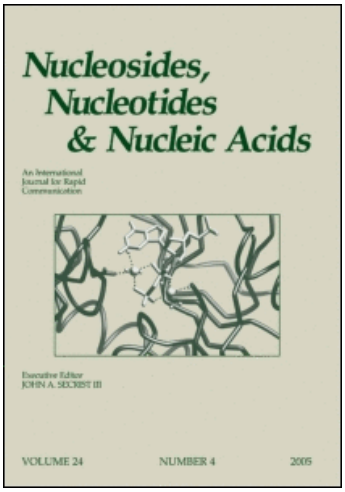

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T. Sudhakar Rao[a]; Weihong Zhang[a]; Haiguang Xiao[a]; Parke Flick[a]; Shiv Kumar[ab]; Satyam Nampalli[a]
[a] Amersham Biosciences, Piscataway, New Jersey, USA [b] Amersham Biosciences, Piscataway, NJ, USA

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Synthesis of Novel Tyrosinyl FRET Cassettes, Terminators, and Their Potential Use in DNA Sequencing

T. Sudhakar Rao, Weihong Zhang, Haiguang Xiao, Parke Flick, Shiv Kumar,* and Satyam Nampalli

Amersham Biosciences, Piscataway, New Jersey, USA

ABSTRACT

Fluorescence resonance energy transfer (FRET) dye labeled cassettes and terminators with one or more donor dyes (fluorescein) and acceptor dye (rhodamine dyes) with benzofuran or tyrosine linker moieties were synthesized. These terminators were evaluated for their energy transfer and DNA sequencing potential using thermostable DNA polymerase.

Key Words: Energy transfer dyes; Terminators; DNA sequencing.

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 human genome project. Recently, we have introduced a four color, Fluorescence Resonance Energy Transfer terminators (FRET)[1] for high throughput DNA sequencing, performed on slab as well as capillary based sequencers. In our continued efforts to develop improved ET (energy transfer) terminators, we have synthesized three different types of new ET-cassettes containing one or two donor dyes and an acceptor dye bridged by either benzofuran or tyrosine linker moieties.

*Correspondence: Shiv Kumar, Amersham Biosciences, 800 Centennial Avenue, Piscataway, NJ 08855-1327, USA; E-mail: shiv.kumar@amersham.com.

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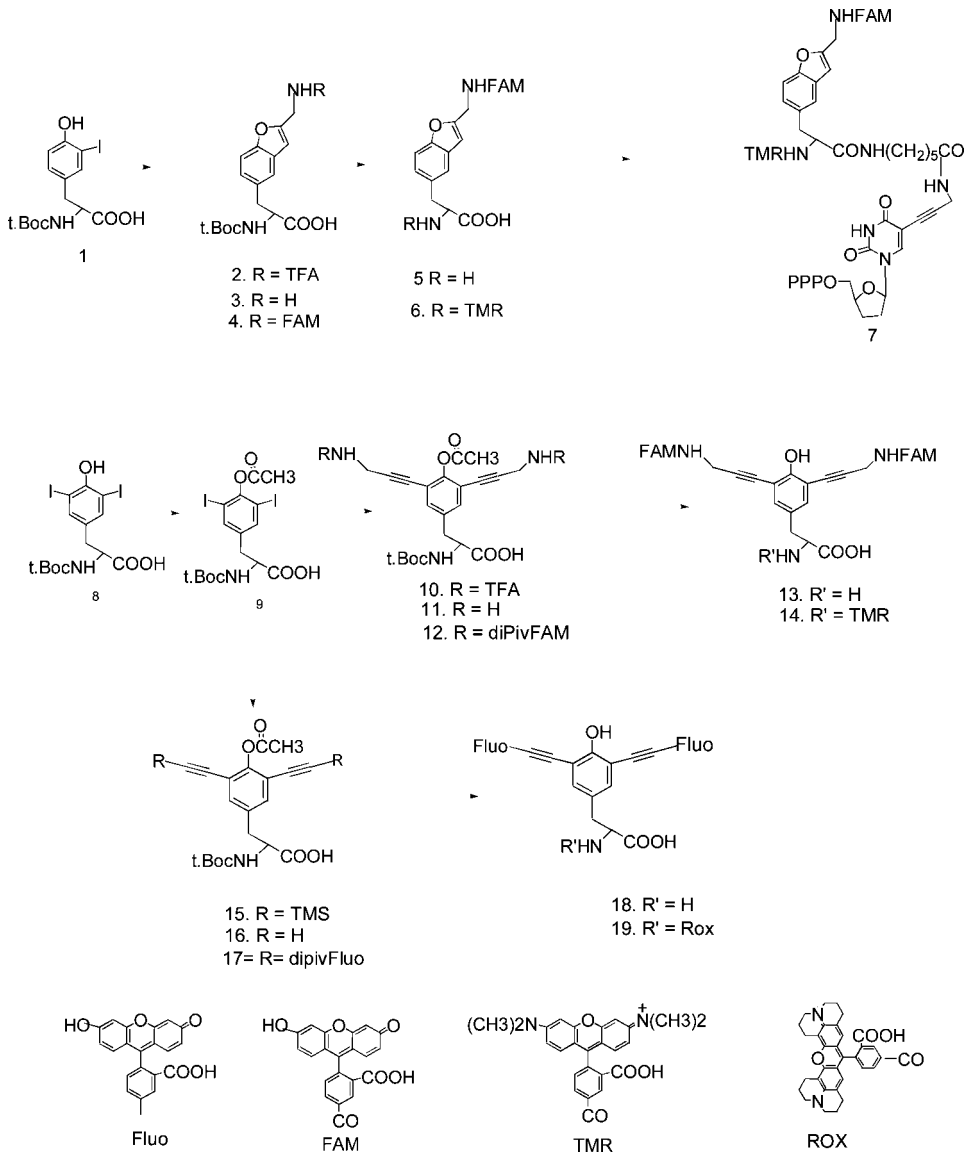

