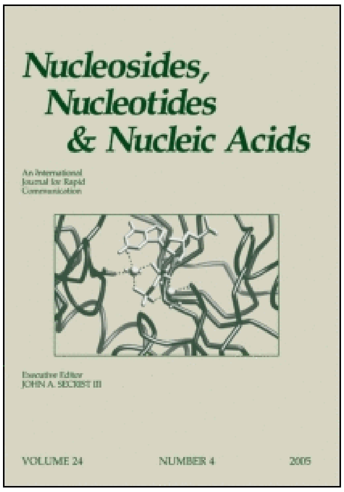

**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Synthesis of Aminomethyl- and Bis-Aminomethyl-Fluorescein Energy Transfer Terminators

Sudhakar Rao Takkellapati[a]; Chung Y. Chen[a]; Vladimir Neschadimenko[a]; Satyam Nampalli[a]; Haiguang Xiao[a]; Jeffrey Wegener[a]; Prasad Dhulipala[a]; Shiv Kumar[a]; Carl Fuller[a]
[a] GE Healthcare, Piscataway, New Jersey, USA

**To cite this Article** Takkellapati, Sudhakar Rao , Chen, Chung Y. , Neschadimenko, Vladimir , Nampalli, Satyam , Xiao, Haiguang , Wegener, Jeffrey , Dhulipala, Prasad , Kumar, Shiv and Fuller, Carl(2007) 'Synthesis of Aminomethyl- and Bis-Aminomethyl-Fluorescein Energy Transfer Terminators', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1467 — 1470

**To link to this Article: DOI:** 10.1080/15257770701542579

**URL:** http://dx.doi.org/10.1080/15257770701542579

*Nucleosides, Nucleotides, and Nucleic Acids*, 26:1467–1470, 2007
Copyright © Taylor & Francis Group, LLC
ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701542579

# SYNTHESIS OF AMINOMETHYL- AND BIS-AMINOMETHYL-FLUORESCEIN ENERGY TRANSFER TERMINATORS

**Sudhakar Rao Takkellapati, Chung Y. Chen, Vladimir Neschadimenko, Satyam Nampalli, Haiguang Xiao, Jeffrey Wegener, Prasad Dhulipala, Shiv Kumar, and Carl Fuller** □ *GE Healthcare, Piscataway, New Jersey, USA*

□ *Synthesis of aminomethyl and bis-aminomethylfluorescein derived energy transfer terminators is described.*

**Keywords** Aminomethyl fluorescein; bis-aminomethyl fluorescein; energy transfer dyes; dye labelled nucleotides; fluorescence; DNA sequencing

## INTRODUCTION

In the last two decades, there has been great interest in the development and application of new and improved technologies in the DNA sequencing area. Energy transfer terminator based fluorescence sequencing has become the method of choice for high-throughput DNA sequencing.[1] In our efforts to develop improved energy transfer (ET) terminators, we have synthesized several ET-terminators with different linkers between donor and acceptor dye. It is found that the energy transfer between two dyes highly depends on the linker in addition to the distance between the dyes and the orientation of the dyes.[2–6] Now we report the synthesis and energy transfer properties of 4′-aminomethyl and 4′, 5′- bis-aminomethylfluorescein energy transfer terminators and their utility in DNA sequencing.

## SYNTHESIS

Synthesis of 4′-aminomethyl-5-carboxyfluorescein ET-terminators is accomplished starting from dipivaloyl-5-carboxyfluorescein **1**. Compound **1** on treatment with 1.2 equivalents of 2-chloro-*N*-(hydroxymethyl)acetamide in the presence of concentrated sulphuric acid predominantly gave protected 4′-aminomethyl-5-carboxyfluorescein[7] derivative **2** along with a

Address correspondence to Sudhakar Takkellapati, GE Healthcare, 800 Centennial Avenue, Piscataway, NJ 08854. E-mail: sudhakar.takkellapati@ge.com

