Novel Calix[4]arene-Based Polymeric Catalysts as Acyltransferase Enzyme Mimics

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ABSTRACT: Monomeric and polymeric calix[4]arenes containing imidazole or triazole functionalities were synthesized. The enzymatic performance of these calix[4]arenes was evaluated by comparing the Michaelis–Menten kinetics for the hydrolysis reaction of *p*-nitrophenyl acetate. The results indicate that the newly synthesized both monomeric and polymeric calix[4]arenes could be used as acyltransferase mimics. The results of monomeric mimics showed that the calix[4]arenes containing imidazole groups are more efficient acyltransferase mimics than triazole-bonded ones. In the case of carboxylic acid-bonded mimic, it was also observed that H-bond contributed to increasing of catalytic yield. The catalytic studies of polymeric mimics demonstrated that the calixarene contents of polymers were very important for their hydrolytic activities. Moreover, the recyclability studies of some polymeric calix[4]arenes revealed that Merrifield resin was the best supporting material for the polymeric catalysts. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 1012–1019, 2012

Key words: calix[4]arenes; polymeric calixarenes; enzyme-mimic; Michaelis–Menten; catalyst; imidazole; triazole

INTRODUCTION

Natural enzymes are macromolecules. The macromolecular structures offer an ideal framework for the construction of versatile and robust catalytic sites. Strong and selective binding of a substrate is attained through a combination of hydrophobic effects and various specific substrate–enzyme interactions such as hydrogen bonding and salt bridges. The macromolecular structure can also create regions where catalyzed reactions occur in a less-than-fully aqueous medium. In native enzymes, however, many difficulties in practical use exist in their sensitive properties such as instability against high temperature, organic solvents, and serious pH conditions (extremely acidic or basic), etc. For this reason, there has been an increasing interest in examining synthetic polymers as enzyme mimics. Significant research efforts have been devoted for over decades to design polymers, which can mimic the catalytic activity of hydrolytic enzymes.[1] Enhancement in the activity of the mimic through the appropriate choice of functional monomers was also demonstrated.[2]

Many successful supramolecular catalysts or artificial enzymes, bearing thiazolium or imidazole moieties, as well as metal complexes as reactive sites, have been reported.[3–7] Recently, studies of calixarene-based enzyme models have attracted more attention; some models have been very successful.[8–11] Calixarene, which is assembled by the base-catalyzed condensation of *p*-alkylphenols with formaldehyde, provides an unique cavity capable of including various guest molecules. Calixarenes and their derivatives are attractive hosts for certain neutral and charged inorganic and organic species in solution, the solid state and in the gas phase as well. It is known that calixarene molecules exhibit a big sterical flexibility by comparing with cyclodextrins, and therefore, there is a possibility to using calixarenes in a wide field of applications. There are many studies dedicated to calixarenes and their derivatives, most of them directed toward the selective complexation of biological substrates. Remarkable progress has been achieved in the synthesis of calixarene derivatives used as enzyme mimetics, chemical sensors, adsorbents, extractants, etc.[12–19]

Schatz and Dospil pioneered the use of a simple enzyme-mimic model consisting of a calix[4]arene without a metal complex.[19] They showed that the attachment of imidazole groups to the calix[4]arene framework yielded an acid-base catalyst that was very effective in hydrolyzing activated esters such as *p*-nitrophenyl benzoate or *p*-nitrophenyl acetate. The authors became interested in using a triazole moiety instead of an imidazole. In general, monomeric derivatives of calixarenes have been utilized as enzyme-mimic models, whereas polymeric calixarenes have not been studied earlier. Hence, the

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authors have prepared polymeric derivatives of imidazole- and triazole-substituted calix[4]arenes to examine their mimetic behaviors. The authors have reported the synthesis and characterization of novel monomeric and polymeric calix[4]arene derivatives (Schemes 1 and 2) bearing imidazole or triazole groups and their use as enzyme mimics for *p*-nitrophenyl ester hydrolysis reactions.

MATERIALS AND METHODS

Instrument and reagent

Starting materials were obtained from commercial suppliers and used without further purification. Dry THF was distilled from the ketyl prepared from sodium and benzophenone. Acetonitrile was dried from calcium hydride and stored under N_2 over molecular sieves (4 Å). Dimethylsulfoxide (DMSO), K_2CO_3, EtOH, KOH, imidazole, 1-*H*-1,2,4-triazole, and methyl-1-*H*-1,2,4-triazole-3-carboxylate 3-glycidoxypropyl triethoxysilane, tetraethoxysilane, Merrifield resin and TentaGel resin, *p*-nitrophenyl acetate (NPA) were used as supplied from Merck or Sigma-Aldrich. Thin layer chromatography (TLC) was performed using silica gel on glass TLC plates (silica gel H, type 60, Merck). In general, solvents were dried by storing them over molecular sieves (Sigma-Aldrich; 4 Å, 8–12 mesh). All aqueous solutions were prepared with deionized water that had been passed through a Millipore Milli-Q Plus water purification system. Column chromatography separations were performed on Merck Silica gel-60 (230–400 mesh).

