Abstract: Starting from the tetrabromide 1a (cone conformation) a number of resorcin[4]arene derivatives (3a, 4a, 5a, 6a) containing an amino function in the side chains have been prepared. Preliminary investigations, including UV, NMR, and MS analyses, of mixtures of the resorcinarene and its potential guests, such as metal cations or amino acids, evidenced promising new properties, depending on the nature of the substituents. Notably, treatment of 1a with pyridine gave readily the corresponding pyridinium salt 7a, capable of interacting with Ga(III) salts in aqueous solution. The same results have been obtained starting from the tetrabromide 1b (1,2-alternate conformation) for two of the above reactions, giving derivatives 3b and 7b.

Key words: macrocycles, molecular recognition, resorcinarenes, receptors, host-guest systems

Calixarenes and resorcinarenes are suitable alternatives to cyclodextrins for the ability of mimicking the molecular recognition processes of the living systems. Three main features play a fundamental role in the recognition process: a) changes in the cavity size and shape; b) chirality; and c) solubility in water, where the biological processes occur.

When calixarenes act as receptors of transition-metal cations, two well-separated main coordination regions are generally identified in their structure: the so-called ‘upper rim’, a π-basic cavity corresponding to the wide side of the aromatic rings system, and the ‘lower rim’, localized at the narrow side among the substituents.

As a part of our studies on modifications of lower rim substituents of resorcin[4]arenes,2 we have reported the preparation of the tetramine 3a (Scheme 1) in a cone conformation, which was shown to interact with Cu(II) cations and to form, as suggested by EPR studies and molecular modeling calculations, a complex in a stoichiometric ratio of 2:1 and in octahedral coordination.3 The synthesis of 3a from the tetrabromide 1a requires the reduction of the readily available tetrazide 2a with an excess of thioacetic acid to give the corresponding tetraamides and subsequent hydrolysis. Since the low yield (18%), requirement of a large amount of thioacetic acid, and a tedious workup make this synthesis unsatisfactory for a multi-gram preparation of 3a, we explored different reductive conditions for 2a. Experiments with R3P/H2O (R = alkyl, aryl) systems (Staudinger reaction)4 failed, with the tetrazide being quantitatively recovered. To overcome the insolubility of 2a in common organic solvents and the precipitation of 3a over the catalyst, we performed the hydrogenolysis (10% Pd/C, 40% w/w, 1 atm, H2) in a 1:1 mixture of THF–EtOH as a solvent, to obtain the tetramine 3a in 90% yield. In contrast, the same solvent system was ineffective for the tetrazide 2b (Scheme 1), in a 1,2-alternate conformation, which in turn required a 1:1 mixture of EtOAc–EtOH to give the corresponding tetramine 3b in 92% yield.

The aminoresorcinarene 3a,b and their intermediate azido derivatives 2a,b, however, are slightly soluble in common
organic solvents. Thus, other different derivatives, where the side chains maintain the NH function, but are enriched with aromatic or pyridine rings to increase the solubility of the macrocycles, were designed. The tetrabromide 1a was used as a synthon for the preparation of these and other derivatives. For instance, the resorcin[4]arene 1a was held at reflux in THF with a large excess of benzylamine – as a result the derivative 4a (Scheme 2) was obtained as the main product in 50% yield, whereas other products (dibenzyl derivatives) could not be separated by conventional methods from the mixture. All attempts to improve the conversion of 1a to 4a, for example, by increasing the amount of amine or performing the reaction in neat benzylamine, failed.

Comparable results in terms of yield (56%) were obtained by the reaction of 1a with 4-(aminomethyl)pyridine giving the product 5a.

Due to the biological role of cobalt\(^{5a,b}\) and the effects of mercury on environment and biology\(^{5c,d}\) we investigated the interaction of the new compounds 4a and 5a with metal cations (Co\(^{2+}\), Hg\(^{2+}\)) by UV/vis spectroscopy. The titration of a solution of CoCl\(_2\) in MeCN at a fixed concentration (5·10\(^{-4}\) M) with solutions of 4a at concentrations ranging from 1·10\(^{-5}\) to 1·10\(^{-3}\) M (Figure 1), allowed to establish the optimal wavelengths \(\lambda_{\text{opt}}\) (Table 1). The nonlinear regression analysis of the titration points at these wavelengths results in the association constants K and in the molar absorption constant \(X_{\text{HG}}\) (Table 1).

