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Mendeleev Commun., 2014, **24**, 82–84
Mendeleev Communications

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

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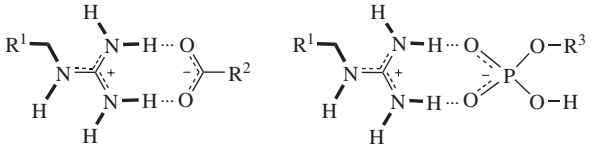
DOI: 10.1016/j.mencom.2014.03.005

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.[1–4] Using macrocyclic platforms such as calixarenes able to spatially organize binding sites makes it possible to obtain effective antibacterial drugs,[5] transfection agents[6] and artificial enzymes.[7]

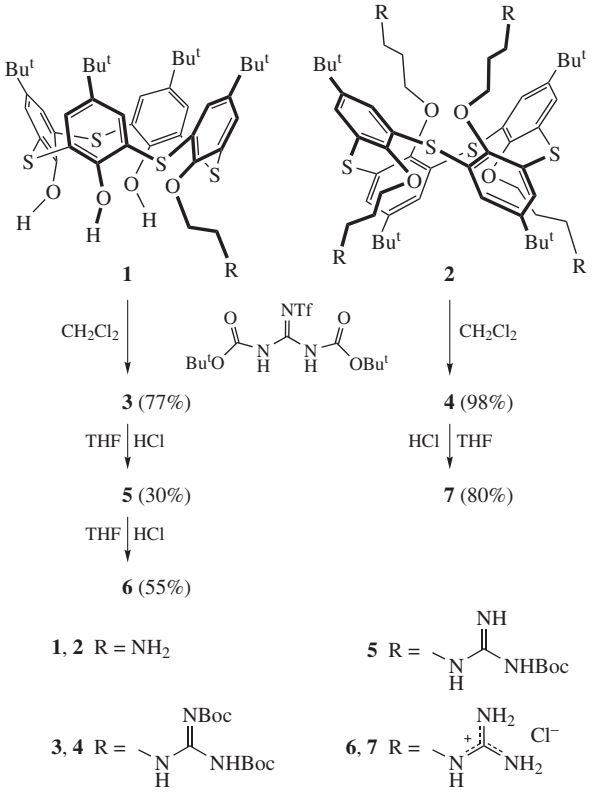
Creation of nanoparticles with surface functional groups providing affinity toward different biomacromolecules attracts attention of many scientists. Such particles offer opportunities to development of drug delivery systems, DNA transfection, diagnostics tools and protein separation.[8–11] Guanidinium fragments provide the greatest affinity of the nanoparticles to polyanionic substrates.[12,13] 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.[1]



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.[14–20]

For the preparation of guanidinium-containing *p-tert*-butylthiacalix[4]arenes the previously described amino derivatives **1** and **2**[21,22] were tested as the starting reactants. Traditional reagents such as 3,5-dimethyl-1*H*-pyrazole-1-carboxamidine nitrate, cyanamide and isothiouronium 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.[23] 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).†



Scheme 1

Interestingly, the rate of removal of the *tert*-butoxycarbonyl protecting groups in **3** and **4** differs greatly. In the case of tetrasubstituted 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

† *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 $NaHSO_4$ (10 ml) and saturated $NaHCO_3$ (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.

in the structure of **3**, which promoted formation of strong hydrogen bonds between the phenolic and guanidinium groups resulting in stabilization of the intermediate structure **5**.

The affinity of water soluble macrocycle **7** to the model polynucleotide (salmon sperm DNA) was evaluated by fluorescence spectroscopy study of the displacement of the fluorescent probe (ethidium bromide, EB) from DNA (for details, see Online Supplementary Materials).[24] Compound **7** effectively interacted with the polyanionic surface of the biopolymer leading to a decrease in the fluorescence of the DNA–EB complex (Figure 1). Typically, interaction of cationic derivatives of the classical calixarene with the double-stranded DNA results in the intercalation of macrocycle into the major groove of macromolecule without distortions in stacking of complementary base pairs and forms the minor groove.[25] In this regard, the fluorescence quenching process can be described as static. To determine the thermodynamic parameters of the interaction, equation (1) was used where $[D_t]$ and $[N_t]$ are the concentration of **7** and the DNA–EB, respectively (Figure 1).[26,27]