Melting points were determined on a Buchi B-540 apparatus. ^{1}H NMR spectra were obtained using a Varian 400 MHz spectrometer operating at 400 MHz at 295 K in $CDCl_3$. IR spectra were recorded on a Perkin Elmer 1605 FTIR spectrometer as KBr Pellets. UV–visible measurements were obtained on PG Instruments T80+ UV–visible recording spectrophotometer. Elemental analyses were performed using a Leco CHNS-932 analyzer. Thermograms were taken by a Seteram thermogravimetric analyzer. The sample weight was at the range of 3–15 mg. Analysis was performed from 21 to 900°C at a heating rate of 10°C/min in an argon atmosphere with a gas flow rate of 20 mL/min. An Orion 410A+ pH meter was used for the pH measurements.

Synthesis

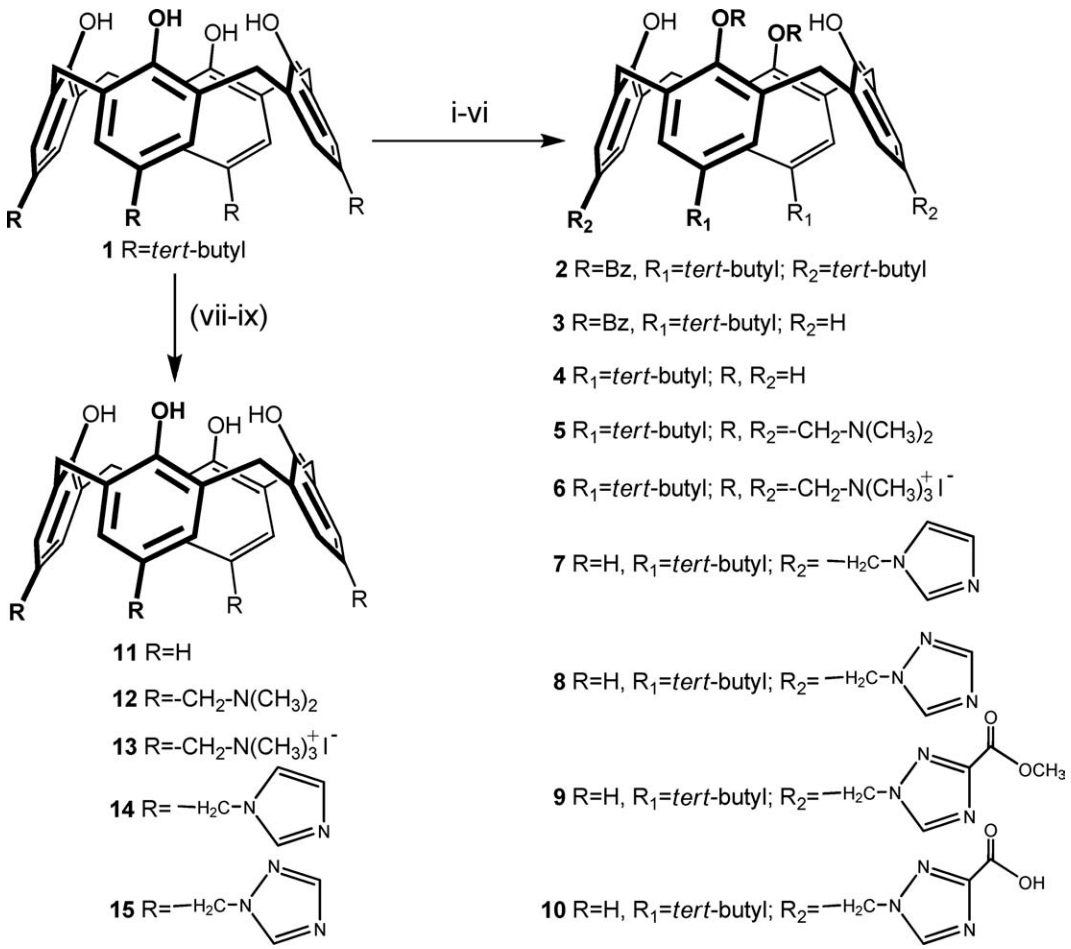
Compounds **1-7** and **11-14** were prepared according to known procedures,[20–26] and the other monomeric calix[4]arenes and their polymeric analogs studied in this work, as illustrated in Schemes 1 and 2, have been synthesized as follows.

General procedure for monomeric triazole-bonded calix[4]arenes (8, 9, and 15)

