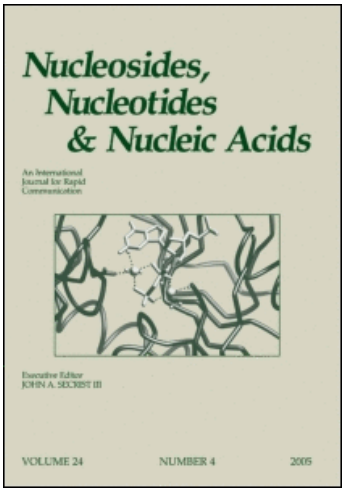

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Synthesis and Properties of New Fluorescein-Labeled Oligonucleotides

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SYNTHESIS AND PROPERTIES OF NEW FLUORESCEIN - LABELED OLIGONUCLEOTIDES

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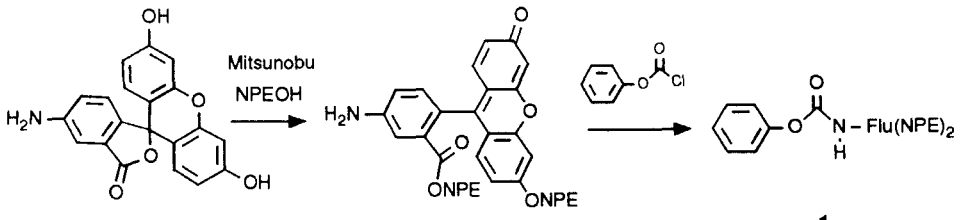
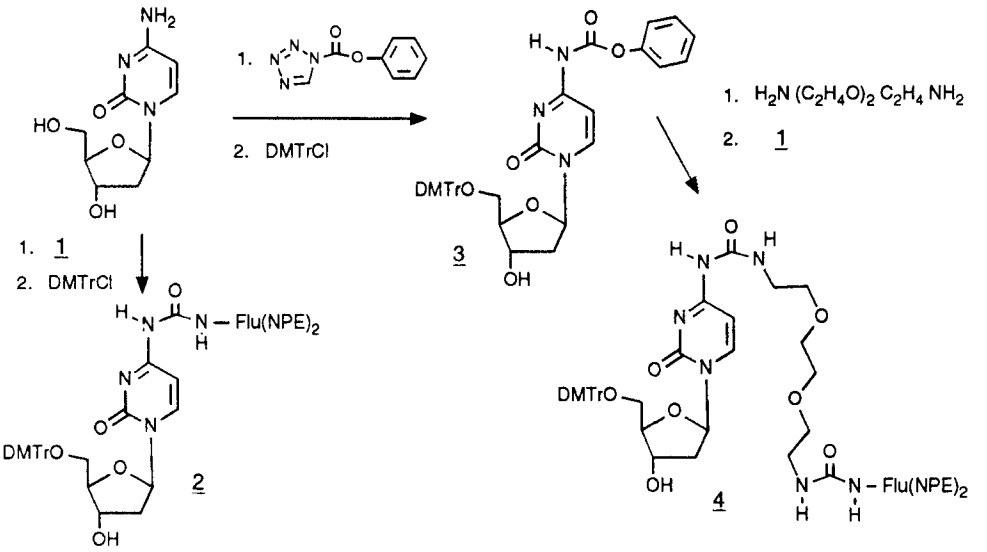
Abstract: 2,4-Dinitroaniline is an efficient intramolecular fluorescence-quencher for fluorescein - labeled oligonucleotides and interacts with the heterocyclic bases on duplex formation. Consequently, intramolecular fluorescence quenching is disturbed in double labeled oligonucleotides of this type, and fluorescein shows strong fluorescence in a duplex form. There is a substential increase of the fluorescence-quantum yield when the marker and quencher is attached to a single guanosine residue. Two kinds of doubly labeled oligonucleotides have been synthesized, using the NPE/NPEOC strategy.

Detection of oligonucleotides plays an important role in biochemistry or in medical diagnostics and has recently been practised by means of fluorescence spectroscopy due to the high sensitivity of this method. The fluorescence anisotropy value increases, when the labeled oligonucleotide forms a duplex.[1,2,3] On the other hand, this paper describes a characteristic increase of the fluorescence quantum yield by hybridisation of a labeled oligonucleotide with a target sequence.