Application of the Job’s graphical method\(^6\) revealed a 1:1 ratio for the host-guest association (Figure 2). In the case of 5a, the titration of the Co(II) cation with aliquots of the resorcinarene did not allow the identification of \(\lambda_{\text{opt}}\); in contrast, the titration of 5a in MeCN \((c = 5·10^{-5} \text{ M})\) with CoCl\(_2\) solutions (1·10\(^{-5}\) to 1·10\(^{-3}\)) revealed the \(\lambda_{\text{min}}\) and \(\lambda_{\text{max}}\) (at 381 and 447 nm, respectively) of the starting UV spectrum to behave as \(\lambda_{\text{opt}}\) (Figure 1 and Table 1). A ratio of 1:1 resulted also in this case by the Job’s graphic (Figure 2).

A similar titration of Hg(II) cation with 5a (at the same concentrations) afforded the \(\lambda_{\text{opt}}\), \(X_{\text{HG}}\), and K values of Table 1. A ratio of 1:1 resulted also in this case by the Job’s graphic (Figure 2). A comparison of association constants \(X_{\text{HG}}\) show that resorcinarene 5a has a better

**Table 1** Features of Complexes of 4a and 5a with Metal Cations

<table>
<thead>
<tr>
<th>Species</th>
<th>(\lambda_{\text{opt}}) (nm)</th>
<th>(X_{\text{HG}})</th>
<th>K·10(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[4a·Co(^{2+})]</td>
<td>574</td>
<td>94 ± 2.5</td>
<td>28.13 ± 4.23</td>
</tr>
<tr>
<td></td>
<td>597</td>
<td>337 ± 7</td>
<td>29.41 ± 4.87</td>
</tr>
<tr>
<td></td>
<td>679</td>
<td>566 ± 10</td>
<td>28.03 ± 1.42</td>
</tr>
<tr>
<td></td>
<td>681</td>
<td>570 ± 9</td>
<td>27.75 ± 1.32</td>
</tr>
<tr>
<td>[5a·Co(^{2+})]</td>
<td>381</td>
<td>3988 ± 29</td>
<td>97.5 ± 17.7</td>
</tr>
<tr>
<td></td>
<td>447</td>
<td>1233 ± 98</td>
<td>88.1 ± 17.7</td>
</tr>
<tr>
<td>[5a·Hg(^{2+})]</td>
<td>381</td>
<td>3925 ± 46</td>
<td>24.05 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>447</td>
<td>1283 ± 48</td>
<td>21.7 ± 4.2</td>
</tr>
</tbody>
</table>

**Scheme 2**

**Figure 1** Examples of titrations of [4a·Co\(^{2+}\)], [5a·Co\(^{2+}\)], and [5a·Hg\(^{2+}\)]
affinity for Co(II) than for Hg(II) and its complex \([5a \cdot \text{Co}^{II}]\) is more stable than \([4a \cdot \text{Co}^{II}]\) as well. Notably, the titration of \(1a\) with \(\text{CoCl}_2\) or \(\text{HgCl}_2\) did not modify the UV spectrum of the pure resorcinarene. In conclusion, the benzylamino \(4a\) and the picolylamino \(5a\) side chains allow the interaction with Co(II) or Hg(II) cations and the formation of complexes with characteristics related to the nature of the substituents. Still in the framework of a research devoted to the preparation of macrocycles with nitrogen features in the side chains, we have reported the preparation of a chiral valinamido resorcinarene with molecular recognition properties towards chiral molecules.\(^7\)

Aiming at the preparation of chiral macrocycles, related to those previously studied, \(1a\) was treated (Scheme 3) with an excess of \((R)-\) or \((S)-\)a-methylbenzylamine to give the two enantiomer products \((R,R,R,R)-6a\) and \((S,S,S)-6a\) (ent-\(6a\)) in a gratifying 45–50% yield, as light yellow solids. To compare the properties of these chiral resorcinarenes with those of valinamido derivatives\(^7\), the ESI mass spectra of mixtures of \(6a\) or ent-\(6a\) with a series of free or derivatized amino acids in MeOH or MeCN, have been analyzed for the presence of additional peaks, as diagnostic for a host/guest complexation. No of such peaks were found, in contrast with the enantioselectivity shown by valinamido resorcinarenes.\(^7\) We may conclude that for the encapsulation to occur the presence of carboxyl groups is required to contribute very likely together with chirality to a hydrogen bond network.

With regard to the solubility in water, among the examples of water-soluble calixarenes,\(^8\) the main studies concern macrocycles featuring ammonium ions, such as the interaction of some calixarenes, with four, six, or eight permethylammonium nuclei at the upper rim, with nucleotides and nucleic acids.\(^9\) Water-soluble calixarenes, containing trimethylammoniomethyl groups in the upper rim (Figure 3), have been also reported\(^10\) as a new inverse phase-transfer catalyst in aldol-type condensation and Michael addition reaction.

