Polyfunctional branched nitrogen-containing *p-tert*-butylthiacalix[4]arene derivatives as efficient agents for packaging calf thymus DNA

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Polyfunctional *p-tert*-butylthiacalix[4]arenes tetrasubstituted at the lower rim with nitrogen-containing fragments in the *cone*, *partial cone*, and *1,3-alternate* conformations were investigated in the processes of calf thymus DNA packaging. The influence of the macrocycle structure and composition on their ability to pack DNA into nano-sized particles was considered. UV spectroscopy and dynamic light scattering results showed that the synthesized *p-tert*-butylthiacalix[4]arenes functionalized at the lower rim with polyamine fragments in the *cone* and *1,3-alternate* conformations can compact calf thymus DNA into nanosized particles (<100 nm). A new type of synthetic receptors based on the *1,3-alternate* stereoisomer of *p-tert*-butylthiacalix[4]arene with ester, hydroxy, and tertiary amino groups was suggested for packaging DNA to nanoparticles of 51—85 nm in size.

Key words: *p-tert*-butylthiacalix[4]arene, synthesis, calf thymus DNA, DNA packaging, dynamic light scattering.

The design of nanosized carrier molecules for the delivery of therapeutic genes is still a very important problem in the field of gene therapy.[1] The *in vitro* and *in vivo* studies showed that nucleic acids have a very low ability to transfect cells, therefore, specially designed nucleic acid carriers are required for the efficient delivery of genes, the so-called gene delivery vectors, both viral and non-viral.[2,3] Non-viral vectors generally do not induce an immune response of the organism, are synthetically available, have an ability to carry genetic material of unlimited size, as well as are relatively safe.[4,5] One of the common approaches used in the development of synthetic non-viral vectors is the functionalization of readily available molecular platforms by receptor groups (amino, ammonium, and guanidinium groups) capable of interacting with nucleic acids. It is known that macrocyclic compounds containing analogous groups efficiently interact with biological substrates. It is therefore not surprising that many biomimetic receptors have been developed using synthetic receptor platforms, such as crown ethers, cryptands, porphyrins, calixarenes, thiacalixarenes and cyclodextrins.[6—8]

As a rule, macromolecules interact with nucleic acids by electrostatic and van der Waals interactions, hydrogen bonds, as well as by hydrophobic and π—π-stacking interactions.[9,10] Such intermolecular interactions make it possible to construct biomimetic supramolecular systems. Analysis of literature data showed[5,11,12] that the most efficient agents for transfection contain in the structure both hydrophobic and hydrophilic sections. Apart from that, functionalized macrocycles, cationic lipids, and polymers were shown to efficiently pack nucleic acids, with some of them being successfully involved in transfection of cells with the efficiency comparable with that of natural polyamines. It was found that the structure and the spatial orientation of functional groups (amino, ammonium, and guanidinium), as well as the length of the alkyl groups of the linker between charged fragments affect the efficiency of both the condensation and the transfection.

Though different functionalized macrocycles can efficiently interact with nucleic acids,[13—15] nonetheless, (thia)calixarenes functionalized at the lower rim seem the most promising as transfection agents. However, the data available at the present moment is insufficient for establishing the influence of the macrocyclic structure of (thia)calixarene on the efficiency of transfection and toxicity, that underlines the importance of further studies necessary for understanding of the structure-function relationship. In this connection, a labile macrocyclic platform of thiacalix[4]arene with a larger cavity as compared to the classic calix[4]arene possesses a greater potential for the development of synthetic transfection agents.

Thus, in the present work we systematized the data on the synthesis of potential agents on the platform of *p-tert*-

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