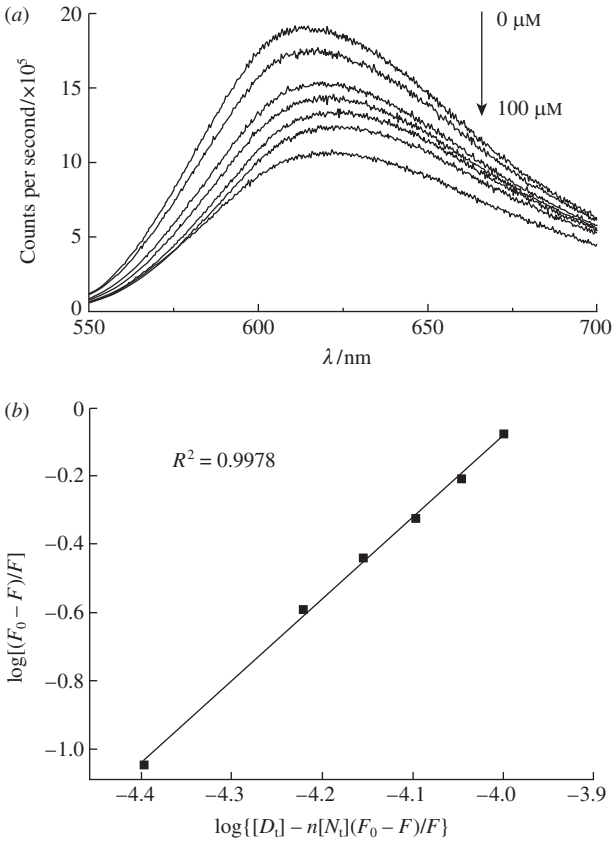


Figure 1 (*a*) The emission spectra of DNA–EB system in the presence of 0, 40, 60, 70, 80, 90, 100 μM of ligand **7**. The arrow shows the emission intensities changes with increasing ligand concentration. (*b*) The plots of $\log[(F_0 - F)/F]$ *vs.* $\log\{[D_t] - n[N_t](F_0 - F)/F_0\}$.

$$\log\frac{F_0 - F}{F} = n\log K_A + n\log\left([D_t] - n[N_t]\frac{F_0 - F}{F}\right). \quad (1)$$

The logarithm of the association constant of thiacalix[4]arene–DNA was found to be 3.96. Interestingly, despite an excess of **7** against DNA in solution [C(DNA) = 4×10^{-6} mol dm^{-3}, C(**7**) =

5,11,17,23-Tetra-tert-*butyl-25,26,27-trihydroxy-28-[2-(*N,N′-*bis*-tert-*butoxycarbonylguanidino)ethoxy]-2,8,14,20-tetrathiacalix[4]arene (cone)* **3**. Yield 77%, mp 264–265 °C (decomp.). IR (Nujol, ν/cm^{-1}): 1266 (COC), 1638 (N–CO), 1718 (C=N), 3331 (NH, OH). ^{1}H NMR ($CDCl_3$) δ: 1.16 (s, 9H, CMe_3), 1.22 (s, 9H, CMe_3), 1.25 (s, 18H, CMe_3), 1.41 (s, 9H, Me_3C^{Boc}), 1.54 (s, 9H, Me_3C^{Boc}), 4.21 (m, 2H, CH_2NH), 4.58 (t, 2H, CH_2O), 7.58 (s, 2H, H_{Ar1}), 7.60 (s, 2H, H_{Ar2}), 7.61 (d, 2H, H_{Ar3}, $^3J_{HH}$ 2.5 Hz), 7.65 (d, 2H, H_{Ar3}, $^3J_{HH}$ 2.5 Hz), 9.21 (t, 2H, N*H*CH_2, $^3J_{HH}$ 5.32 Hz), 11.58 (s, 1H, N*H*Boc). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 28.0, 28.4, 31.0, 31.25, 31.3, 34.1, 34.2, 34.4, 41.2, 76.5, 79.2, 82.9, 120.4, 120.6, 120.9, 135.80, 135.84, 136.0, 136.5, 143.5, 143.85, 149.2, 152.8, 156.2, 156.6, 156.7, 157.8, 163.6. ^{1}H-^{1}H NOESY (the most important cross-peaks): $H^{4'b}/H^{4b}$, $H^{4'b}/H^{4+b}$, H^{4b}/H^{4+b}, $H^{BocNH}/H^{3'}$, H^{BocNH}/H^{5+}. MS (MALDI-TOF), *m/z*: 1044.6 $[M+K]^+$ (calc. $[M]^+$, *m/z*: 1005.4). Found (%): C, 63.76; H, 6.92; N, 4.47; S, 12.12. Calc. for $C_{53}H_{71}N_3O_8S_4$ (%): C, 63.25; H, 7.11; N, 4.18; S, 12.74.

