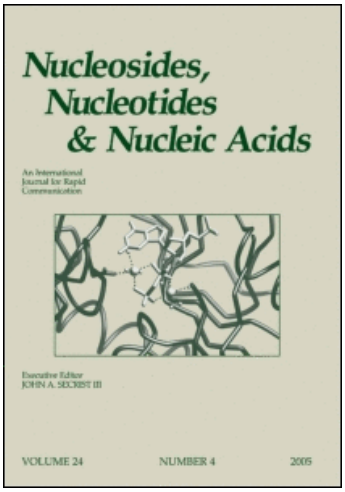

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Synthesis and Energy Transfer Efficiency of FRET Terminators Derived from Different Linkers

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ABSTRACT

A number of different energy transfer dye labeled-cassettes were synthesized using aminoacid based trifunctional linkers and coupled to the propargylamino-substituted dideoxynucleoside-5′-triphosphates (ddNTPs). These terminators were evaluated for their energy transfer efficiency and DNA sequencing potential using thermostable DNA polymerase.

Key Words: Energy transfer dyes; Dye labeled nucleotides; Fluorescence; DNA sequencing.

INTRODUCTION

Dye terminator DNA sequencing[1] involving fluorescently labeled dideoxynucleoside-5′-triphosphates has become the method of choice in almost all the high throughput sequencing facilities. Recent introduction of the fluorescence resonance energy transfer (FRET) labeled primers[2] and terminators[3,4] and thermostable DNA polymerases,[5] which incorporate these terminators efficiently have contribu-

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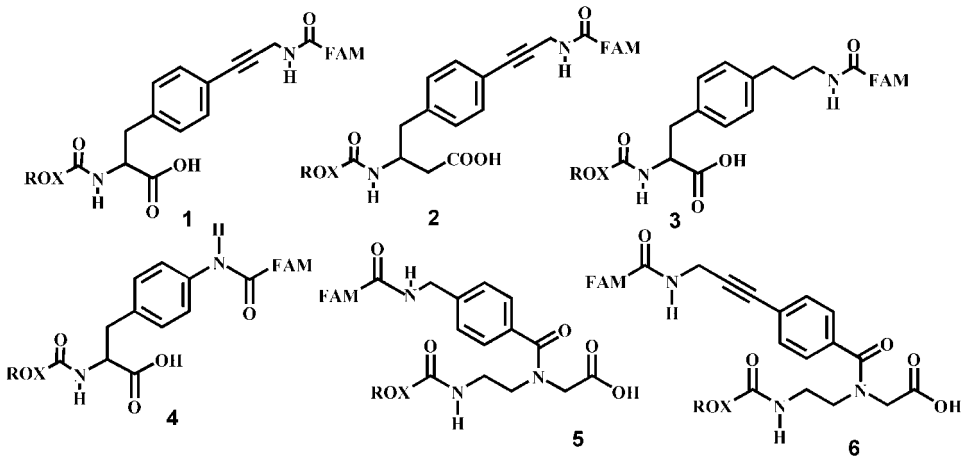
ted to the completion of the first draft of human genome and genomes of several other organisms. In a research program directed towards the development of brighter FRET terminators that would give highly uniform peak heights in DNA sequencing, we have synthesized a number of FRET terminators using different linkers between the donor and acceptor dyes.[3] Energy transfer from the donor to acceptor dye is highly dependent on linker structure, only part of this variation is apparently due to chromophore separation distance and relative orientation. Herein, we present the synthesis of different FRET cassettes and terminators, their fluorescence properties and utility in DNA sequencing.

SYNTHESIS AND RESULTS

The FRET cassettes (Sch. 1) required for the syntheses of FRET terminators (Fig. 1) were conveniently synthesized from the commercially available aminoacids



Scheme 1. FRET cassettes.

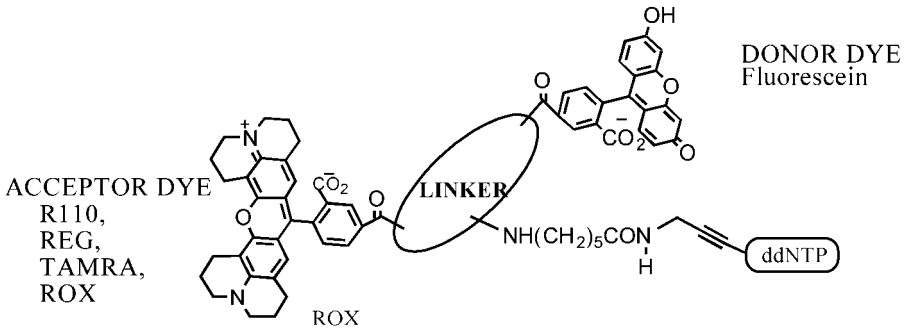


Figure 1. Schematic of trifunctional linker derived FRET terminators.

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Table 1. Energy transfer efficiencies of FRET terminators derived from linkers given in Sch. 1.

