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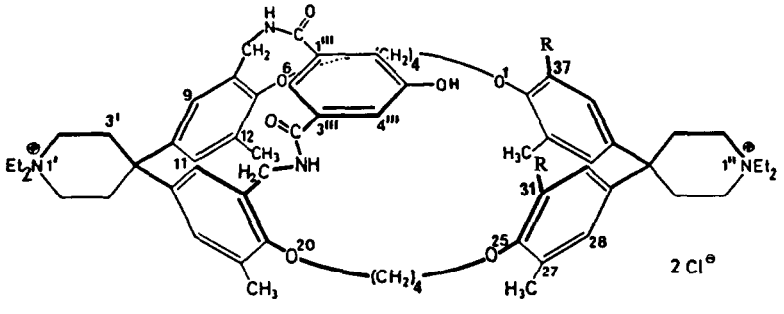
PROGRESS TOWARDS ARTIFICIAL HYDROLASES: SYNTHESIS AND BINDING PROPERTIES OF A WATER-SOLUBLE CYCLOPHANE HOST WITH A PHENOL CAP

Gregor Schürmann[1a] and François Diederich [1b] *
Department of Chemistry and Biochemistry, University of California,
Los Angeles, California 90024 and Max-Planck-Institute for Medical
Research, Abteilung Organische Chemie, D-6900 Heidelberg, FRG.

Abstract: The synthesis of the functionalized macrobicyclic host **1** on the way to the water-soluble artificial esterase **2** is presented. A phenolic nucleophile is properly oriented atop the binding cavity of **1** which forms strong complexes with aromatic guests in aqueous solutions.

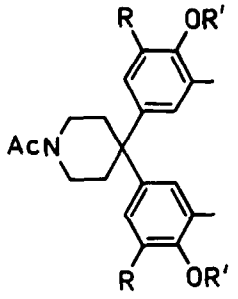
In the past years we have prepared and studied cyclophane-type host structures with apolar binding cavities that complex efficiently organic guest molecules both in the solid state as well as in aqueous and organic solutions.[2] We now wish to report on the synthesis and the binding properties of the functionalized macrobicyclic host **1**, which represents the first target molecule in our research directed towards the synthesis of artificial water-soluble esterases. Despite many efforts during the past decades[3] there is still no fully synthetic molecular system known that acts as an efficient turnover esterase in aqueous solution. Host **1** has been designed to possess an efficient apolar binding cavity in aqueous solution and to have a phenolic nucleophile located atop the cavity in a proper orientation for the transacylation reaction with complexed ester-guests. In compound **2** we intend to introduce two additional imidazoles to promote by general acid-base catalysis especially the second step of the covalently catalyzed ester hydrolysis and to achieve turnover catalysis.[4] Macrobicycle **1** with a protected phenolic residue is the direct precursor in our projected synthesis of the potential catalyst **2**.[5]

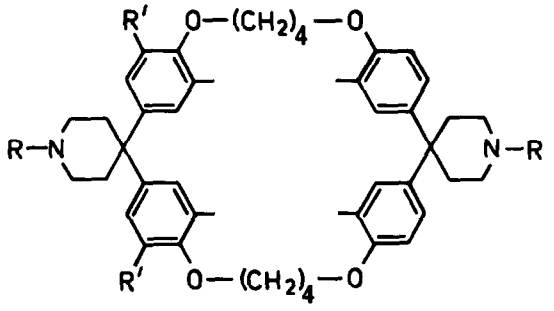


1 R = H

2 R = CH_2–N⟨imidazole⟩

For the synthesis of **1**, the diphenol **3** was dibrominated to give **4** (Br_2 / HOAc; 98%).[6] The reaction of **4** with 1,4-dichlorobutane led to **5** [6] (KOH / n-BuOH; 86%) which was cyclized with the diphenol **3** to give the macromonocycle **6** [6](DMF / Cs_2CO_3; 19%). Reaction of **6** with copper (I) cyanide afforded the dinitrile **7** [6] (DMF, reflux; 83%) which was reduced with BH_3·THF to give the macrocyclic tetramine **8** [6] (94%).

