72%)

5 (R = H; 90%)
6 (R = Bz; 85%)

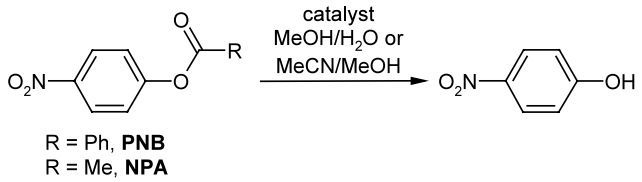
7 (R = H; 70%)
8 (R = Bz; 81%)

Scheme 1. Synthesis of calix[4]arenes **3**–**8**; (i) $AlCl_3$, toluene, rt, 69–98%; (ii) $EtOH/H_2O$, NaOH, 3 days, 80°C, 94–98%; (iii) $CH_2(NMe_2)_2$, THF/HOAc, 48 h, rt, 92–95%; (iv) 1. MeI, DMSO, 1 h, rt; 2. imidazole, 3 h, 80°C, 70–90% (R = H), 72–85% (R = Bz).

* Corresponding author. Tel.: +49-731-502-3153; fax: +49-731-502-2803; e-mail: juergen.schatz@chemie.uni-ulm.de

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01688-4

reactions (e.g. benzoin condensation reactions[7]) can be enhanced by macrocyclic catalysts. As expected, the observed rate accelerations depend strongly on the active site used for catalysis. Usually systems based on catalytic metal complexes exhibit higher rate enhancements compared to metal-free models. Cooperativity of functional groups is important for the catalytic properties of the supramolecular enzyme mimic. In particular imidazole functionalities were often used as an acid–base couple or nucleophile, which can enhance hydrolytic processes or Aldol-type condensation reactions.[8] Recently, calix[*n*]arenes[11] have also come into focus as platforms for enzyme mimics. Catalysis of ester hydrolysis by deshielding of the substrate[12] by complexation in the hydrophobic pocket, or barium complexes



Scheme 2. Hydrolysis of *p*-nitrophenyl benzoate (**PNB**) and acetate (**PNA**) catalysed by macrocycles **3**–**8**.

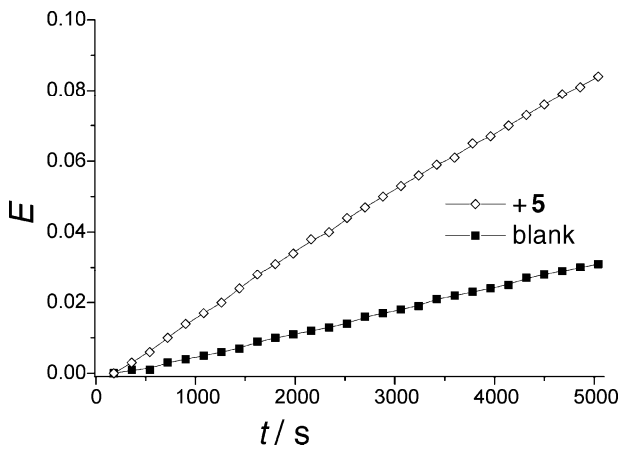


Figure 1. Initial release of *p*-nitrophenol from *p*-nitrophenyl acetate (3.98 mmol L^{-1}) in MeOH/MeCN (9:1) catalysed by calixarene **5** (■, 1.03 mmol L^{-1}) and without added macrocycle (◇) monitored by UV/vis spectroscopy ($\lambda = 410$ nm; $T = 28$°C).

Table 1. Initial rate (v_0) for the release of *p*-nitrophenol from *p*-nitrophenyl benzoate (**NPB**) in MeOH/aq. buffer pH 6.3 (70:30) with addition of various catalysts (c (**NPB**) $= 95.0 \times 10^{-3}$ mol L^{-1}; $T = 30.0$°C)

Cat.	c (cat)[a] (10^{-3} mol L^{-1})	c (**NPB**)/c (cat)	v_0 (10^{-10} mol L^{-1} s^{-1})
–	0.00	0.00	46.4
9	66.0	0.69	62.6
3	47.0	0.51	70.7
5	66.0	0.69	91.2

[a] Per imidazole unit.

of calixcrowns[13–15] is possible. Furthermore, very effective dinuclear metallo-phosphodiesterase models based on calix[4]arenes have been examined recently.[16–18] Here we want to present the synthesis and characterization of simple metal-free enzyme mimics with transacyltransferase activity based on calix[4]arenes (Scheme 1).