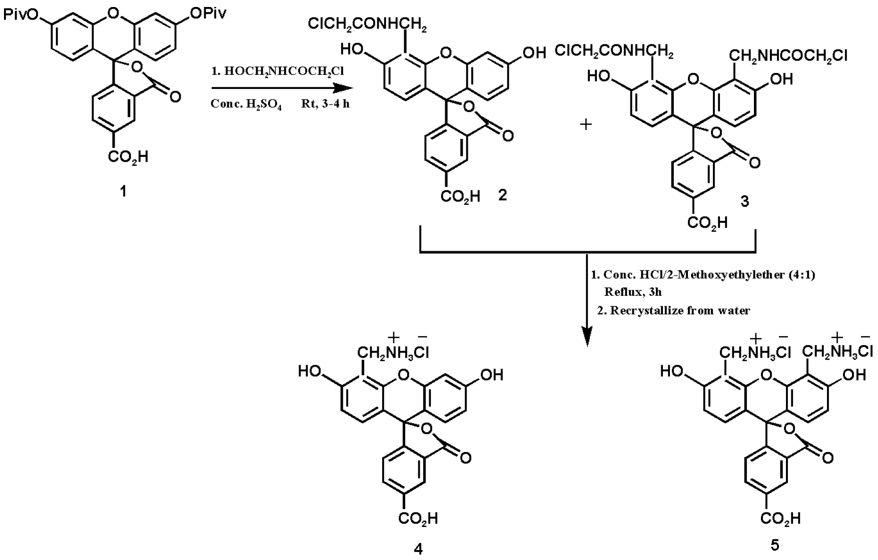

FIGURE 1 Synthesis of 4′-aminomethyl-5-carboxyfluorescein.