A (5 mmol) sample of quaternary salts with CH_3I of **5** and **12** (**6** and **13**) were treated with 56 mmol (for **8** and **9**) or 76 mmol (for **15**) 1-*H*-1,2,4-triazole-3-carboxylate. The reaction mixture was stirred for 3 h at room temperature, the solvents were removed under vacuum, and the residue was dissolved in 250 mL of water. The aqueous solution was extracted twice with 200 mL of ether and neutralized with 10% K_2CO_3 solution, and the precipitate that formed was removed by suction filtration. The product was dried under vacuum and then recrystallized from chloroform to give 3.3 g of **8** (83%), 3.2 g of **9** (75%), and 3.0 g of **15** (81%) as white needles. Compound **8**: Mp: 187°C; δ_H (400 MHz, $CDCl_3$, 295 K): 1.23 (18H, s, *t*-Bu), 3.49 (4H, d, *J* 12.4, $ArCH_2Ar$), 4.24 (4H, d, *J* 12.4, $ArCH_2Ar$), 5.10 (4H, s, $ArCH_2N$), 6.94 (4H, s, ArH), 7.03 (4H, s, ArH), 7.94 (2H, s, *tri*-H), 7.99 (2H, s, *tri*-H); δ_C (400 MHz, $CDCl_3$, 295 K): 152.05, 150.07, 148.57, 144.71, 143.62, 130.69, 129.97, 129.37, 128.90, 128.75, 128.32, 128.29, 125.99, 125.66, 52.29, 42.24, 42.10, 41.06, 40.78, 40.57, 40.46, 40.36, 40.15, 40.06, 39.94, 39.74, 39.53, 34.37, 34.32, 32.16, 32.09, 32.05, 31.86. Anal. Calcd for $C_{42}H_{46}N_6O_4$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.21; H, 6.68; N, 12.04. Compound **9**: Mp: 134°C; ν_{max}/cm^{-1} (KBr): 1732; δ_H (400 MHz, $CDCl_3$, 295 K): 1.15 (18H, s, *t*-Bu), 3.21–4.43 (18H, m, $ArCH_2Ar$, OCH_3, and $ArCH_2N$), 7.07 (4H, s, ArH), 7.32 (4H, s, ArH), 7.24 (2H, s, *tri*-H); δ_C (400 MHz, $CDCl_3$, 295 K): 144.29, 133.76, 130.16, 130.02, 128.45, 128.37, 126.14, 126.10, 79.93, 79.60, 79.27, 68.47, 52.28, 45.05, 42.36, 41.17, 40.89, 40.68, 40.47, 40.26, 40.06, 39.85, 39.64, 34.50, 34.45, 32.15, 32.09, 32.02, 31.98, 31.92, 31.85, 31.79, 31.77, 31.75. Anal. Calcd for $C_{46}H_{50}N_6O_8$: C, 67.80; H, 6.18; N, 10.31. Found: C, 67.87; H, 6.17; N, 10.30. Compound **15**: Mp > 220°C (decomp.); δ_H (400 MHz, $CDCl_3$, 295 K): 3.48 (4H, br, $ArCH_2Ar$), 4.20 (4H, br, $ArCH_2Ar$), 5.13 (8H, s, $ArCH_2N$), 6.78 (4H, br, ArOH), 6.97 (8H, s, ArH), 7.96 and 8.03 (8H, 2s, *tri*-H). Anal. Calcd for $C_{40}H_{36}N_{12}O_4$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.21; H, 4.89; N, 22.47.

Hydrolysis of compound 9 (10)

To a suspension of **9** (3.0 g) in 150 mL of EtOH was added 15% aqueous KOH (10 mL) solution, and the mixture was refluxed for 24 h. After evaporating the solvent under reduced pressure, the residue was mixed with water (50 mL) and acidified with diluted HCl. The precipitates were filtered off, washed with water, and EtOH subsequently, and dried to give **10** (2.8 g) in 95% yield. Mp: 283°C; ν_{max}/cm^{-1} (KBr): 1715; δ_H (400 MHz, $CDCl_3$, 295 K): 1.22 (18H, s, *t*-Bu), 3.50 (4H, d, *J* 13.1, $ArCH_2Ar$), 4.15–4.33 (8H, m, $ArCH_2Ar$



Scheme 1 Synthesis of monomeric calix[4]arenes **2-15**; (i) benzoyl chloride, K_2CO_3, CH_3CN, 91%; (ii) $AlCl_3$, toluene, 80%; (iii) $EtOH/H_2O$, NaOH, 91%; (iv) $CH_2(NMe_2)_2$, THF/HOAc, 82%; (v) 1. MeI, DMSO, 84%, 2. imidazole, 79%; 3. 1,2,4-triazole, 83%; 4. Methyl-1*H*-1,2,4-triazole-3-carboxylate, 75%; (vi) $EtOH/H_2O$, KOH, 95%. (vii) $AlCl_3$, toluene, 78%; (viii) $CH_2(NMe_2)_2$, THF/HOAc, 78%; (ix) 1. MeI, DMSO (88%), 2. imidazole (72%), 3. 1,2,4-triazole (81%).

