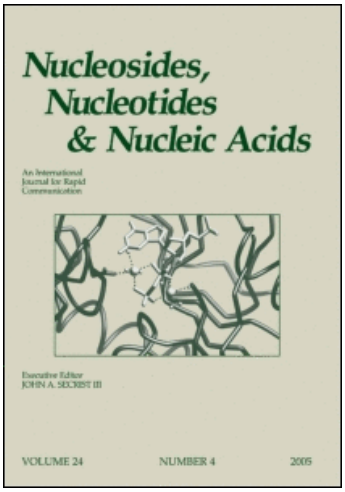

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PNA CONJUGATED TO HIGH-MOLECULAR WEIGHT POLY(ETHYLENE GLYCOL): SYNTHESIS AND PROPERTIES

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□ *The conjugation of a bioactive, fluorescent PNA sequence to high-molecular weight poly(ethylene glycol) (PEG) is described and the properties of the PEG-PNA conjugate are evaluated.*

Keywords Peptide nucleic acids (PNA); oligonucleotide; conjugates; poly(ethylene glycol) (PEG)

Peptide nucleic acids (PNAs) are able to recognize and bind to a specific DNA or RNA strand with high affinity and selectivity, superior to analogous oligonucleotide sequences. These derivatives have high chemical stability, and their resistance to nucleases and proteases make them good candidates as therapeutic agents, diagnostic tools, and probes in molecular biology.[1] However, a practical limitation is their low water solubility and high tendency toward self-aggregation. Moreover, a facile and useful system of delivery is still demanded and several methods have been proposed to improve their cellular uptake, as peptides,[2] cationic liposomes,[3] and lipophilic ligands.[4] In this regard, the conjugation of PNAs with the amphiphilic, atoxic, and largely soluble poly(ethylene glycol)s (PEGs) can be evaluated to increase their solubility in aqueous media, as well as to improve their cell membrane permeability and resistance to enzymatic degradation, using the same approach previously described for oligonucleotides.[5]

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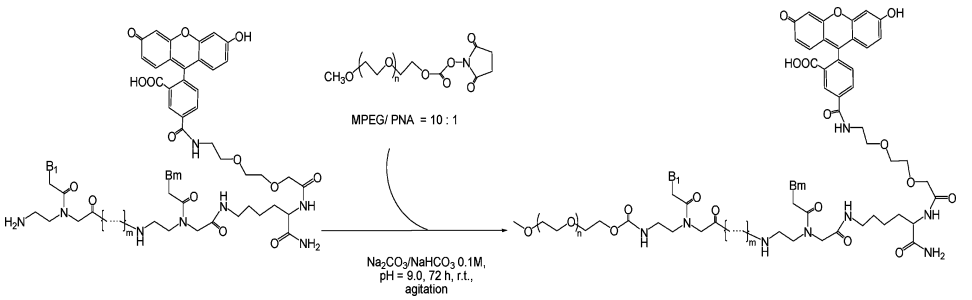

FIGURE 1 Scheme of the synthesis of $mPEG_{5000}$-PNA-Fluo (B_1 to B_m = nucleobases, Fluo = Fluorescein).

Recently, we have optimized the reaction for the conjugation of oligonucleotides to large molecular weight PEG in solution phase, even on a low synthetic scale.[6] The best conditions were first set up on a model dimer of PNA; subsequently, a 17mer PNA of biological interest was prepared by solid phase synthesis and labelled at the *C*-terminus with fluorescein.

Following the established procedure, this PNA was conjugated, in a milligram scale, to high-molecular weight mPEG as reported in Figure 1.

The Fluo-PNA was prepared through a classical solid-phase procedure on a MBHA resin and reverse-phase purification. We designed a PNA in which the fluorescein derivative could be introduced during the solid-phase synthesis using an automated peptide synthesizer. Therefore, a Fmoc-(Boc)L-lysine residue was introduced at the *C*-terminus and the α-group was selectively deprotected and coupled with the Fmoc-protected 2-(2-aminoethoxy)ethoxyacetic acid (AEEA) spacer. After Fmoc removal, carboxyfluorescein was coupled with the terminal amino group of the spacer. The synthesis of the PNA part was then carried out starting from the ε-amino group of lysine using Boc chemistry, without capping steps, in order to avoid the acetylation of fluorescein. The purification was performed using RP-HPLC. The low solubility and the high lipophilicity of this derivative allowed isolation of only a small amount (1.7 mg, 6% yield after purification).

