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Catalytic cyclophanes
VII. Esterase activity of a bisimidazolyl-cyclophane [1]

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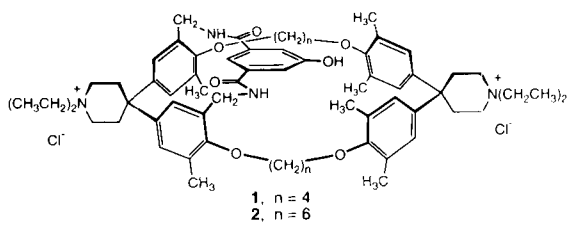
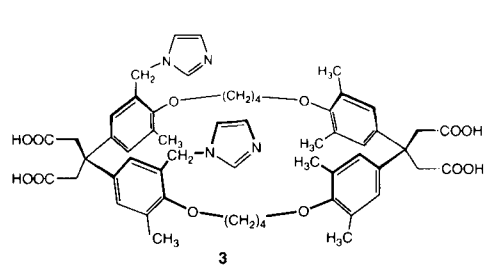
Abstract. We report the synthesis of the novel tetraoxa[6.1.6.1]paracyclophane **3** with two imidazole residues attached to the benzene rings of one of the two diphenylmethane spacers that shape the macrocyclic cavity. Four acetic acid residues diverge from the central carbon atoms of the two spacer units and ensure solubility of **3** in water and binary aqueous solvent mixtures. Cyclophane **3** forms stoichiometric inclusion complexes with nitronaphthyl acetates in aqueous phosphate buffers (pH 8) and catalyzes the hydrolysis of bound substrates under turnover conditions.

Introduction

The ability of enzymes to control reactions and reaction rates originates from the sophisticated employment of noncovalent interactions in complexation and transition state stabilization[2]. To design and synthesize artificial enzymes which also benefit from noncovalent interactions is one of the major challenges for bioorganic chemists[3]. In the development of macrocyclic esterases, we have earlier prepared cyclophanes **1** and **2** with phenolic nucleophiles located on top of the binding site[4]. Although compound **2** was rapidly acylated by bound 4-nitro-1-naphthyl acetate, the deacylation for regenerating **2** was slow and the catalytic turnover very modest in aqueous phosphate buffer at pH 8.



1, n = 4
2, n = 6



3

Imidazoles accelerate the hydrolysis of esters under general acid and general base catalysis[2]. To take advantage of these catalytic mechanisms and to avoid the formation of stable covalent intermediates by transacylation, we turned to the bisimidazolyltetraoxa[6.1.6.1]paracyclophane **3**. We report here on the synthesis of **3** and its turnover capability in the hydrolysis of bound activated substrates. The esterase activity of bisimidazolyl receptors has previously been shown in studies by *Breslow* et al.[5], *Murakami* et al.[6], and *Guthrie* et al.[7]. *Breslow* et al.[5] catalyzed regioselectively the cleavage of cyclic phenyl phosphate esters bound to β-cyclodextrins with two imidazolyl groups attached. *Murakami* et al.[6] used the Cu(II) complex of a bisimidazolyl[10.10]paracyclophane to catalyze the hydrolysis of *p*-nitrophenyl esters with long alkyl chains. The esterase prepared by *Guthrie* et al.[7] is a dimeric steroid with two pendant imidazoles and accelerates the hydrolysis of o-nitrophenyl esters of 3-arylpropionic acids.