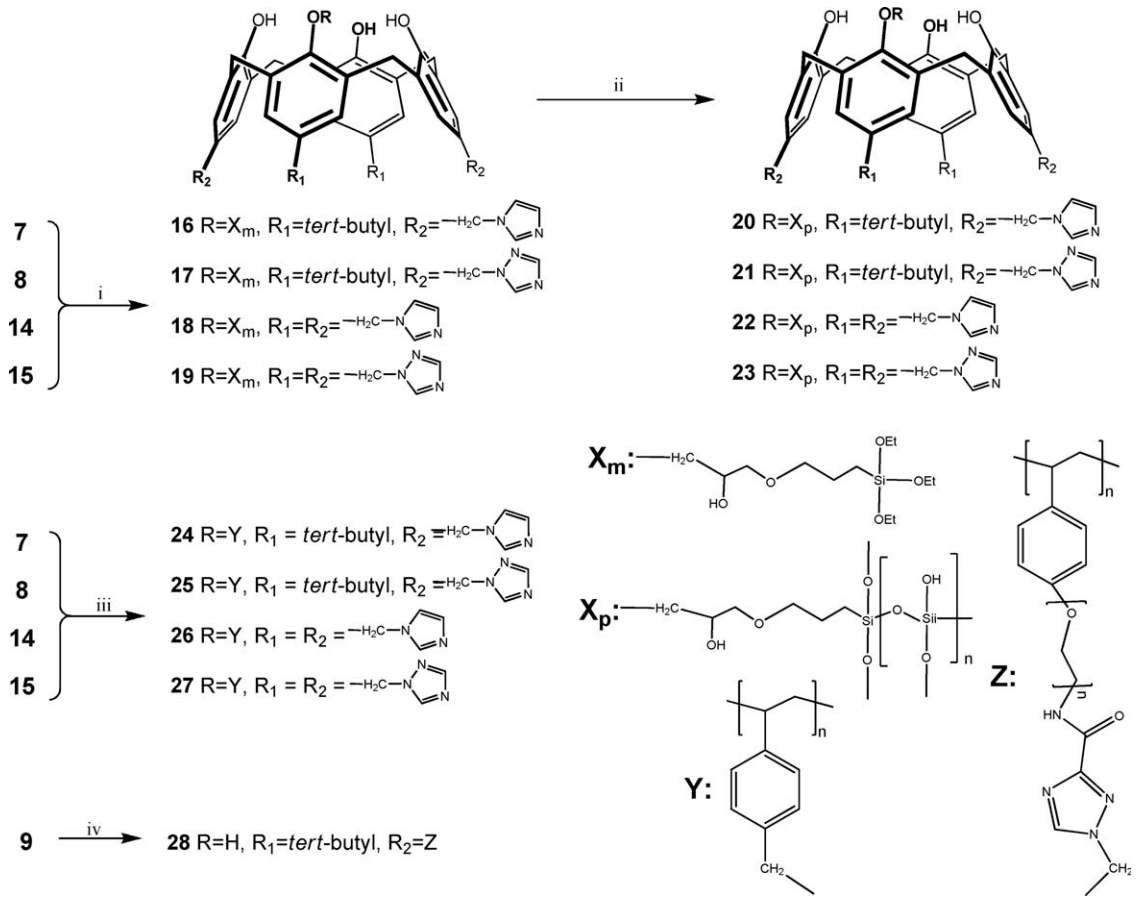
and $ArCH_2N$), 6.95 (4H, s, ArH), 6.99 (4H, s, ArH), 7.06 (2H, s, *tri*-H). Anal. Calcd for $C_{44}H_{46}N_6O_8$: C, 67.16; H, 5.89; N, 10.68. Found: C, 67.23; H, 5.96; N, 10.63.

Preparation of calix[4]arene-based polysiloxanes (20, 21, 22, and 23)

The following general procedure was adopted to transform **7**, **8**, **14,** and **15** into the corresponding calix[4]arene-based polysiloxanes (**20**, **21**, **22,** and **23**). To a solution of compound **7/8/14/15** (1.66 mmol) in dry toluene (20 mL), 3-glycidoxypropyl triethoxysilane (1.66 mmol) was added. After the mixture was stirred at room temperature for 10 h under a nitrogen atmosphere (it was obtained **16**, **17**, **18,** and **19** at this step, respectively), tetraethoxysilane (1.01 mmol), freshly distilled dry toluene (10 mL) and potassium hydroxide (0.1 mL; 1 *M*) were added to reaction mixture. The last mixture was stirred at room temperature for 5 h under nitrogen atmosphere, and then it was further refluxed under streaming dry nitrogen gas for 24 h. Finally, the solid product was filtered and washed in sequence with warm toluene, acetone, methanol, and water thrice. The product was dried at 120°C under vacuum for 3 h to give calix[4]arene-based polysiloxanes **20**, **21**, **22**, and **23**, respectively, and then it was kept in a desiccator until use. Polymer **20**: 0.9 g; IR (ν_{max}, cm^{-1}): 1098, 3345; Found: C, 47.74; H, 6.60; N, 0.89. Polymer **21**: 1.4 g; IR (ν_{max}, cm^{-1}): 1097, 3333; Found: C, 53.36; H, 6.38; N, 4.92. Polymer **22**: 1.2 g; IR (ν_{max}, cm^{-1}): 1075, 3345; Found: C, 51.37; H, 5.43; N, 3.93. Polymer **23**: 1.1 g; IR (ν_{max}, cm^{-1}): 1095, 3329; Found: C, 54.74; H, 5.86; N, 2.96.

