Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Supervisor: doc. PharmDr. Miroslav Miletín, Ph.D.

Consultant: Mgr. Michaela Beranová

Student: Petra Grossová

Title of Thesis: Preparation of conjugates oligonucleotide probes with functional molecules

The aim of this thesis was to find the optimal conditions for conjugation of an oligonucleotide to a fluorescent dye by solid-phase click chemistry. The traditional fluorescent dye Cyanine5 was chosen as the labelling molecule. The effect of the concentration of fluorescent dye, the position of the labelling, the type of solid phase and the reaction time were examined. The deprotection conditions were optimized.

Two series of reactions followed by deprotection and purification were performed. The efficiency of the reaction was evaluated by comparing the peak areas under the curve of conjugated and unconjugated molecules in the chromatograms of the samples after analysis by HCPL with UV detection. Peak integration was performed in LabSolutions software.

The reactions running on the polystyrene solid phase gave the highest yields, with labelling at any position of the chain. For labelling at the 5´-end of the chain, more than 90% efficiency was achieved using all tested solid phases. Furthermore, it was found that a concentration of fluorescent dye 100× lower than that is commonly used was sufficient for the click reaction. The reaction time can be significantly shorter than the standard 24 hours. The reaction reached 94% yield after 10 minutes (5´ CPG500Å, 1mM Cy5), however maximum yield is reached between 1-12 hours. For a more specific determination, a more thorough kinetic study is needed.

Key words: oligonucleotide probes, fluorescent dye, click chemistry