Results and discussion

Synthesis of cyclophane **3**

The synthesis of the bisimidazolylcyclophane **3** is shown in Scheme 1. Several methods to functionalize macrocycle **7** at the two unsubstituted carbon atoms *ortho* to the ether linkages were attempted. Formylation with dichloromethyl methyl ether in the presence of Lewis acid ($AlCl_3$ or $TiCl_4$) yielded starting material or macro-ring cleavage products, depending on the reaction conditions. Acylation with acetyl chloride and $AlCl_3$ also yielded exclusively cleavage of the macrocycle frame. However, chloromethylation with HCHO/HCl in acetic acid provided the desired bis(chloromethyl) compound **8** in good yield (77%). The catalytic functions were attached by reacting **8** with imidazole in acetone in the presence of KOH. The water-soluble target compound **3** was obtained upon hydrolysis of the tetra-ester **9** with methanesulfonic acid in formic acid. Cyclophane **3** was obtained as a hygroscopic solid which was fully characterized as the tetrahydrated bis(methanesulfonate)salt. In the ^{1}H-NMR spectrum, the integration of the singlet at δ 2.70 indicates the presence of 2 equivalents of methanesulfonate per equivalent of cyclophane. This 2:1 ratio was also confirmed by elemental analysis data and indicates, along with the chemical

Table I Observed pseudo-first-order rate constants for 4-nitro-1-naphtholate formation during cleavage of 4-nitro-1-naphthyl acetate (**11**) *in the presence* (k_{obsd}) *and absence* (k_o) *of the synthetic esterases* **1–3** [a].

Cyclophane	[Cyclophane] ($mol \cdot l^{-1}$)	[11] ($mol \cdot l^{-1}$)	k_o (10^{-4} s^{-1})	k_{obsd} (10^{-4} s^{-1})
–	–	$2.0 \cdot 10^{-5}$	0.18	–
Non-turnover conditions				
1	$5.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-5}$		2.43
2	$5.0 \cdot 10^{-4}$	$2.0 \cdot 10^{-5}$		32.1
3	$3.6 \cdot 10^{-4}$	$2.0 \cdot 10^{-5}$		57.0
Turnover conditions [b]				
2	$1.0 \cdot 10^{-5}$	$1.0 \cdot 10^{-4}$		0.25
3	$1.6 \cdot 10^{-5}$	$1.0 \cdot 10^{-4}$		3.20

[a] Aqueous phosphate buffer, pH 8, containing 1% (v/v) Me_2SO, T 293.0K. Product formation was analyzed by monitoring the naphtholate absorption at λ 480 nm. [b] Cyclophane **1** is not a turnover catalyst (Ref. 4).

shifts of the imidazolyl protons, that each imidazole group in **3** is protonated by a methanesulfonic acid molecule. The chemical shift of the imidazole protons 2-H appears at δ 7.30 in the tetra-ester **9**, whereas in the imidazolium rings of **3** · $2MeSO_3H$, the corresponding resonances appear at δ 8.82.

Binding and catalytic studies with cyclophane **3**

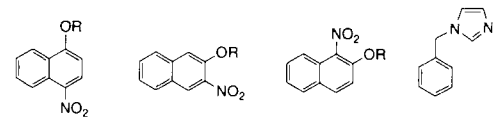
In studies with **3**, we were first interested in calibrating the esterase performance of the new macrocycle against that of two previously prepared phenolic derivatives **1** and **2**. Table I shows that, in phosphate buffer at pH 8, the new cyclophane accelerates the cleavage of 4-nitro-1-naphthyl acetate (**11**) more significantly than **1** and **2** under non-turnover conditions. More importantly, **3** is much more efficient under turnover conditions. However, the hydrolysis of **11** possibly does not take place in the cyclophane cavity, since **3**, unlike **1** and **2**, is not an efficient binder of the apolar 4-nitro-1-naphthol moiety of **11**. ^{1}H-NMR binding studies with **3** and 4-nitro-1-naphthol (**10**) at millimolar concentration ranges showed only insignificant complexation-induced changes in the chemical shifts of the guest protons.