Preparation of Merrifield-supported calix[4]arene polymers (24, 25, 26, and 27)

The following general procedure was adopted to transform **7**, **8**, **14,** and **15** to the corresponding Merrifield-supported calix[4]arene polymers (**24**, **25**, **26,** and **27**). A 2 mmol amount of **7/8/14/15** was dissolved in THF (50 mL). To this solution, 10 mmol NaH (60%) was added. In another flask, 2.0 g of



Scheme 2 Synthesis of polymeric calix[4]arenes **16-28**; (i), 3-glycidoxypropyl triethoxysilane, toluene; (ii) tetraethoxysilane, toluene, KOH; (iii) DMF/THF, NaI, NaH, Merrifield resin (0.8 mmol Cl/g resin); (iv) toluene/methanol, TentaGel resin.

Merrifield's resin (0.8 mmol Cl/1 g resin) was dissolved in DMF (50 mL). To this solution, NaI (2 mmol) was added. Both the mixtures were stirred for half an hour at room temperature separately. After that, the contents of both the flasks were mixed together and heated at 80°C in an inert atmosphere with continuous stirring for 48 h. The cooled contents were filtered through a bed of celite, and the filtrate and dichloromethane washings were combined and concentrated at reduced pressure. Crude product was washed several times with ether to ensure removal of unreacted calixarene derivative **7/8/14/15** and then taken in dichloromethane, neutralized with dilute HCl, washed with water, and dried in Na_2SO_4. The product was dried at 120°C, under vacuum, for 3 h to give Merrifield-supported calix[4]arene polymers **24**, **25**, **26,** and **27**, respectively, and kept in a desiccator before use. Polymer **24**: 2.2 g; IR (ν_{max}, cm^{-1}): 696, 3348; Found: C, 79.97; H, 7.24; N, 0.86. Polymer **25**: 2.1 g; IR (ν_{max}, cm^{-1}): 696, 3341; Found: C, 86.87; H, 7.53; N, 0.87. Polymer **26**: 2.1 g; IR (ν_{max}, cm^{-1}): 696, 3345; Found: C, 84.96; H, 7.17; N, 1.12. Polymer **27**: 2.2 g; IR (ν_{max}, cm^{-1}): 696, 3347; Found: C, 80.54; H, 7.09; N, 2.04.

Preparation of TentaGel-supported calix[4]arene polymer (28)

Compound **9** (0.76 g; 0.96 mmol) was dissolved in a 1 : 1 toluene/MeOH mixture (70 mL) and TentaGel resin (4.8 mmol) was added to solution. After the reaction mixture was refluxed for 38 h, TentaGel resin (4.8 mmol) was added. Then the reaction mixture was heated at reflux for 3 days. The reaction was monitored by TLC and after the substrate had been consumed, the solvent was evaporated under reduced pressure and the residue was triturated with MeOH to remove unreacted **9**. The product was dried at 120°C, under vacuum, for 3 h to give TentaGel-supported calix[4]arene polymer **28** (0.41 g) and kept in a desiccator before use. IR (ν_{max}, cm^{-1}): 3507, 1641. Found: C, 62.84; H, 7.82; N, 0.52.

Enzyme-mimic studies

Hydrolytic activities of both monomeric and polymeric calix[4]arenes were studied using *p*-nitrophenyl acetate (NPA) as a substrate. The hydrolysis

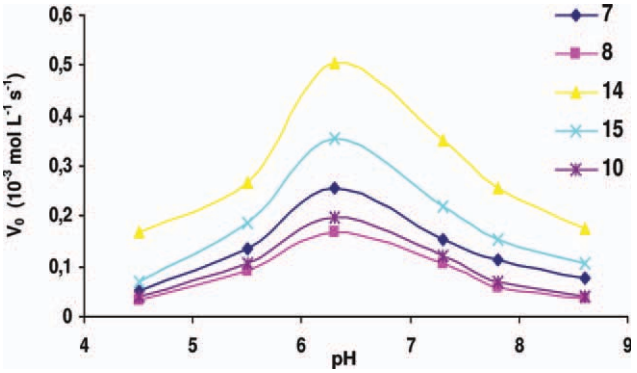


Figure 1 The pH profile of imidazole/triazole-bonded calix[4]arene catalysts in **NPA** hydrolysis. [Catalyst] = 66 × 10^{-5} mol L^{-1}; [NPA] = 95 × 10^{-5} mol L^{-1}; [Buffer] = 0.05 mol L^{-1}; at 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

reaction was monitored spectrophotometrically at 25°C; the *p*-nitrophenolate ion absorbance at 400 nm was followed. The initial rates were measured to avoid interfering effects such as product inhibition at higher rates of product formation. These studies were carried out in homogeneous media with monomeric calix[4]arenes. On the other hand, it was in heterogeneous media with polymeric calix[4]arenes due to their insoluble in methanol/water. The hydrolytic activities of both monomeric and polymeric calix[4]arenes were studied at pH 6.3 and used phosphate buffer solution.

RESULTS AND DISCUSSION

Synthesis of monomeric and polymeric calix[4]arenes

Various diimidazole-, tetraimidazole-, and triazole-substituted monomeric and polymeric calix[4]arene derivatives were synthesized following a multi-step procedure, using *p-tert*-butyl calix[4]arene (**1**)[12–26] as a starting compound in good yield. After removing two or four *tert*-butyl groups of **1** by adapted procedures,[12,13,20] monomeric imidazole- or triazole-substituted calix[4]arenes (**7**,[19] **8, 9, 10, 14**,[20] and **15**) were synthesized via *p*-quinonemethide procedure.[12,13] Attachments were confirmed with spectroscopic methods such as FTIR, ^{1}H NMR, and ^{13}C NMR.

