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## 5'-MODIFIERS

As the use of synthetic oligonucleotides in biomedical research becomes more sophisticated, the need for rapid and simple modification of oligonucleotides becomes increasingly apparent. Because conventional automated synthesis proceeds from 3' to 5', the 5'-terminus is clearly readily available for manipulation. Indeed, the ability to attach a suitable molecule to the 5'-terminus for use as a label is significant in the continuing development of nonradioactive probes and in DNA sequencing and amplification.

A general approach to the modification of the 5'-terminus is to use reagents which would couple to the 5'-hydroxyl of an oligonucleotide. To be most effective, these reagents should be compatible with automated DNA synthesizers. A method which satisfies the above criteria is the use of phosphoramidite reagents which are readily adapted for use in automated synthesizers with little or no modification to existing protocols.

### *5'-Amino-Modifiers*

5'-Amino-Modifiers are  $\beta$ -cyanoethyl (CE) phosphoramidites which, when activated with 1H-tetrazole, can couple to the 5'-terminus of the oligonucleotide in the same time frame and with similar efficiency as nucleoside phosphoramidites. These reagents have the primary amine protected with a monomethoxytrityl (MMT) group. The MMT group should be left on the oligonucleotide for later removal with aqueous acid after reverse phase (RP) purification techniques.

### *5'-Amino-Modifiers TFA*

An alternative to the use of the 5'-Amino-Modifiers, in which the primary amine is protected with the acid-labile MMT group, is the use of the analogues with the primary amine protected with the base-labile trifluoroacetyl (TFA) group. While it is desirable in most cases to use the MMT group to purify the intermediate amino-modified

oligonucleotide by a RP technique, it may be occasionally attractive to use the TFA-analogue in which the protecting group is removed during the ammonia deprotection step. Using the latter strategy, the crude intermediate is reacted with an amine reactive fluorescent label or digoxigenin to give a product which must be purified by HPLC.

### *5'-Phosphorylation*

The Chemical Phosphorylation Reagents can be used with the standard phosphoramidite protocol and have two significant attributes. They contain a DMT group which can be removed to determine coupling efficiency, and their side chains are eliminated with ammonium hydroxide during the removal of the other protecting groups. Our original phosphorylation reagent is not compatible with DMT-on purification techniques. However, the newer CPR II reagent is compatible with RP purification techniques by cartridge or HPLC. These features allow complete compatibility with automated DNA synthesis and make chemical phosphorylation a suitable alternative to enzymatic phosphorylation.

### *5'-Thiol-Modifier*

The 5'-Thiol-Modifier C6 is used to produce a thiol group at the 5'-terminus of a synthetic oligonucleotide. The thiol group can be used to attach a variety of products, including fluorescent tags and enzymes like alkaline phosphatase or horseradish peroxidase. The trityl group used to protect the thiol is not acid labile and therefore can not be removed on a DNA synthesizer using the normal acid deprotection. Final deblocking of the oligonucleotide involves cleavage of the trityl-sulfur bond by oxidation with silver nitrate.

## **5'- OR 3'-MODIFIERS**

### *Thiol-Modifier C6 S-S*

The disulfide thiol modifier may be used for introducing thiol linkages at the 3'- or 5'-terminus. Thiol-Modifier C6 S-S is added conventionally to the 5'-terminus or, for the 3'-terminus, it is added

to any support and then the desired oligonucleotide is synthesized. Dithiothreitol (DTT) is used during deprotection or after purification of the product oligo to cleave the disulfide linkage.

#### *Spacer Phosphoramidites*

The spacer phosphoramidite 9 or 18 is used to insert a mixed polarity 9 or 18 atom spacer arm in an oligonucleotide. These compounds may be added in multiple additions when a longer spacer is required. The spacer phosphoramidite C3 can be added to substitute for an unknown base within a sequence. Spacer C12 adds a 12 carbon hydrophobic section to a sequence. dSpacer is designed to mimic an abasic site in an oligonucleotide.

## SEQUENCE MODIFIERS

#### *dU-based Modifiers*

Products designed to introduce primary amines at specific points along the sequence must be compatible with the techniques of automated synthesis and must not affect normal hybridization. Amino-Modifier-C6-dT has been established to meet all the required criteria. The compound allows the incorporation of a primary amine with a 10 atom spacer at any T site within an oligonucleotide. One to all of the T sites can be modified in this way without significantly affecting hybridization. This is currently the technique of choice for alkaline phosphatase labelling. Amino-Modifier-C2-dT is designed for attaching oligonucleotide cleavage reagents. Carboxy-dT is used for attaching amino labels.

#### *dU-based Labelling Reagents*

Biotin-dT is the best reagent for biotin labelling within the sequence. Similarly, dabcy1-, fluorescein-, or TAMRA-dT can be conveniently used to insert the respective label within a sequence. EDTA-dT can be used to cleave the target strand.

## 5'-LABELLING REAGENTS

All 5'-labelling reagents can be added only once to the 5'-terminus. Although limited in this way, they are typically simpler to use than the equivalent branched reagents in that the reaction times are shorter and they are less susceptible to the effects of moisture.

### *Biotin*

5'-Biotin phosphoramidite is the preferred reagent for adding biotin once to the 5'-terminus. The reagent has a DMT group on the biotin molecule and this makes it compatible with DMT-on purification on cartridges or HPLC.

### *6-FAM, HEX, TET, Cy Dyes*

These fluorescein derivatives are designed for use in instruments for sequencing or genetic analysis with the capability for the detection of multicolored fluorescent tags. The fluorescein derivatives (6-FAM, HEX, TET) are all single isomer products derived from the 6-carboxy fluorescein isomer. The Cy derivatives (Cy3, Cy5) represent the intensely colored and fluorescent cyanine dyes. The Cy phosphoramidites contain an MMT group and, in principal, can be added anywhere in the sequence, as described below for branched labelling reagents.

### *Dabcyl*

The dabcyl group is a universal quencher of fluorescence and this product locates the hydrophobic dabcyl at the 5'-terminus.

### *Psoralen*

Psoralen C2 and C6 phosphoramidites are used for the introduction of psoralen to oligonucleotides. Psoralen is an effective intercalator as well as cross-linking reagent.

## BRANCHED LABELLING REAGENTS AND SUPPORTS

### *Biotin*

Biotin phosphoramidite is capable of branching to allow multiple biotins to be introduced at the 3'- or 5'-terminus while biotin-dT can replace dT residues within the oligonucleotide sequence. BiotinTEG Phosphoramidite contains a 15 atom mixed polarity spacer arm based on triethylene glycol for optimal detection or capture. BiotinTEG-CPG is designed for the direct synthesis of oligonucleotides containing biotin at the 3'-terminus. Biotinylated oligonucleotides are used for the development of diagnostic probes and in DNA sequencing.

### *Fluorescein, 6-FAM-CPG, Fluorescein-dT-CPG*

Fluorescein phosphoramidite is designed to produce, on deprotection and isolation of the derived oligonucleotide, the same fluorescein-type structure as had been previously prepared using fluorescein isothiocyanate (FITC). Our fluorescein phosphoramidite also contains a DMT group to allow quantification of coupling. Fluorescein CPG is used to add the fluorescein label, based on FITC, at the 3'-terminus. 6-FAM-CPG is a single-isomer product derived from 6-carboxy-fluorescein and yields a simpler HPLC profile than fluorescein-CPG. Fluorescein-dT-CPG leads to a product with fluorescein attached to a thymidine analogue at the 3'-terminus, with a free 3'-OH available for further enzymatic reactions.

### *Dabcyl*

The dabcyl molecule acts to quench fluorescence of a dye by forming a non-fluorescent complex. It is particularly useful at the 3'-terminus of oligonucleotides with a fluorophore at the 5'-terminus and containing self-complementarity at the termini. In this way, the fluorescence of a probe is quenched since the stem loop formed favors complex formation. However, fluorescence occurs when the probe becomes linear upon hybridization with the target sequence. Dabsyl

CPG contains a sulfonamide linkage, while dabcyI CPG has an amide linkage to the spacer.

### *Acridine*

Acridine phosphoramidite is designed to produce, on deprotection and isolation, an oligonucleotide containing acridine at any position in the sequence. Acridine CPG is used to add the label at the 3'-terminus. Acridine is an effective intercalating agent.

### *Cholesterol, DNP*

Cholesterol is an effective carrier to enhance cell permeation of antisense oligonucleotides. 2,4-Dinitrophenyl (DNP) labelled oligos may be detected using anti-DNP antibodies.

## **3'-MODIFIERS**

In the design of novel diagnostic probes and in the synthesis of antisense oligonucleotides, there has been interest in the development of reagents for use in modifying the 3'-terminus of oligonucleotides. These modifications may also prove to be useful in the preparation of affinity supports. Oligonucleotides modified at the 3'-terminus resist 3'-exonuclease digestion.

### *3'-Phosphate CPG*

The Chemical Phosphorylation Reagent has proved its utility for phosphorylation of the 3'-terminus. However, the process may be simplified by the use of 3'-Phosphate CPG on which the oligonucleotide may be synthesized directly. After the standard ammonia deprotection, the sulfonylethyl linkage is  $\beta$ -eliminated from the target molecule, leaving a phosphate group at the 3'-terminus.

### *3'-Amino-Modifier CPG*

3'-Amino-Modifier CPG is used in the same way as normal nucleoside CPG for oligonucleotide synthesis. After deprotection,

the product oligonucleotide contains a primary amine at the 3'-terminus. An interesting benefit of this approach is that during the development of a diagnostic probe, the 5'-terminus can also be labelled with  $^{32}\text{P}$  to provide an additional highly sensitive marker. Modification of the 3'-terminus also blocks 3'-exonuclease digestion.

#### *3'-Thiol-Modifier C3 S-S CPG*

Thiol-Modifier C3 S-S CPG is used as the support and then the desired oligonucleotide is synthesized. To the standard ammonium hydroxide used for deprotection, dithiothreitol (DTT) can be added to cleave the disulfide linkage. The deprotection is then carried out in the normal manner. Alternatively, DTT can be omitted during the cleavage and deprotection. The resulting disulfide linkage can then be reduced to the thiol just prior to conjugation.

#### *3'-Spacer C3 CPG*

This simple product has found use as a reagent for blocking oligonucleotides from polymerase extension.

#### *Glyceryl CPG*

Glyceryl CPG has been used to generate oligonucleotides containing a 3'-aldehyde or carboxylate group.

#### *3'-Carboxylate Photolabile C6 CPG*

This is the first example of a product designed to release a fully protected oligonucleotide into solution by photochemical cleavage. In this case, a carboxylic acid group is also available for further conjugation reaction. Alternatively, this product can be used to introduce a 3'-carboxylate after cleavage and deprotection is carried out.

#### *3'-Amino Photolabile C6 CPG*

In a manner similar to the 3'-Carboxylate Photolabile C6 CPG, 3'-Amino Photolabile C6 CPG is designed to release a fully protected

oligonucleotide containing a 3'-amino group into solution by photochemical cleavage. This strategy allows more specificity in the subsequent conjugation reaction.

## RNA SUPPORTS FOR 3'-MODIFICATION

Interest in the development of antisense oligonucleotides has focused attention on the ability to attach marker and carrier molecules to the 3'-terminus. The 3'-hydroxyl group, though accessible for chemical modification, does not support specific reaction at that position because of the presence of competitive reactive centers. One approach to 3'-modification is to prepare an oligonucleotide with a ribonucleoside terminus, using an RNA support. Oxidation of the 2',3'-diol cleaves the 2'-3' bond and generates reactive aldehyde groups which are available for specific chemical manipulation.

Other labelling reagents may be added periodically. Their uses are varied and a Technical Bulletin will be produced for each product. In addition, full technical details of these and all newer products will be posted on our web site.

*Note On Monomer Catalog Numbers*

The last two digits of the catalog numbers are shown in this booklet as "xx". Please consult Pages 78-83 for information concerning available pack sizes for these products. The code for Glen Research products is shown below:

xx = 95	50µmoles
xx = 90	100µmoles
xx = 01	0.1g
xx = 02	0.25g
xx = 05	0.5g

*Other Instrument Types*

All minor bases, RNA products and modifiers are packaged for ABI instruments. If you would like another type of vial add the following to the end of the catalog number:

Biosearch Cyclone	E
Biosearch Expedite	E
MilliGen 7500	M
Beckman Oligo 1000	B
Pharmacia Gene Assembler	P

For reasons of quality assurance, we will no longer transfer powders or oils from stock Applied Biosystems vials to vials for other instruments. Powders may be hygroscopic and electrostatic, making transfer difficult, and oils have to be dissolved and the solvent evaporated. For best performance, it is preferable for the customer to dissolve the product and immediately transfer the solution to the correct instrument vial. Consequently, the unusual product will be delivered in an industry-standard septum-capped vial along with a clean dry vial for the appropriate instrument. In turn, we will monitor product usage for each instrument type and will attempt to have stock prepackaged for the most popular configurations.

*Storage and Solution Preparation*

Glen Research modifiers are packaged in septum-capped vials under argon. The compounds should be stored desiccated at 4°C or below

and, in the case of viscous oils, the vials should remain upright to allow the oil to gather at the bottom. Solutions should be prepared at the same concentration as used for nucleoside monomers. For Applied Biosystems and other DNA synthesizers, prepare a 0.1M solution by adding 1mL of anhydrous acetonitrile per 100 $\mu$ moles of the compound. Individual Certificates of Analysis show the dilution required for larger packs to produce a 0.1M solution. *For Beckman and Expedite instruments, dissolve 100 $\mu$ moles in 1.5mL of anhydrous acetonitrile. For larger packs, multiply the volume required for a 0.1M solution by 1.5 to give the approximately 0.067M solution used by these instruments.* Allow the liquid to sit for approximately 5 minutes, swirling occasionally to ensure complete dissolution. Unless otherwise noted the solutions are stable in acetonitrile for 3-5 days.

### *Instrument Preparation*

These products are packaged in or supplied with vials designed to fit directly on most commercial synthesizers on a spare monomer port. Before installing the modifier or labelling reagent, flush the line liberally with anhydrous acetonitrile to clean and dry the line. Quickly install the vial containing the modifier or labelling reagent. Prime the line to the valve block with the reagent solution. (Flushing the line with the reagent solution puts valuable reagent into the waste container.)

