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SUMMIT
SOFIA UNIVERSITY MARKING MOMENTUM FOR INNOVATION AND TECHNOLOGICAL TRANSFER

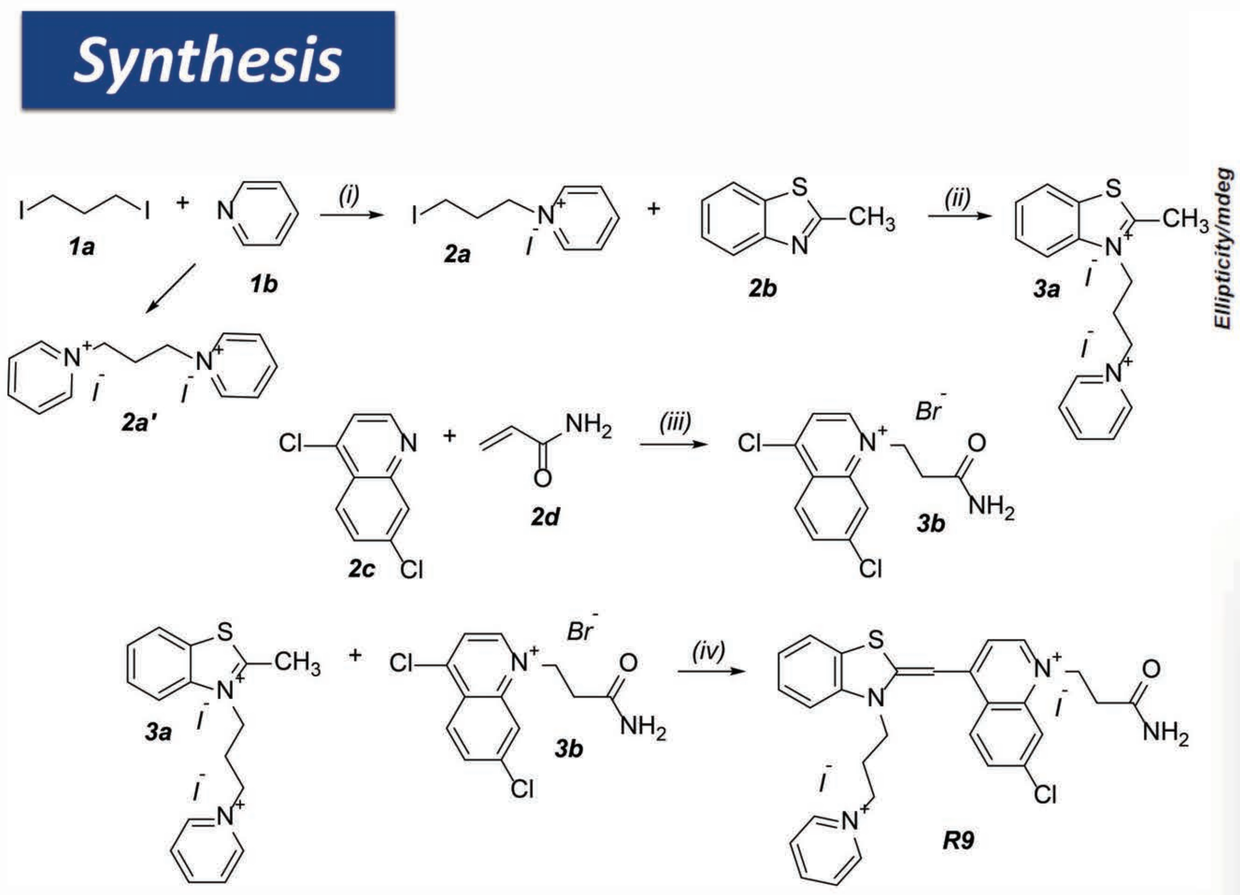
SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