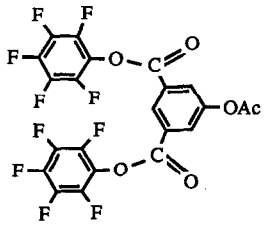




	R	R'
3	H	H
4	Br	H
5	Br	$(CH_2)_4$-Cl

	R	R'
6	Ac	Br
7	Ac	CN
8	Et	CH_2NH_2
9	Et	$CH_2NHCOCH_3$

Cyclization of **8** with the bis(pentafluorophenyl ester) **10** led to the macrobicycle **11** (CH_2Cl_2, high dilution). The acetylated phenol **11** could not be isolated since the acetoxy residue hydrolized rapidly upon work up: chromatography of the crude product of the cyclization reaction on silica from chloroform/ ethanol (10:1; saturated with NH_3) afforded the macrobicyclic phenol **12** [6] in 20% yield. Quaternization of **12** in pure ethyl iodide followed by ion exchange chromatography (Cl^-) led to the hygroscopic target compound **1** (91%) which crystallized out of methanol / ether and which was analyzed as a dihydrate.[7]



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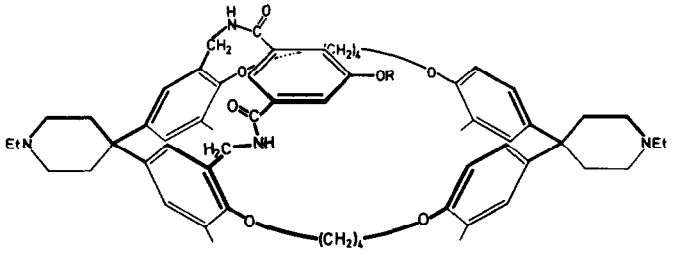

11 R = CH_3CO

12 R = H

Before continuing the synthetic transformations leading from **1** to **2** we wished to elucidate if the phenolic nucleophile is properly oriented for attacking a complexed ester-guest. When designing **1** and **2**, examinations of CPK molecular models indicated that a location of the phenol ring atop the binding cavity would be sterically more favorable than a location of the ring in the opposite direction above the piperidinium unit. The models also indicated that the location of the phenol ring in a direction about perpendicular to the mean plane of the tetraoxaparacyclophane is very unfavorable for steric reasons. Only in a position atop the cavity, according to the models, can the two amide linkages take a strain-free transoid conformation in planar conjugation with the phenol ring. By ^{1}H NMR,we obtained strong experimental support for an exclusive location of the nucleophile atop the binding cavity of **1**. One highly resolved AMX-system is obtained for the two Ar-C*H*$_2$ - N*H* -CO- linkages of **1** and of **12** at 80- 500 MHz (T = 303 - 393 K; Me_2SO-d_6), which indicates that the phenolic residue takes preferably one unique orientation. If the phenol ring was oriented exclusively in the direction opposite to the cavity above the piperidinium unit, a strong upfield shift of the protons 9-H and 3'-H would be expected. We observe, however, no upfield shift of 3'-H and 9-H of **1** and **12** as compared to the positions of these protons in **7** and **9** [6]. The strongest support for the location of the phenol ring on the cavity side was obtained from ^{1}H NOE difference spectroscopy (360 MHz, CD_3CN, 303K). Upon irradiation of 4'''-H of **1**, an enhancement of signals is observed exclusively for the protons 28-H, 30-H, and 31-H.

The host-guest complexation analysis with **1** in aqueous solution demonstrated that the phenol ring does not interfere with the binding of aromatic guests in the macrocyclic cavity. The critical micelle concentration (cmc) of **1** in an aqueous borate buffer (0.1056 M boric acid and 0.1 M NaOH) at pH = 10.5 was determined by ^{1}H NMR as cmc $\approx 1.5 \times 10^{-3}$ mol·L^{-1}.[2d] Below this concentration we studied the complexation of benzene and naphthalene derivatives by monitoring the ^{1}H NMR complexation shifts (500 MHz, 303K). In the aqueous buffer at pH 10.5 with [**1**] = [guest] = 5×10^{-4} mol·L^{-1}, we observed considerable complexation of sodium 2-naphthalenesulfonate and of 1-(trimethylammonio)naphthalene fluorosulfonate. In a mixture of this buffer with methanol (10:1) under otherwise identical conditions, we observed complexation between **1** and p-methyl-benzonitrile, p-nitrotoluene, and 6-methoxy-2-naphthonitrile. Complexation was indicated in each study by considerable upfield complexation shifts of the protons of the guest and by specific up and downfield shifts of the protons of the host.[8] The observed complexation shifts support the location of the aromatic guests in the plane of the cavity of **1** passing through the two diphenylmethane carbon atoms and perpendicular to the mean molecular plane of the tetraoxaparacyclophane unit. From the observed complexation shifts, the association constants K_a of the 1:1 complexes of **1** are estimated to be in the same range as the K_a - values of comparable macromonocyclic hosts without a cap.[2d]

In accord with the favorable location of the phenolic nucleophile atop the cavity, preliminary studies indicate, that the UV-monitored cleavage of p-nitrophenyl acetate (2×10^{-5} mol·L^{-1}) in the presence of **1** (5×10^{-4} mol·L^{-1}) is considerably accelerated in an aqueous borate buffer at pH 8.5 (293K) as compared to the reaction in the absence of **1**. A detailed investigation of the acceleration of the transacylation of esters in the presence of **1** and the synthetic transformation of **1** into target molecule **2** are now under way.

