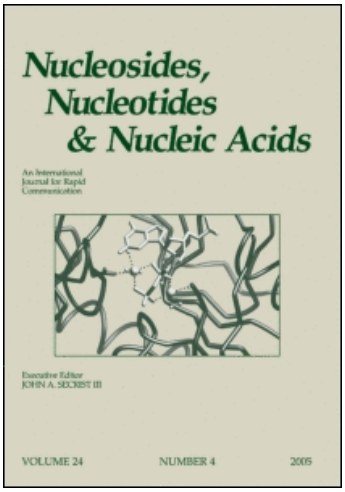

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NOVEL FLUOROPHORES FOR LABELING OF NUCLEOSIDES AND OLIGONUCLEOTIDES

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□ *Five novel fluorophores have been synthesized and their comparative fluorescence has been studied in different organic solvents and aqueous solutions of inorganic ions. Out of these, two highly sensitive fluorophores have been used to label phosphoramidites and oligodeoxyribonucleotides. The fluorescently labelled amidites and oligodeoxyribonucleotides showed good fluorescence signals.*

Keywords Fluorophores; nucleosides; oligonucleotides

INTRODUCTION

Fluorescent probes are now in widespread use in various formats of DNA/RNA assays, which exploit the high affinity and specificity of nucleic acid hybridization and/or the possibility of target amplification.[1,2]

Fluorescent molecules can be covalently attached to oligonucleotides[3,4] by various enzymatic[5] or chemical methods[6,7] through various active sites on bases, sugars (3′, 5′) or phosphate units. We have synthesized five novel fluorophores, viz. 6-(6-isobutyrylamino-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid (**1**), 6-(6-dimethyl-amino-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid (**2**), 6-(6-benzoyl amino-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid (**3**), 6-(6-amino-1-oxo-1H, 3H-benzo[de]isoquinolin-2-yl)-hexanoic acid (**4**) and 6-(6-amino-1H,3H-benzo[de] isoquinolin-2-yl)-hexanoic acid (**5**) and labelled nucleosides, bearing primary aliphatic amino group at C-4/C-5 position of bases and oligodeoxyribonucleotides using fluorescent amidites.

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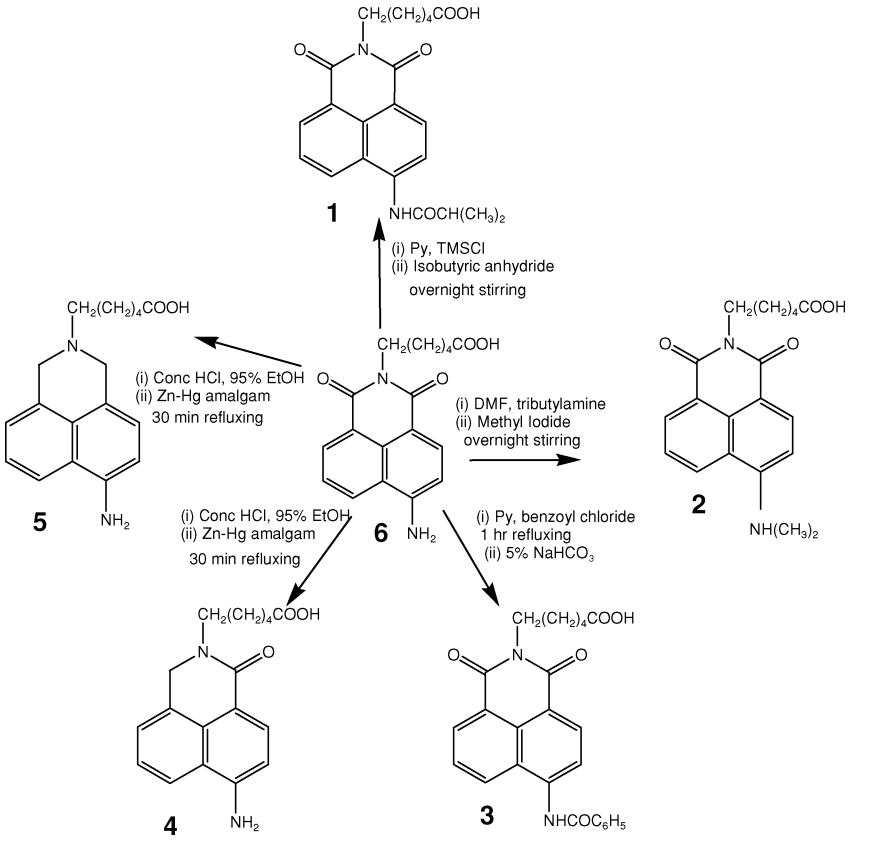

FIGURE 1 Synthesis of fluorophores 1–5.