Figure 1. Synthesis of energy transfer cassettes and terminators using benzofuran and tyrosine based linkers.

Benzofurano ET Cassette/Terminator (6/7). Synthesis of the benzofurano ET-cassette was accomplished starting from iodotyrosine **1**. The starting material **1** on reaction with TFA-propargylamine in the presence of CuI and tetrakis triphenyl phosphine palladium(0) gave **2**. Removal of TFA group from **2** followed by treatment with FAM-NHS ester afforded the single dye containing linker molecule (**4**). Compound **4** on reaction with trifluoroacetic acid followed by TMR-NHS

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ester yielded the energy transfer (ET) dye cassette (**6**), which on conjugation with 11-ddUTP gave the target ET-dye-terminator (**7**).

Bis-Propargylamino-FAM-Tyrosine-TMR ET Cassette (14). Synthesis of the ET-cassette containing two donor dyes and an acceptor dye, started from diiodo tyrosine **8**. The phenolic hydroxyl group of **8** was protected with acetyl group prior to Heck coupling since it can form a furan ring under the reaction conditions. Thus compound **8** on reaction with acetic anhydride gave acetyl derivative **9**, which on coupling with TFA-propargyl amine gave bis propargylamino derivative **10**. Removal of TFA groups from **10** followed by reaction with dipiv-FAM-NHS ester gave bis-FAM derivative **12**. Deprotection of Piv and t.Boc groups from compound **12** was accomplished by treating with NH_4OH followed by trifluoroacetic acid to give **13**, which on reaction with TMR-NHS ester afforded the ET-cassette **14**.

Bis-acetylene-FAM-Tyrosine-ROX-ET Cassette (19). The further rigidified cassette construct, which comprises direct covalent attachment of two donor dyes via acetylenic groups and the acceptor dye to the α-amino functionality was synthesized from **9**. Heck reaction of **9** with TMS-acetylene in the presence of Pd(II) afforded the bis-acetylenic derivative **15**. Removal of TMS groups from **15** was accomplished with sodium methoxide. Since the acetyl group was also removed during this process, the phenolic group was re-protected by treating with acetic anhydride. Compound **16** thus obtained was subjected to a bis-Heck reaction with 5-iodo fluorescein to give **17**. Removal of piv and t.Boc groups followed by conjugation with ROX-NHS ester gave the desired ET-dye cassette **19**.

FRET measurement studies on the three types of novel compounds synthesized (**7**, **14** and **19**) were performed using the fluorescence spectrometer (Photon Technology International). Compounds **7** and **14** exhibited 3 and 2 as their PET (Percent Energy Transfer) values, respectively, while **19** failed to show ET due to quenching. Evaluation of Compound **7** in DNA sequencing experiments using Thermo Sequenase™ II, showed it to be a substrate with less intense fluorescent signals compared to FAM-Phe-TMR-11-ddUTP.

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