### *Coupling Reaction*

Modification reagents can be used without adjusting the coupling time for DNA synthesis which is typically 15 - 60 seconds. They can also be used with a longer coupling in an RNA synthesis. Labelling reagents containing both CE phosphoramidite and DMT groups are sluggish to react due to steric hindrance and should be coupled with a reaction time similar to RNA synthesis, i.e., 12 - 15 minutes. Because of this slow reaction with the 5'-hydroxyl group of the oligonucleotide, it is imperative to use anhydrous acetonitrile as diluent, to work quickly to minimize absorption of atmospheric moisture, and to use high quality anhydrous activator solution. These

reagents can be added in single or multiple additions at the 3'- or 5'-terminus and even within the sequence.

### *Deprotection*

If special deprotection steps are required, these are noted under the individual items. Usually, these reagents are compatible with DMT-on purification techniques.

### *Purification*

The modified or labelled oligonucleotide may be purified using a Poly-Pak™ cartridge, HPLC or gel electrophoresis. Poly-Pak purification is accomplished using the standard DMT-ON procedure. Reverse Phase (RP) HPLC may be performed on a modified oligonucleotide before or after attachment of the label. If purification is desired prior to label attachment, the DMT or MMT group should not be removed from the oligonucleotide as the lipophilic character of these groups aids in HPLC purification. RP HPLC purification is best accomplished using a C18 column. Oligos labelled with lipophilic groups like fluorescein or acridine can be purified "label-on" after removal of the DMT group on the synthesizer which also gives an indication of labelling yield.

### *Note On Catalog Numbers for Supports*

The last two digits of the catalog numbers in the supports section are shown as "xx". The last pages of the booklet show available pack sizes. The code for supports is shown below:

xx = 01	0.1g
xx = 10	1.0g
xx = 42	Pack of 4 0.2µmole columns
xx = 41	Pack of 4 1µmole columns

### *Other Instrument Types*

All minor bases, RNA products and modifiers are packaged for use on ABI instruments. If you would like a column for use on another

type of instrument, add the following to the end of the catalog number:

Biosearch Cyclone	E
Biosearch Expedite	E
Biosearch 8000 Series	E
MilliGen 7500	E

(Please inquire for availability of columns for other instrument types.)

### *Preparation for Synthesis using Modification Supports*

Modifier CPGs are off-white powders packaged in bulk or industry-standard DNA synthesis columns. They should be stored under the conditions recommended on the Certificate of Analysis. Since the synthesizer assumes that the 3'-base is attached to the CPG, an arbitrary base should be entered at the 3'-terminus and the 3'-terminal base of the target sequence should be entered as the second base.

### *Coupling Reactions on Modification Supports*

Columns should be used in a manner identical to normal protected nucleoside synthesis columns since the CPG contains a DMT group.

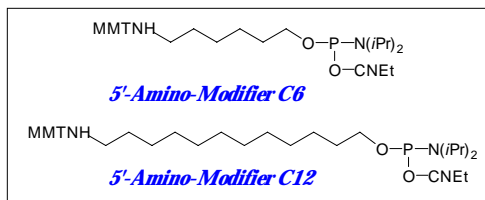
### *Deprotection of Oligonucleotides Modified at the 3'-Terminus*

The final DMT group may be removed on the synthesizer or it may be retained to aid in purification using a reverse phase technique. Ammonium hydroxide treatment for a minimum of 2 hours is used to cleave the modified oligonucleotide from the support prior to removal of all other protecting groups, including the base-labile Fmoc protecting group, with the normal deprotection conditions.

### *Purification of Oligonucleotides Modified at the 3'-Terminus*

The modified oligonucleotide may be purified using a Poly-Pak cartridge, HPLC or gel electrophoresis. Poly-Pak cartridge purification is accomplished using the standard DMT-on procedure.

Poly-Pak is a trademark of Glen Research Corporation

***5'-Amino-Modifier C3***

5'-Amino-Modifier C3 has been discontinued. The replacement is 5'-Amino-Modifier-C3 TFA (10-1923-xx) shown on Page 19.

***5'-Amino-Modifier C6***

Name: 6-(4-Monomethoxytritylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1906-xx

Formula:  $C_{35}H_{48}N_3O_3P$   
M.W.: 589.76

***5'-Amino-Modifier C12***

Name: 12-(4-Monomethoxytritylamino)dodecyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1912-xx

Formula:  $C_{41}H_{60}N_3O_3P$   
M.W.: 673.92

***Introduction***

5'-Amino-Modifiers are designed for use in automated synthesizers to functionalize the 5'-terminus of a target oligonucleotide. The primary amine can be used to attach a variety of products to the oligonucleotide, including fluorescent tags<sup>1,3</sup>, biotin<sup>2</sup>, alkaline phosphatase<sup>4</sup>, and EDTA<sup>5</sup>. The shorter carbon chain linkers may be

used to attach compounds where proximity to the oligonucleotide poses no problem. The longer carbon chain linkers have specific applications in affinity chromatography where the oligonucleotide must be adequately spaced from the surface, and for labelling with biotin<sup>2</sup> or fluorescent tags<sup>1,3</sup> where interaction with the oligonucleotide, or the duplex it forms, may quench some of the fluorescence.

### *Use of 5'-Amino-Modifiers*

Solution preparation and use of 5'-Amino-Modifiers is described on Page 10.

#### *Deprotection*

Due to the increased potential for thermally initiated side reactions during the deprotection of modified oligonucleotides, it is recommended that the ammonium hydroxide treatment be carried out at a lower temperature than is used for unmodified oligonucleotides. The following procedure has proved to be satisfactory. After adding the normal volume of ammonium hydroxide, leave the solution for 1 hour at room temperature followed by at least 17 hours at approximately 40°C. (Note: The ammonium hydroxide should be stored in the refrigerator.)

The MMT protecting group of the 5'-Amino-Modifiers can be removed on the synthesizer by deblocking until the yellow color elutes totally (typically 5 min.). The solution of MMT cation produced by acid deprotection is yellow and is not well quantified by trityl monitors. The method used to determine coupling efficiency is described below. However, for maximum amine reactivity, it is preferable to retain the MMT group for later removal with aqueous acid.

### *Purification*

The modified oligonucleotide may be purified using a Poly-Pak cartridge, HPLC or gel electrophoresis. Poly-Pak cartridge purification is accomplished using the trityl-on procedure. Reverse Phase (RP) HPLC may be performed either before or after attachment of the label. If purification is desired prior to label attachment, the MMT group should not be removed from the oligonucleotide as the lipophilic character of the MMT group aids in HPLC purification. RP HPLC purification is best accomplished using a C18 column.

If the Poly-Pak cartridge technique is used for purification, the MMT removal on the cartridge with 2% aqueous trifluoroacetic acid is not reliably efficient. For cartridge or RP HPLC, the MMT group is removed by treating the purified oligonucleotide with acetic acid:water (80:20) at room temperature for 1 hour.

### *References*

1. B.A. Connolly and P. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. B.S. Sproat, B.S. Beijer, P. Rider, and P. Neuner, *Nucleic Acids Res.*, 1987, **15**, 4837.
3. R. Zuckerman, D. Corey, and P. Shultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
4. P. Li, et al., *Nucleic Acids Res.*, 1987, **15**, 5275.
5. G.B. Dreyer and P.B. Dervan, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 968.

### *Determination of Coupling Efficiency*

This procedure is designed specifically for the determination of coupling efficiency. It should not be used for routine deprotection. The determination is based on a comparison of the absorbance of MMT cation at 472nm against the absorbance of the previous DMT cation at 497nm. The ratio factor (RF) of the absorbance of standard solutions is:

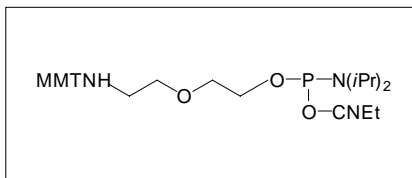
$$\text{RF} = \frac{\text{A-DMT}(497)}{\text{A-MMT}(472)} = 1.33$$

*Procedure*

1. Carry out synthesis (1 $\mu$ mole) in the trityl-on mode.
2. Collect the last DMT solution in a 100mL volumetric flask and make up to the mark with 0.1M toluenesulfonic acid in anhydrous acetonitrile (TSA). Measure the absorbance (A1) at 497nm, after zeroing the instrument at 600nm with TSA.
3. After the synthesis is complete, remove the synthesis column and manually deblock the MMT group with aliquots of the normal deblocking mix until all yellow color has eluted. This process takes up to 15 minutes. Collect the solution in a 100mL volumetric flask and make up to the mark with TSA. Measure the absorbance (A2) at 472nm.
4. Calculate the coupling efficiency using the formula:

$$\text{Coupling (\%)} = \frac{\text{A2}}{\text{A1}} \times \text{RF} \times 100$$

Note: Due to incomplete deblocking of the MMT group, this procedure yields a coupling efficiency determination about 5% below the actual coupling efficiency.



### *5'-Amino-Modifier 5*

Name: 2-[2-(4-Monomethoxytrityl)aminoethoxy]ethyl)-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1905-xx

Formula:  $C_{33}H_{44}N_3O_4P$   
M.W.: 577.71

### *Use of 5'-Amino-Modifier 5*

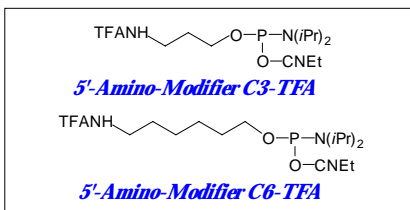
This product is a useful alternative to 5'-Amino-Modifier C6 but contains a five atom mixed polarity spacer designed for better performance of the derived amino-modified oligonucleotide in aqueous conditions. Solution preparation and use of 5'-Amino-Modifier 5 is described on Page 10.

### *Deprotection*

Deprotection conditions are identical to the alkylamino-modifiers and are described on Page 15.

### *Purification*

Purification procedures are identical to the alkylamino-modifiers and are described on Page 16.



### **5'-Amino-Modifier C3-TFA**

Name: 6-(Trifluoroacetylamino)propyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1923-xx

Formula:  $\text{C}_{14}\text{H}_{25}\text{F}_3\text{N}_3\text{O}_3\text{P}$   
M.W.: 371.34

### **5'-Amino-Modifier C6-TFA**

Name: 6-(Trifluoroacetylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1916-xx

Formula:  $\text{C}_{17}\text{H}_{31}\text{F}_3\text{N}_3\text{O}_3\text{P}$   
M.W.: 413.42

### **Use of 5'-Amino-Modifier TFA**

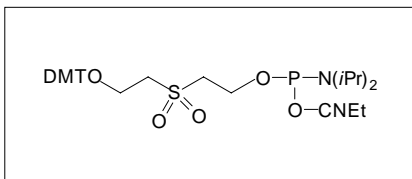
Amino-Modifier TFA is used when there is no need to purify the derived amino-modified oligonucleotide. Solution preparation and use of 5'-Amino-Modifier TFA is described on Page 10.

### *Deprotection*

The base-labile TFA protecting group of the 5'-Amino-Modifier TFA is removed during the standard ammonium hydroxide deprotection.

### *Purification*

The primary amino group of the crude amino-modified oligonucleotide may be reacted with a labelling compound prior to purification. The labelled product is then purified using RP HPLC.



### *Chemical Phosphorylation Reagent*

Name: 2-[2-(4,4'-Dimethoxytrityloxy)ethylsulfonyl]ethyl-(2-cyanoethyl)-(N,N-diisopropyl)- phosphoramidite

Cat. No.: 10-1900-xx

Formula:  $C_{34}H_{45}N_2O_7PS$   
M.W.: 656.77

### *Use of Chemical Phosphorylation Reagent for 5'-Phosphorylation*

The use of this reagent is an alternative to enzymatic techniques for oligonucleotide phosphorylation<sup>1</sup> with the advantage of allowing determination of phosphorylation efficiency.

Solution preparation and use of Chemical Phosphorylation Reagent is described on Page 10.

### *Deprotection*

The DMT group should be removed on the synthesizer by the standard deblocking method to determine coupling efficiency. The standard ammonium hydroxide treatment is used to cleave the modified oligonucleotide from the support and to remove all other protecting groups as well as the sulfonylethyl group. Note that the DMT group is eliminated with the sulfonylethyl group during ammonium hydroxide deprotection, rendering this product incompatible with reverse phase chromatographic purification techniques.

### ***Use of Chemical Phosphorylation Reagent for 3'-Phosphorylation*** (Note: See also 3'-Phosphate CPG on Page 56)

Chemical Phosphorylation Reagent has proved to be fast and convenient for chemical phosphorylation of the 5'-terminus of oligonucleotides. In addition, this reagent has proved its utility for simple phosphorylation of the 3'-terminus. It is introduced as the first addition to any nucleoside support, followed by normal synthesis of the target oligonucleotide. After the standard ammonium hydroxide deprotection, the linkage decomposes and is  $\beta$ -eliminated from the target molecule, leaving a phosphate group at the 3'-terminus.

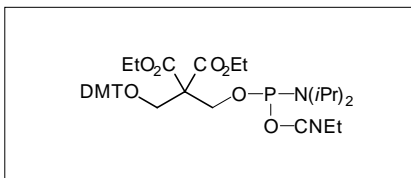
The final DMT group may be removed on the synthesizer or it may be retained to aid in purification. If the DMT group is retained, it may be removed on a purification cartridge or, following purification, by treating the oligonucleotide with acetic acid:water (80:20) at room temperature for 1 hour.

### ***Purification of 3'- or 5'-Phosphates***

Oligonucleotides with 5'-phosphates may be purified using either HPLC or electrophoresis. Ion-exchange HPLC or polyacrylamide gel electrophoresis, using conventional methods, are recommended. If chromatographic purification is considered to be unnecessary, the phosphorylated oligonucleotide can be conveniently desalted on a purification cartridge.