Since some water-soluble resorcin[\(n\)]arenes have been reported in the recent literature,\(^11\) the tetrapyridinium salt \(7a\) (Scheme 4), obtained in almost quantitative yield just by dissolving \(1a\) in anhydrous pyridine at room temperature, represents a nice example to obtain water-soluble resorcin[4]arenes. The 1,2-alternate isomer \(7b\) gave identical results. Notably, the reaction takes place in the NMR tube as well, by adding pyridine to a solution of the bromo derivative \(1a\) or \(1b\) dissolved in CDCl\(_3\); after decantation of the liquid, the precipitate is washed with CCl\(_4\) and is ready for NMR measurements in D\(_2\)O.

**Scheme 3**
Both the compounds were soluble enough in D$_2$O to allow some preliminary complexation studies, using Ga(III) as a guest in place of Fe(III) cation.\textsuperscript{12,13} \textsuperscript{1}H NMR titration of the macrocycle 7a in D$_2$O at 80 °C with Ga$_2$(SO$_4$)$_3$ resulted in a series of plots showing the chemical shift variations (Δδ in Hz) of the proton signals as a function of the added Ga(III) solution (Table 2).\textsuperscript{13}

The molar ratio of the cation to the resorcinarene in the complex could not be determined as there was no defined change in the slope of the curves. However, the different effects of the Ga(III) cation on the protons (Table 2) of the macrocycle suggested the existence of a main interaction site in the lower rim, in the space defined by the aromatic methoxy groups. Indeed, the substituents for the presence of a positive charge are not expected to contribute to complexation as, for instance, the side chains containing carboxyl groups.\textsuperscript{13}

All the synthesized compounds were fully characterized by unambiguous assignments of all the signals in the \textsuperscript{1}H and \textsuperscript{13}C NMR spectra (see experimental part); in particular the more complex distribution pattern was diagnostic for the resorcinarenes 3b and 7b in the 1,2-alternate forms: aromatic protons, 2:2; aromatic methoxy groups, 2:2:2:2; aliphatic (and pyridinium, in compound 7b) protons, 1:2:1.\textsuperscript{3} Mass spectra and elemental analyses confirmed the assigned structures. Notably, the mass fragmentation of the amino derivatives 3a–5a followed a different pathway from that usually given by resorcinarenes. The mass fragmentation of the bromo derivative 1a, for example, is characterized by the loss of one side chain to give a tropium like ion [M – CH$_2$CH$_2$Br$^+$]; a neutral fragment HCH$_2$Br can be lost from a second chain of this ion, affording again an odd mass ion. In contrast, in compounds such as 4a, 5a, and 6a, the nitrogen drives the fragmentation and the chain is only partially lost; in the case of 6a a reaction with loss of styrene can be hypothesized:

\[
\text{CHCH}_2\text{CH}_2\text{NHCH(Me)Ph} \rightarrow \text{CHCH}_2\text{CH}_2\text{NH}_2 + \text{H}_2\text{C=CHPh}
\]

In summary, the tetrabromide 1a has been used as starting material for the preparation of the new resorcin[4]arenes 4a–6a, containing an amino group in their side chains. Compounds 4a and 5a have been shown to act as host macrocycles for metal cations (Co$^{2+}$ and Hg$^{2+}$) in aqueous solution and differences between the two resorcinarenes towards the same cation as well of the two metal ions towards the same resorcinarene have been noticed. Conversely, in resorcinarenes 6a and ent-6a, the chiral centre was shown not to be sufficient for an interaction with free or derivatized amino acids. Finally, the precursor 1a was made soluble in water by simple treatment with pyridine and the possible interaction sites of the tetraammonium derivative 7a have been investigated by \textsuperscript{1}H NMR titration. A few of these reaction have been repeated on the 1,2-alternate isomer 1b.

In conclusion, the resorcinarene scaffold demonstrated once more high versatility in the modification of its substituents for a promising modulation of its receptorial properties.

Melting points were determined with a Büchi B-545 apparatus and are uncorrected. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were determined with a Varian Gemini instrument, using tetramethylsilane as an internal standard in the reported solvents. Fast atom bombardment mass spectra were determined on a VG 7070EQ spectrometer, ESI-MS spectra were obtained using a Thermo Finnegan LCQ Deca XP-Plus ion-trap mass spectrometer equipped with an electrospray ionization (ESI) source. Conditions: source voltage = +5.0 kV; sheath gas = 25 AU (Arbitrary Units); auxiliary gas = 10 AU; capillary voltage = +40.0 V; capillary temperature = 200 °C; tube lens offset = +15 V. Optical rotations were measured with a Perkin–Elmer 243 polarimeter at 21 °C. Elementary analyses were obtained using a Carlo Erba EA 1108 Elemental Analyzer.