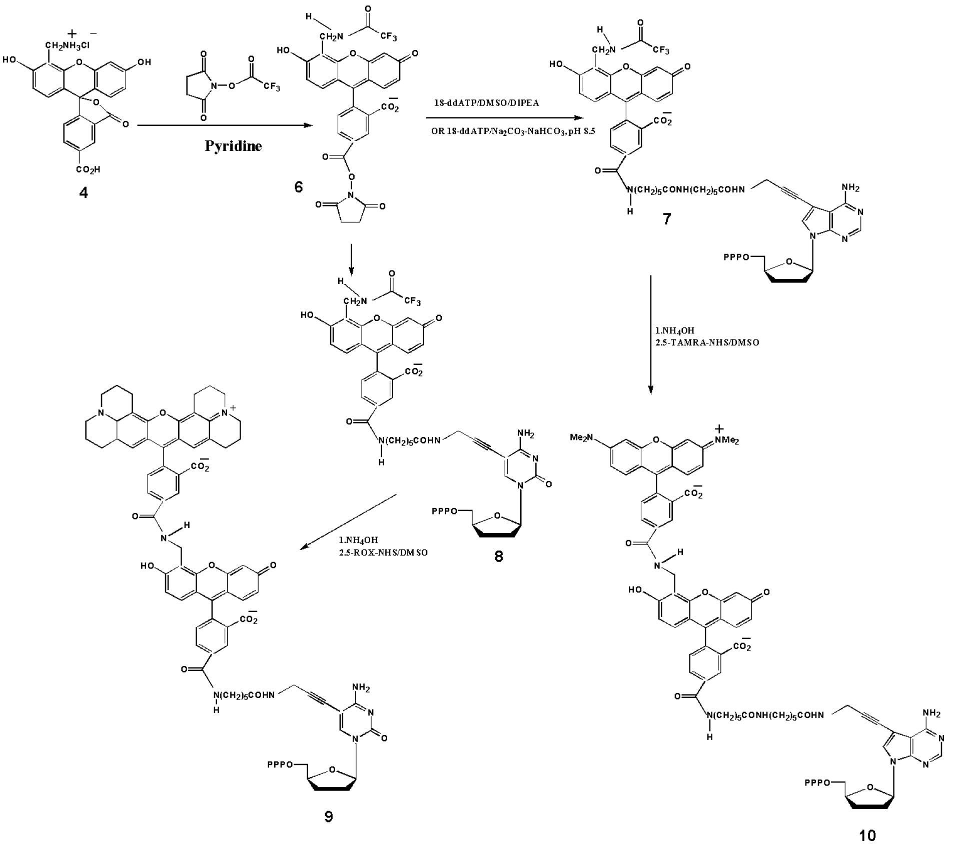

FIGURE 2 Synthesis of 4′-aminomethyl-5-carboxyfluorescein ET-terminators.

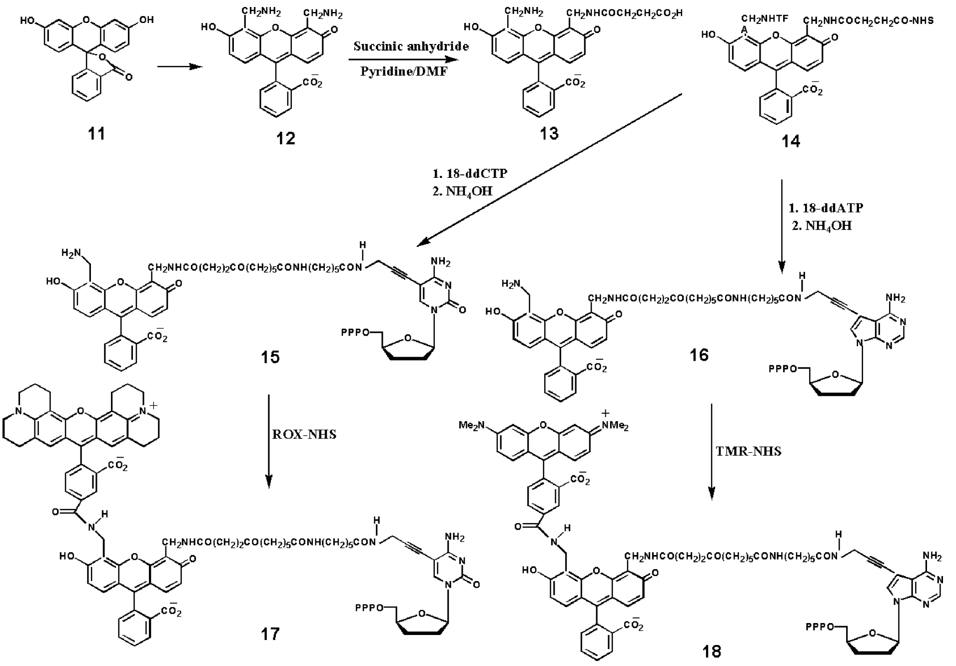

**FIGURE 3** Synthesis of 4′,5′-bis-aminomethylfluorescein ET-terminators.

small amount of disubstituted derivate **3** (Figure 1). Deprotection of **2** and **3** is accomplished by refluxing with 1 M hydrochloric acid to give 4′-aminomethyl- (**4**) and 4′, 5′-bis-aminomethyl-5-carboxyfluorescein 5. After deprotection aminomethyl derivative **4** is isolated by crystallization. However, this crystallized 4′-aminomethyl-5-carboxyfluorescein contained a small amount of bis-aminomethyl derivative **5** which is completely removed by preparative HPLC methods.

4′-Aminomethyl-5-carboxyfluorescein **4** is converted to protected active NHS ester **6** in one step by our recently developed method utilizing TFA-NHS reagent.[8] Compound **6** on reaction with ddATP or ddCTP with an extended linker arm, in anhydrous DMSO or carbonate/bicarbonate buffer gave single dye labeled nucleotides **7** and **8** (Figure 2) in very high yield. Deprotection of the amino functionality of **7** and **8** followed by treatment with 5-TMR- or 5-ROX-NHS ester in DMSO afforded pure ET-terminators **9** and **10** after subjecting to ion-exchange and reverse phase column chromatography.