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References and Notes

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4) Impressive enhancements of the rates of transacylation have been reported; see ref. 3d and 3h. An efficient way to acccelerate the second step of the covalently catalyzed ester-hydrolysis, however, has still to be established for artificial molecular esterases.

5) For the transformation **1→2**, the phenolic group will be protected as methanesulfonate. Bromomethylation at C-31,37, reaction with imidazole, deprotection with K_2CO_3 and ion exchange (Cl^-) should give **2.**

6) Elemental analysis and spectroscopic data (IR, 500 MHz ^1H NMR, EI-MS; FAB-MS and 2D COSY for the macrobicycles) support the structures proposed for all new compounds.

7) **1**: mp: 258° C (dec). FAB-MS (m-nitrobenzyl alcohol) for $C_{64}H_{84}Cl_2N_4O_7$ (M^+ = 1090.57): 1089.7 (5%, M^+-1), 1062.7 (43%, M^+-C_2H_4), 1061.7 (74%, M^+-C_2H_5), 1055.7 (31%, M^+-Cl), 1020.7 (68%, M^+-2Cl), 1019.7 (100%, M^+-H-2Cl), 991.6 (58%, M^+-C_2H_5-2Cl). IR (KBr): υ = 3270 (N-H) and 1655 (C=O) cm^{-1}. ^1H NMR (1D and 2D COSY, 500 MHz, CD_3CN, 303K, J[Hz]): δ = 1.18 and 1.19 (2t, J =7.1; each 6H, CH_3CH_2N), 1.55 -1.6 (m; 2H, 3-*H*), 1.85 -2.3 (m; 6H, 3-*H*, 4-*H*), 2.01 (s; 6H, 27-CH_3), 2.21 (s; 6H, 12-CH_3), 2.45 - 2.95 (m; 8H, 3'-*H*, 3"-*H*), 3.05 - 3.45 (m; 16H, 2'-*H*, 2"-*H* , CH_3CH_2N), 3.45 - 3.55 and 3.6 - 3.7 (2 m; each 2H, 5-*H*), 3.7 - 3.9 and 4.0 - 4.15 (2m; each 2H,2-*H*), 3.84 (A part of AMX, J =14.2 and < 1.0; 2H, CH_2-NH), 5.31 (M part of AMX, J = 14.2 and 10.2; 2H, CH_2-NH), 6.81 (d, J =8.4; 2H, 31-*H*), 6.91 (d, J = 8.4; 2H, 30-*H*), 6.92 (s; 2H, 28-*H*), 7.14 (s; 1H, 2"'-*H*), 7.16 (d, J =2; 2H, 11-*H*), 7.62 (s; 2H, 4"'-*H*), 8.40 (s; 2H, 9-*H*), 9.10 (X-part of AMX,J = 10.2 and < 1.0; 2H, CH_2-N*H*).

8) Examples of complexation shifts (upfield shift =+$\Delta\delta$): p-tolunitrile: $\Delta\delta$ = +0.22(CH_3),+0.43 (2-*H*), +0.31 (3-*H*); 2-methoxy-6-naphthonitrile: $\Delta\delta$ = +0.51 (CH_3), +1.10 (5-*H*); ≈+0.2 to ≈+0.8 (1,3,4,7,8-*H*). Selected protons of host **1** in the solution of the **1**·2-methoxy-6-naphthonitrile complex: $\Delta\delta$ = ≈+0.20 (3,4-*H*), +0.07 (12-CH_3), -0.10 (3"-*H*), +0.45(5-*H*), +0.2 (2-*H*), -0.18 (30-*H*), -0.14 (28-*H*), -0.08 (11-*H*), -0.06 (9-*H*). From the complexation shifts at [H] = [G] = 5 x 10^{-4} mol·L^{-1} and from $\Delta\delta$ at saturation binding observed for the guests in complexes of related hosts,[2] the association constants of 1:1complexes are estimated as K_a ≈ 10^2 - 10^3 L·mol^{-1} (benzene guests) and K_a ≈ 10^3 - 10^4 L·mol^{-1} (naphthalene guests).

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