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Reviewer: Jiří Míšek, PhD.

Thesis Title: **Synthesis of novel helquats and their properties**

The submitted thesis deals with the development of the library of compounds based on novel helical dicationic scaffolds called helquats and the subsequent screening of the helquat library in order to discover new nonlinear optics materials and fluorescent probes for biomedically relevant analytes. The thesis is split into six sections.

Introduction covers the most important areas of this multidisciplinary project. It is written in a concise and informative way. The introduction section on cationic dyes contains a number of structures of dyes and fluorophores along with their applications however no spectral characteristics are provided. The reviewer would appreciate at least a brief discussion of structure-spectral characteristics properties, as the understanding of how structure of organic molecules is related to spectral properties is essential for design of new functional small molecule sensors. Also, in the section describing small molecules binding in the minor groove of DNA I would expect mentioning the name of Peter Dervan as he has had a major contribution to this area with his pyrrole-imidazole polyamides. There is also a number abbreviation (e.g. DDQ, CBF_4, DMAP, CPL, HPLC etc.) that appears in the text without a corresponding explanation in the abbreviation section. Page 37 contains a structure of heparin orange that is incorrect.

Aims of the thesis are clearly stated. The following section Results and discussion is split into two subsections. The first describes synthesis of helquats and their derivatisation. The second subsection is about applications of the prepared library. The author must have put a considerable effort to synthesize and purify the extensive library of cationic organic molecules that is surely not a trivial task. It is stated that more than 500 derivatives were synthesized and screened however Experimental section contains characterization of less then 100 compounds. It is thus not clear whether the rest of the library was synthesized by someone else or they were not all included in the thesis. I was personally intrigued with the result related to the DNA fluorescent sensor called Helidye1 and the subsequent structure-

activity relationship. It us truly remarkable how a very small structural changes can affect binding and spectral properties of the dye. Also, interesting phenomenon is helical chirality of the dye that is obviously essential for the function.

The section Conclusion is to the point and Experimental part is also well organized with complete characterization of compounds mentioned in the text of the thesis. The last section References and notes suffers from just minor inconsistencies (e.g. reference 186 on page 69 is provided for therapeutic levels of heparin. The actual reference is on quantum yield measurements.)

Overall, Vishwas D. Joshi proved that he can conduct high level research and write about it and thus I have no reservation to recommend his thesis for the defense.

Questions:

1. How do you explain the observation from Table 1 that increasing reaction temperature suppressed the formation of the side product **3s**.

2. Preparation of compound **5** was accompanied with difficulties with the second alkylation of methylpyridine ring. Provided explanation suspects the steric hindrance of methylpyridyl unit. However alkylation of compound **7** with two methylpyridine rings proceeded smoothly. Can you please explain this observation?

3. Quantum yield of HeliDye1 in the presence of DNA was estimated to be around 3%. DAPI fluorescent probe has a quantum yield in the presence of DNA around 90%.[1] Given the similar extinction coefficient of both dyes, how do you explain both dyes seem to glow with same intensity in Figure 74A first column?

In Prague, January 15, 2018

Jiří Míšek

[1] Kapuscinki J. et al. Nucleic Acid Research **1978**, *5*, 3775.