Scheme 1



Scheme 2

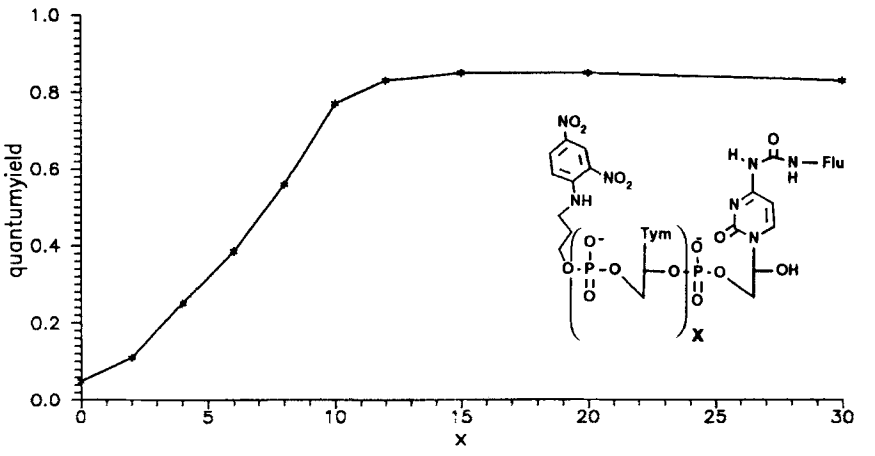
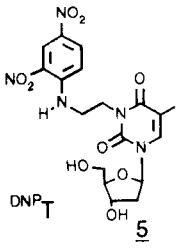


Figure 1: Fluorescence quantum yield relative to fluorescein (1.0) at pH 8

Table 1: Fluorescence quantum yield relative to fluorescein (1.0) at pH 8

Sample concentration $5*10^{-7}$ M , 25 °C	quantum yield
5'- d (AAA GGG AAC AAA AG ^{Flu}C ^{DNP}T GGG TA) -3'	0.16
5'- d (AAA GGG AAC AAA AG ^{Flu}C ^{DNP}T GGG TA) -3' 3'- d (TTT CCC TTG TTT TC G A CCC AT) -5'	0.27
5'- d (ACT GCT G ^{DNP}T ^{Flu}C GAT TTC CCA C) -3'	0.19
5'- d (ACT GCT G ^{DNP}T ^{Flu}C GAT TTC CCA C) -3' 3'- d (TGA CGA C A G CTA AAG GGT G) -5'	0.43



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The NPE-group is a useful protection for the fluorescein moiety using phosphoramidite chemistry.[2,3] The modified fluorescein building block **1** is a versatile compound for the synthesis of fluorescence labeled nucleosides and nucleotides (Scheme 1).

2'-Deoxy-cytidine can either been labeled directly with **1** via an urea function to **2** or the intermediary carbamate **3**[4] is modified with a diaminospacer[5] before coupling with **1** leading to **4** (Scheme 2).

2,4-Dinitroaniline is an efficient fluorescence quencher for fluorescein. We have synthesized oligonucleotides, labeled with fluorescein at the nucleobase at the 3'-end and with dinitroaniline at the 5'- terminus separating these functions with oligothymidylate spacers. The fluorescence quantum yield is reduced to the half of the unquenched labeled oligonucleotide if an hexameric thymidylate has been built in (Figure 1). This result indicates that a short range mechanism of fluorescence quenching exists, which affords a direct contact of the quencher with the dye within the fluorescence lifetime of fluorescein.

Furthermore 2,4-dinitroaniline interacts as a planar and hydrophobic label with the heterocyclic bases when a dinitroaniline labeled oligonucleotide forms a duplex, seen seen directly by a 25 % hyperchromicity at the melting temperature of the oligonucleotide on detection at 360 nm. Probably dinitroaniline acts here as an intercalator. These interactions disturbe the quenching properties, which led us to synthesize doubly labeled oligonucleotides at adjacent nucleotide units. The fluorescence marker was attached at the amino-position of 2'-deoxy-cytidine and the 2,4-dinitroaniline quencher was introduced in the N^3-position of thymidine (**5**). In this case duplex formation is associated with an increase of fluorescence quantum yield of about two-fold (Table 1).