The synthesis of imidazole substituted calix[4]arenes **3**–**8** as possible acid–base catalysts starting from *p*-*tert*-butylcalix[4]arene (**1**) was possible in high overall yields by a five- or four-step procedure for the catalysts **3**, **5**, **7** and **4**, **6**, **8**, respectively. Selective acylation of **1** with benzoyl chloride by adapted procedures[19–22] gave rise to calix[4]arenes **2** bearing ester functionalities at positions 25,26,27-, 25,27- (distal), or 25, 26- (proximal) at the lower rim of the macrocycle.

Exploiting the difference in reactivity of the free phenol units and the phenol esters, *tert*-butyl groups could be removed selectively by treating the compounds with $AlCl_3$ in toluene as an acceptor. Now, the introduction of the imidazole moiety succeeded following the *p*-quinonemethide route[19] already established for the tetraimidazolylmethylcalix[4]arene.[23] This protocol yielded mono-imidazole **4** and distal substituted calix[4]arene **6** in the partial cone, and macrocycle **8** in an alternate conformation. As expected, hydrolysis of the esters was possible by heating the calixarenes for 3 days at 80°C with an excess of NaOH in $EtOH/H_2O$ solution.[20] Selective removal of the *tert*-butyl groups and introduction of the heterocycle as described before gave calixarenes **3**, **5**, and **7** in the cone conformation, as proven by ^{13}C absorptions of 32–33 ppm for the methylene bridges which are in agreement with the de Mendoza rule.[24]

The initial rate ($v_0 \equiv d(c_{PNB})/dt$) for the release of *p*-nitrophenol from *p*-nitrophenyl benzoate (**PNB**) was determined by UV/vis spectroscopy at λ_{max} 410 and 450 nm, respectively (errors $\leq 5\%$, cf. Scheme 2 and Fig. 1). Initial rates were measured to avoid interfering effects such as product inhibition at higher rates of product formation. In an aqueous reaction medium (Table 1) the initial release of **PNB** was slightly increased by the addition of methyl imidazole (**9**) used for comparison.

Attachment of one nucleophilic group onto the upper rim of the calix[4]arene resulted in a further increase of the initial reaction rate. The macrocyclic skeleton improved the hydrolysis by 13% compared with the non-macrocyclic catalyst and by 52% towards the blank hydrolysis. Addition of diimidazole calix[4]arene **5** bearing the catalytic groups in a distal arrangement at a similar effective concentration of imidazole units doubled the initial reaction rate indicating some kind of cooperativity of the catalytic sites. At the chosen pH 6.3 roughly 50% of the imidazole units are protonated[8] and therefore both imidazole groups can act as an acid–base catalysts for the ester hydrolysis in aqueous solution.

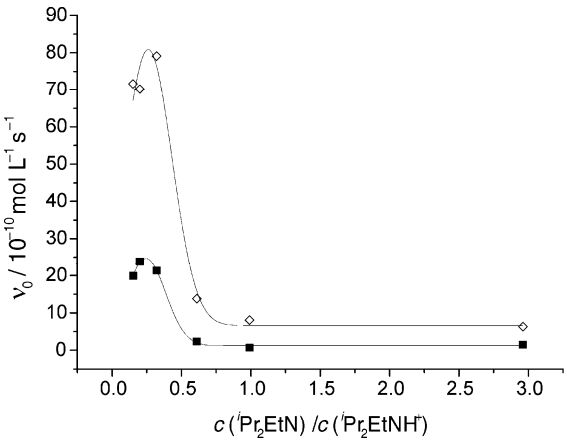


Figure 2. Initial rate (v_0) for the release of *p*-nitrophenol from *p*-nitrophenyl acetate in MeOH/MeCN (9:1) catalysed by calixarene **8** (◇) and without added macrocycle (■) as a function of added buffer (c (**8**) = 1.03×10^{-3} mol L^{-1}, c (**NPA**) = 13.2×10^{-3} mol L^{-1}; T = 28°C).