5,11,17,23-Tetra-tert-*butyl-25,26,27,28-tetrakis[3-(*N,N′-*bis*-tert-*butoxycarbonylguanidino)propoxy]-2,8,14,20-tetrathiacalix[4]arene (1,3-alternate)* **4**. Yield 98%, mp 147–148 °C. IR (Nujol, ν/cm^{-1}): 1264 (COC), 1636 (N–CO), 1717 (C=N), 3334, (NH). ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 36H, CMe_3), 1.25 (m, 6H, $CH_2CH_2CH_2$), 1.51 (s, 36H, Me_3C^{Boc}), 1.52 (s, 36H, Me_3C^{Boc}), 3.31 [m, 8H, NCH_2(CH_2)$_2$], 3.97 [t, 8H, OCH_2(CH_2)$_2$, $^3J_{HH}$ 7.8 Hz], 7.39 (s, 8H, H_{Ar}), 8.31 [t, 4H, N*H*(CH_2)$_3$, J_{HH} 4.6 Hz], 11.48 (s, 4H, N*H*Boc). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 27.9, 28.0, 28.3, 31.5, 34.3, 37.6, 65.2, 79.2, 83.2, 127.1, 128.2, 146.3, 153.2, 156.1, 156.3, 163.6. ^{1}H-^{1}H NOESY (the most important cross-peaks): H^{4b}/H^7, H^{4b}/H^8, H^{4b}/H^9, H^{4b}/H^{10}. MS (MALDI-TOF), *m/z*: 1941.6 $[M+Na]^+$ (calc. $[M]^+$, *m/z*: 1917.0). Found (%): C, 60.04; H, 7.69; N, 8.92; S, 6.29. Calc. for $C_{96}H_{148}N_{12}O_{20}S_4$ (%): C, 60.10; H, 7.78; N, 8.78; S, 6.69.

Compounds **5** *and* **6**. Concentrated hydrochloric acid (2 ml) was added to the solution of 0.50 g of compound **3** in THF. The reaction mixture was stirred for 24 h (**5**) or 72 h (**6**). Then the solvent was evaporated *in vacuo* and water (20 ml) was added to the reaction mixture, the precipitate was filtered off and washed with water. The crude residue obtained was dried in dessicator under reduced pressure, purified by column chromatography ($CHCl_3$: MeOH, 9:1) and dried under reduced pressure.

5,11,17,23-Tetra-tert-*butyl-25,26,27-trihydroxy-28-[2-(*N-tert-*butoxycarbonylguanidino)ethoxy]-2,8,14,20-tetrathiacalix[4]arene (cone)* **5**. Yield 30%, mp >216 °C (decomp.). IR (Nujol, ν/cm^{-1}): 1265 (COC), 1636 (N–CO), 1716 (C=N), 3335 (NH, OH). ^{1}H NMR ($CDCl_3$) δ: 0.63 (br. s, 9H, CMe_3), 1.22 (s, 9H, CMe_3), 1.32 (s, 18H, CMe_3), 1.41 (s, 9H, Me_3C^{Boc}), 4.12 (br. s, 2H, CH_2NH), 5.09 (br. s, 2H, CH_2O), 6.77 (br. s, 2H, H_{Ar1}), 7.57 (s, 2H, H_{Ar2}), 7.61 (d, 2H, H_{Ar3}, $^3J_{HH}$ 2.0 Hz), 7.67 (d, 2H, H_{Ar3}, $^3J_{HH}$ 2.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 27.8, 30.4, 31.0, 31.2, 31.3, 31.47, 31.5, 33.9, 34.1, 122.3, 123.1, 123.7, 130.2, 134.0, 136.3, 136.4, 142.0, 152.5, 157.2. ^{1}H-^{1}H NOESY (the most important cross-peaks): $H^{4'b}/H^{4b}$, $H^{4'b}/H^{4+b}$, H^{4b}/H^{4+b}, $H^{BocNH}/H^{3'}$, H^{BocNH}/H^{5+}. MS (MALDI-TOF), *m/z*: 906.5 $[M+H]^+$, 928.5 $[M+Na]^+$, 944.5 $[M+K]^+$ (calc. $[M]^+$, *m/z*: 905.4). Found (%): C, 63.23; H, 6.82; N, 4.52; S, 13.94. Calc. for $C_{48}H_{63}N_3O_6S_4$ (%): C, 63.61; H, 7.01; N, 4.64; S, 14.15.