The triazole-bonded calix[4]arene derivatives were firstly synthesized in this study. For this aim, the first, the reaction of quaternary ammonium derivative **6** with 1-*H*-1,2,4-triazole and methyl-1-*H*-1,2,4-triazole-3-carboxylate gave disubstituted calix[4]arene triazoles **8** and **9** in 83 and 75% yields in DMSO, respectively. Moreover, to see the catalytic effect of increasing of substitution, quaternary ammonium derivative **13** was converted into tetrasubstituted calix[4]arene triazole **15** in 81% yield, in DMSO. On the other hand, to comprehend the H-bond effect on catalysis, carboxylic acid derivative **10** was obtained in 95% yield by the hydrolysis of compound **9** with 15% aqueous KOH in ethanol.

From the ^{1}H NMR data, it was concluded that **8** and **15** adopted a *cone* conformation[12,13] (due to the presence of doublets at 3.49 and 4.24 ppm, J = 12.4 Hz, and 3.48 and 4.20 ppm, J = 12.6 Hz for $ArCH_2Ar$ protons, respectively) and the substitution of triazole ester groups onto **9** to be completed (due to the presence of multiplets at 3.21–4.43 ppm for $ArCH_2N$ and OCH_3 groups). Comparison of IR spectra of **9** and **10** confirmed the hydrolysis of **9** due to the appearing of carboxylic acid carbonyl band at 1715 cm^{-1}, while the disappearing of ester carbonyl band at 1732 cm^{-1}.

Polymeric calix[4]arenes such as polysiloxane-based (**20, 21, 22,** and **23**), Merrifield-supported (**24, 25, 26,** and **27**) and TentaGel-supported (**28**), were also prepared from their monomeric analogs according to previously described synthetic procedures.[19,20] Polysiloxane-based calix[4]arenes were synthesized at two steps. The monomeric derivatives were initially treated with 3-glycidoxypropyl triethoxysilane. Polymerization was performed using tetraethoxysilane and KOH and provided polysiloxane-based calix[4]arenes. Merrifield- and TentaGel-supported calix[4]arenes were prepared by immobilizing monomeric derivatives to Merrifield and TentaGel resins, respectively. All polymeric calix[4]arenes were obtained as solid powders that were insoluble in water and organic solvents such as MeOH, CH_2Cl_2, DMF, and acetone.

The products were characterized by FTIR, TGA, and elemental analysis. Thermogravimetric analyses of polymeric calix[4]arenes were performed. From these data, it is revealed that the prepared calix[4]arenes are strong polymers to heat although their preparation process requires slightly a long synthetic route leading to cost. According to the carbon, hydrogen, or nitrogen content of polymers **20-28**, it was concluded that the bonded calixarene amounts were found to be approximately 0.159, 0.586, 0.351, 0.376, 0.153, 0.104, 0.100, 0.203, and 0.162 mmol, respectively. In addition from the FTIR results given in experimental section, it was observed that all monomeric calix[4]arenes transformed to their polymeric analogs.

Catalytic studies with monomeric calix[4]arenes

First, the hydrolytic activity of monomeric calix[4]arenes as a function of the pH was studied. Bell-shaped profiles (Fig. 1) were exhibited with a maximum of k_{cat} at pH 6.3. This circumstance indicates that almost 50% of the imidazole or triazole units were protonated approximately at pH 6.3.[19] Therefore, imidazole

TABLE I
Michaelis–Menten Kinetic Data of Monomeric Imidazole/Triazole-Substituted Calix[4]arene Catalysts (7, 8, 10, 14, and 15) in NPA Hydrolysis[a]

Catalyst	k_{cat} (s^{-1})	K_M (10^{-5} mol L^{-1})	k_{cat}/K_M (10^3 mol L^{-1} s^{-1})	k_{cat}/k_{uncat}[b]	K_{TS} (10^{-5} mol L^{-1})
7	0.020	10.4	0.19	5.0	2.3
8	0.011	18.8	0.059	2.8	7.4
10	0.013	16.0	0.081	3.3	5.4
14	0.128	1.8	7.11	32	64.1
15	0.054	4.2	1.3	14	35.4

[a] [Catalyst] = 66×10^{-5} mol L^{-1}; [NPA] = 95×10^{-5} mol L^{-1}; [Buffer] = 0.05 mol L^{-1}; at pH = 6.3; at 25°C.
[b] $k_{uncat} = 0.0040$ s^{-1}.

or triazole groups can act as acid–base catalysts for the ester hydrolysis in MeOH/aqueous buffer. Moreover, the presence of a bell-shaped profiles suggests that calix[4]arenes bearing imidazole or triazole groups work as bifunctional acid–base catalysts.