### ***Reference***

1. T. Horn and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.



### *Chemical Phosphorylation Reagent (CPR II)*

Name: [3-(4,4'-Dimethoxytrityloxy)-2,2-dicarboxyethyl]propyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1901-xx

Formula:  $C_{39}H_{51}N_2O_9P$   
M.W.: 722.82

The DMT protecting group of the Chemical Phosphorylation Reagent (10-1900-xx) can not be used for DMT-on purification. If the DMT group is intentionally left on the oligonucleotide, it is eliminated along with the sulfonyl ethyl group to produce the 5'-phosphate during the ammonium hydroxide deprotection.

A novel phosphorylation reagent, CPR II, has been described<sup>1</sup>. It contains a DMT group which can be removed on the synthesizer to determine phosphorylation yield. The side chain is then completely eliminated during ammonium hydroxide deprotection. Alternatively, with this reagent, the DMT group can be left on the oligonucleotide and used for RP purification. The DMT group is removed with aqueous acid and the side chain is eliminated after brief treatment with aqueous ammonium hydroxide to yield the 5'-phosphate. This novel phosphorylation reagent clearly offers great potential for rapid purification of oligonucleotide 5'-phosphates based on the popular DMT-on technique using disposable cartridges such as Poly-Pak cartridges.

### *Use of CPR II for 5'-Phosphorylation*

The coupling rate of CPR II is slower than for regular deoxynucleoside phosphoramidites and the coupling wait step should be increased to 6 minutes (360 seconds). In addition, the capping step in the last cycle should be omitted to avoid some reversal of coupling. This applies to all instruments regardless of whether the capping step is before or after oxidation. This instability to the capping mix means that CPR II should not be used for 3'-phosphorylation.

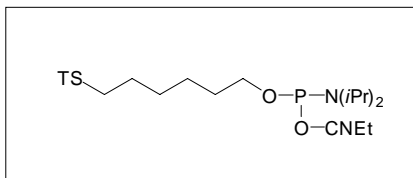
### *Purification*

The DMT group of CPR II can be retained for use in purification or can be removed to determine coupling yield. If no purification is planned for the phosphorylated oligonucleotide, the final DMT group should be removed. (Use two detritylation steps separated by a trityl flush step for complete DMT removal.) The oligonucleotide should then be cleaved and deprotected normally. After isolation, the phosphorylated oligonucleotide can be simply desalted and used. Purification of phosphorylated oligonucleotides can be achieved by ion-exchange HPLC or gel electrophoresis.

A simple reverse phase purification strategy is also possible. After normal cleavage and deprotection with ammonium hydroxide, the oligonucleotide can be purified by reverse phase cartridge or HPLC. On a cartridge, the DMT group is removed using 2% aqueous trifluoroacetic acid for 5 minutes. The phosphorylated oligonucleotide is eluted from the cartridge in the normal manner. If HPLC is used, the DMT group is removed with acetic acid/water (4:1) for 1 hour at room temperature and the sample is dried. In both cases, the final elimination of the side chain is achieved with water/ammonium hydroxide (2:1) for 15 minutes.

### *Reference*

1. A. Guzaev, H. Salo, A. Azhaye, and H. Lonnberg, *Tetrahedron*, 1995, **51**, 9375-9384.



### ***5'-Thiol-Modifier C6***

Name: (S-Trityl-6-mercaptohexyl)-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1926-xx

Formula:  $C_{34}H_{45}N_2O_2S$   
M.W.: 576.78

### ***Use of 5'-Thiol-Modifier C6***

The 5'-Thiol-Modifier C6 is used to produce a thiol group at the 5'-terminus of a synthetic oligonucleotide<sup>1</sup>. The thiol group can be used to attach a variety of products to the oligonucleotide, including fluorescent tags<sup>1,3</sup>, biotin<sup>2</sup>, and alkaline phosphatase<sup>4</sup>.

Solution preparation and use of 5'-Thiol-Modifiers is described on Page 10. *Carry out the oxidation step of the final cycle with 0.02M Iodine solution to minimize oxidative cleavage of the trityl-S linkage.*

### ***Deprotection and Purification***

The trityl group used to protect the thiol is not acid labile and therefore can not be removed on a DNA synthesizer using the normal acid deprotection. Cleavage of the oligonucleotide from the support and removal of the base protecting groups are carried out with ammonium hydroxide in the normal manner. If purification is desired, it should be done before removing the trityl group. The presence of the trityl group allows standard trityl-on reverse phase (RP) purification techniques to be used.

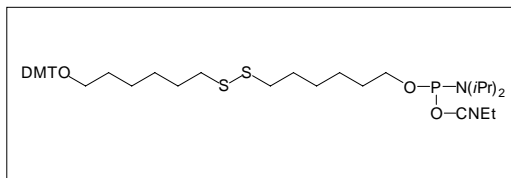
Final deblocking of the oligonucleotide involves cleavage of the trityl-sulfur bond. This is accomplished by oxidation with silver nitrate with the excess silver nitrate being precipitated with dithiothreitol (DTT). Excess DTT can be removed by extraction with ethyl acetate, by desalting or by ethanol precipitation.

### *Procedure*

1. Deprotect with ammonium hydroxide in the normal manner.
2. Purify the trityl containing oligonucleotide by HPLC or Poly-Pak cartridge.
3. Evaporate the product solution to dryness.
4. Suspend the product in 0.1M triethylammonium acetate (TEAA), pH6.5 at a concentration of approximately 100 A260 units/mL.
5. Add 0.15 volumes of 1M aqueous silver nitrate solution, mix thoroughly, and leave to react at room temperature for 30 minutes.
6. Add 0.20 volumes of 1M aqueous DTT solution, mix thoroughly, and leave at room temperature for 5 minutes.
7. Centrifuge the suspension to remove the silver DTT complex. Remove the supernatant. Wash the precipitate with 1 volume of 0.1M TEAA. Centrifuge and combine the supernatant with the first volume. (Alternatively, vortex the suspension and apply to a desalting column equilibrated with conjugation buffer.)
8. Remove the excess DTT from the supernatant by desalting on either a Poly-Pak cartridge (see Note on Page 29) or NAP-25 column and proceed directly to the conjugation reaction. (If not used immediately, the free thiol oligonucleotide must be stored under an inert atmosphere to avoid oxidative dimerization to the disulfide.)

### *References*

1. B.A. Connolly and P. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. B.S. Sproat, B.S. Beijer, P. Rider, and P. Neuner, *Nucleic Acids Res.*, 1987, **15**, 4837.
3. R. Zuckerman, D. Corey, and P. Shultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
4. P. Li, et al., *Nucleic Acids Res.*, 1987, **15**, 5275.



### *5'-Thiol-Modifier C6 S-S*

Name: 1-O-Dimethoxytrityl-hexyl-disulfide, 1'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1936-xx

Formula:  $C_{42}H_{61}N_2O_5PS_2$   
M.W.: 769.05

The conjugation of enzymes, especially horseradish peroxidase, to oligonucleotides has become very significant in the production of diagnostic probe systems. These developments, along with continued interest in labelling with thiol-specific tags, have prompted us to rethink routes to thiol-modified oligonucleotides.

Thiol-Modifiers for the production of sulfhydryl groups at the 5'-terminus have been readily available commercially, including from Glen Research. These products are based<sup>1,2,3</sup> on blocking the thiol group during synthesis with a trityl protecting group. Although this procedure has been successfully used, several problems exist including a low level of oxidative detritylation during oligonucleotide synthesis and the use of silver nitrate during final deblocking. A recent note<sup>4</sup> describes a procedure to modify a 5'-amino-modified oligonucleotide to a thiol using N-acetyl-DL-homocystein thiolactone. Synthetic routes to oligonucleotides containing 3'-thiols have also been described<sup>5,6,7</sup> utilizing solid supports containing disulfide linkages. 3'-Thiol-modified oligonucleotides are especially interesting in cases where a different label is desired for the 5'-terminus.

### ***Use of 5'-Thiol-Modifier C6 S-S***

Thiol-Modifier S-S is an alternative to 5'-Thiol-Modifier C6 but can be used at the 5'- or 3'-terminus. Solution preparation and use of 5'-Thiol-Modifier C6 S-S is described on Page 10. *If the reagent is added only at the 5'-terminus, carry out the oxidation step of the final cycle with 0.02M Iodine solution to avoid oxidative cleavage of the disulfide linkage. If used at the 3'-terminus, all oxidation steps should use 0.02M Iodine solution.*

### ***Thiol Group at the 5'-Terminus***

#### ***DMT-off Synthesis***

Add the Thiol-Modifier S-S at the 5'-terminus of the oligonucleotide in the automated DMT-off synthesis mode. The DMT release from the last cycle can be used to determine coupling efficiency. To the standard ammonium hydroxide used for deprotection, add dithiothreitol (DTT) to a concentration of 0.05M. Carry out deprotection in the normal manner (typically 55°C/16h). This procedure removes the base protecting groups and cleaves the disulfide linkage to generate the 5'-thiol. Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures.

#### ***DMT-on Synthesis***

Add the Thiol-Modifier S-S at the 5'-terminus of the oligonucleotide in the automated DMT-on synthesis mode. Carry out deprotection in the normal manner. Purify the trityl containing oligonucleotide on a Poly-Pak cartridge omitting the 2% TFA step. Evaporate the product solution to dryness. Cleave the disulfide linkage using 100 mM DTT, pH 8.3 - 8.5, at room temperature for 30 minutes<sup>8</sup>. Desalt the oligonucleotide on a Poly-Pak cartridge. Be sure to include a 10 mL rinse of 5% acetonitrile (ACN) in 0.1 M TEAA. This will remove any residual DTT that is bound to the cartridge without any loss of oligo. Elute the oligo from the Poly-Pak cartridge as usual with 20% ACN in water. (The DMT containing thiol will remain attached to the cartridge.)

### *Thiol Group at the 3'-Terminus*

Use Thiol-Modifier C3 S-S CPG as support or add the Thiol-Modifier C6 S-S to any nucleoside support and then synthesize the desired oligonucleotide. *To avoid oxidative cleavage of the disulfide linkage, all oxidation steps should use 0.02M Iodine solution.* To the standard ammonium hydroxide used for deprotection, add dithiothreitol (DTT) to a concentration of 50mM. Carry out deprotection in the normal manner (typically 55°C/8h min.). This procedure removes the base protecting groups and cleaves the disulfide linkage to generate the 3'-thiol. Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures. Alternatively, omit the DTT from the ammonium hydroxide. Just prior to conjugation, cleave the disulfide with 100mM DTT/ pH 8.3 - 8.5/RT/30min. Desalt on a desalting column equilibrated with conjugation buffer. Continue to the conjugation reaction. *See also 3'-Thiol-Modifier C3 S-S CPG on Page 55.*

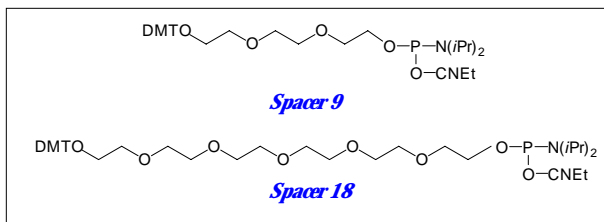
### *References*

1. B.A. Connolly and R. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. B.A. Connolly, *Nucleic Acids Res.*, 1987, **15**, 3131-3139.
3. N.D. Sinha and R.M. Cook, *Nucleic Acids Res.*, 1988, **16**, 2659.
4. A. Kumar, S. Advani, H. Dawar, and G.P. Talwar, *Nucleic Acids Res.*, 1991, **19**, 4561.
5. R. Zuckermann, D. Corey, and P. Schultz, *Nucleic Acids Res.*, 1987, **15**, 5305.
6. K.C. Gupta, P. Sharma, S. Sathyanarayana, and P. Kumar, *Tetrahedron Lett.*, 1990, **31**, 2471-2474.
7. U. Asseline, E. Bonfils, R. Kurfurst, M. Chassignol, V. Roig, and N.T. Thuong, *Tetrahedron*, 1992, **48**, 1233-1254.
8. Gregg Morin, Geron Corporation, Personal Communication.

*Notes:*

Thiol-modified oligonucleotides should be kept either under an inert atmosphere or in a solution containing DTT (10mM) to avoid oxidative disulfide formation. The DTT can be extracted from the solution using ethyl acetate or removed by desalting on a Poly-Pak cartridge prior to conjugation.

When desalting an oligonucleotide from a solution containing DTT on a Poly-Pak cartridge, it is necessary to include a 10 mL rinse of 5% acetonitrile (ACN) in 0.1 M TEAA. This will remove any residual DTT that is bound to the cartridge without any loss of oligo. Elute the oligo from the Poly-Pak cartridge as usual with 20% ACN in water.

***Spacer Phosphoramidite 9***

Name: 9-O-Dimethoxytrityl-triethyleneglycol, 1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1909-xx

Formula:  $C_{36}H_{49}N_2O_7P$   
M.W.: 652.77

***Spacer Phosphoramidite 18***

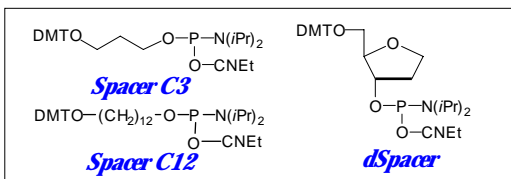
Name: 18-O-Dimethoxytrityl-hexaethyleneglycol, 1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1918-xx

Formula:  $C_{42}H_{61}N_2O_{10}P$   
M.W.: 784.93

***Use of Spacer 9 and 18 Phosphoramidites***

Spacer molecules have been used to bridge sections of oligonucleotides where no appropriate binding is possible, as well as to space tags further away from the oligonucleotide. An other application is in the preparation of oligonucleotides containing hairpin loops made of polyethylene glycol<sup>1,2,3</sup>. Naturally occurring hairpin and cruciform structures are known to exist in regions which function as regulation and promotion sites. The loop length can be varied by consecutive additions of Spacer Phosphoramidites.