Table 2. Initial rate (v_0) for the release of *p*-nitrophenol from *p*-nitrophenyl acetate (NPA) in MeCN/MeOH (90:10) with addition of various catalysts (c (cat.) = 2.05×10^{-3} mol L^{-1} per imidazole unit, c (**NPA**) = 13.2×10^{-3} mol L^{-1}; buffer: c (iPr$_2$EtNH$^+$) = 20.3×10^{-3} mol L^{-1}, c (iPr$_2$EtN) = 63.8×10^{-3} mol L^{-1}; T = 28.0°C)

Cat.	v_0 (10^{-10} mol L^{-1} s^{-1})	$v_{0\ rel}$
–	21.5	≡1.0
1	19.6	0.9
9	38.1	1.8
3	29.9	1.4
4	40.8	1.9
5	55.3	2.6
6	68.7	3.2
7	51.2	2.4
8	84.8	3.9

Owing to the limited solubility especially of *p-tert*-butylcalix[4]arene (**1**) needed for comparison, the hydrolysis was also studied in an organic solvent. In this case, *p*-nitrophenyl acetate (**NPA**) was used as substrate. Fig. 2 summarizes the dependence of v_0 for the release of nitrophenyl from NPA from the added buffer.

Both with and without added catalyst, a bell-shaped profile typical for an acid–base catalysis could be observed. At the optimum buffer composition, calix[4]arene **8** increased the initial rate by a factor of 4. This acceleration compared to the reaction without added macrocycle was even higher when moving away from optimum conditions. In this case, the hydrolysis was accelerated about 9–11 times.

Addition of the parent *p-tert*-butylcalix[4]arene (**1**) bearing no catalytic groups did not affect the reaction rate (Table 2). A possible host–guest inclusion of the **NPA** in the cavity of the calixarene does neither support nor interfere with the hydrolytic process. Methyl imidazole (**9**) and mono-substituted calix[4]arenes **3** and **4** exhibited only a slight improvement. In the organic reaction medium used now, the solvent can compete efficiently with the substrate and the productive host–guest binding is less favoured as compared to the situation in aqueous solution.

Surprisingly, proximal and distal substituted calix[4]arenes **5**–**8** improved the hydrolysis in a similar way; the acylated species (**6**, **8**) exhibited higher catalytic ability than calixarenes **5** and **7**, bearing only hydroxyl groups at the lower rim. This result is in favour of a mechanism for the catalysis involving the imidazole groups of the calixarenes and not acylation at the lower rim as observed for a barium complex of a calixcrown.[15] This is also supported by time-dependent NMR studies which gave no indication for an acylation at the lower rim and accumulation of such an intermediate above a 3–5% level in the reaction mixture.

Assuming a nucleophilic assistance of the imidazole moieties of the calixarenes, semi-empirical (PM3) optimization[25] of such intermediates for the ester hydrolysis (Fig. 3) suggests that both calix[4]arene **5** and **7** is flexible enough to adopt a conformation suitable for the productive interaction with **NPA** and

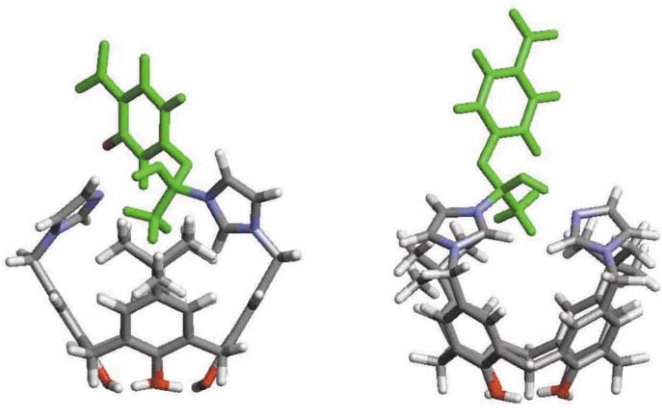


Figure 3. Molecular structures of the assumed intermediates for the ester hydrolysis of **NPA** catalysed by calix[4]arenes **5** (left, Erel = 0.0 kcal mol–1) and **7** (right, Erel = 0.6 kcal mol–1) obtained by semi-empirical geometry optimization (PM3).[25]

catalysis stems mainly from cooperative action of the functional groups attached at the wide rim of the calix[4]arene skeleton. In agreement with the kinetic data, the intermediate derived for the calixarene **5** is slightly (–0.6 kcal mol^{-1}) more stable than the corresponding intermediate calculated for calixarene **7**.