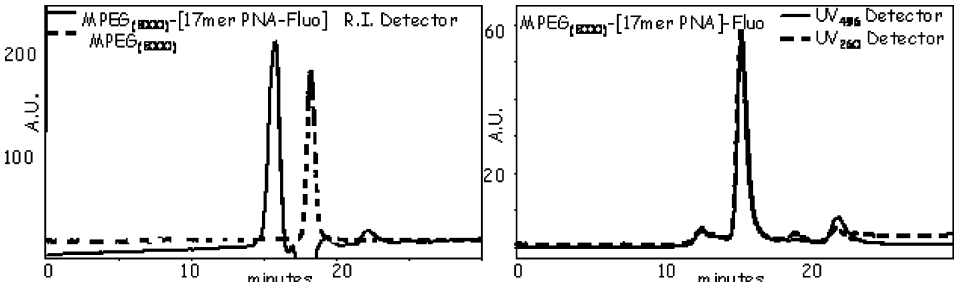

FIGURE 2 GPC analysis of $mPEG_{5000}$-PNA-Fluo.

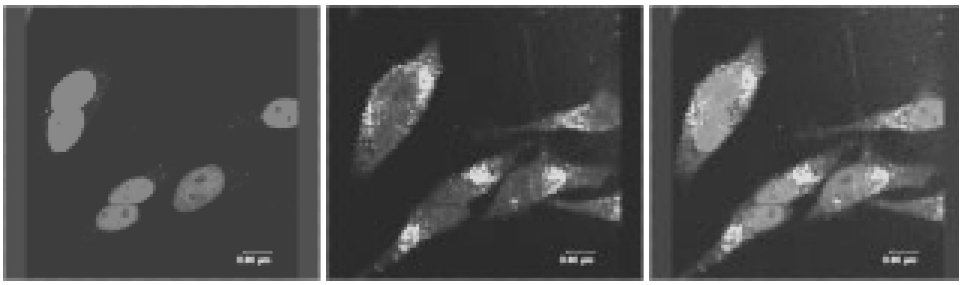

FIGURE 3 Confocal microscopy images of HeLa cells treated with $mPEG_{5000}$-PNA-Fluo.

The purified, fluorescent PNA 17mer was conjugated in a water solution buffered at pH 9.0, containg 25% acetonitrile, with a 10-fold excess of $mPEG_{5000}$ (M.W. = 5000 Da) activated with an N-hydroxysuccinyl moiety, leading to the formation of a final urethane bond. After the reaction, an almost complete solubilization of the starting, insoluble PNA was observed, confirming the solubilizing effect of the linked PEG chain. The GPC analysis of the recovered sample confirmed the efficiency of the PEGylation procedure; the use of the refractive index and the UV absorbance allowed recognition of both PEG and Fluo-PNA components in the same final chromatographic peak (Figure 2). The residual, unreacted PEG could be effectively removed using gel-filtration chromatography, as demonstrated by the large difference observed between the retention times of starting PEG and final PEG-PNA.

The capacity of this new $mPEG_{5000}$-PNA-Fluo to penetrate the cellular membrane was investigated in HeLa cells, using confocal laser microscopy. Figure 3 shows the images obtained with HeLa cells treated for 24 h with 1 μM $mPEG_{5000}$-PNA-Fluo. The panels (from left to right) show the nuclei stained with propidium iodide, the green fluorescence emitted by the conjugated PNA, and a superimposed image displaying both red and green fluorescence light. It can be seen that the PEGylated PNA localizes mainly in the cytoplasm, but to a lesser extent also in the nucleus. We observed that the non-PEGylated PNA analog also displays the capacity to enter into HeLa cells, but the image obtained shows a spotted distribution, probably due to the fact that the PNA alone strongly self-aggregates (not shown). Detailed analyses of the uptake properties of PEGylated PNAs will be reported elsewhere.

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