### ***Spacer Phosphoramidite C3***

Name: 3-O-Dimethoxytrityl-propyl-1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1913-xx

Formula: C<sub>34</sub>H<sub>44</sub>N<sub>2</sub>O<sub>5</sub>P  
M.W.: 578.69

### ***Spacer Phosphoramidite C12***

Name: 12-O-Dimethoxytrityl-dodecyl-1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1928-xx

Formula: C<sub>42</sub>H<sub>61</sub>N<sub>2</sub>O<sub>5</sub>P  
M.W.: 704.93

### ***dSpacer Phosphoramidite***

Name: 5'-O-Dimethoxytrityl-1',2'-Dideoxyribose-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1914-xx

Formula: C<sub>35</sub>H<sub>45</sub>N<sub>2</sub>O<sub>6</sub>P  
M.W.: 620.73

### ***Use of Spacer C3, Spacer C12, and dSpacer Phosphoramidites***

Spacer Phosphoramidites C3 and C12 can be added to substitute for unknown bases within a sequence. dSpacer<sup>4,5</sup> can be used to mimic

abasic sites in an oligonucleotide. Abasic sites in DNA are generated chemically or enzymatically by selective hydrolysis of the glycosidic linkage. The resulting apurinic/apyrimidinic sites lack coding information and lead to misincorporation of bases by polymerases and may play an important role in mutagenesis. Abasic sites are rather unstable and are susceptible to  $\beta$ -elimination causing chain scission. The instability of the natural abasic site can be eliminated by the substitution of dSpacer which is the reduced form of the natural abasic site

Solution preparation and use of the Spacer Phosphoramidites are described on Page 10.

#### *Deprotection*

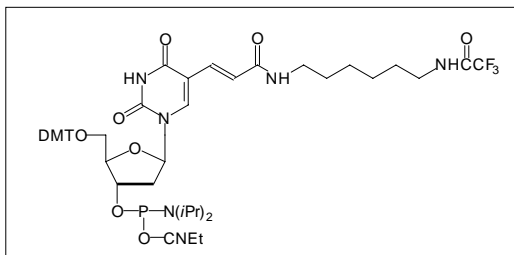
Oligonucleotides containing Spacer Phosphoramidites are deprotected under normal conditions.

#### *Purification*

There is no need for any changes to regular purification and isolation schemes.

#### *References*

1. M. Durard, K. Chevrie, M. Chassignol, N.T. Thuong, and J.C. Maurizot, *Nucleic Acids Res.*, 1990, **18**, 6353.
2. M. Salunkhe, T.F. Wu, and R.L. Letsinger, *J. Amer. Chem. Soc.*, 1992, **114**, 8768-8772.
3. N.G. Dolinnaya, M. Blumenfeld, I.N. Merenkova, T.S. Oretskaya, N.F.Krynetskaya, M.G. Ivanovskaya, M. Vasseur, and Z.A. Shabarova, *Nucleic Acids Res.*, 1993, **21**, 5403-5407.
4. M. Takeshita, C.N. Chang, F. Johnson, S. Will, and A.P. Grollman, *J. Biol. Chem.*, 1987, **262**, 10171-10179.
5. M.W. Kalnik, C.N. Chang, A.P. Grollman, and D.J. Patel, *Biochemistry*, 1988, **27**, 924-931.



### *Amino-Modifier C6 dT*

Name: 5'-Dimethoxytrityl-5-[N-(trifluoroacetylaminohexyl)-3-acrylimido]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

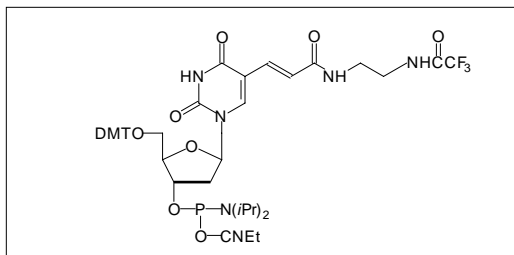
Cat. No.: 10-1039-xx

Formula:  $C_{50}H_{62}N_6O_{10}F_3P$   
M.W.: 995.05

Amino-Modifier C6 dT<sup>1</sup> and its corresponding labelled products Biotin-, Fluorescein-, TAMRA-, and Dabcyl-dT have proved to be optimal for amino-modifying and labelling oligonucleotides within the sequence. The resulting labelled oligonucleotide has standard hybridization characteristics<sup>2</sup> and the amino-modified oligonucleotide is ideal for attaching large molecules like alkaline phosphatase<sup>3,4</sup>.

Duplexes formed from oligos containing T labelled at the C-5 position were compared<sup>2</sup> to similar oligos where the label and spacer were attached at the N-4 position of C. Duplexes containing a modified T base exhibited normal melting behavior, while those containing a modified C base in some cases did not.

Biotin-, Fluorescein-, TAMRA-, and Dabcyl-dT are the labelled



### *Amino-Modifier C2 dT*

Name: 5'-Dimethoxytrityl-5-[N-(trifluoroacetyl aminoethyl)-3-acrylimido]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

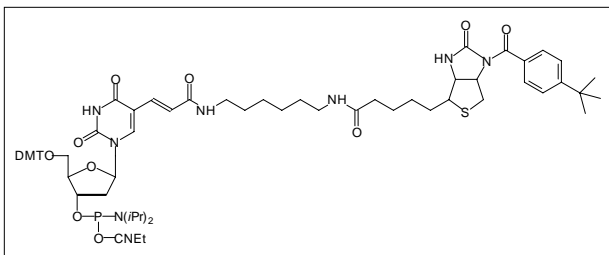
Cat. No.: 10-1037-xx

Formula:  $C_{46}H_{54}N_6O_{10}F_3P$   
M.W.: 938.94

versions of Amino-Modifier C6 dT and exhibit the same desirable hybridization attributes.

In contrast to Amino-Modifier C6 dT where the label is designed to be placed where it cannot interact with the double stranded oligonucleotide, the C2 version is designed for the attachment of molecules like EDTA or alkylating reagents which can cut the complementary strand or double strand. EDTA-C2-dT is the analogue of Amino-Modifier C2 dT that contains the triethyl ester of EDTA. The esters are hydrolyzed during deprotection using 0.4M methanolic sodium hydroxide, as described on Page 73.

Carboxy-dT is used when conjugation of an amino-containing tag is desired.



### *Biotin-dT*

Name: 5'-Dimethoxytrityl-5-[N-(((4-*t*-butylbenzoyl)-biotinyl)-aminohexyl)-3-acrylimido]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1038-xx

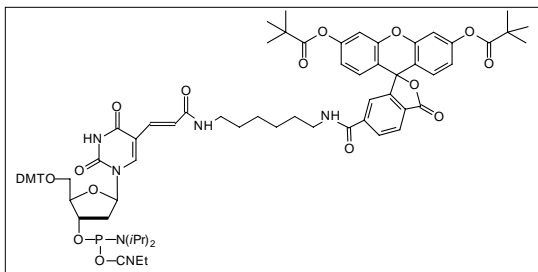
Formula: C<sub>69</sub>H<sub>89</sub>N<sub>8</sub>O<sub>12</sub>PS  
M.W.: 1285.55

### *Deprotection and Purification*

Amino-Modifier dT reacts in a manner identical to normal phosphoramidite monomers and the trifluoroacetyl protecting group on the primary amine is removed during the standard ammonium hydroxide deprotection step.

The *t*-butylbenzoyl group, used in Biotin-dT to add solubility to the product and to protect the biotin, is removed during the standard ammonium hydroxide deprotection step.

Oligos containing Fluorescein-dT can be deprotected with ammonium hydroxide using standard conditions, which also remove the protecting groups on the fluorescein section. Similarly, Dabcyl-dT is deprotected under standard conditions with ammonium



### *Fluorescein-dT*

Name: 5'-Dimethoxytrityloxy-5-[N-((3',6'-dipivaloylfluoresceinyl)-amino)hexyl]-3-acrylimido]-2'-deoxyUridine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1056-xx

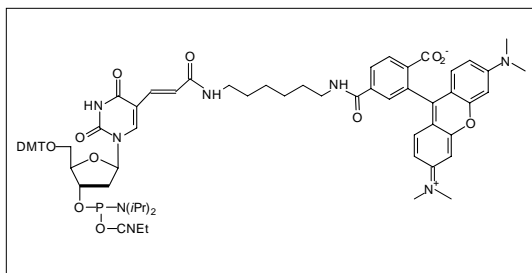
Formula:  $C_{79}H_{89}N_6O_{17}P$   
M.W.: 1425.57

hydroxide. The DabcyI group does not require further protecting groups.

TAMRA-dT has to be deprotected under very mild conditions to safeguard the labile TAMRA section. We recommend the use of UltraMild monomers and the use of potassium carbonate in methanol for deprotection, as described on Page 72. An alternative procedure using t-butylamine/methanol/water (1:1:2), which allows the use of regular monomers, has also been described.<sup>5,6</sup>

The ethyl esters of EDTA-C2-dT<sup>7</sup> are hydrolyzed during mild deprotection with the sodium hydroxide in methanol procedure shown on Page 73.

The methyl ester of Carboxy-dT is hydrolyzed during deprotection



### *TAMRA-dT*

Name: 5'-Dimethoxytrityloxy-5-[N-((tetramethylrhodaminy)-aminohexyl)-3-acrylimido]-2'-deoxyUridine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1057-xx

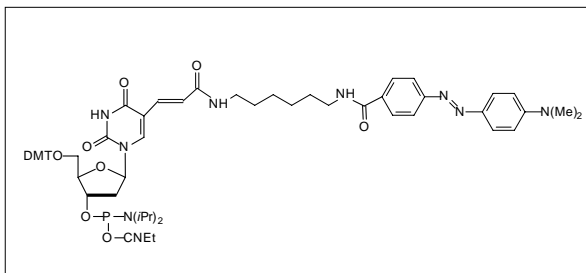
Formula:  $C_{73}H_{83}N_8O_{13}P$   
M.W.: 1311.48

with the sodium hydroxide in methanol procedure shown on Page 73 and can be coupled directly to a molecule containing a primary amino group by a standard peptide coupling<sup>8</sup> or *via* the intermediate N-hydroxy-succinimide (NHS) ester.

Purification of the modified synthetic oligonucleotides is accomplished using standard procedures: purification cartridge, HPLC or gel electrophoresis.

#### *Procedure for Coupling to Carboxyl Group*

Dissolve the carboxyl modified oligonucleotide (0.05 $\mu$ moles) in 50 $\mu$ L of a 0.5M aqueous solution of the desired amino component, containing 0.025mmol of N-ethyl-N(3-dimethylaminopropyl)-carbodiimide. Incubate the reaction mixture at 4°C for 6 hours.



### *Dabcyl-dT*

Name: 5'-Dimethoxytrityloxy-5-[(N-4'-carboxy-4-(dimethylamino)-azobenzene)-amino]hexyl-3-acrylimido]-2'-deoxyUridine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

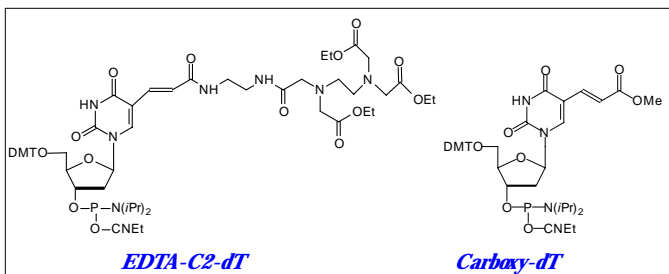
Cat. No.: 10-1058-xx

Formula:  $C_{63}H_{76}N_9O_{10}P$   
M.W.: 1150.32

Purify the conjugated oligonucleotide by RP HPLC.

### *References*

1. J.L. Ruth, C. Morgan, and A. Pasko, *DNA*, 1985, **4**, 93.
2. J. Telsner, K.A. Cruickshank, L.E. Morrison, and T.L. Netzler, *J. Am. Chem. Soc.*, 1989, **111**, 6966.
3. E. Jablonski, E.W. Moomaw, R.H. Tullis, and J.L. Ruth, *Nucleic Acids Res.*, 1986, **14**, 6115.
4. J.G. Farmar and M. Castaneda, *Biotechniques*, 1991, **11**, 588-589.
5. B. Mullah and A. Andrus, *Tetrahedron Lett.*, 1997, **38**, 5751-5754.
6. S.L. Woo, S.M. Menchen, and S. Fung, 1993, US Patent No. 5,231,191.
7. G.B. Dreyer and P.B. Dervan, *Proc Natl Acad Sci U S A*, 1985, **82**, 968-72.

***EDTA-C2-dT***

Name: 5'-Dimethoxytrityl-5-[N-ethylenediaminetriethylacetate, monoacetyl aminoethyl-3-acrylimido]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1059-xx

Formula:  $C_{60}H_{81}N_8O_{16}P$   
M.W.: 1201.32

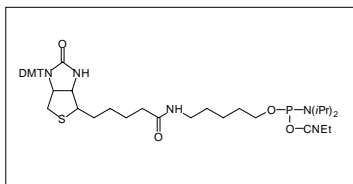
***Carboxy-dT***

Name: 5'-Dimethoxytrityl-5-[3-methyl acrylate]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1035-xx

Formula:  $C_{43}H_{51}N_4O_{10}P$   
M.W.: 814.88

8. U. Asseline, E. Bonfils, R. Kurfurst, M. Chassignol, V. Roig, and N.T. Thuong, *Tetrahedron*, 1992, **48**, 1233.



### *5'-Biotin Phosphoramidite*

Name: [1-N-(4,4'-Dimethoxytrityl)-biotinyl-6-aminoethyl]-2-cyanoethyl-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-5950-xx

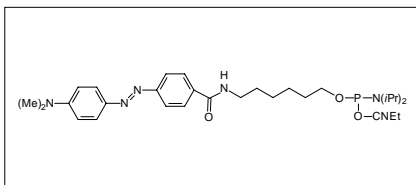
Formula:  $C_{46}H_{64}N_5O_6PS$   
M.W.: 846.08

The performance features of 5'-Biotin Phosphoramidite are listed below:

- It is, of course, freely soluble in acetonitrile.
- It can be used with the standard cycle of all instruments (although it will benefit from up to a 3 minute coupling time).
- Because of the short coupling time, it is more robust than our other biotin products with better performance in higher moisture situations and, therefore, has a better lifetime on the synthesizer.
- Because of the DMT group on the biotin, the coupling yield can still be determined on the synthesizer.

