

## Guanidine-equipped thiacalix[4]arenes: synthesis, interaction with DNA and aggregation properties

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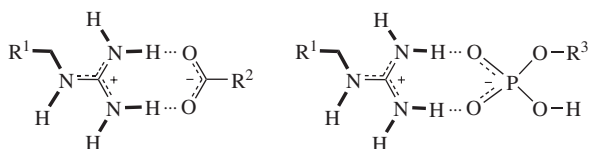
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DOI: 10.1016/j.mencom.2014.03.005

New tetrakis-guanidinium-containing *p*-*tert*-butylthiacalix[4]arene forms monodisperse nanoparticles with diameter 44 nm in the concentration range of 40–100 μM. Its association constant with model DNA was evaluated as  $pK_a = 3.96$  by fluorescent intercalator displacement assay.

Molecular design of synthetic receptors that can effectively interact with anionic and polyanionic biomacromolecules is an important goal of supramolecular chemistry.<sup>1–4</sup> Using macrocyclic platforms such as calixarenes able to spatially organize binding sites makes it possible to obtain effective antibacterial drugs,<sup>5</sup> transfection agents<sup>6</sup> and artificial enzymes.<sup>7</sup>

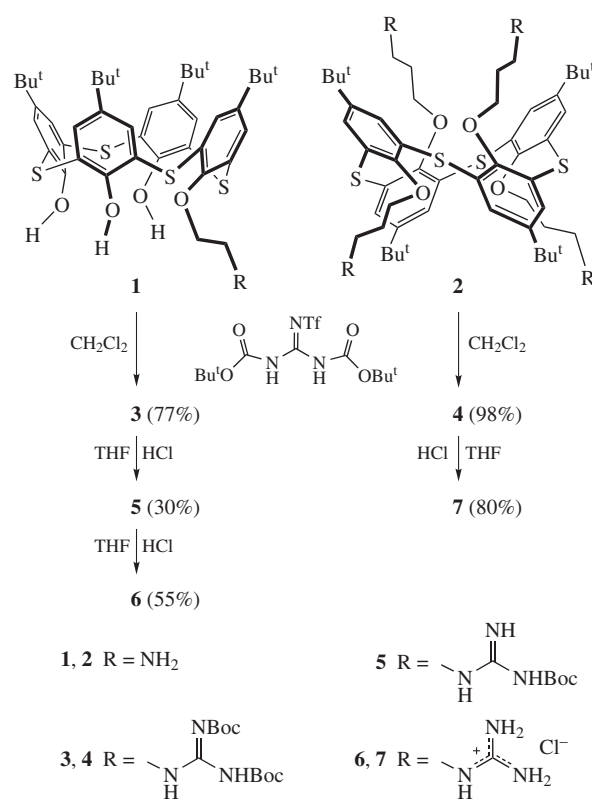
Creation of nanoparticles with surface functional groups providing affinity toward different biomacromolecules attracts attention of many scientists. Such particles offer opportunities to development of drug delivery systems, DNA transfection, diagnostics tools and protein separation.<sup>8–11</sup> Guanidinium fragments provide the greatest affinity of the nanoparticles to polyanionic substrates.<sup>12,13</sup> The efficiency of interaction of a guanidinium moiety with anionic substrates, such as carboxylate and phosphate groups, is due to their geometrical and charge complementarity.<sup>1</sup>



Despite the relevance, there are no examples in the literature describing the synthesis of guanidinium-containing thiacalix[4]arenes which have a greater conformational and synthetic potential against classical calix[4]arenes.<sup>14–20</sup>

For the preparation of guanidinium-containing *p*-*tert*-butylthiacalix[4]arenes the previously described amino derivatives **1** and **2**<sup>21,22</sup> were tested as the starting reactants. Traditional reagents such as 3,5-dimethyl-1*H*-pyrazole-1-carboxamide nitrate, cyanamide and isothiuronium salts previously used for introduction of the guanidinium fragments into non-macrocyclic derivatives appeared to be ineffective in the case of derivatives **1** and **2**. In all the cases, a complex mixture of partially functionalized products was obtained.

To access the target compounds, we turned to more effective diprotected triflyl guanidines.<sup>23</sup> In the two-step synthesis, amines **1**, **2** were treated with *N,N'*-bis(*tert*-butoxycarbonyl)-*N''*-triflylguanidine and then the resulting Boc-derivatives **3**, **4** were deprotected using hydrochloric acid (Scheme 1).<sup>†</sup>



Scheme 1

Interestingly, the rate of removal of the *tert*-butoxycarbonyl protecting groups in **3** and **4** differs greatly. In the case of tetra-substituted at the lower rim *p*-*tert*-butylthiacalix[4]arene **4**, the deprotection proceeded within 24 h affording a water soluble compound **7** in high yield. In the case of compound **3** with free phenolic groups at the lower rim, the reaction mixture after 24 h consisted of the target compound **6** along with the mono-Boc-derivative **5** in the ratio **5**:**6** of 45:55. Compound **5** was isolated by preparative column chromatography and characterized.

Such an unexpected difference in the reactivity of compounds **3** and **4** can be explained by the presence of free phenolic groups

<sup>†</sup> Compounds **3** and **4**. The stoichiometric amount of *N,N'*-bis(*tert*-butoxycarbonyl)-*N''*-triflylguanidine in 20 ml of dichloromethane was added to the solution of 1.00 g of corresponding compound **1**, **2** in 40 ml of dichloromethane in one portion at room temperature. After 24 h, the

mixture was washed with 2 M aqueous NaHSO<sub>4</sub> (10 ml) and saturated NaHCO<sub>3</sub> (10 ml). Each aqueous layer was extracted with dichloromethane (2×10 ml). The combined organic phases were washed with brine (10 ml), dried (MS 3 Å), filtered and concentrated under reduced pressure.