RESULTS AND DISCUSSION

The present work is an effort towards the development of highly sensitive fluorophores, **1–5** (Figure 1). We have chosen **1** and **2**, because of their high sensitivity, for labelling of nucleosides, amidites and oligodeoxyribonucleotides. The fluorescence of **1** and **2** was recorded in organic and inorganic media, like dioxane, methanol-water (50:50) and 1 M aqueous solution of NaCl, KCl, $MgSO_4$ and $NaHCO_3$. Fluorophore **1** showed higher degree of fluorescence in inorganic media than **2**, and hence it may be a better choice for biological systems. For recording fluorescence of phosphoramidites of these labelled nucleosides (10 μM solutions), excitation wavelengths were fixed at 397 nm for 2′-deoxyuridine and 368 nm for 2′-deoxycytidine, respectively. The emissions were recorded at 465 nm. Phosphoramidite of labelled 2′-deoxycytidine showed higher fluorescence than phosphoramidite of labelled 2′-deoxyuridine. In general, phosphoramidites have shown higher sensitivity than labelled nucleosides.

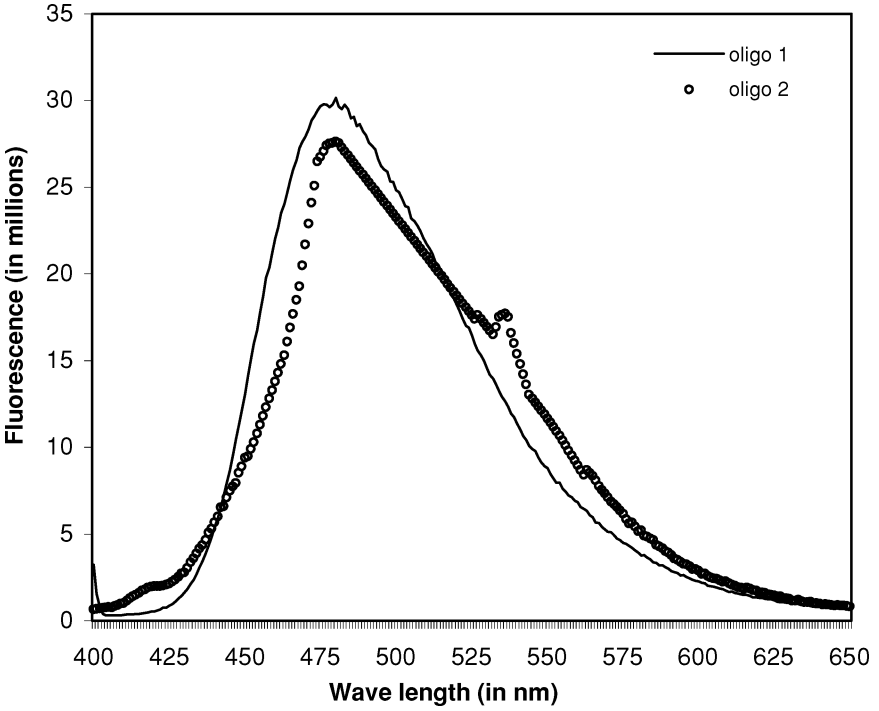

FIGURE 2 Emission spectra of labelled oligonucleotides.

Fluorescence of both the labelled oligonucleotides, at 0.03 OD each, was studied in phosphate buffer (0.01 M; pH 7.1). d(U*GTGGGTTAAGA), oligo-1 and d(C* CTTAACCCACT), oligo-2 were excited at 397 nm and 368 nm, respectively and scanned in the range of 400–650 nm. These labeled oligonucleotides have shown an appreciable degree of fluorescence (Figure 2) and hence can be used for various purposes in molecular biology.

EXPERIMENTAL

Synthesis of Labeled Nucleosides and Phosphoramidites

The fluorophores **1–5** were synthesized using standard chemistry as shown in Figure 1 from compound **6**, synthesis of which has been reported earlier by us.[7] Compound 5′-*O*-dimethoxytrityl-4-*N*-(tris-4, 9, 13-triazatridecane-1-yl)-2′-deoxycytidine was synthesized according to a published procedure[8] in 74% yield.

The fluorophores were attached to nucleosides bearing linker arms.[9] The p-nitrophenol activated fluorophores **1** and **2** (311 mg, 1 mmol each), were added to nucleosides (1 mmol each) suspended in DMF (5 mL) separately and stirred overnight. The products formed were purified

chromatographically. The fluorescently labelled nucleosides were finally converted to their respective phosphoramidites[10] in 60–65% yield using standard protocols.

Synthesis of Labeled Oligodeoxyribonucleotides

The oligonucleotides have been synthesized on Pharmacia LKB Gene Assembler Plus on 0.20 μmol scale using standard protocols. The DMT group from LCAA-CPG attached 11-mer, DMT-d(GTGGGTTAAGA) was removed using dichloroacetic acid in DCM. The labeled phosphoramidite was added onto the column. The coupling yield of this step was 98.9% as detected by trityl analysis.[9] This phosphite-triester was oxidized to phosphotriester using iodine in THF, 2,6-lutidine and water. The fluorescently labelled oligonucleotide, d(U*GTGGGTTAAGA), was delinked from the LCAA-CPG support by ammonia treatment and purified on reversed phase HPLC using 0.1 M triethylammonium acetate and acetonitrile. The above procedure was repeated with support bound DMT-d(CTTAACCCACT) using the respective amidite to synthesize fluorescently labelled oligonucleotide, d(C*CTTAACCCACT).

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