**CAUTION!** 5'-Biotin phosphoramidite can be added only once to the 5'-terminus of an oligonucleotide. However, the DMT group on the biotin can be used in RP cartridge and HPLC purification techniques.

5'-Biotin Phosphoramidite is sold under license from Zeneca PLC.



### *5'-DabcyI Phosphoramidite*

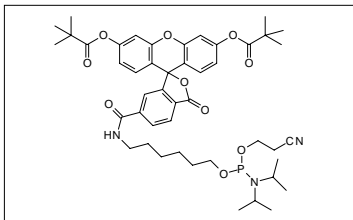
Name: 6-(N-4'-carboxy-4-(dimethylamino)-azobenzene)-aminohexyl-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-5912-xx

Formula:  $C_{30}H_{45}N_6O_3P$   
M.W.: 568.69

When the dabcyI group is spatially adjacent to a fluorophore, fluorescence is quenched. This property has been used in the preparation of molecular beacon probes which form a stem loop structure with fluorescence quenched until they become linear on hybridization to the target, returning the natural fluorescence. DabcyI is also a very hydrophobic molecule and the use of 5'-DabcyI Phosphoramidite locates it at the 5'-terminus where it can aid in purification using reverse phase techniques.

5'-DabcyI Phosphoramidite can be used with the standard cycle of all DNA synthesizers. However, as with all minor bases and most labelling reagents, it will benefit from an extended coupling time of 3 minutes. Although there is no DMT group to remove from 5'-DabcyI Phosphoramidite, the synthesis should be carried out DMT-on. Purification of oligos labelled with 5'-dabcyI alone is easily done using the Poly-Pak LABEL-on technique. Most oligos containing dabcyI will be doubly labelled and special cartridge techniques or RP HPLC will be needed for purification.



### *5'-Fluorescein Phosphoramidite (6-FAM)*

Name: [(3',6'-dipivaloylfluoresceinyl)-6-carboxamidohexyl]-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

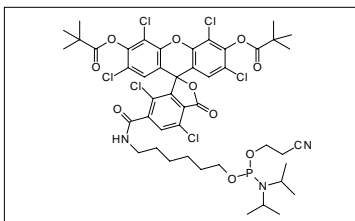
Cat. No.: 10-5901-xx

Formula:  $C_{46}H_{58}N_3O_{10}P$   
M.W.: 843.95

### *Use of Fluorescein Phosphoramidites*

Fluorescein labelled oligonucleotides have found applications in DNA sequencing and amplification, as well as techniques for genetic analysis. 5'-Fluorescein phosphoramidite is used to label the 5'-terminus during oligonucleotide synthesis. The product contains no 4,4'-dimethoxytrityl (DMT) group and can be added only once at the 5'-terminus, thereby terminating synthesis. This product is prepared using the 6-carboxyfluorescein isomer with a straight chain 6 carbon linker. Therefore, only one fluorescein-oligo peak is detected on RP HPLC.

5'-Fluorescein phosphoramidites can be used with the standard cycle of all DNA synthesizers. However, as with all minor bases and most labelling reagents, they will benefit from an extended coupling time of 3 minutes.



### *5'-Hexachloro-Fluorescein Phosphoramidite (HEX)*

Name: [4,7,2',4',5',7'-hexachloro-(3',6'-dipivaloylfluoresceinyl)-6-carboxamidohexyl]-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

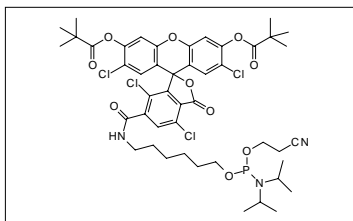
Cat. No.: 10-5902-xx

Formula:  $C_{46}H_{52}N_3O_{10}Cl_6$   
M.W.: 1050.62

To take advantage of the multicolor detection capability of modern DNA sequencers and genetic analyzers, further derivatives of 5'-fluorescein phosphoramidite with shifted absorbance and emission maxima are of interest. The tetrachloro- and hexachloro-fluorescein phosphoramidites are the first two in a series of fluorescein analogues. The use of these products is identical to their fluorescein parent. The spectral characteristics of these dyes are detailed on Page 46.

### *Deprotection*

In general, dye-labelled oligonucleotides can be deprotected at room temperature in concentrated ammonium hydroxide for a minimum of 24 hours, or shorter times which are appropriated for the protecting groups on the monomers being used. FAM labelled oligos can be heated to 55°C in ammonium hydroxide for extended periods of time



### ***5'-Tetrachloro-Fluorescein Phosphoramidite (TET)***

Name: [4,7,2',7'-tetrachloro-(3',6'-dipivaloylfluoresceinyl)-6-carboxamidohexyl]-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-5903-xx

Formula:  $C_{46}H_{54}N_3O_{10}Cl_4P$   
M.W.: 981.73

but TET and Cy-3 labelled oligos are less stable and will tolerate only a few hours at 55°C. HEX and Cy-5 labelled oligos must be deprotected at room temperature and the ammonia should be removed immediately after deprotection.

### ***Purification***

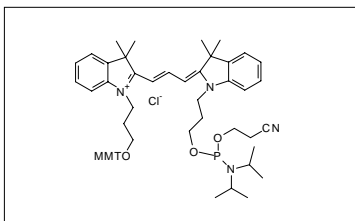
Purify the dye-labelled oligonucleotide using cartridge or HPLC purification. The cartridge procedure is simple and effective.

### ***Sample Preparation***

1. Dry the dye-labelled oligonucleotide and dissolve the residue in 1mL 0.1M Triethylammonium acetate (TEAA).

### ***Cartridge Preparation***

2. Connect a syringe to the female luer of the cartridge and have the male luer terminate in a waste vessel.



### *Cy3-CE Phosphoramidite*

Name: Indodicarbocyanine 3, 1'-O-(4-monomethoxytrityl)-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

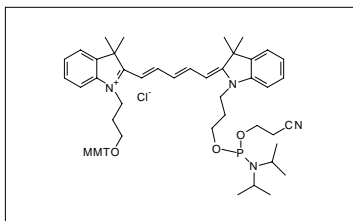
Cat. No.: 10-5913-xx

Formula:  $C_{58}H_{70}N_4O_4PCl$   
M.W.: 953.64

3. Flush the cartridge with 2mL acetonitrile followed by 2mL 2M TEAA.

#### *Purification Procedure*

4. Load the sample solution from step 1 onto the cartridge. Collect the eluted fraction and again push it through the cartridge.
5. Flush the cartridge with 5mL 8% acetonitrile in 0.1M TEAA.
6. Flush the cartridge with 2mL of deionized water.
7. Elute the purified oligonucleotide by flushing the cartridge with ~2mL 20% acetonitrile. Collect the eluted fractions. The first 4 drops of eluent can be discarded and the product is normally in the next 1mL. Use the color of the product to determine the extent of elution from the cartridge.
8. Determine the  $A_{260}$  units and store any unused oligonucleotide as a lyophilized solid or in neutral aqueous media at  $-20^{\circ}C$ .



### *Cy5-CE Phosphoramidite*

Name: Indodicarbocyanine 5, 1'-O-(4-monomethoxytrityl)-1-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

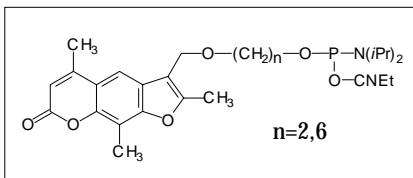
Cat. No.: 10-5915-xx

Formula:  $C_{60}H_{72}N_4O_4PCl$

M.W.: 979.68

### *Fluorescence Properties of Fluorescein, TAMRA and Cy dyes*

	Absorbance Maximum	Emission Maximum	Color
Fluorescein	494nm	525nm	Green
Tetrachloro-Fluorescein	521nm	536nm	Orange
Hexachloro-Fluorescein	535nm	556nm	Pink
TAMRA	565nm	580nm	Rose
Cy3	552nm	570nm	Red
Cy5	643nm	667nm	Violet



### *Psoralen C2*

Name: 2-[4'-(Hydroxymethyl)-4,5',8-trimethylpsoralen]-ethyl-1-O-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Cat. No.: 10-1982-xx

Formula:  $C_{26}H_{35}N_2O_6P$   
M.W.: 502.55

### *Psoralen C6*

Name: 2-[4'-(Hydroxymethyl)-4,5',8-trimethylpsoralen]-hexyl-1-O-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

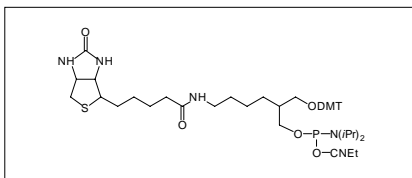
Cat. No.: 10-1983-xx

Formula:  $C_{30}H_{43}N_2O_6P$   
M.W.: 558.65

Psoralen C2 Phosphoramidite is added to the 5' terminus to serve as a cross-linking reagent in double-stranded oligonucleotides<sup>1</sup>. To complement psoralen C2 labelling, psoralen C6 phosphoramidite can cross-link with a triplex oligonucleotide strand<sup>2</sup>. Mild deprotection (*e.g.*, ammonium hydroxide for 24 hours at room temperature) is recommended. Psoralen labelled oligonucleotides can be purified by cartridge using the DMT-on procedure (omitting the 2% TFA step).

### *References*

1. U. Pieleas and U. Englisch, *Nucleic Acids Res.*, 1989, **17**, 285.
2. M. Takasugi, A. Guendouz, M. Chassignol, J.L. Decout, J. Lhomme, N.T. Thuong, and C. Helene, *Proc. Natl. Acad. Sci. U.S.A.*, 1991, **88**, 5602-5606.



### *Biotin*

Name: 1-Dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1953-xx

Formula:  $C_{47}H_{66}N_5O_7PS$   
M.W.: 876.10

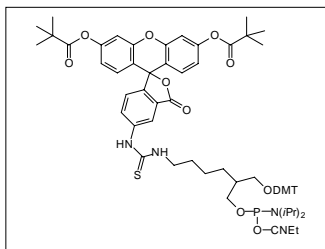
An alternative to the use of a biotin-NHS ester procedure is the coupling of a biotin phosphoramidite during oligonucleotide synthesis<sup>1</sup>. The requirements below are incorporated into this biotin phosphoramidite:

1. The product is soluble in acetonitrile.
2. The product includes a DMT group for cartridge purifications, necessary because of the potential for cross contamination in HPLC purifications.
3. The product is also capable of branching to allow multiple additions at the 3'- or 5'-terminus for probe development.

The branched spacer used in this biotin phosphoramidite has been described<sup>2</sup>. The use of this phosphoramidite is virtually identical to a nucleoside phosphoramidite with a **12-15 minute** coupling optimal.

### *References*

1. A.J. Cocuzza, *Tetrahedron Lett.*, 1989, **30**, 6287-6290.
2. P.S. Nelson, M. Kent, and S. Muthini, *Nucleic Acids Res.*, 1992, **20**, 6253-6259.



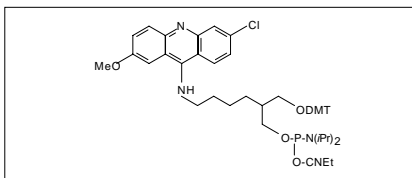
### *Fluorescein*

Name: 1-Dimethoxytrityloxy-2-(N-thiourea-(di-O-pivaloyl-fluorescein)-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1963-xx

Formula:  $C_{68}H_{79}N_4O_{12}PS$   
M.W.: 1207.50

Synthesis of fluorescein labelled sequencing primers is the primary use for a fluorescein phosphoramidite. Fluorescein phosphoramidite is designed to produce, on deprotection and isolation of the derived oligonucleotide, the same fluorescein-type structure as had been previously prepared with fluorescein isothiocyanate (FITC). Fluorescein phosphoramidite is unstable to light and should be used immediately the solution is prepared. A **12-15 minute** coupling is recommended. This product contains a DMT group to allow quantification of coupling. We recommend that the DMT group should be removed using the DMT-off cycle, since fluorescein labelled oligos have a mobility similar to DMT-on oligos. Cartridge purification using the procedure described on Page 45 works well. (If 2% TFA is used to remove the DMT group during the purification of a DMT-on fluorescein labelled oligo, it is necessary to add base to restore the fluorescence.)



### *Acridine*

Name: 1-Dimethoxytrityloxy-2-(N-acridinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

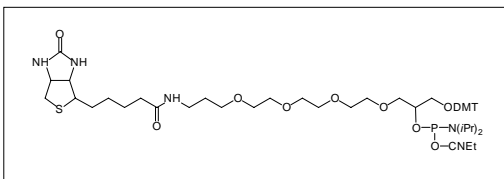
Cat. No.: 10-1973-xx

Formula:  $C_{51}H_{60}N_4O_6PCl$   
M.W.: 891.53

Acridine is an effective intercalating agent as well as being a lipophilic carrier molecule. Acridine labelled oligonucleotides may be expected to be used in antisense research. Acridine phosphoramidite contains a DMT group to allow quantification of coupling and we recommend removal using a DMT-off cycle. A **12-15 minute** coupling is optimal.

The secondary amino linkage attaching the acridine portion of the molecule to the spacer arm is less stable to ammonium hydroxide than usually encountered. The mild deprotection scheme described on Page 73 has been used effectively as has deprotection with ammonium hydroxide at room temperature for 24h.

Acridine labelled oligos have a mobility similar to DMT-on oligos and cartridge purification using the procedure described on Page 45 works well.



