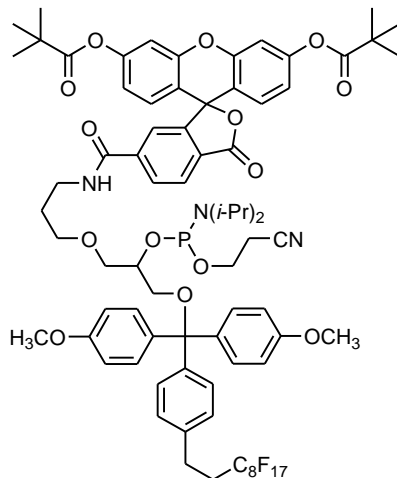


FDMT-5'-Fluorescein II CEP
Product No. FL 1700

Product Information



$C_{77}H_{79}F_{17}N_3O_{14}P$
Mol. Wt.: 1624.41

For the installation of fluorescein into oligonucleotides in cases where a temporary, highly hydrophobic group would aid purification.

Introduction. Highly fluorinated organic compounds are both hydrophobic and lipophobic, preferring instead to associate with other fluorinated substances. Organic molecules that have both an organic domain and a perfluoroalkyl domain (e.g., a linear perfluoroalkyl "ponytail") are known as *fluorous molecules*,¹ (not to be confused with *fluorescent* molecules!) and may be separated from non-fluorous molecules by interaction with fluorinated separation media such as Fluoro-Pak columns.^{2,3} Fluorous-fluorous interactions are strong and selective ("like dissolves like").

We have previously introduced Fluorous Affinity Purification of Oligonucleotides, a higher affinity alternative to DMT-on reversed-phase cartridge purification.² It relies on the strong interaction of fluorous-tagged oligonucleotides with the fluorous adsorbent in Fluoro-Pak columns. The fluorous tag took the form of a fluorous dimethoxytrityl (FDMT) group, which was installed using the appropriate FDMT-bearing nucleoside phosphoramidite. After fluorous purification on Fluoro-Pak columns with on-column detritylation, high recoveries of oligonucleotides were obtained, free from failure sequences, even with 100-mers.² The FDMT group also facilitates RP-HPLC purification.⁴

We have now developed new reagents that may impact the fluorescent probe field, where purification is often problematic because the desired labeled material may not be well-separated from undesired by-products. Fluorous-tagged oligonucleotides are retained strongly on fluorous or RP-HPLC adsorbents, more-so than molecules that bear dyes or normal DMT groups. *Regardless of the structure of the oligonucleotide, the fluorous tag dominates, consistently moving such molecules to longer retention times and therefore away from non-fluorous-tagged by-products. The fluorous tail "wags the dog".*

Fluorous Fluorophores, including FDMT-5'-Fluorescein II CEP: Dyes such as fluorescein are often introduced into oligonucleotides using a non-DMT-bearing phosphoramidite such as 5'-Fluorescein CEP ("6-FAM", BA 0054, see Figure 1.), which places a 6-carboxyfluorescein residue at the 5'-terminus and does not allow further extension. We now offer two fluorous-tagged fluorescein phosphoramidites. Fluorous 5'-Fluorescein CEP ("Fluorous 6-FAM", FL 1710), where a permanently-attached fluorous tail is present, allows the isolation of all oligonucleotides that bear a fluorescein moiety using fluorous or reversed-phase adsorbents. The fluorous tail may also enhance contact quenching with hydrophobic quenchers, especially those that have fluorous tails, also available from Berry & Associates (e.g., Fluorous 3'-Dabcyl CPG, FL 1800).

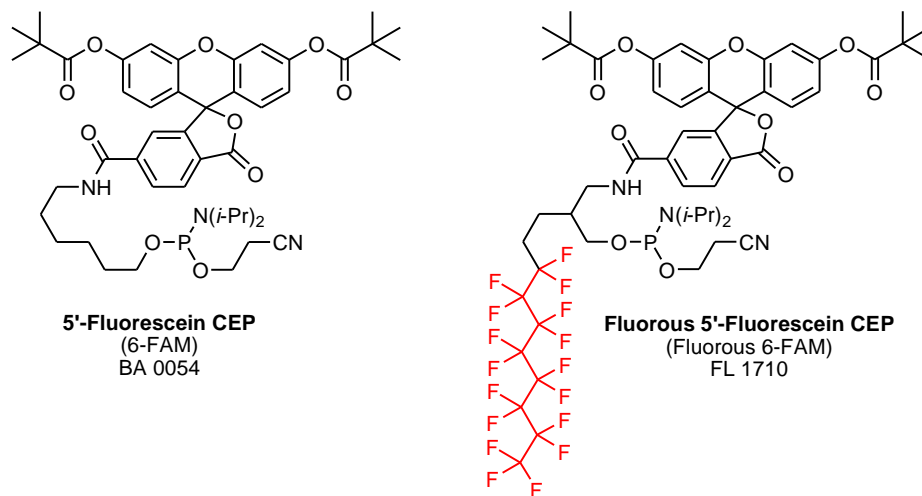


Figure 1. Non-DMT Bearing Fluorescein Phosphoramidites.