5,11,17,23-Tetra-tert-*butyl-25,26,27-trihydroxy-28-(2-guanidinoethoxy)-2,8,14,20-tetrathiacalix[4]arene hydrochloride (cone)* **6**. Yield 55%, mp >232 °C (decomp.). IR (Nujol, ν/cm^{-1}): 1265 (COC), 1716 (C=N), 3335 (NH, OH). ^{1}H NMR ($CDCl_3$) δ: 0.70 (br. s, 9H, CMe_3), 1.20 (s, 9H, CMe_3), 1.30 (s, 18H, CMe_3), 3.91 (br. s, 2H, CH_2NH), 4.51 (br. s, 2H, CH_2O), 7.53 (s, 2H, H_{Ar2}), 7.57 (br. s, 2H, H_{Ar3}), 7.65 (br. s, 4H, H_{Ar3}). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 30.5, 31.4, 31.45, 34.1, 31.2, 31.3, 123.1, 129.1, 135.3, 135.6, 136.2, 157.0. ^{1}H-^{1}H NOESY (the most important cross-peaks): H^{4b}/H^3, $H^{4'b}/H^{3'}$, $H^{4'b}/H^3$. MS (MALDI-TOF), *m/z*: 806.5 $[M-Cl]^+$ (calc. $[M]^+$, *m/z*: 841.3). Found (%): C, 61.03; H, 6.62; N, 4.82; S, 14.97. Calc. for $C_{43}H_{56}N_3O_4S_4Cl$ (%): C, 61.36; H, 6.59; N, 4.99; S, 15.24.

5,11,17,23-Tetra-tert-*butyl-25,26,27,28-tetrakis(3-guanidinopropoxy)-2,8,14,20-tetrathiacalix[4]arene tetrahydrochloride (1,3-alternate)* **7**. Concentrated hydrochloric acid (2 ml) was added to the solution of 0.50 g of compound **4** in THF. The reaction mixture was stirred for 24 h. Then the solvent was evaporated *in vacuo* and water (20 ml) was added to the reaction mixture, the precipitate was filtered off and washed with water. The white powder obtained was dried in dessicator under reduced pressure. Yield 80%, mp 246–247 °C. IR (Nujol, ν/cm^{-1}): 1266 (COC), 1664 (NH_2), 3178 (=NH). ^{1}H NMR (DMSO-d_6) δ: 1.21 (s, 36H, CMe_3), 1.59 (m, 8H, $CH_2CH_2CH_2$), 3.18 [m, 8H, NCH_2(CH_2)$_2$], 3.93 [m, 8H, OCH_2(CH_2)$_2$], 7.31 (br. s, 16H, C^+NH_2), 7.44 (s, 8H, H_{Ar}), 7.66 (m, 4H, CH_2N*H*). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 28.8, 31.0, 33.9, 37.6, 67.6, 127.1, 127.8, 129.9, 145.5, 157.0, 157.2. ^{1}H-^{1}H NOESY (the most important cross-peaks): H^{4b}/H^7, H^{4b}/H^8, H^{4b}/H^9, H^3/H^7. MS (MALDI-TOF), *m/z*: 1117.4 $[M-3HCl-Cl]^+$ (calc. $[M]^+$, *m/z*: 1262.5). Found (%): C, 52.95; H, 6.88; N, 13.12; S, 9.79. Calc. for $C_{56}H_{88}N_{12}O_4S_4Cl_4$ (%): C, 53.24; H, 7.02; N, 13.30; S, 10.15.

= (40–100)×10^{-6} mol dm^{-3}], the associate stoichiometry **7**:DNA = = 2.38:1. This is probably due to the ability of compound **7** to form nanoparticles which participate in the recognition. To confirm this assumption, the dynamic light scattering (DLS) method was used. It was found that within the studied range of concentrations the macrocycle **7** exists as monodisperse nanoparticles (PDI = 0.24) with size of 44.5 nm. The addition of DNA to these nanoparticles decreases the particle size (14.9 nm) and increases the polydispersity (PDI = 0.41). This is obviously due to the dissociation of the nanoparticles providing more efficient interaction with the polyanionic surface of DNA.

In conclusion, the herein obtained thiacalix[4]arene cationic derivatives seem promising for further application as receptors for recognition and transfer of various polyanionic substrates like DNA, RNA, proteins, *etc*. The present study can help to create efficient and safer vectors based on macrocyclic platform for gene therapy.

This work was supported by the Federal Program 'Research and Scientific-Pedagogical Personnel of Innovative Russia' for 2009–2013 (no. 16.740.11.0472), the Russian Foundation for Basic Research (grant nos. 12-03-00252-a, 13-04-97061 and 13-03-12055_ofi_m).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2014.02.005.

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Received: 19th August 2013; Com. 13/4186