### ***BiotinTEG***

**Name:** 1-Dimethoxytrityloxy-3-O-(N-biotinyl-3-aminopropyl)-triethyleneglycolyl-glycerol-2-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

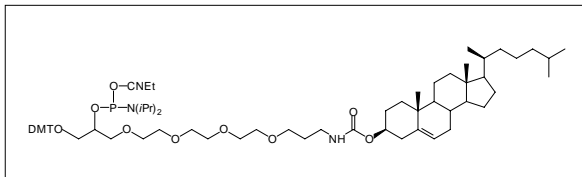
**Cat. No.:** 10-1955-xx

**Formula:** C<sub>52</sub>H<sub>76</sub>N<sub>5</sub>O<sub>11</sub>PS  
**M.W.:** 1010.24

A biotin phosphoramidite which can be added only to the 5'-terminus<sup>1,2</sup> is a useful if incomplete answer to biotinylation needs. An improvement is the use of a branched structure<sup>3</sup> which can be placed in one or several additions at the 3'- or 5'-terminus. A biotin phosphoramidite containing an additional mixed polarity triethyleneglycol (TEG) spacer would be an excellent design<sup>4</sup> for flexibility of addition as well as optimal capture by avidin or streptavidin. A **12-15 minute** coupling is recommended for BiotinTEG. See note on Page 53.

### ***References***

1. A.M. Alves, D. Holland, and M.D. Edge, *Tetrahedron Lett.*, 1989, **30**, 3089.
2. R.T. Pon, *Tetrahedron Lett.* 1991, **32**, 1715-1718.
3. K. Misiura, I. Durrant, M.R. Evans, and M.J. Gait, *Nucleic Acids Res.*, 1990, **18**, 4345.
4. C. Levenson, C. Chang, and F.T. Oakes, *US Patent 4,914,210*, 1990.



### *Cholesteryl-TEG*

Name: 1-Dimethoxytrityl-3-O-(N-cholesteryl-3-aminopropyl)-triethyleneglycol-glycerol-2-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

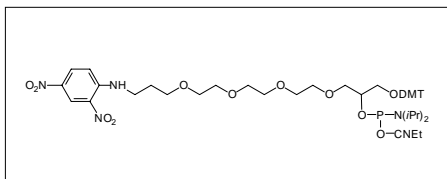
Cat. No.: 10-1975-xx

Formula:  $C_{70}H_{106}N_3O_{11}P$   
M.W.: 1196.60

Potential therapeutic oligonucleotides must permeate the cell membrane for optimal activity. The addition of lipophilic groups to an oligonucleotide would be expected to enhance activity. The use of cholesteryl oligos and the consequent improvement in activity has been described<sup>1,2</sup>. At 0.1M concentration cholesteryl-TEG is not reliably soluble in acetonitrile, tending to oil out. It is soluble in acetonitrile at 0.04M concentration. A diluent vial containing acetonitrile/THF is provided with this product. An extended coupling time of **12-15 minutes** is optimal. Cholesteryl labelled oligonucleotides are very lipophilic and are easily purified DMT-off by RP HPLC or Poly-Pak cartridge. See note on Page 53.

### *References*

1. C. Mackellar, D. Graham, D.W. Will, S. Burgess, and T. Brown, *Nucleic Acids Res.*, 1992, **20**, 3411-3417.
2. C.A. Stein, R. Pal, A.L. Devico, G. Hoke, S. Mumbauer, O. Kinstler, M.G. Sarnadharan, and R.L. Letsinger, *Biochemistry*, 1991, **30**, 2439-2444.



## ***DNP-TEG***

Name: 1-Dimethoxytrityl-3-O-(N-(2,4-dinitrophenyl)-3-aminopropyl)-triethyleneglycolyl-glyceryl-2-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite

Cat. No.: 10-1985-xx

Formula:  $C_{48}H_{64}N_5O_{13}P$   
M.W.: 950.00

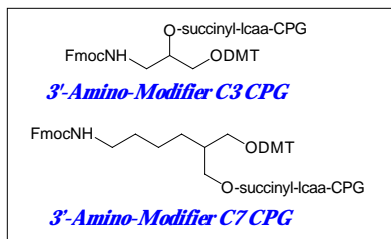
A new analytical test based on detection of 2,4-dinitrophenyl (DNP) labelled oligonucleotides with anti-DNP antibodies has been proposed<sup>1,2</sup>. The branched TEG spacer in DNP-TEG phosphoramidite means that it can be added once or several times<sup>2</sup> to the 3'- or 5'-terminus. An extended coupling time of **12-15 minutes** is optimal. DNP labelled oligos have a mobility similar to DMT-on oligos and cartridge purification (omitting the 2% TFA step) works well.

### *Note:*

For optimal yield, oligos containing Biotin-TEG, Cholesteryl-TEG or DNP-TEG at the 5'-terminus should be prepared DMT-on, with DMT removal after cleavage and deprotection.

### ***References***

1. D.W. Will, C.E. Pritchard, and T. Brown, *Carbohydrate Research*, 1991, **216**, 315-322.
2. J. Grzybowski, D.W. Will, R.E. Randall, C.A. Smith, and T. Brown, *Nucleic Acids Res.*, 1993, **21**, 1705-1712.

***3'-Amino-Modifier C3 CPG***

Name: (1-Dimethoxytrityloxy-3-fluorenylmethoxycarbonylamino-propan-2-succinoyl)-long chain alkylamino-CPG

Cat. No.: 20-2950-xx

***3'-Amino-Modifier C7 CPG***

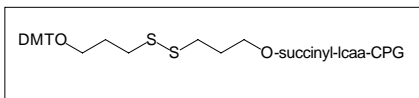
Name: (1-Dimethoxytrityloxy-3-fluorenylmethoxycarbonylamino-hexan-2-methylsuccinoyl)-long chain alkylamino-CPG

Cat. No.: 20-2957-xx

3'-Amino-Modifier CPG is designed to introduce a primary amine to the 3'-terminus of a target oligonucleotide. The primary uses of 3'-amino-oligonucleotides are for subsequent labelling as diagnostic probes and to generate an oligonucleotide resistant to 3'-exonuclease activity<sup>1</sup>. Handling of these products is described on Page 12.

***Reference***

1. J.G. Zendejui, K.M. Vasquez, J.H. Tinsley, D.J. Kessler, and M.E. Hogan, *Nucleic Acids Res.*, 1992, **20**, 307.



### ***3'-Thiol-Modifier C3 S-S CPG***

Name: 1-O-Dimethoxytrityl-propyl-disulfide, 1'-succinoyl-long chain alkylamino-CPG

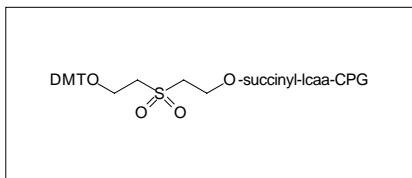
Cat. No.: 20-2933-xx

#### ***Thiol Group at the 3'-Terminus***

3'-Thiol-Modifier C3 S-S CPG is designed to introduce a thiol group to the 3'-terminus of a target oligonucleotide<sup>1</sup>. Synthesis using these supports is described on Page 12. *To avoid oxidative cleavage of the disulfide linkage, all oxidation steps should use 0.02M Iodine solution.* To the standard ammonium hydroxide used for deprotection, add dithiothreitol (DTT) to a concentration of 50mM. Carry out deprotection in the normal manner (typically 55°C/8h). This procedure removes the base protecting groups and cleaves the disulfide linkage to generate the 3'-thiol. Isolate, desalt and, if necessary, purify the thiol-modified oligonucleotide using standard procedures. Alternatively, omit the DTT from the ammonium hydroxide. Just prior to conjugation, cleave the disulfide with 100mM DTT/ pH 8.3 - 8.5/RT/30minutes. Desalt on a column equilibrated with conjugation buffer. Continue to the conjugation reaction. *See Thiol-Modifier C6 S-S on Page 26.* Thiol-modified oligonucleotides should be kept either under an inert atmosphere or in a solution containing DTT (10mM) to avoid disulfide formation. DTT can be extracted from the solution using ethyl acetate or removed by desalting prior to conjugation. *See Note on Page 29.*

#### ***Reference***

1. A. Kumar, S. Advani, H. Dawar, and G.P. Talwar, *Nucleic Acids Res.*, 1991, **19**, 4561.



### *3'-Phosphate CPG*

Name: 2-[2-(4,4'-Dimethoxytrityloxy)ethylsulfonyl]ethyl-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-2900-xx

### *Phosphorylation at the 3'-Terminus*

The use of this reagent is an alternative to enzymatic techniques for oligonucleotide phosphorylation<sup>1</sup>. Synthesis using these supports is described on Page 12.

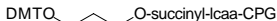
### *Purification of 3'-Phosphates*

Oligonucleotides with 3'-phosphates may be purified DMT-on using cartridges and HPLC or DMT-off using gel electrophoresis. If purification is considered to be unnecessary, the phosphorylated oligonucleotide can be conveniently desalted on a purification cartridge.

See also Chemical Phosphorylation Reagent on Page 20.

### *Reference*

1. T. Horn and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.



### ***3'-Spacer C3 CPG***

Name: (1-Dimethoxytrityloxy-propanediol-3-succinoyl)-long chain alkylamino-CPG

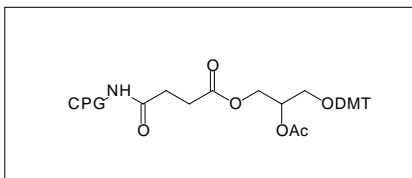
Cat. No.: 20-2913-xx

### ***Use of 3'-Spacer C3 CPG***

Some sequencing strategies as well as PCR probes require the 3' terminus of an oligonucleotide to be blocked from allowing polymerase extension. This may be achieved by modifying the 3'-terminus with a phosphate group, a phosphate ester, or using an inverted 3'-3' linkage. However, side reactions during deprotection of the oligonucleotide or enzymatic impurities may free the 3'-hydroxyl group to a small extent. So far, the 3'-propyl phosphate formed using 3'-Spacer C3 CPG has proved to be the simplest and most effective non-nucleosidic blocker of the 3'-terminus.

### ***Purification***

Oligonucleotides with 3'-propyl phosphates may be purified using the standard procedures used for unmodified oligonucleotides.



### *Glyceryl CPG*

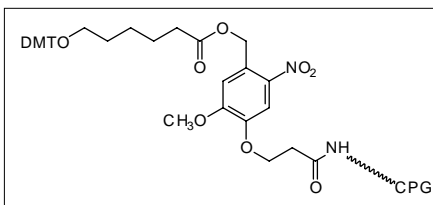
Name: 3-[(4,4'-Dimethoxytrityloxy)glyceryl-1-succinoyl]-long chain alkylamino-CPG

Cat. No. 20-2902-xx

A support has been described<sup>1</sup> for the preparation of oligonucleotides with a 3'-phosphoglyceryl terminus. The terminus is readily oxidized by sodium periodate to form a 3'-phosphoglycaldehyde. The aldehyde may be further oxidized to the corresponding carboxylic acid. Either the aldehyde or the carboxylate may be used for subsequent conjugation to amine-containing products. The authors were specifically interested in the products from the radical cleavage of oligonucleotides by DNA targeting molecules like bleomycin, enediyne antibiotics and transition metal complexes.

### *Reference*

1. H. Urata and M. Akagi, *Tetrahedron Lett.*, 1993, **34**, 4015-4018.



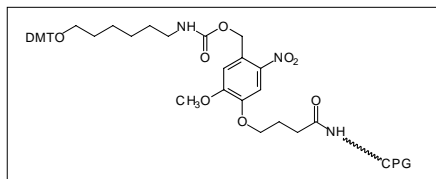
### *3'-Carboxylate Photolabile C6 CPG*

Cat. No. 20-4090-xx

3'-Carboxylate Photolabile C6 CPG is a universal, photolabile support for the preparation of an oligonucleotide 3'-carboxylate with or without the base protecting groups<sup>1,2</sup>. The research goals which culminated in the development of the support are detailed below:

- Oligonucleotides containing 3'-terminal carboxylic acids should be isolated in normal yield by ammonium hydroxide deprotection.
- Photolytic cleavage from the support should result in the release of a fully protected oligonucleotide 3'-carboxylic acid.
- Photolytic cleavage from the support should occur under conditions which lead to minimal damage of the product oligonucleotide.
- The support should be independent of the base at the 3'-terminus, i.e., a universal support, the first 3'-base being added during the first phosphoramidite coupling cycle.

A similar strategy<sup>3</sup> allows 3'-alkylamino oligonucleotides to be prepared using 3'-Amino Photolabile C6 CPG. After conventional synthesis, oligonucleotides can be cleaved from the support photochemically to release the fully protected 3'-amino oligonucleotides into solution. Conjugation of a hapten to the amino group can then be carried out in organic solution. This avoids the need for the vast excess of hapten which is used routinely in conjugation reactions with fully deprotected oligonucleotides in aqueous solution, typically at pH 9. It also makes removal of excess hapten much more facile.



### ***3'-Amino Photolabile C6 CPG***

Cat. No. 20-4091-xx

The conditions chosen for irradiation of the product oligonucleotides have been shown<sup>1</sup> to cause less than 1% thymidine dimer formation, as a measure of photoinduced damage. The yields of the oligonucleotides isolated by photo cleavage are reported to be about 30% less than those from ammonium hydroxide cleavage. Photolytic cleavage is carried out using Hg/Xe lamp at 400nm. Alternatively, a transilluminator (long wavelength UV) may be used.

### ***Deprotection***

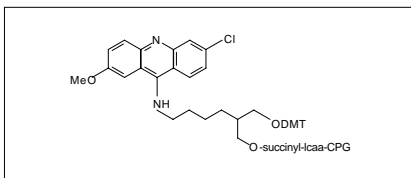
For photolysis conditions, refer to the following references for complete details. For hydrolytic cleavage and complete deprotection, carry out the deprotection procedure with sodium hydroxide described on Page 73.

### ***References***

1. D.J. Yoo and M.M. Greenberg, *J. Org. Chem.*, 1995, **60**, 3358-3364.
2. H. Venkatesan and M.M. Greenberg, *J. Org. Chem.*, 1996, **61**, 525-529.
3. D.L. McMinn and M.M. Greenberg, *Tetrahedron*, 1996, **52**, 3827-3840.







