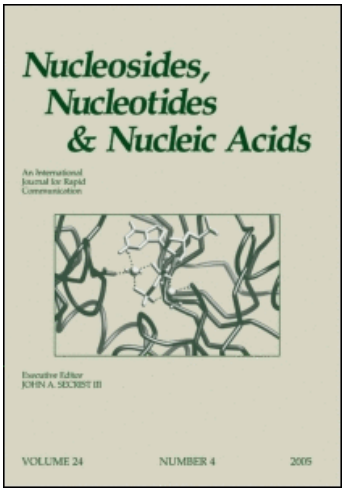

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FOUR COLOR FRET DYE NUCLEOTIDE TERMINATORS FOR DNA SEQUENCING

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ABSTRACT

The synthesis of four color set of energy transfer-dye terminators (**8a**–**8d**) starting from *p*-iodo-β-phenylalanine was accomplished and their utility in the sequencing reactions has been evaluated.

In the last decade, there has been great interest in the development of powerful new technologies in the DNA sequencing area. This is mainly attributed to human genome project. In this process fluorescence based sequencing has become the dominant method for high-throughput DNA sequencing (1,3). There are a few reports in the literature on the energy transfer dye-labeled primers (4a–c) and terminators (5). Recently, we developed a four-color set of ET (energy transfer) terminators as powerful DNA sequencing reagents, synthesized from a rigid and linear linker (**1**) derived from 4-iodo-α-phenylalanine (6). In order to investigate the ET properties of the terminators derived from a higher homologue of **1**, a four color set of ET terminators (**8a**–**8d**) were synthesized from β-phenylalanine.

The synthesis of the four-color ET-dye cassettes (containing a donor dye and acceptor dye) was accomplished starting from *t*.Boc-*p*-iodo-β-phenylalanine (**2**). Compound **2** on coupling with *N*-TFA-propargylamine in the presence of tetrakis (triphenylphosphine)palladium(0) and copper iodide (7) gave *N*-Boc-*p*-(trifluoro-acetamidopropargyl)-β-phenylalanine (**3**) in 81% yield. Removal of trifluoroacetyl group by the treatment with ammonium hydroxide followed by

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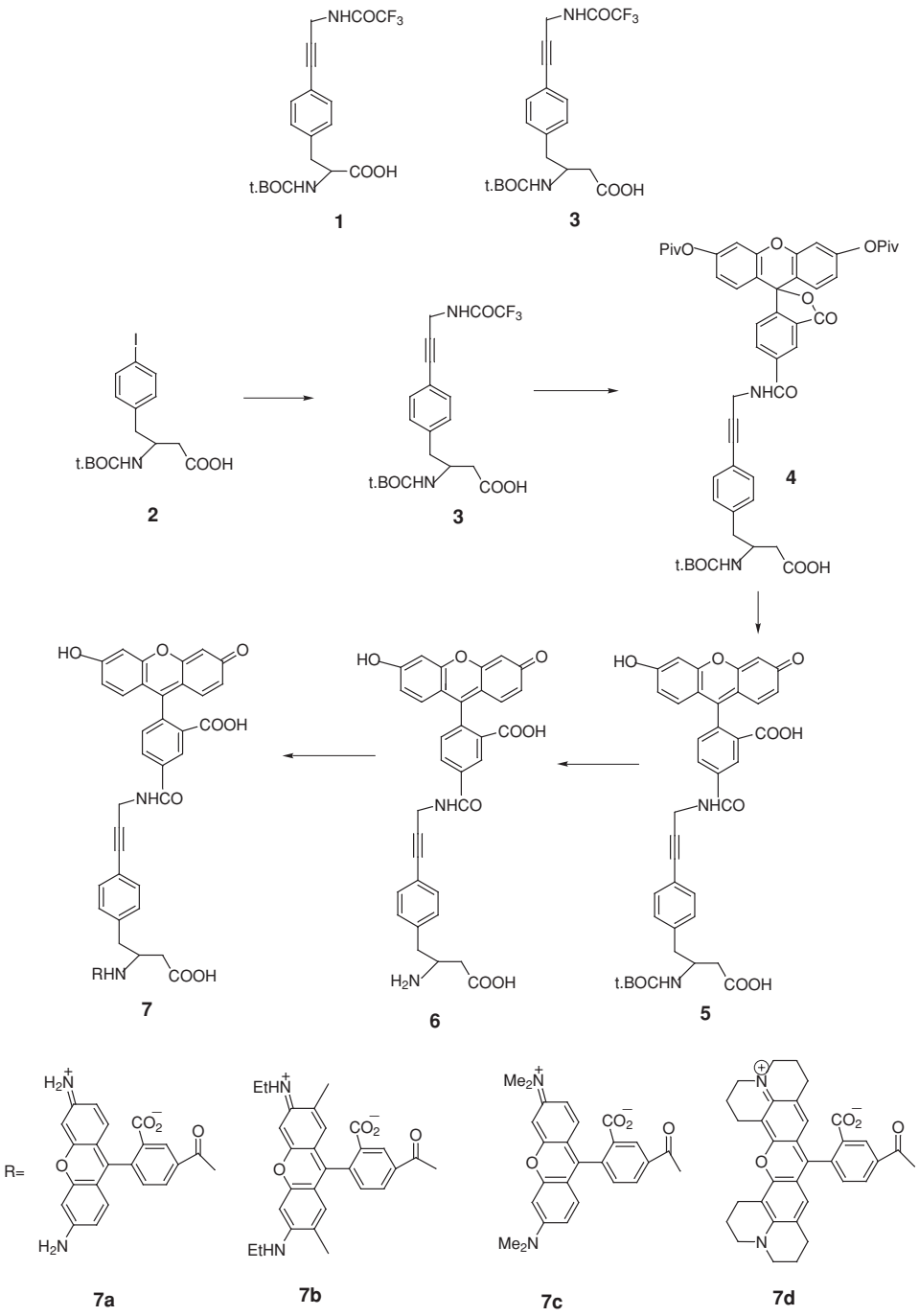

Scheme I.