Synthesis of 4′, 5′-bis-aminomethylfluorescein is accomplished starting from fluorescein **11** in a similar manner as described for 4′-aminomethyl-5-carboxyfluorescein, using 2.1 equivalents of 2-chloro-*N*-(hydroxymethyl)-acetamide followed by deprotection. 4′ , 5′-Bisaminomethyl fluorescein **12** is selectively converted to mono succinyl derivative by treating with 1.2 equivalents of succinic anhydride in pyridine and DMF (Figure 3). Compound **12** on reaction with TFA-NHS gave the protected active NHS ester **14**, which served as a key intermediate for preparing bis-aminomethylfluorescein ET-terminators. Compound **14** on treatment with ddATP or ddCTP with extended linker arm, followed by deprotection of the amine group and reaction with 5-TMR- or 5-ROX-NHS ester gave the ET-terminators **17** and **18** in high yield.

## RESULTS

The ET properties of all the terminators prepared in the current study are determined by measuring the energy transfer efficiency on a fluorometer. These studies revealed that the ET-terminators derived from aminomethyl fluorescein and bis-aminomethylfluorescein are 2.5 times brighter than the existing commercially available DYEnamic ET terminators.[4] These terminators are also evaluated for their ability to act as polymerase substrates and their effectiveness in the sequencing reactions. All these terminators are found to be good substrates for polymerases and the sequencing data obtained is comparable to that obtained with DYEnamic ET terminators.

## REFERENCES

1. a) Rosenblum, B.B.; Lee, L.G.; Spurgeon, S.L.; Khan, S.H.; Menchen, Heiner, C.R.; Chen, S.M. New dye-labeled terminators for improved DNA sequencing patterns. *Nucleic Acids Res.* **1997**, 25, 4500–4504.
   b) Kumar, S. Fluorescent dye nucleotide conjugates for DNA sequencing. In *Modified Nucleotides, Synthesis and Applications*; Loakes, D., Ed.; Research Signpost, Trivandrum (India), **2002**, 87–100.
2. Nampalli, S.; Khot, M.; Kumar, S. Fluorescence energy transfer terminators for DNA sequencing. *Tetrahedron Lett.* **2000**, 41, 8867–8871.
3. Rao, T.S.; Nampalli, S.; Zhang, W.; Xiao, H.; Kumar, S. Synthesis of novel piperidinyl linker based energy transfer terminators and their potential use in DNA sequencing. *Nucleosides Nucleotides Nucleic Acids* **2005**, 24, 801–804.
4. Rao, T.S.; Nampalli, S.; Lavrenov, K.; Zhang, W.; Xiao, H.; Nelson, J.; Kumar, S. Four color FRET dye nucleotide terminators for DNA sequencing. *Nucleosides Nucleotides Nucleic Acids* **2001**, 20, 673–676.
5. Nampalli, S.; Zhang, W.; Rao, T.S.; Xiao, H.; Kotra, L.P.; Kumar, S. Unnatural amino acid derived FRET cassettes, terminators and their DNA sequencing potential. *Tetrahedron Lett.* **2002**, 43, 1999–2003.
6. Kumar, S.; Nampalli, S.; Finn, P.; Rao, T.S.; Chen, C-Y.; Flick, P.K.; Fuller, C.W. Synthesis and energy transfer efficiency of FRET terminators derived from different linkers. *Nucleosides,Nucleotides Nucleic Acids* **2003**, 22, 1595–1598.
7. Kirkemo, C.L.; Shipchandler, M.T. Fluorescent polarization assay for ligands using aminomethylfluorescein derivatives as tracers. US Patent 4,510,251, April 9, 1985.
8. Rao, T.S.; Sekher, P.; Nampalli, S.; Kumar, S. TFA-NHS as bifunctional protecting agent: simultaneous protection and activation of amino carboxylic acids. *Tetrahedron Lett.* **2002**, 43, 7793–7795.