

Reviewer #3:

In this manuscript, the authors developed a method to synthesize 3rd generation dendrimers starting from thiocalixarene cores. Based on different core conformations, the final 3rd generation dendrimers also got different conformations: cone, partial cone, and 1,3-alternate. The authors investigated the effects of conformations on the dendrimer properties and found that the 3rd generation dendrimer originating from the 1,3-alternate core has the least hemolytic toxicity. Other information including polymer characterization and polymer-DNA complexation were also provided to prove that the polymer can successfully bind with DNAs.

The content in this manuscript is clear, detailed, and rigorous. Before accepting this manuscript, I recommend the authors answer the following questions:

Response:

Dear Reviewer! Thank you very much for carefully reading and reviewing our paper! We are very pleased that you have given it such a high rating.

Comment 1: Why was the calf thymus DNA selected in this study? Is it special when compared to other DNAs for example, plasmid DNA? Is this new PAMAM working on RNA binding and delivery?

Response: Our previous study [10.3390/ijms222111901] described the interaction of the first-generation **PAMAM-calix-dendrimers** with salmon sperm DNA. In the present study, we chose calf thymus DNA as having a significantly higher molecular mass. Thus, we have strongly proved the compactification of even high molecular weight DNA by **PAMAM-calix-dendrimers** into small size particles (up to 200 nm). The similar effect of our synthesized compounds on DNAs differing significantly in molecular weight gives us prerequisites for extrapolation of these properties to plasmid DNA as well, but we believe that this requires additional research. The effect of **PAMAM-calix-dendrimers** on RNA should also be the subject of a separate study, since the difference between RNA and DNA is quite significant. We are likely to observe binding of RNA by **PAMAM-calix-dendrimers** via electrostatic interactions, but we cannot yet predict this effect based on the present study. Overall, this is a very interesting work, and we plan to expand the range of research objects in the future. Thanks to the Reviewer for valuable advice.

Comment 2: Zeta potential is needed to evaluate the cationic content of the polymer or polymer/DNA complex.

Response: As requested by the Reviewer, we measured zeta potentials. The discussion of the obtained results has been added to the text:

The electrokinetic potentials of DNA complexes (10 μ M in nucleotide base pairs) with the third-generation **PAMAM-calix-dendrimers** indicated the stability of the formed associates (Table S4). The resulting complexes are generally positively charged (electrokinetic potential ranges from +30.5 to +40.0 mV), with the exception of complexes with very low concentrations of **PAMAM-calix-dendrimers** (1 μ M, range from –19.8 to –28.3 mV) (Figures S79–S90). This high positive charge on G3 **PAMAM-calix-dendrimers** without DNA (Table S4) put them on par with cationic polymers with excellent abilities in transfection [73].

A corresponding experimental section has also been added to the Electronic Supplementary Information

1.9. Electrokinetic Potentials.

Electrokinetic (ζ) potentials were determined by electrophoretic light scattering on the Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Samples were prepared for the DLS measurements and transferred with the syringe to the disposable folded capillary cell for measurement. The ζ potentials were measured using the Malvern M3-PALS method and averaged from five measurements.

Comment 3: The agarose gel electrophoresis (AGE) is an easy way to visualize the complexation ability of the polymers at different polymer/DNA (N/P) ratios (either molar or mass ratios). An example of AGE protocol can be found in this literature: Acta biomaterialia 9 (1), 4726-4733

Response:

In this study, the complexation of **PAMAM-calix-dendrimers** with DNA was strongly confirmed by UV-Vis, fluorescence and CD spectroscopy. The mechanism of interaction has also been hypothesized and proven. DNA compactization by **PAMAM-calix-dendrimers** was proven by DLS and TEM. The methods used definitely confirmed complexation and aggregate formation. We fully agree with the Reviewer about the importance of using agarose gel electrophoresis. According to the first recommendation of the reviewer, we plan to further study the interaction of **PAMAM-calix-dendrimers** with plasmid DNA and RNA using agarose gel electrophoresis. Once again, we thank the Reviewer for recommending the publication (Acta biomaterialia 9 (1), 4726-4733), which helped us to further evaluate the quality of our work. We were pleased to cite it in the manuscript.