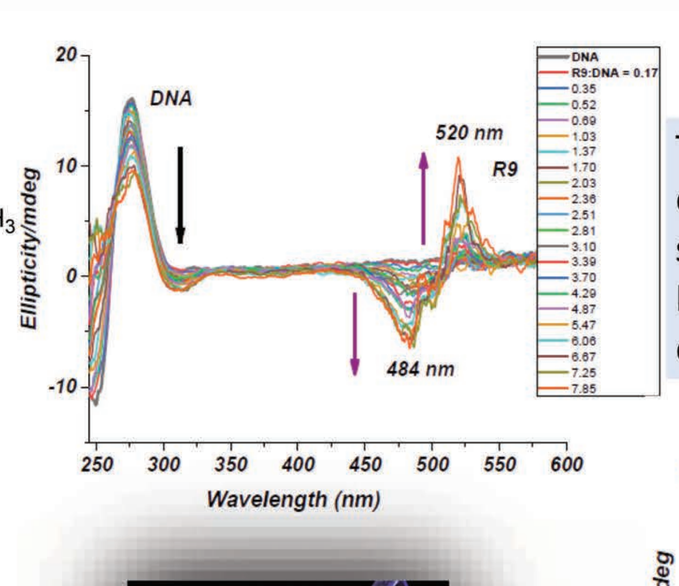
Nanoconfined Monomethine Cyanine Dye – Fluorogenic Probe for Cells Staining and Nucleic Acids Visualization

The development of fluorescence-based methods for bioassays and medical diagnostics requires the design and synthesis of specific markers for targeting biological micro objects. However, biomolecular recognition in real cellular systems is not always as selective as desired. **A new concept for creating fluorescent biomolecular probes is demonstrated, utilizing a fluorogenic dye and biodegradable, biocompatible nanomaterials.** The synthesis of a new dicationic asymmetric monomethine cyanine dye with benzo[d]thiazolium-N-propionamide and a chloroquinoline end groups is presented. The photophysical properties of the newly synthesized dye have been examined by the combined application of spectroscopic and theoretical methods. The applicability of the dye as a fluorogenic nucleic acid probe has been proven by UV-VIS spectroscopy and fluorescence titration. The dye–nucleic acid interaction mode was investigated by UV-Vis and CD spectroscopy. The newly synthesized dicationic dye, like other similar fluorogenic structures, has limited permeability, which restricts its use as a probe for RNA and DNA. To enhance cellular delivery, we utilized a patented technology that employs solid, insoluble lipid nanoparticles. This method ensures the complete introduction of the dye into cells while minimizing activity outside the cells. In our study involving two human cell lines, we observed improved penetration through the cell membrane and distinctive selectivity in visualizing nucleic acids within the cytoplasm and nucleus.