Lineweaver–Burk plots were created from different substrate concentration experiments of monomeric calix[4]arenes as enzyme mimics. The Michaelis–Menten kinetic data and results obtained from these plots were summarized in Table I. In Michaelis–Menten kinetics, a lower value of K_M and a higher value of k_{cat} indicate a stronger substrate binding and effective catalysis. The results showed that the order of K_M values of mimics was **8** > **10** > **7** > **15** > **14**, whereas the order was reversed in the case of k_{cat}. As expected, the data clearly revealed that tetrasubstituted mimics **14** and **15** were more effective catalysts than the disubstituted mimics **7, 8,** and **10**. More catalytic sites could enhance the hydrolytic activity of the mimics. It was also concluded that imidazole-functionalized mimics **7** and **14** showed stronger catalytic effect than triazole-functionalized **8, 10,** and **15** on the hydrolysis of **PNA**. The results also indicated that the ditriazole-substituted calix[4]arene **10** was slightly more efficient as a catalyst than its analog **8**. The efficiency can be attributed to the H-bonding effect from the carboxyl group of **10** that enhances the hydrolytic power when compared with **8**. Moreover, both k_{cat}/K_M and k_{cat}/k_{uncat} values demonstrated that the order of the catalytic efficiencies of catalysts were **14** > **15** > **7** > **10** > **8**.

In addition, the authors have calculated the K_{TS}, which is the dissociation constant of the substrate–catalysis transition-state complex (Table I). The data indicate that 14 and 15 form more stabilized transition-state complexes than the other mimics. Thus, this result supports the hydrolytic activity values of the monomeric mimics described above. In this study, according to plausible mechanism, at pH 6.3, the H-bonding of protonated nitrogen atoms in imidazole or triazole moieties with substrate and the nucleophilic attack of nonprotonated nitrogen atoms induced the transformation of the substrate to a phenolate anion. The hydrolysis is completed by addition of the water molecules to the carbonyl group (Fig. 2).

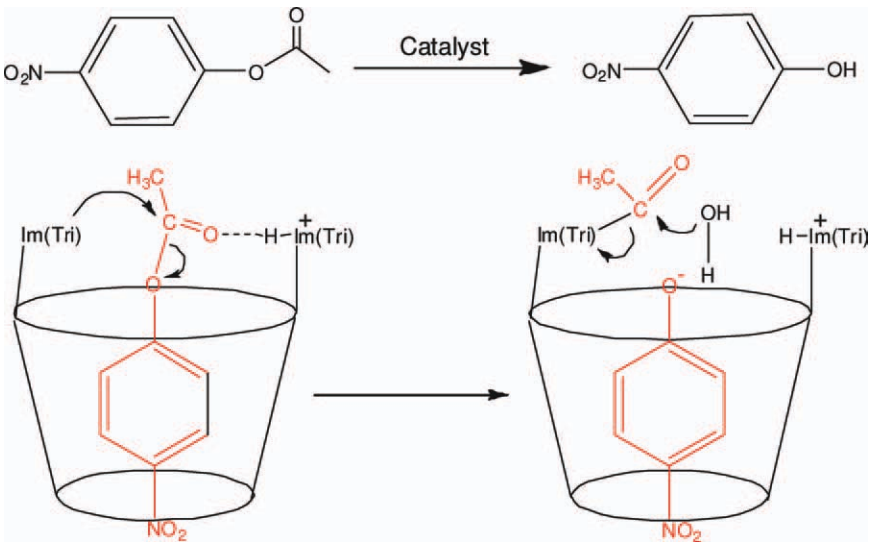


Figure 2 Plausible catalytic reaction mechanism of ester hydrolysis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

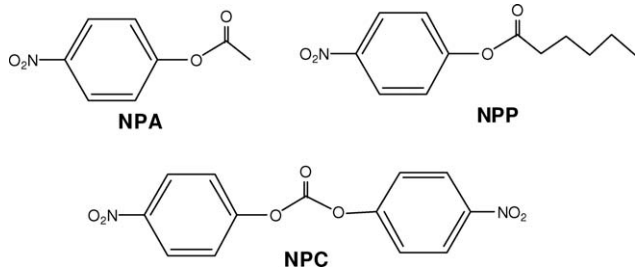


Figure 3 Activated ester substrates used this study.

Substrate effect

A series of reactions involving monomeric mimics with different substrates (Fig. 3) was also carried out. The results illustrated in Table II show that the rate acceleration (k_{cat}/k_{uncat}) order was **NPA** > **NPP** > **NPC** by all of the monomeric mimics. In the case of **NPP**, the decreasing acceleration of hydrolysis may be due to the steric hindrance and flexibility of the palmitate tail of **NPP**. In addition, the steric effect may be true for **NPC**, because **NPC** is almost twofold in size compared with **NPA**. In the case of **NPC**, more product inhibition could be contributing to the decrease in hydrolysis acceleration because twofold more products occur from **NPC** than that of other substrates.