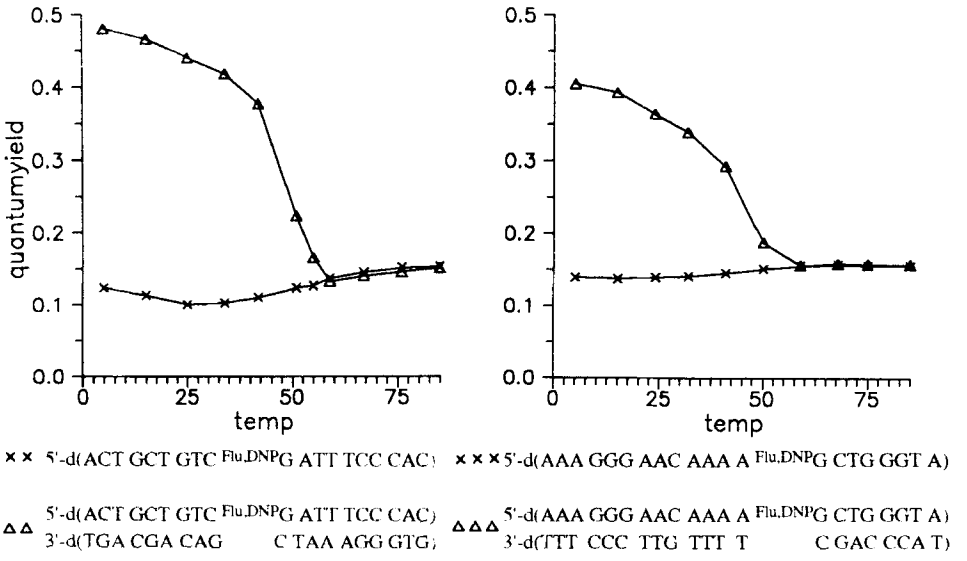
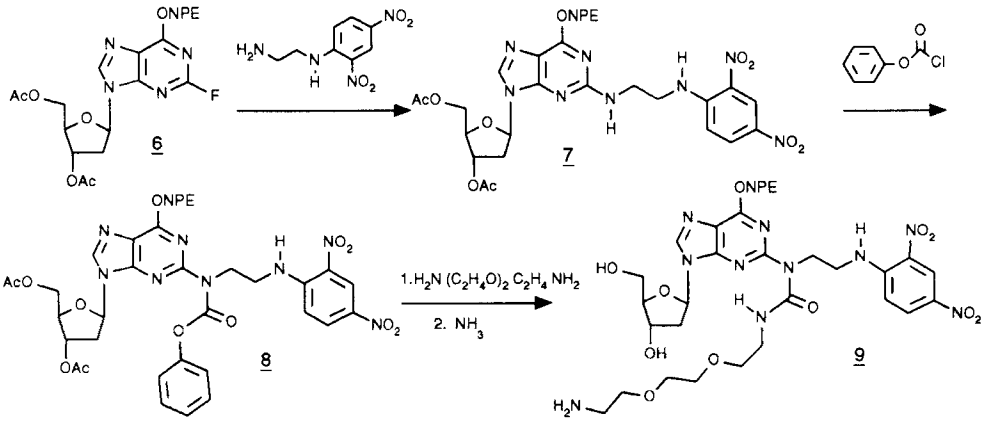
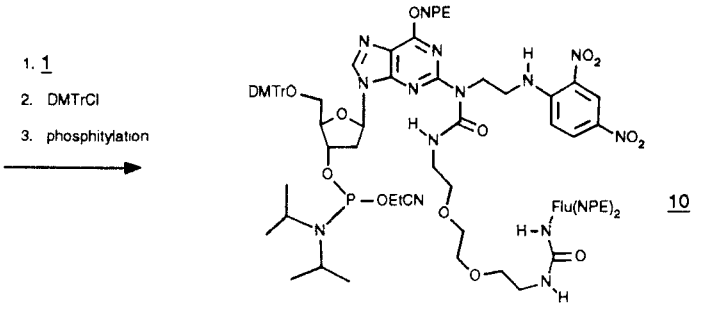


Figure 2: Fluorescence quantum yield relative to fluorescein (1.0) at pH 8

There is also the possibility to bind fluorescein and 2,4-dinitroaniline to a single base. Starting from 2'-deoxy-inosine derivative **6**, a series of reactions **6** - **10** led to an interesting modification of the 2-amino group of guanosine, carrying the interacting fluorophor and quencher at a substantial distance but still in the same molecule.





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The fluorescence quantum yields increase now about 4 - 5 fold, when labeled oligonucleotides of this kind form a duplex with the target sequences. This effect is clearly seen from a big change of the quantum yields of the labeled duplexes, compared to the quantum yields of the single-strands (Figure 2).

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