^{1}H-NMR titrations showed, however, that other nitronaphthols with different substitution patterns form 1:1 inclusion complexes with **3**. For example, 3-nitro-2-naphthol (**12**) was bound at 293K by **3** in D_2O/Me_2SO-d_6 (60:40 v/v) with an association constant K_a 440 $l \cdot mol^{-1}$ (ΔG −3.5 kcal · mol^{-1}) and in D_2O/MeCN-d_3 (70:30 v/v) with K_a 90 $l \cdot mol^{-1}$ (ΔG −2.6 kcal · mol^{-1}). A weaker complex is formed by **3** and 1-nitro-2-naphthol (**14**) in D_2O/Me_2SO-d_6 (60:40 v/v) (K_a 100 $l \cdot mol^{-1}$; ΔG −2.7 kcal · mol^{-1}). The inclusion of these substrates into the cavity is clearly supported by the observed complexation-induced changes in chemical shift of the guest resonances. From the titration data in the D_2O/Me_2SO-d_6 mixture, we calculated the following upfield shifts $\Delta\delta_{sat}$ (ppm) for protons of bound **12**: 1.18 (1-H), 1.77 (4-H), 2.46 (5-H), and for protons of bound **14**: 1.68 (4-H), 1.63 (5-H).

Table II Observed pseudo-first-order rate constants for the formation of nitronaphtholate during the cleavage of nitronaphthyl acetates in the presence (k_{obsd}) *and absence* (k_o) *of catalysts* **3** *and* **16** [a].

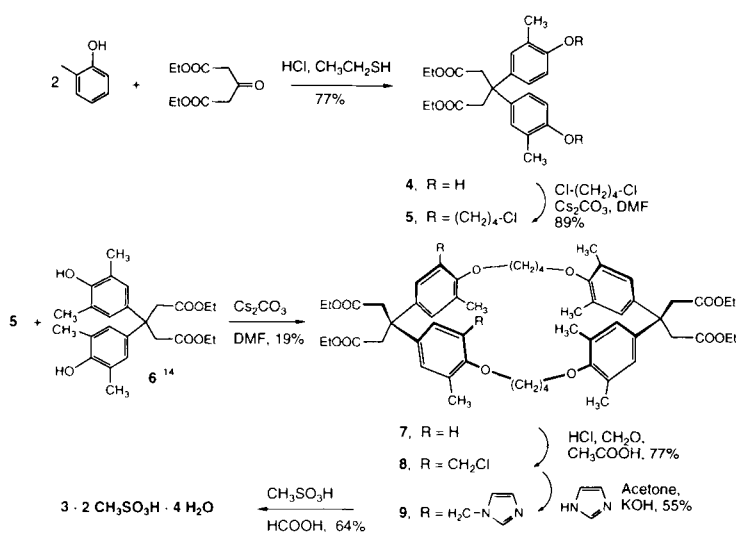
Substrate	Catalyst	[Substrate] ($mol \cdot l^{-1}$)	[catalyst] ($mol \cdot l^{-1}$)	k_o or k_{obsd} (10^{-5} s^{-1})	$(k_{obsd} - k_o)/k_o$
13	–	$5.0 \cdot 10^{-4}$	–	1.06	
13	**16**	$5.3 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$	2.17	1.1
13	**3**	$5.4 \cdot 10^{-4}$	$7.3 \cdot 10^{-5}$	11.2	9.6
15	–	$3.4 \cdot 10^{-4}$	–	6.36	
15	**16**	$5.5 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$	14.8	1.3
15	**3**	$5.4 \cdot 10^{-4}$	$7.3 \cdot 10^{-5}$	36.0	4.7

[a] Aqueous phosphate buffer solution, pH 8, containing 25% (v/v) Me_2SO, T 293.0 K. Product formation was analyzed by monitoring the naphtholate absorption at λ 480 nm.



When the hydrolysis of 3-nitro-2-naphthyl acetate (**13**) was studied under turnover conditions in aqueous phosphate buffer, pH 8, containing 25% (v/v) Me_2SO, **3** was found to be a better catalyst than 2.6 equivalents[8] of a comparison compound, *N*-benzylimidazole (**16**) (Table II). The pseudo-first-order rate constant for the hydrolysis of **13** (0.5 mM) catalyzed by **3** (0.073 mM. k_{obsd} $1.12 \cdot 10^{-4}$ s^{-1}) is one order of magnitude larger than those for the hydrolysis catalyzed by **16** (0.19 mM, k_{obsd} $2.17 \cdot 10^{-5}$ s^{-1}) or by the buffer (k_o $1.06 \cdot 10^{-5}$ s^{-1}).