In summary, imidazole substituted calix[4]arenes are easily accessible acid–base catalysts with acyltransferase activity which show rate enhancements up to a factor of 10, as tested for the release of *p*-nitrophenol from *p*-nitrophenolates.

Supplementary material

Details about geometry optimization of the intermediates of calixarenes **5** and **7** with **NPA** are available as Supplementary Material.

Acknowledgements

This research was supported by the Fonds der chemischen Industrie and the Deutschen Forschungsgemeinschaft. Generous support by Professor Dr. G. Maas is gratefully acknowledged.

References

1. Murakami, Y.; Kikuchi, J.-i.; Hisaeda, Y.; Hayashida, O. *Chem. Rev*. **1996**, *96*, 721–758.
2. Dugas, H. *Bioorganic Chemistry*; Springer Verlag: New York, Berlin, Heidelberg, 1996.
3. Kirby, A. J. *Angew. Chem*. **1996**, *108*, 770–790; *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 707–724.
4. Easton, C. J.; Lincoln, S. F. *Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry*; Imperial College Press: London, 1999; Chapter 4; pp. 101–132.
5. Breslow, R.; Dong, S. D. *Chem. Rev*. **1998**, *98*, 1997–2011.
6. Diederich, F. *Cyclophanes*; The Royal Society Chemistry: Cambridge, 1994.
7. Ikeda, H.; Horimoto, Y.; Nakata, M.; Ueno, A. *Tetrahedron Lett*. **2000**, *41*, 6483–6487.
8. Breslow, R. *J. Mol. Catal*. **1994**, *91*, 161–174.
9. Tee, O. S.; Gadosy, T. A. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2307–2311.
10. Diederich, F.; Chao, I. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 335–338.
11. Mandolini, L.; Ungaro, R.; Eds. *Calixarenes in Action*; Imperial College Press: London, 2000.
12. Shinkai, S.; Shirahama, Y.; Tsubaki, T.; Manabe, O. *J. Am. Chem. Soc*. **1989**, *111*, 5477–5478.
13. Cacciapaglia, R.; Casnati, A.; Mandolini, L.; Ungaro, R. *J. Am. Chem. Soc*. **1992**, *114*, 10956–10958.
14. Cacciapaglia, R.; Mandolini, L. *Chem. Soc. Rev*. **1993**, 221–231.
15. Cacciapaglia, R.; Mandolini, L.; Arnecke, R.; Böhmer, V.; Vogt, W. *J. Chem. Soc., Perkin Trans. 2* **1998**, 419–423.
16. Molenveld, P.; Stikvoort, W. M. G.; Kooijman, H.; Spek, A. L.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem*. **1999**, *64*, 3896–3906.
17. Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem*. **1999**, *64*, 6337–6341.
18. Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev*. **2000**, *29*, 75–86.
19. Gutsche, D. C. *Calixarenes Revisited*; Royal Society of Chemistry: Cambridge, 1998.
20. Berthalon, S.; Regnouf-de-Vains, J.-B.; Lamartine, R. *Synth. Commun*. **1996**, *26*, 3103–3108.
21. Dalbavie, J.-O.; Regnouf-de-Vains, J.-B.; Lamartine, R.; Lecocq, S.; Perrin, M. *Eur. J. Inorg. Chem*. **2000**, 683–691.
22. Gutsche, D. C.; Kanamathareddy, S. *J. Org. Chem*. **1995**, *60*, 6070–6075.
23. Gutsche, D. C.; Nam, K. C. *J. Am. Chem. Soc*. **1988**, *110*, 6153–6162.
24. Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Sanchez, C. *J. Org. Chem*. **1991**, *56*, 3372–3376.
25. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, K. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. GAUSSIAN98, Revision A.7–A.9, Gaussian, Inc., Pittsburgh, PA, 1998.