### Scheme 4

![Scheme 4](image-url)

4a–6a, containing an amino group in their side chains.

In summary, the tetrabromide 1a has been used as starting material for the preparation of the new resorcin[4]arenes 4a–6a, containing an amino group in their side chains. Compounds 4a and 5a have been shown to act as host macrocycles for metal cations (Co$^{2+}$ and Hg$^{2+}$) in aqueous solution and differences between the two resorcinarenes towards the same cation as well of the two metal ions towards the same resorcinarene have been noticed. Conversely, in resorcinarenes 6a and ent-6a, the chiral centre was shown not to be sufficient for an interaction with free or derivatized amino acids. Finally, the precursor 1a was made soluble in water by simple treatment with pyridine and the possible interaction sites of the tetraammonium derivative 7a have been investigated by \textsuperscript{1}H NMR titration. A few of these reaction have been repeated on the 1,2-alternate isomer 1b.

In conclusion, the resorcinarene scaffold demonstrated once more high versatility in the modification of its substituents for a promising modulation of its receptorial properties.
To a solution of the tetrabromide $1b$ (396 mg, 0.386 mmol) in anhyd DMF (10 ml) was added NaN$_3$ (504 mg, 7.73 mmol) and the mixture was stirred for 15 h at 65°C. The mixture was cooled, added to sat. aq NaHCO$_3$ (10 ml), and extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with brine (50 ml), dried (MgSO$_4$), filtered, and concentrated under vacuum. The residue was purified by silica gel chromatography ($CHCl_3$–$n$-hexane, 9:1) to give $2b$ (242 mg, 71%) as a vitreous solid.

Table 2 Selected Proton Signals and $\Delta\delta$ in $^1$H NMR Titration of 7a with Ga$_2$(SO$_4$)$_3$.

<table>
<thead>
<tr>
<th>Proton</th>
<th>$\delta$</th>
<th>$\Delta\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-internal</td>
<td>7.06 (s)</td>
<td>-31.3</td>
</tr>
<tr>
<td>H-external</td>
<td>6.25 (s)</td>
<td>18.6</td>
</tr>
<tr>
<td>CH</td>
<td>4.12 (t, $J = 8$ Hz)</td>
<td>-8.3</td>
</tr>
<tr>
<td>OMe</td>
<td>3.45 (s)</td>
<td>9.2</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>2.83 (q, $J = 7.5$ Hz)</td>
<td>-11.2</td>
</tr>
</tbody>
</table>

Table 2 Selected Proton Signals and $\Delta\delta$ in $^1$H NMR Titration of 7a with Ga$_2$(SO$_4$)$_3$.

The other signals underwent $\Delta\delta$ shifts comparable with those due to dilution effects.

The synthesis of this compound was performed by Botta et al.

(d, C-25, C-26, C-27, C-28), 123.35 (s, C-1, C-3, C-7, C-9, C-13, C-15, C-19, C-21), 122.95 (2 d, C-3', C-5’), 96.62 (d, C-5, C-11, C-17, C-23), 56.06 (q, CH2N+), 52.18 (t, CH2N), 47.38 (t, CH2NPy), 32.45 (d, CH), 32.25 (t, CH).


Anal. Calcd for C49H60N8O8: C, 77.36; H, 8.38; N, 4.63. Found: C, 77.89; H, 8.72; N, 4.68.

r-2c-8c-14c-20-Tetra[(N-α-methylbenzyl)amino]ethyloctacyclo[19.3.1.13.7.19.13.115.19]octacosa-2,4,6,10,12,16,18,22,24-octyl Octamethyl Ether (6a and ent-6a)

To a stirred solution of the tetrabromide 1a (256 mg, 0.25 mmol) in anhyd THF (75 ml) was added (R)-α-methylbenzylamine (2.42 g, 20 mmol). The mixture was stirred at r.t. for 1 h and held at reflux for 18 h. The solution was evaporated under reduced pressure and the residue was treated with a minimum amount of THF, filtered, and washed with Et2O (3 × 5 ml) to give (R,R,R,R)-6a (136 mg, 46%) as a light yellow solid; mp 218–220 °C; [α]D +37.31 (c = 0.52, CHCl3).