When the nitro group in the substrate is on C-3 rather than C-1 of the naphthalene moiety, the binding strength decreases, as suggested by the results of the ^{1}H-NMR titrations executed with **12** and **14** (see above). Along with this decrease in binding strength, the $(k_{obsd} - k_o)/k_o$ ratio (4.7) for the hydrolysis of 1-nitro-2-naphthyl acetate **15** in the presence and absence of **3** also decreases, compared to the ratio measured for the hydrolysis of **13**



Scheme 1.

(9.6). The similar $(k_{obsd} - k_o)/k_o$ ratios (1.1 and 1.3) for the hydrolysis of **13** and **15** catalyzed by *N*-benzylimidazole (**16**) indicate that the enhancement of the hydrolysis by imidazolyl units is similar for both substrates. Therefore, the origin of a smaller $(k_{obsd} - k_o)/k_o$ ratio for **15** than for **13** (9.6 *vs.* 4.7) should come from the difference in binding to the catalyst. The observation of Michaelis–Menten kinetics obviously would have been a more rigorous demonstration of the relevance of substrate binding to catalysis[4]. However, the limited solubility of both substrates and catalyst **3** prevented a study of the initial rate as a function of either substrate or catalyst concentration at higher concentration ranges than those shown in Table II. Under the conditions of Table II, only a minor amount of substrate is complexed, as suggested by the binding studies discussed above. Solubility problems prevented assessment of the full catalytic advantage of **3** over **16**.

Conclusion

Our studies show that the new bisimidazolyl-cyclophane **3** is a better catalyst for the cleavage of nitronaphthyl esters than the phenolic derivatives **1** and **2** under both non-turnover and turnover conditions. Unfortunately, important studies such as the demonstration of Michaelis–Menten kinetics and the investigation of the pH-rate profile for the bisimidazole catalysis[5] could not be executed due to the limited solubility of both **3** and the substrates that bind in its cavity in aqueous buffers and binary aqueous buffer mixtures. Despite these limitations, the studies with **3** have clearly underlined the catalytic potential of bisimidazolyl-cyclophanes in the hydrolysis of esters under turnover conditions, and the development of a new generation of such catalysts with improved solubility and enhanced binding and catalytic properties is now under way.

Experimental

Commercial chemicals were used directly unless otherwise noted. Dimethylformamide (DMF) was dried over basic alumina for 3 days before use. Dichloromethane (CH_2Cl_2) was dried over neutral alumina. Column chromatography was performed on silica gel (SiO_2, Kieselgel 60, 70-230 mesh) from E. Merck. Analytical thin-layer chromatography (TLC) was conducted on E. Merck silica gel 60 F-254 precoated plates.

Melting points were measured on a Büchi (Dr. Tottoli) apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on Bruker AF 200 or AM 500 spectrometers. Chemical-shift values are reported relative to tetramethylsilane (TMS), either as an internal standard, or by using the known chemical shift values of solvent peaks relative to TMS as a reference. IR spectra were recorded on a Perkin–Elmer 1600 Series FT-IR instrument. Elemental analyses were performed at Spang Microanalytical Laboratory (Eagle Harbour, Michigan). Electron-impact (EI) mass spectra (70 eV) were determined on either an AEI Model MS 902 or Model MS-9 mass spectrometer. Fast-atom-bombardment (FAB) mass spectra (matrix: *m*-nitrobenzyl alcohol) were recorded on a VG ZAB-SE or AEI Model MS-9 instrument. In the synthesis section below, *m/z* values followed by relative intensities are given. For recrystallizations, compounds were dissolved completely in one solvent. A second solvent in which the compound was insoluble was added dropwise until the mixture became cloudy. This solution was then heated again to clarity. The saturated solution was cooled to 0–5°C in an ice–salt mixture, and a spatula was used to scratch the wall of the flask occasionally until the crystallization process started. The names of new compounds were proposed by Chemical Abstracts Services (CAS), Columbus, Ohio.