FRET Terminators	Energy Transfer Efficiency
DYEnamic ET Terminators (**1**)	
FAM-Phe-ROX-11-ddCTP	19%
FAM-Phe-TAMRA-11-ddATP	16%
FAM-Phe-REG-11-ddUTP	26%
FAM-Phe-R110-11-ddGTP	8%
Homo-β-Phe-derived terminators (**2**)	30–50% as bright as DYEnamic ET terminator set
Propylamino-saturated linker derived terminators (**3**)	50% as bright as DYEnamic ET terminator set
p-amino-Phe-derived ET terminators (**4**)	~1.5 times brighter than DYEnamic ET terminator set
Peptide derived cassettes and terminators (**5–6**)	Failed to display fluorescence emissions as a result of quenching

containing either two amino and one acid group functionalities (**4**) or chemically converted to contain two amino groups (**1–3 & 5–6**). The amino groups were used to attach the donor and acceptor dyes and the acid group was used to attach the dideoxynucleoside-5′-triphosphates. The energy transfer efficiency of cassettes and terminators depends upon the linker constitution, distance between the dyes and relative dipole-induced-dipole orientation of the participating donor and acceptor dyes in the transition state. The energy transfer efficiency/brightness of the terminators derived from different linkers (Sch. 1) was measured and the results are provided in Table 1. The four-color set of FRET terminators (DYEnamic™ ET terminators) derived from phenylalanine derived ET cassette (**1**), in combination with ThermoSequenase™ II DNA polymerase, was developed for high throughput DNA sequencing on slab gels as well as capillary sequencers.[3]

It is clear from the FRET data (Table 1) that small differences in linker structure makes a significant difference in energy transfer efficiency. The 30–50% less energy transfer efficiency from phenylalanine to homo-β-phenylalanine is surprising, which may be due to the different orientation of dyes in the terminator structure. The *para*-propylamino phenylalanine derived terminators also showed 2-fold weaker fluorescence and peptide (aminoethyl glycine) derived cassettes and terminators did not show any fluorescence emission from the acceptor dye when excited at 488 nm in aqueous solutions. The terminators derived from structures **1–4** generated satisfactory DNA sequencing data.

CONCLUSION

We have designed and synthesized six different constructs for FRET studies. Their energy transfer efficiencies and DNA sequencing potential was studied in

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DNA polymerase catalyzed dye terminator sequencing reactions. Of these constructs, phenylalanine derived terminators were developed for DNA sequencing on slab gel as well as capillary sequencers to give excellent peak uniformity, read-length and accuracy in excess of 1000 bases.

REFERENCES

1. Sanger, F.; Nicklen, S.; Coulson, A.R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad Sci. USA **1977**, *74*, 5463–5467.
2. Ju, J.; Ruan, C.; Fuller, C.W.; Glazer, A.N.; Mathies, R.A. Fluorescence energy transfer dye-labeled primers for DNA sequencing and analysis. Proc. Natl. Acad Sci. USA **1995**, *92*, 4347–4351; b) Rosenblum, B.B.; Lee, L.G.; Spurgeon, S.L.; Khan, S.H.; Menchen, S.M.; Heiner, C.R.; Chen, S.M. New dye-labeled terminators for improved DNA sequencing patterns. *Nucleic Acids Res.* **1997**, *25*, 4500–4504.
3. a) Nampalli, S.; Khot, M.; Kumar, S. Fluorescence energy transfer terminators for DNA sequencing. Tetrahedron Letters. **2000**, *41*, 8867–8871; b) Nampalli, S.; Khot, M.; Nelson, J.R.; Flick, P.K.; Fuller, C.W.; Kumar, S. Fluorescence resonance energy transfer dye nucleotide terminators: A new synthetic approach for high-throughput DNA sequencing. Nucleosides, Nucleotides & Nucleic Acids **2001**, *20*, 361–367; c) Nampalli, S.; Zhang, W.; Sudhakar Rao, T.; Xiao, H.; Kotra, L.P.; Kumar, S. Unnatural amino acid derived FRET cassettes, terminators and their DNA sequencing potential. Tetrahedron Letters **2002**, *43*, 1999–2003; d) Finn, P.J.; Sun, L.; Nampalli, S.; Xiao, H.; Nelson, J.R.; Mamone, J.A.; Grossmann, G.; Flick, P.K.; Fuller, C.W.; Kumar, S. Synthesis and application of charge-modified dye-labeled dideoxynucleoside-5′-triphosphates to 'direct-load' DNA sequencing. Nucleic Acids Research **2002**, *30*, 2877–2885.
4. Lee, L.G.; Spurgeon, S.L.; Heiner, C.R.; Benson, S.C.; Rosenblum, B.B.; Menchen, S.M.; Graham, R.J.; Constantinescu, A.; Upadhya, K.G.; Cassel, J.M. New energy transfer dyes for DNA sequencing. Nucleic Acids Res. **1997**, *25*, 2816–2822.
5. Tabor, S.; Richardson, C.C. A single hydroxyl moiety in Pol I-type DNA polymerases is responsible for distinguishing between deoxy- and dideoxyribonucleotides. Proc. Natl. Acad Sci. USA **1995**, *61*, 6339–6343; b) Reeve, M.A.; Fuller, C.W. A thermostable DNA polymerase for DNA sequencing. Nature **1995**, *376*, 796–797.

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