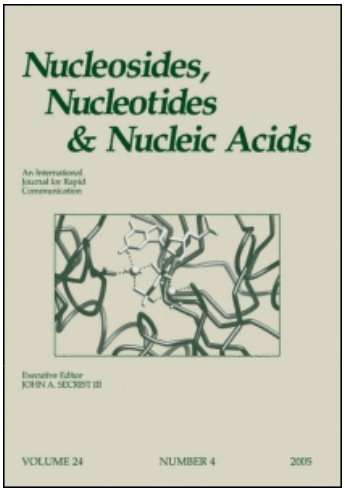

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Synthesis of Novel Piperidinyl Linker Based Energy Transfer Terminators and Their Potential Use in DNA Sequencing

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SYNTHESIS OF NOVEL PIPERIDINYL LINKER BASED ENERGY TRANSFER TERMINATORS AND THEIR POTENTIAL USE IN DNA SEQUENCING

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▫ *Synthesis of novel piperidinyl linker based ET cassettes and terminators is described. These novel terminators are evaluated in the DNA sequencing experiments using thermostable DNA polymerase.*

Keywords Energy Transfer Dyes, Dye Labelled Nucleotides, Fluorescence, DNA Sequencing

INTRODUCTION

In the last decade, there has been great interest in the development and application of new powerful and improved technologies in the DNA sequencing area. This is mainly attributed to the human genome project. In this process fluorescence based sequencing has become the dominant method for high-throughput DNA sequencing. Until a few years ago, DNA sequencing instruments have utilized four-color single dyes as fluorescent labels.[1,2] Recently, we have introduced a four-color energy transfer dye nucleotide terminators kit for high throughput DNA sequencing (containing a donor dye and acceptor dye cassette) based on α-phenylalanine linker.[3] These ET-terminators are superior to the single-dye terminators. In our efforts to develop improved more efficient ET (energy transfer) terminators, we have synthesized several ET-terminators with different linkers between donor and acceptor dye.[4–6] It is found that the energy transfer between the dyes highly depends on the linker in addition to the distance between the dyes and the orientation of the dyes. Now herein we report the synthesis of novel piperidinyl ET-terminators and their utility in DNA sequencing.

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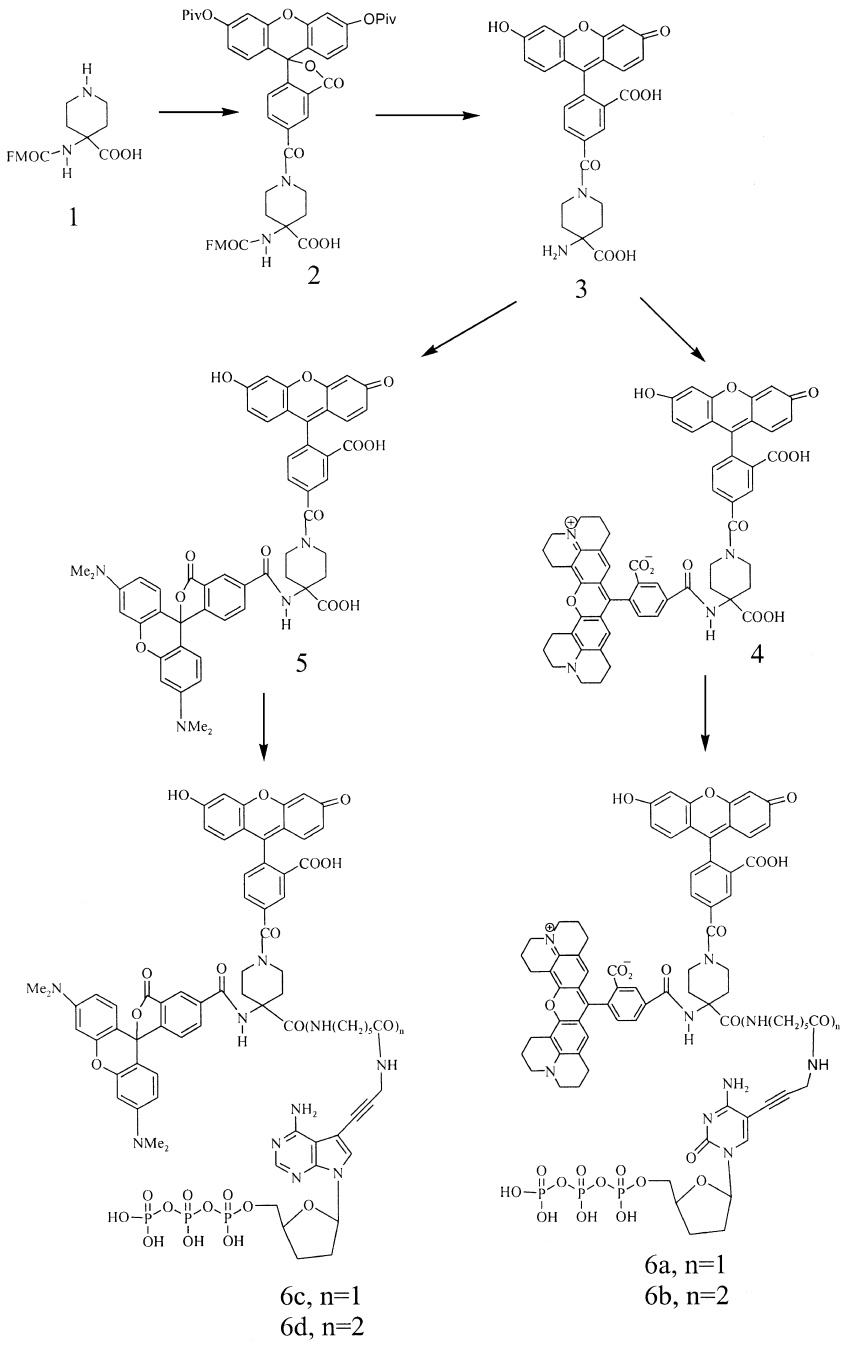