1H NMR (CDCl3): δ = 7.62 (4 × 2 H, br d, J = 7 Hz, H-2, H-6), 7.43 (4 × 3 H, m, H-3, H-4, H-5), 6.85 (4 H, s, H-5, H-11, H-17, H-23), 4.27 (4 H, q, J = 6 Hz, 4 NCCH3), 4.25 (4 H, t, J = 7.5 Hz, 4 CH2), 3.55 (12 H, s, 4 OCH3), 3.46 (12 H, s, 4 OCH3), 3.24 (4 H, t, J = 8 Hz, H-25, 4 NCCH3), 6.14 (4 H, s, H-25, H-26, H-27, H-28), 6.37 (4 H, s, H-5, H-11, H-17, H-23), 4.37 (4 H, q, J = 6 Hz, 4 NCCH3), 3.77, 3.74, 3.63, 3.61 (4 s, OCH3), 2.99, 2.93 (2 H, dq, J = 6 Hz, 2 CH2N+), 2.60, 2.53 (2 H, m, 2 CH), 1.85 (12 H, d, J = 6 Hz, 4 CH2), 1.85 (12 H, d, J = 6 Hz, 4 CH2).

r-2c-8c-14c-20-Tetra(2-pyridinoethyl)pentacyclo[19.3.1.13.7.19.13.115.19]octacosa-2,4,6,10,12,16,18,22,24-octyl Octamethyl Ether Tetrabromide (7a)

A solution of the tetrabromide 1a (205 mg, 0.2 mmol) in anhyd pyridine (5 ml) was stirred at r.t. for 30 min till a precipitate was completely formed. The solid was filtered through a Celite pad, washed with Et2O (3 × 10 ml), and finally dried under high vacuum to give the tetrabromopyridinium salt 7a (249 mg, 94%).

1H NMR (D2O, 80 °C): δ = 8.61 (4 × 2 H, br d, J = 6 Hz, H-2', H-6'), 8.41 (4 × 1 H, br t, J = 7.5 Hz, H-4'), 7.88 (4 × 2 H, br d, J = 6.5 Hz, H-3', H-5'), 6.70 (4 H, br s, H-5, H-26, H-27, H-28), 6.43 (4 H, s, H-5, H-11, H-17, H-23), 6.55 (2 H, br q, J = 6 Hz, CH2N), 0.52 (1 H, t, J = 7.5 Hz, CH2), 3.63 (24 H, s, 8 OCH3), 2.63 (2 H, br q, J = 6 Hz, CH2).

13C NMR (D2O, 80 °C): δ = 155.82 (s, C-4, C-6, C-10, C-12, C-16, C-18, C-22, C-24), 145.55, 144.11, 127.64 (d, 2 d, 2 d, C-2', C-3', C-4', C-5', C-6'), 122.95 (d, 2 d, 2 d, C-25, C-26, C-27, C-28), 123.50 (s, C-1, C-3, C-7, C-9, C-13, C-15, C-19, C-21), 97.59 (d, d, 10, C-5, C-11, C-17, C-23), 56.90, 56.82 (s, OCH3), 34.17 (t, CH2), 31.45 (d, CH).


Anal. Calcd for C49H60N8O8: C, 76.73; H, 7.8; N, 4.71. Found: C, 76.81; H, 7.84; N, 4.74.

The same procedure with (S)-α-methylbenzylamine gave (S,S,S,S)-6a in 49% yield; yellow solid; mp 218–220 °C; [α]D –35.33 (c = 0.37, CHCl3).

1H and 13C NMR and ESI-MS (+) spectra were identical with those of (R,R,R,R)-6a.


ESI Mass Spectral Analysis

In a typical experiment, the resorcinarene 6a (10 µg/ml) and the respective amino acid A (see below, in 61% excess) in MeCN were injected in the mass spectrometer. The amino acids used were L-alanine (MW 89), L-alanine methyl ester (MW 103), D-phenylalanine (MW 165), D-phenylalanine ethyl ester (MW 193), L-tyrosine (MW 181), and L-tyrosine methyl ester (MW 195). The ESI mass spectrum was analyzed for the presence of peaks, such as [6a+H+A]+, [6aNa+H+A]+, [6aNaH+Na+H/2]+, etc.
shift variations ($\Delta\delta$ in Hz) of the proton signals was measured with reference to the values in the $^{1}H$ NMR spectrum of the pure resorcicarnarene. The $^{1}H$ NMR spectra of the mixtures were recorded at 80 °C to gain a better resolution. As a result, a series of plot showing for each signal the relative $\Delta\delta$ as a function of the amount of metal ion added were constructed as an information source of the interaction sites (Table 2).

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