Faculty of Science
CHARLES UNIVERSITY

Department of
ORGANIC CHEMISTRY

Prague, August 11, 2022

Subject: PhD Thesis Report

Author: Miroslav Kuba
Reviewer: Jiří Míšek, Ph.D.

Thesis Title: Novel Fluorescent Nucleotides for Metabolic Labelling and for the Construction of DNA Probes

As the title indicates, the thesis aim was to develop synthetic nucleosides and nucleotides for fluorescent labelling of DNA in vitro and in vivo. In particular, in vivo metabolic fluorogenic labelling of DNA would represent a major achievement in the field, as it would allow on-line monitoring of DNA synthesis and subsequent changes with spatiotemporal resolution.

The author started the quest with the preparation of synthetic deoxycytidine with a modification based on a mimic of a fluorescent protein fluorophore. The synthetic nucleotide was incorporated into DNA by enzymatic means. This incorporation was associated with a marked increase in fluorescence. Experiments with modified ssDNA and protein binders showed only modest response in the emission spectra. Cell experiments were not performed. In further experiments, four more fluorescent modifications of nucleotides were successfully synthesized and tested. The xanthylium-derived nucleotide showed a spectacular increase in fluorescence upon incorporation into DNA. This characteristic was utilized in the development of a new protocol for RT-PCR. Unfortunately, this nucleotide was too toxic for the cellular assay.

Next, a nucleotide modification based on known DNA-binding fluorophore thiazole orange was prepared. Interestingly, cellular experiments have shown that the modified nucleotide incorporates into DNA with the marked change in fluorescence lifetime compared to the free nucleotide. This characteristic has been successfully exploited for real-time fluorescence lifetime imaging of DNA synthesis in living cells. This discovery provides a new tool for molecular biology with a potentially broad range of applications.

The red-emitting BODIPY fluorophore was usedas another fluorescent modification. However, this modification turned out to have less favorable photophysical and biological characteristics for the intended applications. The last fluorescent modification explored was the silicon rhodamine dye that is compatible with high-resolution fluorescent microscopy techniques. Indeed, this fluorescent nucleotide triphosphate enabled high resolution imaging of genomic DNA in cells.

Department of Organic Chemistry
Faculty of Science
Charles University
Hlavova 8, Prague 2, 128 43, Czech Republic

Assist. Prof. Jiří Míšek, Ph.D.
misek@natur.cuni.cz
T: +420 221 951 578

IČO: 00216208
DIČ: CZ00216208

orgchem@natur.cuni.cz

The thesis itself is well written in good English, concise and comprehensive. The experimental section is thorough and documents the fact that the author carried out majority of the experiments that spans from organic synthesis to biochemistry and molecular biology. Thus, it is clear that the author gained a substantial amount of knowledge and skills in the field of organic chemistry, chemical biology and even photophysics of organic molecules during his PhD work. Therefore, I undoubtedly recommend this thesis for the defense.

Questions:

1. Chromophore **34** and its nucleoside derivative dC^{Trp} contain double bond that readily photoisomerizes under daylight. The corresponding triphosphate $dC^{Trp}TP$ does not show this beaviour. What is the rational for the observed difference? Can xanthylium derivative **43** also isomerize around exocyclic double bond?

2. What is the explanation for the marked increase in fluorescence of xanthylium derivative $dT^{NNIR}TP$ upon incorporation in DNA?

3. Xanthylium and thiazole orange fluorophores are cationic, however the nature of the corresponding anion is not revealed neither in the theoretical nor in the experimental section. Do you know what the anion is?

Jiří Míšek, Ph.D.

Department of Organic Chemistry
Faculty of Science
Charles University
Hlavova 8, Prague 2, 128 43, Czech Republic

Assist. Prof. Jiří Míšek, Ph.D.
misek@natur.cuni.cz
T: +420 221 951 578

IČO: 00216208
DIČ: CZ00216208

orgchem@natur.cuni.cz