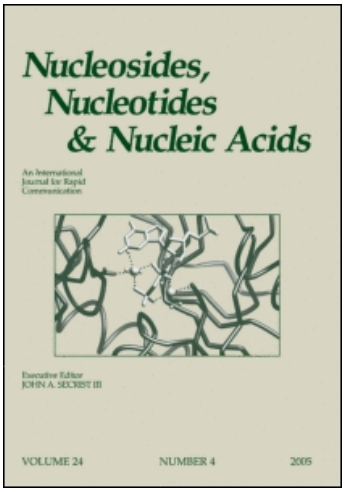

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Synthesis and Biological Evaluation of Nucleoside Triphosphates Incorporating an Oxyamino Function for "Post-amplification Labelling"

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NUCLEOSIDE TRIPHOSPHATES INCORPORATING AN OXYAMINO FUNCTION FOR "POST-AMPLIFICATION LABELLING"

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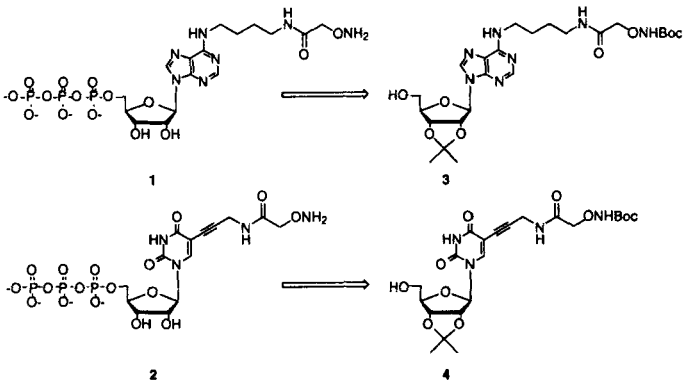
ABSTRACT: We report a novel strategy for Post-Amplification Labelling based upon coupling nucleotide incorporating an oxyamino function with a fluorophore bearing an aldehydic group.

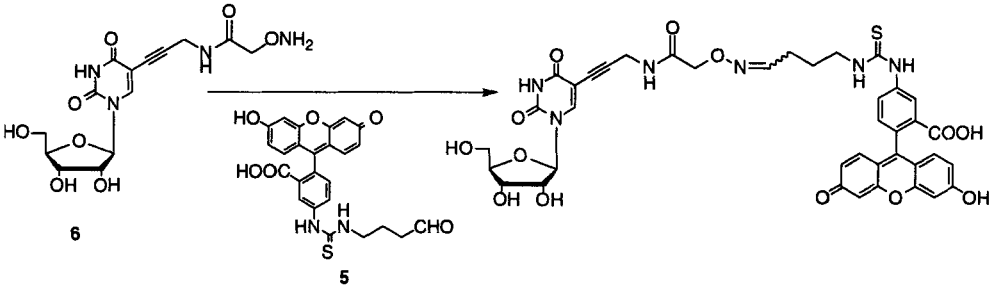
Recent progress in nucleic acids detection for diagnostic purposes have clearly established the importance of labeling strategy to reach high sensitivity and specificity. In combination with nucleic acid amplification techniques, very powerful diagnostic assays have been developed. One of the most commonly applied systems involves incorporation of nucleotides bearing reporter groups (fluorescent, luminescent). However, these bulky reporter groups can affect the amplification efficiency and specificity.

A new strategy called "Post-Amplification Labelling" has been developed as an alternative procedure for labeling amplified RNA target without affecting the amplification



efficiency and incorporation fidelity. In this approach activated nucleotides are reacted with a fluorescent label after their incorporation. In our strategy, the coupling reaction involves nucleotides (**1**, **2**) containing the reactive oxyamino group and an aldehyde linked to a fluorescent label[1].

The triphosphate derivatives **1** and **2** were prepared from the suitably protected nucleosides, respectively **3** and **4** using Eckstein's procedure[4]. The efficiency and the selectivity of the coupling reaction were demonstrated at the nucleoside level. Reaction of the aldehydic fluorescent label **5**, prepared in two steps from fluorescein isothiocyanate, with the unprotected nucleoside **6** afforded quantitatively the corresponding oxime ether.



6 **5**

Incorporation of the modified nucleotide **2** was studied using T7 RNA polymerase with various amounts of the nucleotide. HPLC analysis following enzymatic digestion (P1 nuclease and alkaline phosphatase) of the transcripts indicated that the oxyamino triphosphate was quantitatively incorporated. The oxyamino modified transcripts formed reacted with high efficiency with the fluorescent label **5**. Using the GeneChip BIOMER-16S, antisense probe array (Affymetrix, Santa Clara, CA), resequencing of 16S rRNA of Mycobacteria was achieved with a sequence base-call accuracy for the analysed region equal to 99.4%.

In conclusion the oxyamino and aldehyde functions appear to be good candidates for the efficient coupling reaction required for post-amplification-labelling.

REFERENCES

(1) The high efficiency and selectivity of such oxyamino-aldehyde coupling reaction has been demonstrated at the nucleoside and oligonucleotide levels[2,3].

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