Another strategy for installing a dye such as fluorescein is to use a DMT-bearing phosphoramidite such as 5'-Fluorescein II CEP ("6-FAM II", BA 0253F). The DMT group may be used to quantify coupling efficiency or serve as a purification handle. If greater affinity for a hydrophobic adsorbent is required for purifications, we offer FDMT-5'-Fluorescein II CEP (FL 1700, Figure 2) which bears a fluorous DMT (FDMT) group. The highly hydrophobic FDMT group dominates the adsorptive properties of the molecules, allowing separation from non-FDMT-bearing materials using HPLC (fluorous or RP) or cartridge-based

methods (FluoroPak or RP columns). The FDMT group can be removed using the same methods that are used with DMT groups, and on-column detritylation may be employed.

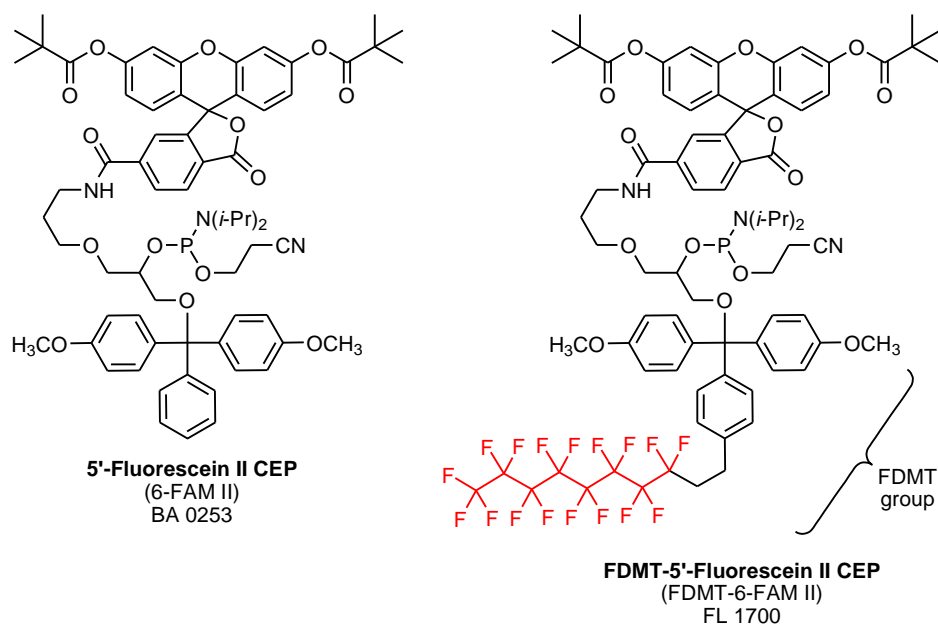


Figure 2. DMT-Containing Fluorescein Phosphoramidites.

Using FDMT-5'-Fluorescein II CEP: For oligonucleotide synthesis, the phosphoramidite should be diluted with dry acetonitrile at concentrations recommended by the synthesizer manufacturer. In our hands, standard coupling times were not sufficient, but ~90% yield could be obtained with extended coupling (i.e. 15 min). Standard deprotection conditions can be used (i.e. ammonium hydroxide, 55 °C, 2 h).

Literature:

- (1) *Handbook of Fluorous Chemistry*; Gladysz, J. A.; Curran, D. P.; Horváth, I. T., Eds.; Wiley-VCH: Weinheim, **2004**.
- (2) Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K.-Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114-7122.
- (3) Fluoro-Pak is a trademark of Berry & Associates, Inc. Products for fluororous affinity purification and fluororous tagging of oligonucleotides are subject to patent applications filed by Berry & Associates, Inc. Further, the use of these products is licensed under U.S. Patents 6,673,539, 6,156,896; 5,859,247; and 5,777,121 and one or more pending patents owned or controlled by Fluorous Technologies, Inc.

(4) Fluorous-tagged oligonucleotides are highly retained on both fluorous and traditional reversed-phase adsorbents, allowing easy separation from non-fluorous-tagged oligos and by-products.