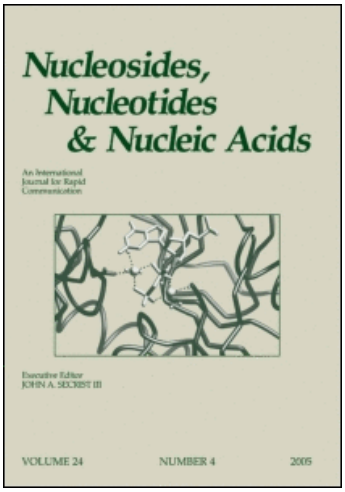

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Synthesis and Cellular Uptake of Fluorescently Labeled Oligodeoxynucleotide Prodrugs

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SYNTHESIS AND CELLULAR UPTAKE OF FLUORESCENTLY LABELED OLIGODEOXYNUCLEOTIDE PRODRUGS.

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ABSTRACT: In this paper we described the synthesis on solid support of 5'-fluorescein labeled dodecathymidylates having internucleoside phosphodiester or phosphorothioate linkages temporary masked with enzymolabile S-pivaloyl-2-thioethyl (tBuSATE) groups.

One of the possible ways to enhance the cellular uptake of ODNs is to reduce the number of negative charges that are associated with internucleoside linkages present in the backbone. After intracellular delivery, pro-ODNs may be converted by cellular esterases to parent ODNs able to activate RNase H.

We report a preparation and cellular uptake data of five fluorescent pro-dodecathymidylates, having a number of tBuSATE phosphotriester or thionophosphotriester linkages ranging from four to eight.

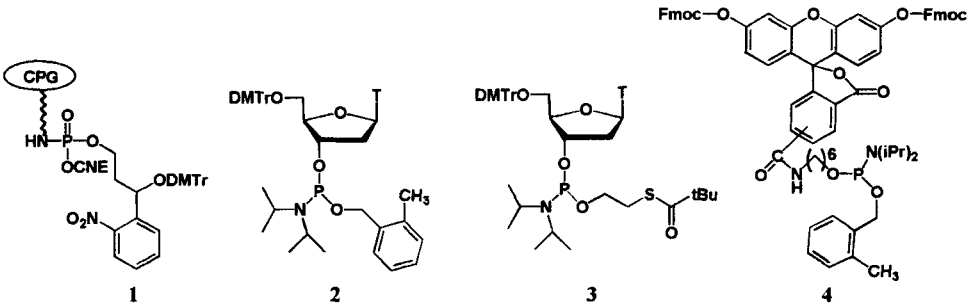

FIG.1: Building blocks and photolabile solid support.

Due to the lability of SATE masking groups in strong basic or nucleophilic conditions, a solid phase synthesis was developped which included the use of photolabile solid support[1] **1**, thymidine 3'-O-phosphoramidite having a tBuSATE group[2] **3** or an *o*-methylbenzyl group **2** in place of the regular 2-cyanoethyl one and Fmoc-protected carboxyfluorescein phosphoramidite derivative **4 (FIG.1)**. During the aqueous iodine oxidation steps, *o*-methylbenzyl group was selectively removed yielding phosphodiester linkages[3]. Removal of Fmoc protecting groups was achieved upon short DBU treatment[4] of supported pro-ODN. Pro-ODN was cleaved from the solid support upon U.V. irradiation.

The capacity of these pro-ODNs to be taken up, in absence of any helper, by HeLa cells was investigated by fluorescence microscopy.

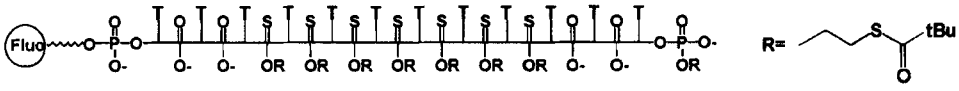

FIG.2: Pro-dodecathymidylate containing eight tBuSATE masking groups.

The most lipophilic pro-dodecathymidylate (**FIG.2**) was found to be the more efficiently taken up by HeLa cells after 15-30 min incubation at 37°C. These observations applied also to the four other pro-ODNs but to a lesser extent. It appears that uptake depends on several factors including the number and distribution of tBuSATE masking groups, aqueous solubility, structure of the triester internucleoside links and lipophilicity. Mechanism(s) involved for the internalization of these oligonucleotide prodrugs is(are) currently under investigation.

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