### ***Acridine CPG***

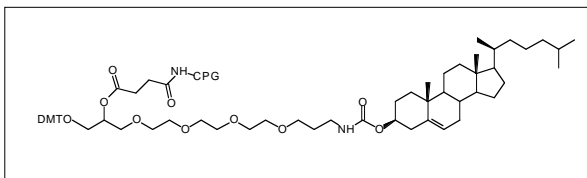
Name: 1-Dimethoxytrityloxy-2-(N-acridinyl-4-aminobutyl)-propyl-3-O-succinyl-long chain alkylamino-CPG

Cat. No.: 20-2973-xx

### ***Acridine Labelling at the 3'-Terminus***

Acridine-CPG is used to introduce an acridine molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. For mild cleavage and deprotection, follow the procedure described on Page 73.

See also Acridine phosphoramidite on Page 50.



### *Cholesteryl-TEG CPG*

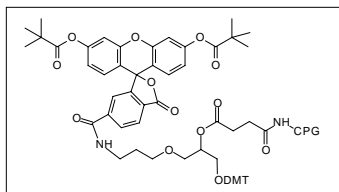
Name: 1-Dimethoxytrityloxy-3-O-(N-cholesteryl-3-aminopropyl)-triethyleneglycol-glycerol-2-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-2975-xx

### *Cholesterol Labelling at the 3'-Terminus*

Cholesteryl-TEG CPG is used to introduce a cholesterol molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. Cleavage of the oligonucleotide from the support requires 2 hours at room temperature.

See also Cholesteryl-TEG phosphoramidite on Page 52.



### ***3'-(6-FAM) CPG 1000***

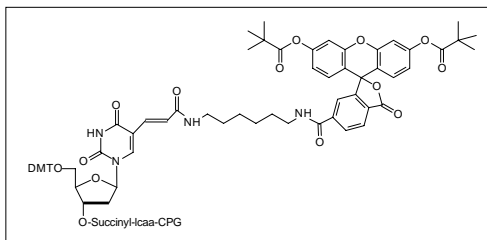
Name: 1-Dimethoxytrityloxy-3-[O-(N-carboxy-(di-O-pivaloyl-fluorescein)-3-aminopropyl)]-propyl-2-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-2961-xx

### ***Fluorescein Labelling at the 3'-Terminus***

3'-(6-FAM) CPG is used to introduce a fluorescein molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. This product is derived from pure 6-carboxy-fluorescein and is attached via an amide linkage, giving an oligo product which is much simpler to purify by HPLC. This product blocks the 3'-terminus from polymerase extension and exonuclease digestion. Cleavage of the oligonucleotide from the support requires 2 hours at room temperature.

See also 6-FAM phosphoramidite on Page 42.



### ***3'-Fluorescein-dT CPG***

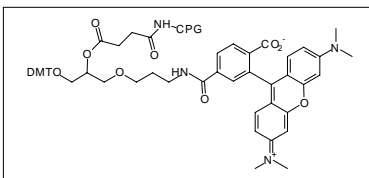
Name: 5'-Dimethoxytrityloxy-5-[N-((3',6'-dipivaloylfluoresceinyl)-amino)hexyl]-3'-acrylimido]-2'-deoxyUridine-3'-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-2056-xx

### ***Fluorescein-dT Labelling at the 3'-Terminus***

Fluorescein-dT CPG is used to introduce a Fluorescein-dT molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support. Cleavage and deprotection are carried out using normal procedures.

See also Fluorescein-dT phosphoramidite on Page 36.



### ***3'-TAMRA CPG***

Name: 1-Dimethoxytrityloxy-3-[O-(N-carboxy-(Tetramethyl-rhodamine)-3-aminopropyl)]-propyl-2-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-5910-xx

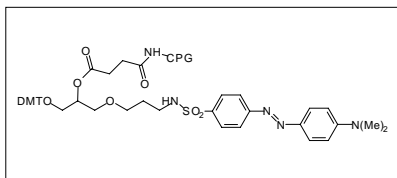
### ***TAMRA Labelling at the 3'-Terminus***

TAMRA CPG is used to introduce a TAMRA molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. TAMRA CPG has to be deprotected under very mild conditions to safeguard the labile TAMRA section. We recommend the use of UltraMild monomers and the use of potassium carbonate in methanol for deprotection, as described on Page 72. An alternative procedure using *t*-butylamine/methanol/water (1:1:2), which allows the use of regular monomers, has also been described.<sup>1,2</sup>

See also TAMRA-dT phosphoramidite on Page 37.

### ***References***

1. B. Mullah and A. Andrus, *Tetrahedron Lett*, 1997, **38**, 5751-5754.
2. S.L. Woo, S.M. Menchen, and S. Fung, 1993, US Patent No. 5,231,191.



### ***3'-Dabsyl CPG***

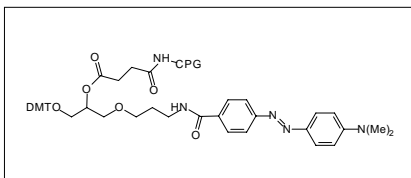
Name: 1-Dimethoxytrityloxy-3-[O-(N-4'-sulfonyl-4-(dimethylamino)-azobenzene)-3-aminopropyl]-propyl-2-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-5911-xx

### ***Dabsyl Labelling at the 3'-Terminus***

Dabsyl CPG is used to introduce a dabsyl molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. Cleavage of the oligonucleotide from the support requires 2 hours at room temperature.

This product contains a sulfonamide linkage and differs from 3'-Dabsyl CPG 1000 on Page 69 which contains an amide linkage. 3'-Dabsyl CPG is provided as a higher loaded support (up to ~80  $\mu\text{moles/g}$ ) which would restrict the length of oligo to about a 35mer.



### ***3'-DabcyI CPG 1000***

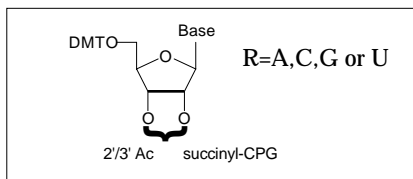
Name: 1-Dimethoxytrityloxy-3-[O-(N-4'-carboxy-4-(dimethylamino)-azobenzene)-3-aminopropyl]-propyl-2-O-succinoyl-long chain alkylamino-CPG

Cat. No.: 20-5912-xx

### ***DabcyI Labelling at the 3'-Terminus***

DabcyI CPG is used to introduce a dabcyI molecule to the 3'-terminus of the product oligonucleotide. Oligonucleotide synthesis proceeds in a manner analogous to the use of a normal nucleoside support with the modifications noted on Page 12. Cleavage of the oligonucleotide from the support requires 2 hours at room temperature.

See also DabcyI-dT phosphoramidite on Page 38 and 5'-dabcyI phosphoramidite on Page 41.

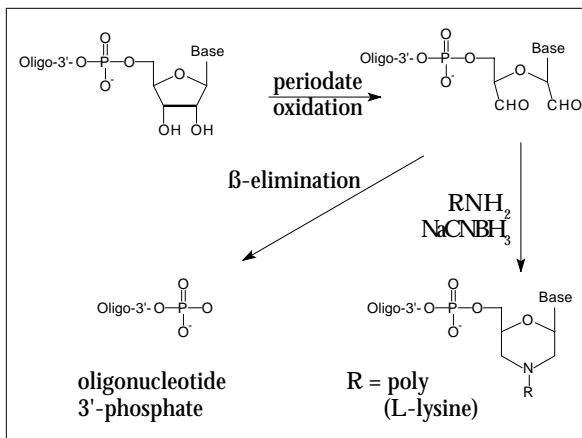


### *RNA Supports for DNA Modification*

Interest in the development of anti-sense oligonucleotides has focused attention on the ability to attach marker and carrier molecules to the 3'-terminus. The 3'-hydroxyl group, although accessible for chemical modification, does not support specific reaction at that position because of the presence of competitive reactive centers. It may be possible to design a support which would allow the protected oligonucleotide to be released while all other protecting groups remain intact. Chemical modification reactions could then proceed at the free 3'-hydroxyl group. To our knowledge, no such support has been described.

One approach to 3'-modification is to prepare an oligonucleotide with a ribonucleoside terminus. Periodate oxidation of the 2',3'-diol cleaves the 2'-3' bond and generates reactive aldehyde groups which are available for specific chemical manipulation, as shown in Figure 1.

Two papers<sup>1,2</sup> describe the attachment of a ribonucleoside to the 3'-terminus of an oligodeoxynucleotide using T4 RNA ligase. Following periodate oxidation, the cleaved ribose ring can be converted to a morpholino ring by the addition of a primary amine. In this way, the authors describe the attachment of poly (L-lysine) at the 3'-terminus of an anti-sense oligonucleotide. The addition of the macromolecular carrier significantly improved intracellular delivery of the anti-sense oligonucleotide. The use of an RNA support allows the direct incorporation of a 3'-ribonucleoside.

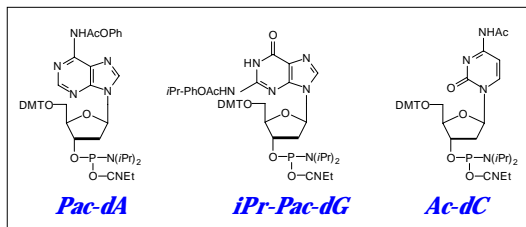


**Figure 1:** Reactions of 3'-Terminal Ribonucleoside

Similarly, other molecules could be attached through a primary amine, creating the opportunity to label oligonucleotides at the 3'-terminus. Also, the possibility of direct attachment of an oligonucleotide to an amino support to form an affinity support exists.

### References

1. M. Lemaitre, B. Bayard, and B. Lebleu, *Proc. Nat. Acad. Sci. USA*, 1987, **84**, 648.
2. M. Lemaitre, C. Bisbal, B. Bayard, and B. Lebleu, *Nucleosides & Nucleotides*, 1987, **6**, 311.



### *Procedure for UltraMild Deprotection of Oligodeoxynucleotides*

Glen Research is pleased to offer a set of monomers using phenoxyacetyl (Pac) protected dA, 4-isopropyl-phenoxyacetyl (iPr-Pac) protected dG, and acetyl (Ac) protected dC. These monomers can be used with sensitive labelling reagents such as TAMRA, Cy5 and HEX since cleavage and deprotection can be carried out in 4 hours at room temperature with ammonium hydroxide or with 0.05M potassium carbonate in anhydrous methanol as described below.

1. Carry out the synthesis of oligonucleotides containing labile bases or tags DMT-off.
2. Open the synthesis column and transfer the support to a suitable reaction vial.
3. Treat the support with 1mL of 0.05M potassium carbonate in anhydrous methanol for a minimum of 4 h at room temperature. (For oligos with a high G content, overnight is preferred.)
4. Pipette the supernatant from the support and neutralize with 1.5mL of 2M triethylammonium acetate.

*Either:*

5. Desalt the oligonucleotide using normal procedures, lyophilize the resulting product and store the oligonucleotide at  $-20^{\circ}\text{C}$ .

*Or:* Follow steps 5a. and b. on Page 73.

### *Procedure for Mild Deprotection of Oligodeoxynucleotides*

Some linkages, modified bases, or modifiers may require more gentle deprotection conditions than the normal assault with ammonium hydroxide. The following procedure, described for a 0.2  $\mu$ mole synthesis, is mild and leads logically to Poly-Pak type DMT-on purification. This procedure has been used for oligonucleotides modified with Carboxy-dT (Page 39) and acridine (Page 50, 63).

1. Carry out the synthesis of oligonucleotides containing modified bases DMT-on, and oligonucleotides labelled with, for example, psoralen or acridine DMT-off.
2. Open the synthesis column and transfer the support to a suitable reaction vial.
3. Treat the support with 1mL of 0.4M methanolic sodium hydroxide (methanol:water, 4:1) for 17h at room temperature.
4. Pipette the supernatant from the support and neutralize with 1.5mL of 2M triethylammonium acetate.

*Either:*

5. Desalt the oligonucleotide using normal procedures, lyophilize the resulting product and store the oligonucleotide at  $-20^{\circ}\text{C}$ .

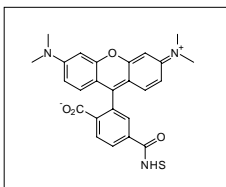
*Or:*

- 5a. Dilute the neutralized solution with 13.5mL of water (to bring the methanol content to about 5%). Apply the diluted oligonucleotide solution to a prepared purification cartridge and carry out the standard purification scheme. (If the oligonucleotide is labelled and contains no DMT group, skip the 2% TFA wash.)
- 5b. Elute the purified oligonucleotide and lyophilize the resulting product. Store the oligonucleotide at  $-20^{\circ}\text{C}$ .

*General Procedure for Labelling of Amino-Modified Oligonucleotides*

This general procedure can be used to conjugate amino-modified oligonucleotides with active ester or isothiocyanate derivatives of fluorescent dyes which are not suitable for use as cyanoethyl phosphoramidites. At pH 9, conjugation occurs virtually exclusively at the amino group and not at all at the exocyclic amino groups of the nucleosides.

1. Dissolve the product from a 0.2  $\mu\text{mole}$  synthesis of amino-modified oligonucleotide (i.e., approximately 0.1 - 0.2  $\mu\text{moles}$  of free primary amines) in 0.7mL of sterile distilled water.
2. Add 0.1mL of 10X conjugation buffer (1M  $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ , pH9).
3. Freshly prepare a 10mg/mL solution of active ester in DMF. Add 0.2mL of the solution to the reaction mixture.
4. Allow the mixture to stand at least 2 hours. (Note: Overnight reaction may be more convenient.)
5. Desalt the reaction mixture on a Poly-Pak cartridge or a column containing Sephadex G-25 or G-50 to remove the excess label. Purify the product using RP HPLC if necessary.



### ***TAMRA NHS Ester***

Name: Tetramethylrhodamine, N-hydroxysuccinimide ester

Cat. No.: 50-5910-xx

Formula:  $C_{29}H_{25}N_3O_7$   
M.W.: 527.53

Because rhodamine derivatives are not sufficiently stable to survive conventional deprotection, these can be attached to amino-modified oligonucleotides using post-synthesis labelling techniques. This product is the activated carboxylate, N-hydroxysuccinimide (NHS) ester, of the rhodamine dye in solution in DMSO. It is conjugated with an amino-modified oligonucleotide in sodium carbonate/bicarbonate buffer at pH 9. Although this technique is time consuming and places demands on the final purification to remove unconjugated dye, it is nevertheless routine and successful.