Kinetic and binding studies

A Sartorius 4503 microbalance and Eppendorf or Gilson micropipettes were used for sample preparation in binding and catalytic studies. Substrates for these studies prepared according to published procedures are 4-nitro-1-naphthol (**10**)[9], 4-nitro-1-naphthyl acetate (**11**)[9], 3-nitro-2-naphthol (**12**)[10], 3-nitro-2-nitronaphthyl acetate (**13**)[10], 1-nitro-2-naphthol (**14**)[11], 1-nitro-2-naphthyl acetate (**15**)[11], and *N*-benzylimidazole (**16**)[12]. All ^{1}H-NMR titration data was obtained on a Bruker AM 500 spectrometer at fast host/guest exchange conditions. The data were evaluated using a non-linear least-squares curve-fitting program[13]. For solubility reasons, titrations were performed in non-buffered solvent mixtures: the use of phosphate buffer as in the catalytic studies led to precipitate formation at the higher concentration ranges needed for meaningful binding studies.

The pH-8 phosphate buffer (*I* 0.19) used in kinetic studies was prepared by mixing 5.5 ml of 0.066M KH_2PO_4 and 94.5 ml of 0.066M Na_2HPO_4, and was stored for a maximum of 7 days. The UV/Vis absorption of the reaction products, such as 3-nitro-2-naphtholate, was monitored at λ 480 nm with a Varian Cary 2300 spectrophotometer. The absorption data as a function of time was evaluated with a non-linear least-squares curve-fitting program to give pseudo-first-order product formation rate constants k_o or k_{obsd} (s^{-1}) and calculated UV/Vis absorptions at 100% reaction completion (A_∞). For complete and detailed operating procedures in the kinetic studies, see the Experimental Section in Ref. 4.

3,3-Bis(4-hydroxy-3-methylphenyl)pentanedioic acid diethyl ester (**4**)

In an ice-water bath, 30.3 g (0.15 mol) of diethyl 3-oxopentanedioate and 64.9 g (0.60 mol) of 2-methylphenol were mixed with 8 ml of ethanethiol. Gaseous HCl, dried with concd. H_2SO_4, was bubbled through the reaction mixture for 1 h at 0–10°C and for 4 h at 20°C. After the reaction mixture was left standing overnight, gaseous HCl was bubbled through the mixture for one further hour. The product slowly started crystallizing out and was isolated by filtration and washed with hexane/ethyl-acetate (2:1). Drying *in vacuo* gave 46.1 g (77%) of **4**, which was recrystallized for elemental analysis from acetone/hexane: m.p. 173.0–173.5°C. IR (KBr): ν (O-H) 3382, (C=O) 1729 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.99 (t, *J* 7.0 Hz, 6 H), 2.17 (s, 6 H), 3.44 (s, 4 H), 3.88 (q, *J* 7.0 Hz, 4 H), 4.74 (s, 2 H), 6.62 (d, *J* 7.0 Hz, 2 H), 6.83 (dd, *J* 8.4 and 2.3 Hz, 2 H), 6.87 (d, *J* 2.3 Hz, 2 H). MS 400 (M^+, 24), 313 (100). Anal. calcd. for $C_{23}H_{28}O_6$ (400.48): C 68.98, H 7.05; found: C 68.92, H 7.18%.