SCHEME 1 Synthesis of piperidinyl ET terminators.

RESULTS AND DISCUSSION

The synthesis of the ET cassettes (containing a donor dye and acceptor dye) was accomplished starting from *N*-FMOC-piperidinyl-1, 1-amino carboxylic acid (**1**). Compound **1** on reaction with dipivaloyl-5-carboxyfluorescein acid chloride (the acid chloride was prepared by treating the protected carboxyfluorescein with oxalyl chloride) gave 4-*N*-(dipivaloylfluorescein-5-carbonyl)-*N*-FMOC-piperidinyl-1, 1-amino carboxylic acid (**3**) in 80% yield. Removal of the FMOC group and pivaloyl groups in **2** was accomplished by treating with piperidine, to give 4-*N*-(fluorescein-5-carbonyl) piperidinyl-1, 1-amino carboxylic acid (**3**) single dye cassette in quantitative yield, which served as the common intermediate for the preparation of double dye cassettes. Compound **3** on reaction with 5-carboxy-X-rhodamine-(5-ROX) and 5-carboxytetramethylrhodamine-(5-TMR) NHS esters independently in DMSO in the presence of excess *N*, *N*-diisopropylethylamine afforded the double dye cassettes, 4-*N*-(fluorescein-5-carbonyl)-*N*-ROX-piperidinyl-1, 1-amino carboxylic acid **4** and 4-*N*-(fluorescein-5-carbonyl)-*N*-TMR-piperidinyl-1, 1-amino carboxylic acid **5**, in 30 to 40% yield (Scheme 1). The double-dye cassettes thus obtained were activated with disuccinimidyl carbonate in the presence of 4-(dimethylamino) pyridine and coupled with the nucleoside triphosphates containing C-11 and C-18 linker arms to give the final ET terminators **6a–6d*** (5-FAM-piperidine-ROX-11-ddCTP, 5-FAM-piperidine-ROX-18-ddCTP, 5-FAM-piperidine-TMR-11-ddATP and 5-FAM-piperidine-TMR-18-ddATP, Scheme 1).

The ET measurements of the terminators **6a–6d** on a fluorometer revealed that these novel terminators are 2–2.5 times brighter than the existing commercially available DYEnamic ET terminators. Evaluation of Compounds **6a–6d** in DNA sequencing experiments using Thermo Sequenase™ II DNA polymerase demonstrated that these are good substrates for polymerases and displayed brighter fluorescence signals.

CONCLUSION

The synthesis of ET-dye cassettes based on functionalized piperidine was accomplished and these ET-cassettes were conjugated with dideoxynucleoside triphosphates containing C11 and C18 linker arm to give the final ET dye terminators. These terminators were evaluated for their utility in the sequencing reactions in comparison with those commercially available DYEnamic ET terminators. It was found that the brightness of these novel terminators is considerably higher (2–2.5 times) than those DYEnamic ET terminators.

*The ET terminators are characterised by UV and mass spectral data.

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