### ***Labelling Protocol***

For a 0.2  $\mu$ mole synthesis of an amino-modified oligo:

1. Dissolve oligo in 500  $\mu$ L of conjugation buffer.
2. Add 6  $\mu$ L of TAMRA/DMSO solution (~ 6 fold excess).
3. Vortex mixture and incubate at 37  $^{\circ}$ C in the dark for 1-2 hrs.
4. Separate oligo-TAMRA conjugate from salts and free TAMRA by size exclusion on a NAP-10 column or equivalent.

***Desalting using a NAP-10 column***

5. Equilibrate NAP column with approximately 10 ml of 50 mM TEAA buffer pH 7.
6. Load reaction mix on column and let flow into the column.
7. Add 0.5 ml TEAA buffer and let flow into column.
8. Elute oligo TAMRA conjugate with  $\leq 1.5$  ml TEAA buffer.
9. Collect conjugate and dry it in a vacuum concentrator.
10. Conjugate may be further purified by RP HPLC or PAGE to separate labelled from unlabelled oligonucleotides.

## ***General Procedure for Labelling of Thiol-Modified Oligonucleotides***

### *Step 1a: Oligo Sulfhydryl activation (Trityl-protected oligo)*

Dissolve trityl-protected oligo (15-25  $A_{260}$  units or 0.2  $\mu$ mole synthesis) in 0.1M TEAA (100  $A_{260}$  units/mL). Add 0.15 volume 1M  $AgNO_3$ , vortex to mix. Let stand for 30 min. Add 0.20 volumes 1M DTT, vortex to mix. Let stand for 20 min.

### *Step 1b: Oligo Sulfhydryl activation (Oligo-disulfide)*

Dissolve oligo disulfide (15-25  $A_{260}$  units) in 0.25mL 100mM DTT, pH 8.3 - 8.5. Incubate at room temperature for 30 minutes.

### *Step 2: Removal of DTT and other reaction byproducts and change to conjugation buffer*

Load entire sample on a NAP-10 (or equivalent) column equilibrated with 50mM sodium phosphate, pH 6.0. Allow to drip through. Add 0.75mL of 50mM sodium phosphate, pH 6.0 and allow to drip through. Elute with 1mL sodium phosphate pH 6.0. Collect for conjugation.

### *Step 3: Conjugation*

Dissolve thiol reactive ligand in sodium phosphate, pH 6.0 ( $\approx$  20mM final concentration). *If thiol reactive ligand is not soluble in water dissolve in appropriate solvent (DMF or DMSO) at the same concentration.* Add 0.2mL thiol reactive ligand to activated, desalted thiol modified oligo from step 2. Vortex and incubate at RT for 2-4 hours or overnight at 4°C.

### *Step 4: Purification*

Purify oligo conjugate by PAGE, RP HPLC or ion exchange HPLC.

Item	Catalog No.	Pack
<i><b>5'-Amino-Modifiers</b></i>		
5'-Amino-Modifier C6	10-1906-90	100µm
	10-1906-02	0.25g
5'-Amino-Modifier C12	10-1912-90	100µm
	10-1912-02	0.25g
5'-Amino-Modifier 5	10-1905-90	100µm
	10-1905-02	0.25g
5'-Amino-Modifier C3-TFA	10-1923-90	100µm
	10-1923-02	0.25g
5'-Amino-Modifier C6-TFA	10-1916-90	100µm
	10-1916-02	0.25g
Chemical Phosphorylation Reagent	10-1900-90	100µm
	10-1900-02	0.25g
CPR II	10-1901-90	100µm
	10-1901-02	0.25g
5'-Thiol-Modifier C6	10-1926-90	100µm
	10-1926-02	0.25g
<i><b>5'- or 3'-Modifiers</b></i>		
Thiol-Modifier C6 S-S	10-1936-90	100µm
	10-1936-02	0.25g
Spacer Phosphoramidite 9	10-1909-90	100µm
	10-1909-02	0.25g
Spacer Phosphoramidite 18	10-1918-90	100µm
	10-1918-02	0.25g
Spacer Phosphoramidite C3	10-1913-90	100µm
	10-1913-02	0.25g
Spacer Phosphoramidite C12	10-1928-90	100µm
	10-1928-02	0.25g
dSpacer Phosphoramidite	10-1914-90	100µm
	10-1914-02	0.25g
<i><b>Sequence Modifiers</b></i>		
Amino-Modifier C6 dT	10-1039-90	100µm
	10-1039-02	0.25g
	10-1039-05	0.5g

Item	Catalog No.	Pack
Amino-Modifier C2 dT	10-1037-90	100µm
	10-1037-02	0.25g
	10-1037-05	0.5g
Biotin-dT	10-1038-95	50µm
	10-1038-90	100µm
	10-1038-02	0.25g
Fluorescein-dT	10-1056-95	50µm
	10-1056-90	100µm
	10-1056-02	0.25g
TAMRA-dT	10-1057-95	50µm
	10-1057-90	100µm
	10-1057-02	0.25g
Dabcyl-dT	10-1058-95	50µm
	10-1058-90	100µm
	10-1058-02	0.25g
EDTA-C2-dT	10-1059-95	50µm
	10-1059-90	100µm
	10-1059-02	0.25g
Carboxy-dT	10-1035-90	100µm
	10-1035-02	0.25g
	10-1035-05	0.5g
<b><i>5'-Labelling Reagents</i></b>		
5'-Biotin Phosphoramidite	10-5950-95	50µm
	10-5950-90	100µm
	10-5950-02	0.25g
5'-Dabcyl Phosphoramidite	10-5912-95	50µm
	10-5912-90	100µm
	10-5912-02	0.25g
5'-Fluorescein Phosphoramidite (6-FAM)	10-5901-95	50µm
	10-5901-90	100µm
	10-5901-02	0.25g
5'-Hexachloro-Fluorescein Phosphoramidite (HEX)	10-5902-95	50µm
	10-5902-90	100µm
	10-5902-02	0.25g

Item	Catalog No.	Pack
5'-Tetrachloro-Fluorescein Phosphoramidite (TET)	10-5903-95	50µm
	10-5903-90	100µm
	10-5903-02	0.25g
Cy3 Phosphoramidite	10-5913-95	50µm
	10-5913-90	100µm
	10-5913-02	0.25g
Cy5 Phosphoramidite	10-5915-95	50µm
	10-5915-90	100µm
	10-5915-02	0.25g
Psoralen C2 Phosphoramidite	10-1982-90	100µm
	10-1982-02	0.25g
Psoralen C6 Phosphoramidite	10-1983-90	100µm
	10-1983-02	0.25g
<i><b>Branched Labelling Reagents</b></i>		
Biotin Phosphoramidite	10-1953-95	50µm
	10-1953-90	100µm
	10-1953-02	0.25g
Fluorescein Phosphoramidite	10-1963-95	50µm
	10-1963-90	100µm
	10-1963-02	0.25g
Acridine Phosphoramidite	10-1973-95	50µm
	10-1973-90	100µm
	10-1973-02	0.25g
BiotinTEG Phosphoramidite	10-1955-95	50µm
	10-1955-90	100µm
	10-1955-02	0.25g
Cholesteryl-TEG Phosphoramidite	10-1975-95	50µm
	10-1975-90	100µm
	10-1975-02	0.25g
DNP-TEG Phosphoramidite	10-1985-95	50µm
	10-1985-90	100µm
	10-1985-02	0.25g

Item	Catalog No.	Pack
<b><i>3'-Modifiers</i></b>		
3'-Amino-Modifier C3 CPG, 0.2 $\mu$ mole	20-2950-42	Pk/4
3'-Amino-Modifier C3 CPG, 1 $\mu$ mole	20-2950-41	Pk/4
3'-Amino-Modifier C3 CPG, bulk	20-2950-01	0.1g
	20-2950-10	1.0g
3'-Amino-Modifier C7 CPG, 0.2 $\mu$ mole	20-2957-42	Pk/4
3'-Amino-Modifier C7 CPG, 1 $\mu$ mole	20-2957-41	Pk/4
3'-Amino-Modifier C7 CPG, bulk	20-2957-01	0.1g
	20-2957-10	1.0g
3'-Thiol-Modifier C3 S-S CPG, 0.2 $\mu$ mole	20-2933-42	Pk/4
3'-Thiol-Modifier C3 S-S CPG, 1 $\mu$ mole	20-2933-41	Pk/4
3'-Thiol-Modifier C3 S-S CPG, bulk	20-2933-01	0.1g
	20-2933-10	1.0g
3'-Phosphate CPG, 0.2 $\mu$ mole	20-2900-42	Pk/4
3'-Phosphate CPG, 1 $\mu$ mole	20-2900-41	Pk/4
3'-Phosphate CPG, bulk	20-2900-01	0.1g
	20-2900-10	1.0g
3'-Spacer C3 CPG, 0.2 $\mu$ mole	20-2913-42	Pk/4
3'-Spacer C3CPG, 1 $\mu$ mole	20-2913-41	Pk/4
3'-Spacer C3CPG, bulk	20-2913-01	0.1g
	20-2913-10	1.0g
Glyceryl CPG, 0.2 $\mu$ mole	20-2902-42	Pk/4
Glyceryl CPG, 1 $\mu$ mole	20-2902-41	Pk/4
Glyceryl CPG, bulk	20-2902-01	0.1g
	20-2902-10	1.0g
3'-Carboxylate Photolabile CPG, 0.2 $\mu$ mole	20-4090-42	Pk/4
3'-Carboxylate Photolabile CPG, 1 $\mu$ mole	20-4090-41	Pk/4
3'-Carboxylate Photolabile CPG, bulk	20-4090-01	0.1g
	20-4090-10	1.0g
3'-Amino Photolabile CPG, 0.2 $\mu$ mole	20-4091-42	Pk/4
3'-Amino Photolabile CPG, 1 $\mu$ mole	20-4091-41	Pk/4
3'-Amino Photolabile CPG, bulk	20-4091-01	0.1g
	20-4091-10	1.0g

Item	Catalog No.	Pack
<i><b>3'-Labelling</b></i>		
BiotinTEG CPG, 0.2 $\mu$ mole	20-2955-42	Pk/4
BiotinTEG CPG, 1 $\mu$ mole	20-2955-41	Pk/4
BiotinTEG CPG, bulk	20-2955-01	0.1g
	20-2955-10	1.0g
Fluorescein CPG, 0.2 $\mu$ mole	20-2963-42	Pk/4
Fluorescein CPG, 1 $\mu$ mole	20-2963-41	Pk/4
Fluorescein CPG, bulk	20-2963-01	0.1g
	20-2963-10	1.0g
6-FAM CPG, 0.2 $\mu$ mole	20-2961-42	Pk/4
6-FAM CPG, 1 $\mu$ mole	20-2961-41	Pk/4
6-FAM CPG, bulk	20-2961-01	0.1g
	20-2961-10	1.0g
Fluorescein-dT CPG, 0.2 $\mu$ mole	20-2056-42	Pk/4
Fluorescein-dT CPG, 1 $\mu$ mole	20-2056-41	Pk/4
Fluorescein-dT CPG, bulk	20-2056-01	0.1g
	20-2056-10	1.0g
TAMRA CPG, 0.2 $\mu$ mole	20-5910-42	Pk/4
TAMRA CPG, 1 $\mu$ mole	20-5910-41	Pk/4
TAMRA CPG, bulk	20-5910-01	0.1g
	20-5910-10	1.0g
Dabsyl CPG, 0.2 $\mu$ mole	20-5911-42	Pk/4
Dabsyl CPG, 1 $\mu$ mole	20-5911-41	Pk/4
Dabsyl CPG, bulk	20-5911-01	0.1g
	20-5911-10	1.0g
Dabcyl CPG, 0.2 $\mu$ mole	20-5912-42	Pk/4
Dabcyl CPG, 1 $\mu$ mole	20-5912-41	Pk/4
Dabcyl CPG, bulk	20-5912-01	0.1g
	20-5912-10	1.0g
Acridine CPG, 0.2 $\mu$ mole	20-2973-42	Pk/4
Acridine CPG, 1 $\mu$ mole	20-2973-41	Pk/4
Acridine CPG, bulk	20-2973-01	0.1g
	20-2973-10	1.0g

Item	Catalog No.	Pack
Cholesteryl-TEG CPG, 0.2 $\mu$ mole	20-2975-42	Pk/4
Cholesteryl-TEG CPG, 1 $\mu$ mole	20-2975-41	Pk/4
Cholesteryl-TEG CPG, bulk	20-2975-01	0.1g
	20-2975-10	1.0g
<b><i>RNA Supports for DNA Modification</i></b>		
A-CPG 500, bulk	20-3303-01	0.1g
	20-3303-02	0.25g
	20-3303-10	1.0g
A-CPG 500, 0.2 $\mu$ mole column	20-3403-42	Pk/4
A-CPG 500, 1 $\mu$ mole column	20-3403-41	Pk/4
C-CPG 500, bulk	20-3310-01	0.1g
	20-3310-02	0.25g
	20-3310-10	1.0g
C-CPG 500, 0.2 $\mu$ mole column	20-3410-42	Pk/4
C-CPG 500, 1 $\mu$ mole column	20-3410-41	Pk/4
G-CPG 500, bulk	20-3320-01	0.1g
	20-3320-02	0.25g
	20-3320-10	1.0g
G-CPG 500, 0.2 $\mu$ mole column	20-3420-42	Pk/4
G-CPG 500, 1 $\mu$ mole column	20-3420-41	Pk/4
T-CPG 500, bulk	20-3330-01	0.1g
	20-3330-02	0.25g
	20-3330-10	1.0g
T-CPG 500, 0.2 $\mu$ mole column	20-3430-42	Pk/4
T-CPG 500, 1 $\mu$ mole column	20-3430-14	Pk/4
TAMRA NHS Ester <i>(solution in anhydrous DMSO)</i>	50-5910-66	60 $\mu$ L

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