3,3-Bis[4-(4-chlorobutoxy)-3-methylphenyl] pentanedioic acid diethyl ester (**5**)

A total of 127 g (1 mol) of 1,4-dichlorobutane was added slowly at 80°C to a mixture of 20.0 g (0.05 mol) of **4**, 32.6 g (0.10 mol) of Cs_2CO_3, and 27.6 g (0.20 mol) of K_2CO_3 in 250 ml of dried DMF. The reaction mixture was stirred for 24 h and then cooled to 20°C. The inorganic salts were removed by filtration through celite, and the solution was evaporated to dryness. The residual oil was chromatographed on SiO_2 (hexane/ethyl-acetate 7:3 then 6:4) to yield 25.7 g (89%) of **5** as a pale-yellow oil. IR (neat): ν(C=O) 1734 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.99 (t, *J* 7.1 Hz, 6 H), 1.9–2.0 (m, 8 H), 2.13 (s, 6 H), 3.45 (s, 4 H), 3.63 (t, *J* 6.3 Hz, 4 H), 3.87 (q, *J* 7.1 Hz, 4 H), 3.96 (t, *J* 5.6 Hz, 4 H), 6.66 (d, *J* 8.5 Hz, 2 H), 6.87 (d, *J* 2.0 Hz, 2 H), 6.91 (dd, *J* 8.5 and 2.0 Hz, 2 H). HRMS (M^+, $C_{31}H_{42}O_6Cl_2$) calcd.: 580.2358; obsd.: 580.2318.

5,14,20,29,32,37-Hexamethyl-7,12,22,27-tetraoxapentacyclo [26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,-37-dodecaene-2,2,17,17-tetraacetic acid tetraethyl ester (**7**)

A mixture of 11.8 g (0.027 mol) of 3,3-bis(4-hydroxy-3,5-dimethylphenyl)pentanedioic acid diethyl ester (**6**)[14], 16.0 g (0.027 mol) of **5**, 19.7 g (0.06 mol) of Cs_2CO_3, and 16.6 g (0.12 mol) of K_2CO_3 was stirred in 3 l of dried DMF at 90°C for 1 day. After the reaction mixture was cooled to 20°C, the inorganic salts were removed by filtration over celite and the solution was evaporated to dryness. The crude product was chromatographed (SiO_2, hexane/ethyl-acetate 4:1) to yield 4.78 g (19%) of **7** as a white solid; m.p. 142.0–143.0°C (hexane). IR (KBr): ν (C=O) 1730 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (t, *J* 7.1 Hz, 6 H), 0.99 (t, *J* 7.1 Hz, 6 H), 1.9–2.0 (m, 8 H), 2.01 (s, 6 H), 2.11 (s, 12 H), 3.42 (s, 4 H), 3.46 (s, 4 H), 3.8–3.9 (m, 12 H), 4.02 (t, *J* 7.1 Hz, 4 H), 6.65 (d, *J* 8.6 Hz, 2 H), 6.65 (s, 4 H), 6.79 (d, *J* 2.3 Hz, 2 H), 6.85 (dd, *J* 8.6 and 2.3 Hz, 2 H). FAB MS 936 (M^+). Anal. calcd. for $C_{56}H_{72}O_{12}$ (937.19): C 71.77, H 7.74; found: C 71.98, H 7.89%.

5,29-Bis(chloromethyl)-14,20,32,33,36,37-hexamethyl-7,12,22,27-tetra-oxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetraacetic acid tetraethyl ester (**8**)

Acetic acid (40 ml) followed by 5 ml of concd. HCl was added to an ice-cold mixture of 3.4 g (0.004 mol) of **7** and 19 ml (0.25 mol) of a 37% (w/w) aqueous solution of formaldehyde. Gaseous HCl was then bubbled through the stirred solution for 2 h at 0–5°C and for another 2 h at 20°C. The crude product was isolated by filtration as a white solid, which was dissolved in $CHCl_3$ and washed with saturated NaCl until washing liquors showed neutral pH. The organic solution was dried over $MgSO_4$; evaporation then yielded 2.9 g (77%) of crude solid **8**, which was used without further purification in the subsequent conversion to **9**. IR (KBr): ν (C=O) 1722 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12 H), 1.95–2.0 (m, 8 H), 2.05–2.15 (m, 18 H), 3.44 (s, 4 H), 3.47 (s, 4 H), 3.8–3.9 (m, 12 H), 3.95 (m, 4 H), 4.49 (s, 4 H), 6.66 (s, 4 H), 6.81 (s, 2 H), 6.93 (s, 2 H). $C_{58}H_{74}Cl_2O_{12}$ (MW 1034.14); FAB MS 1032 (M^+).