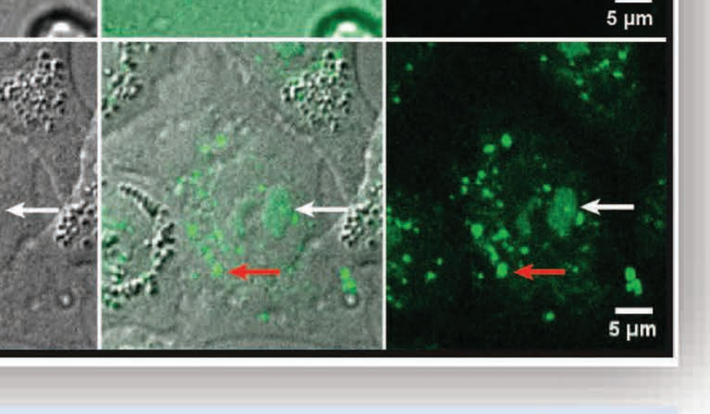
Synthesis

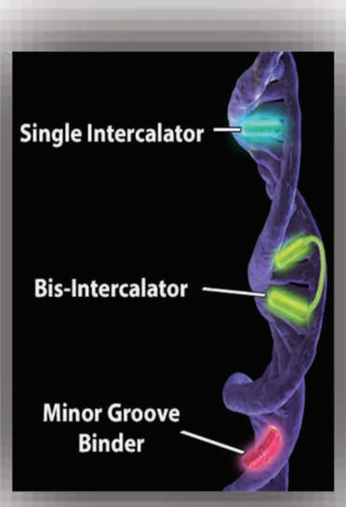


Circular Dichroism

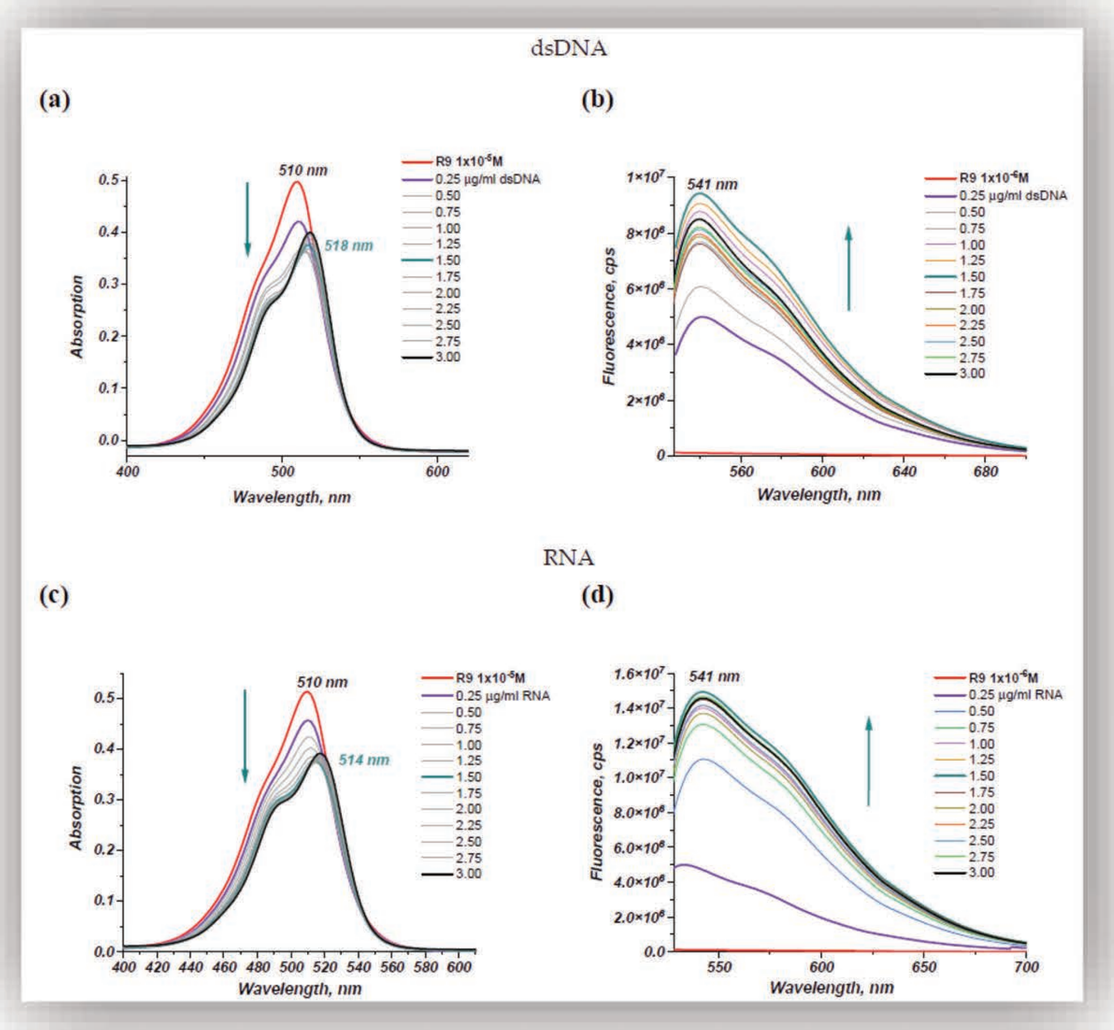


The **R9** dye molecule is achiral and does not exhibit an intrinsic CD spectrum, but in the presence of DNA and RNA an induced circular dichroism (ICD) peak is observed.





Interaction of R9 dye with DNA and RNA



Cellular Dye Uptake

Fate of CellInject lipid particle system, loaded with dye **R9**, on *HeLa Kyoto* cells.

Faculty of Chemistry and Pharmacy

Head of the research group
Prof. Sonia Ilieva
Members of the group
Assoc. Prof. Aleksey Vasilev
Assoc. Prof. Christo Tzachev
Assoc. Prof. Diana Cheshmedzhieva
Assistant Stefaniya Gaydarova

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