Catalytic studies with polymeric calix[4]arenes

The authors have also examined enzyme-mimic efficiencies of various polymeric calix[4]arenes. The calculated k_{cat} and K_M values (Table III) for polysiloxane-based calix[4]arenes showed that the imidazole-substituted polysiloxanes **20** and **22** had higher efficiencies than triazole-substituted **21** and **23**. These results were in agreement with that of monomeric calix[4]arene catalysts. But this circumstance was reversed for the polymers **20** and **21**; the triazole-substituted polymer **21** was a better mimic than the imidazole-substituted polymer **20**. The higher catalytic activity may be due to the disubstituted polymer **21** having more calixarene content than **20**. In addition, tetrasubstituted polysiloxanes (**22** and **23**) were better polymeric mimics than their disubstituted analogs (**20** and **21**) although polymers **22** and **23** have less calixarene content than polymers **20** and **21**. As expected, this result proved that the catalytic group amounts of polymers were also effective on catalysis. In addition, k_{cat}/K_M values confirmed that the acceleration ratios of polysiloxane-based calix[4]arene catalysts were as explained above.

Merrifield- and TentaGel-supported calix[4]arene polymers (**24, 25, 26,** and **28**) were also used as enzyme mimics. The results (Table IV) were similar with that of polysiloxane polymers (**20, 21, 22,** and **23**). The higher catalytic efficiency of tetrasubstituted triazole polymer **27** compared with imidazole-substituted **26** may be explained by the calixarene content. Interestingly, disubstituted TentaGel-supported polymer **28** showed better catalytic efficiency than **24, 25,** and **26**. This circumstance may due to a combination of calixarene contents of polymers, H-bonding of –NH moieties and both the H-bonding and the nucleophilic contribution of unbonded amino groups on TentaGel parts of polymer **28**.

Recyclability studies with polymeric calix[4]arenes

The recovery and recyclability of the polymeric mimics were also examined. The activities of the catalysts were tested by the hydrolysis of **NPA**. After each reaction, the catalyst particles were filtered, washed, and dried. When the particles were reused, the recycled polysiloxane-based and TentaGel-supported calix[4]arene polymeric mimics (**20** and **28**, respectively) suffered a dramatic decrease in activity

TABLE II
Rate Accelerations (k_{cat}/k_{uncat}) Obtained Using Different Substrates[a]

Catalyst	NPA	NPP	NPC
7	4.6	3.2	1.4
8	2.5	1.9	1.3
10	2.9	2.1	1.3
14	28	6.4	1.5
15	12	4.7	1.4

[a] [Catalyst] = 66×10^{-5} mol L^{-1}; [Buffer] = 0.05 mol L^{-1};
[b] $k_{uncat} = 0.0040\ s^{-1}$, at pH = 6.3 and 25°C.

TABLE III
Michaelis–Menten Kinetic Data of Polysiloxane-based Calix[4]arene Catalysts (20–23) in NPA Hydrolysis[a]

Catalyst	20	21	22	23
k_{cat} (s^{-1})	1.38	1.62	3.23	2.15
K_M (10^{-5} mol L^{-1})	33.2	27.7	14.1	21.4
k_{cat}/K_M (10^3 mol L^{-1} s^{-1})	4.16	5.85	22.9	10.0

[a] Polymer amount = 10 mg; [NPA] = 95×10^{-5} mol L^{-1}; [Buffer] = 0.05 mol L^{-1}; pH = 6.3 at 25°C.

TABLE IV
Michaelis–Menten Kinetic Data of Merrifield- and TentaGel-supported Calix[4]arene Catalysts (24–28) in NPA Hydrolysis[a]

Catalyst	24	25	26	27	28
k_{cat} (s^{-1})	1.54	1.31	1.69	3.15	2.61
K_M (10^{-5} mol L^{-1})	29.9	33.7	27.0	15.6	18.8
k_{cat}/K_M (10^3 mol L^{-1} s^{-1})	5.15	3.89	6.26	20.2	13.9

[a] Polymer amount = 10 mg; [NPA] = 95×10^{-5} mol L^{-1}; [Buffer] = 0.05 mol L^{-1}; pH = 6.3 at 25°C.

after three cycles. The drop in activity was attributed to a cleavage of the –Si–O– and the $–OCH_2CH_2O–$ chains in the polymers. However, the recyclability of the Merrifield-supported catalyst (**24**) under the same conditions was successful. After three cycles, only a small decrease in activity (from 97 to 82%) was observed.

CONCLUSIONS

The authors have synthesized new calix[4]arene-based catalysts with imidazole/triazole moieties as acyltransferase enzyme mimics for the hydrolysis of *p*-nitrophenyl esters. From the Michaelis–Menten kinetic values of monomeric calix[4]arene derivatives, it was concluded that the calix[4]arenes containing imidazole groups more efficient acyltransferase mimics than triazole-bonded ones. Detailed studies such as pH and different substrate effects on catalysis were also performed with some selected monomeric calix[4]arene mimics. The pH results indicated that the suitable pH was 6.3 and both imidazole- and triazole-bonded calix[4]arenes were bifunctional acid–base catalysts. From the different substrate tests showed that the hydrolysis of *p*-nitrophenyl acetate was most efficient catalytic reaction with selected calix[4]arene mimics. Moreover, it was observed that H-bond contributed to increasing of catalytic yield. The enzyme-mimic studies of polymeric mimics revealed that the calixarene contents of all of the calix[4]arene-based polymers were very important for their hydrolytic activities. Further studies with polymeric calix[4]arenes have also shown that Merrifield resin is a superior support for these catalysts, as it can be recycled with a minimum loss of activity.

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