5,29-Bis(1H-imidazol-1-ylmethyl)-14,20,32,33,36,37-hexamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetraacetic acid tetraethyl ester (**9**)

Powdered KOH (1.5 g, 0.027 mol) was added to a stirred solution of 30 ml of acetone containing 1.9 g (0.028 mol) of imidazole. After 20 min, 2.9 g (0.0028 mol) of dichloride **8** were added, and the mixture was stirred for 1 day at 20°C. Benzene was added, and the organic solution was washed with saturated NaCl until washing liquors showed neutral pH, and it was then dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on SiO_2 with $CHCl_3$ followed by $CHCl_3/CH_3OH$ (97:3); 1.7 g (55%) of 9; m.p. 170.5–171°C (dec.)·IR (KBr): ν (C=O) 1728 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.9–1.0 (2 t, *J* 7.1 Hz, 12 H), 1.9–2.0 (m, 8 H), 2.10 (s, 12 H), 2.18 (s, 6 H), 3.36 (s, 4 H), 3.48 (s, 4 H), 3.71 (t, *J* 6.2 Hz, 4 H), 3.76 (t, *J* 6.0 Hz, 4 H), 3.83 (q, *J* 7.1 Hz, 4 H), 3.87 (q, *J* 7.1 Hz, 4 H), 4.96 (s, 4 H), 6.52 (s, 2 H), 6.66 (s, 2 H), 6.71 (s, 4 H), 6.83 (bs, 4 H), 7.30 (s, 2 H). FAB MS 1097.56 (MH^+, 100). Anal. calcd. for $C_{64}H_{80}O_{12}N_4 \cdot 1H_2O$ (1115.39): C 68.92, H 7.41, N 5.02; found: C 69.16, H 7.47, N 4.81%.

5,29-Bis(1H-imidazol-1-ylmethyl)-14,20,32,33,36,37-hexamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetraacetic acid (**3**)

Methanesulfonic acid (0.74 g, 7.75 mmol) was added to 60 ml of 88% aqueous formic acid containing 1.7 g (1.55 mmol) of **8**. The reaction mixture was stirred under reflux for 1 day. The light-brown solution was cooled to 20°C, and ether was added. A white solid precipitated within two days from the solution, while the flask wall was occasionally scratched. Recrystallization from methanol/ether gave 1.24 g (64%) of **3** as the tetrahydrated bis(methanesulfonate) salt **3**·$2CH_3SO_3H \cdot 4H_2O$; m.p. 157.0–158.0°C. IR (KBr): ν (O-H) 2950, (C=O) 1722 cm^{-1}. ^{1}H NMR (200 MHz, MeOH-d_4): δ 1.85–2.05 (b, 8 H), 2.11 (s, 18 H), 2.69 (s, 6 H, $CH_3SO_3^-$), 3.51 (s, 8 H), 3.81 (m, 4 H), 3.92 (m, 4 H), 5.36 (s, 4 H), 6.78 (s, 4 H), 6.98 (bs, 2 H), 7.20 (bs, 2 H), 7.39 (bs, 4 H), 8.82 (s, 2 H); FAB MS 985 (MH^+, 100). Anal. calcd. for $C_{56}H_{64}O_{12}N_4 \cdot 2CH_3SO_3H \cdot 4H_2O$ (1247.40): C 55.85, H 6.30, N 4.49; found: C 55.69, H 6.37, N 4.58%.

Acknowledgement

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