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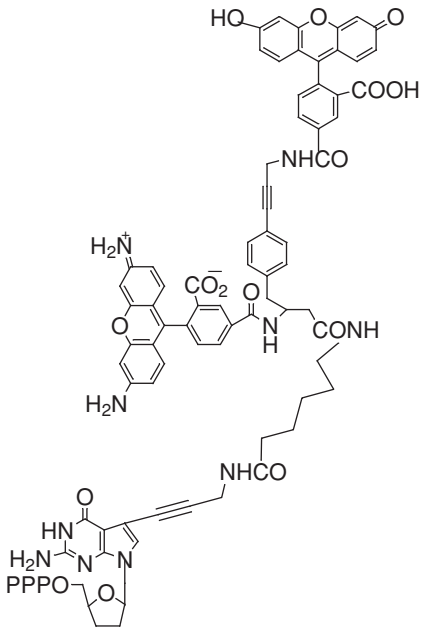
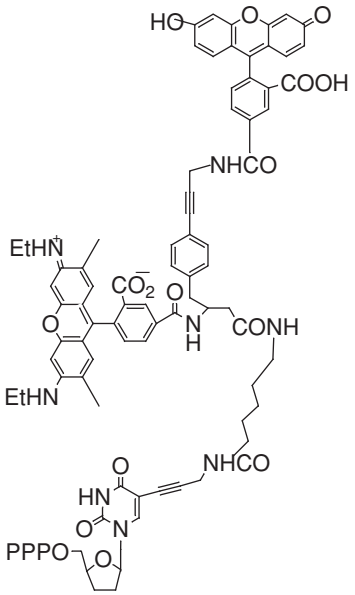
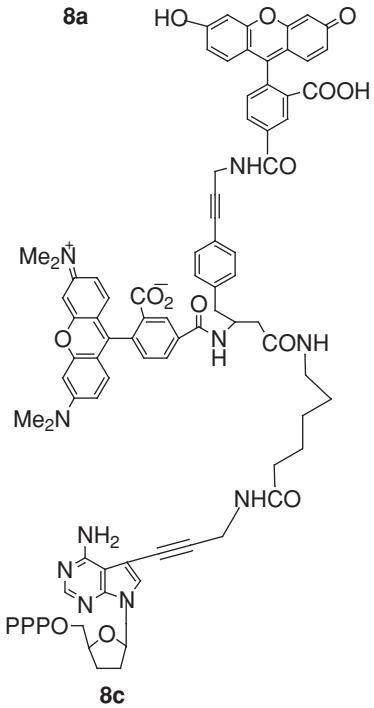


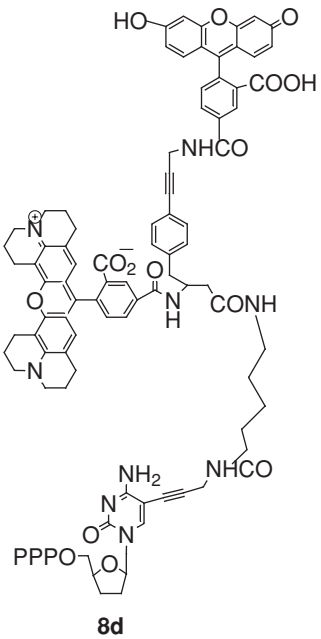
8a



8b



8c



8d

Scheme II.

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reaction with dipivaloyl-5-carboxyfluorescein-NHS ester in DMF in the presence of *N*,*N*-diisopropylethylamine afforded *N*-Boc-*p*-(propargylamido-dipiv-5-carboxyfluorescein)-β-phenylalanine (**4**) in 50% yield. Deprotection of the pivaloyl groups in compound **4** with 30% ammonium hydroxide, followed by treatment with 1:1 aqueous trifluoroacetic acid gave the fluorescein single dye cassette **6** (63%) which served as the common intermediate for all four ET dye cassettes. Compound **6** on reaction with R110-, R6G-, TAMRA-, and ROX-NHS esters in DMSO in the presence of excess *N*,*N*-diisopropylethylamine afforded the ET-dye cassettes **7a–7d** (Scheme I) in 52–65% yield. The ET cassettes thus obtained were activated with disuccinimidyl carbonate in the presence of DMAP and coupled with the nucleoside triphosphates containing C-11 linker arm (generated by reacting either 5-propargylamino pyrimidine nucleotides or 7-propargylamino-7-deazapurine nucleotides with aminocaproic acid NHS ester) to give the final four ET terminators **8a–8d** (FAM-β-F-R110-ddGTP, FAM-β-F-R6G-ddUTP, FAM-β-F-TAMRA-ddATP, FAM-β-F-ROX-ddCTP, Scheme II).

All the four ET-dye terminators synthesized were evaluated in DNA sequencing experiments using Thermo Sequenase II DNA polymerase. These experiments showed that the brightness of the dye terminators derived from β-phenylalanine is considerably lower than those derived from α-phenylalanine. It appears that a small structural change (in this case only one extra methylene group) altered the relative orientation of the donor dye to acceptor dye of the molecule, thereby reducing the energy transfer.

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