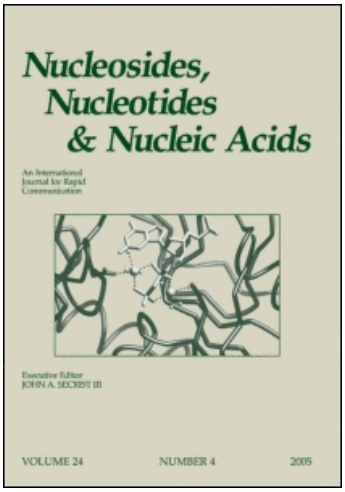

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A Convenient Method for Labeling Oligonucleotide Analogs by a Fluorogenic Tag for Direct In Vitro and In Vivo Visualization of Carboxyesterase Activity

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A CONVENIENT METHOD FOR LABELING OLIGONUCLEOTIDE ANALOGS BY A FLUOROGENIC TAG FOR DIRECT IN VITRO AND IN VIVO VISUALIZATION OF CARBOXYESTERASE ACTIVITY

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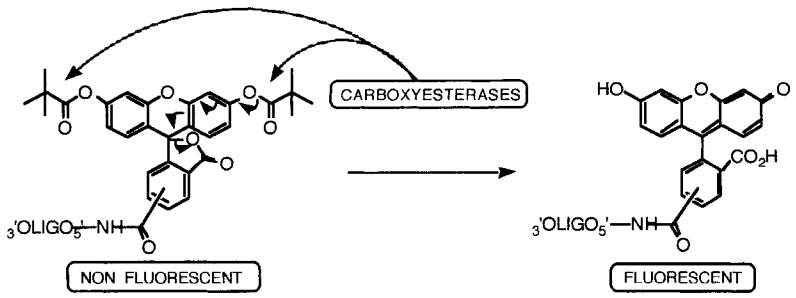
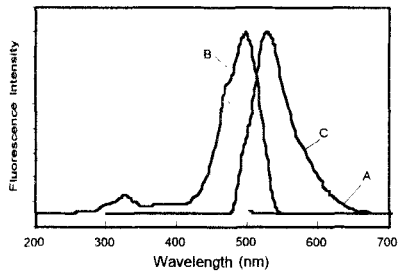
ABSTRACT: The synthesis of two oligonucleoside phosphorothioate and methylphosphonate analogs functionalized with a fluorogenic tag is described. The fluorescent conjugate formation is demonstrated in cell culture medium, cell extracts and in human fibroblasts.

The prooligonucleotide concept has been extensively developped during these last years[1,2]. In this approach, a step of activation by cellular esterases, in particular carboxyesterases, is necessary for intracellular delivery of active oligos. In the aim of monitoring and locating carboxyesterase activity in the cell, a method of labeling oligos with a fluorogenic tag has been designed.

In this study, the two phenolic functions of the carboxyfluorescein were protected by pivaloyl groups yielding a non fluorescent lactone derivative. Cleavage of the ester groups by the carboxyesterases would induce lactone opening to the fluorescent xanthen-3-one tautomer.



Two nuclease-resistant phosphorothioate (PS^-) and methylphosphonate ($P\text{-}CH_3$) oligonucleotide 18-mers complementary to the *c-myc* oncogene mRNA were attached to the diprotected fluorescein derivative via an amino linker at the 5'-end of the oligomer[3]. The fluorogenic conjugate formation was performed in buffer solution at pH 9[4] and the labeled oligos were purified by HPLC. They were characterized by ES (PS^-) or MALDI-TOF ($P\text{-}CH_3$) mass spectrometry.



(A) emission spectrum of the fluorogenic conjugate (PS^-) before incubation in cell extracts; B) excitation and (C) emission spectra of the conjugate (PS^-) upon incubation for 15h in cell extracts

The two tagged oligos were incubated with the pig liver esterase (PLE), in culture medium (CM), in total CEM cell extract (TCE) and the stability of the pivaloyl groups was evaluated by spectrofluorometry. The excitation and emission spectra of oligonucleotide conjugates have shown the fluorogenic substrate becoming fluorescent upon carboxyesterase mediated hydrolysis in the different studied mediums.

The labeled oligo (PS^-) was microinjected in the cytoplasm of human Hs68 fibroblasts and fluorescence microscopy studies were performed. The conjugate rapidly migrated into the nucleus .The persistence of the fluorescence in the nucleus after 16 hours indicated the stability of the link between the fluorescein dye and the oligonucleotide.

The designed fluorogenic probe could be useful to investigate the uptake and the behavior of the prooligonucleotide inside the cells.

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