The
Glen Report
Newsletter

GLEN RESEARCH
part of Maravai LifeSciences

Volume 33.1 | May 2021

New Product – MGB Eclipse® CPG

MGB Eclipse® pairs a minor groove binding (MGB) tripeptide with a dark quencher. The MGB significantly enhances hybridization, while the Eclipse® has a broad absorption range that quenches many of the most common fluorophores, including FAM, HEX, TET and Yakima Yellow®. Together, MGB Eclipse® is an attractive group for the synthesis of hydrolysis probes, the most common probe detection method for qPCR.

Otherwise known as MGB TaqMan®, TaqMan® MGB and TaqMan® MGB-NFQ probes, MGB Eclipse® hydrolysis probes are effective and have been widely used for close to twenty years. They work in the same way as traditional TaqMan® probes. 5'-Fluorophore and 3'-quencher dual-labeled short oligonucleotide probes complementary to the DNA targets are degraded by Taq DNA polymerase during the primer extension portions of PCR, which results in a linear relationship between fluorescence and amplified PCR product. With the additional MGB, melting temperatures are notably increased, allowing probes of shorter length. This is particularly attractive for mismatch discrimination.[1]

MGB Eclipse® hydrolysis probes have been used in a wide range of applications, including pathogen detection, SNP detection, viral load quantification and mutation detection. These probes continue to be popular.[2] Using RT-qPCR, MGB Eclipse® hydrolysis probes were recently used to quantify the transcripts of three different proteins to understand how the presence of a common gut bacterium influences porcine immune cells.[3] In another investigation, similar probes were used to study cell free amounts of three mitochondrial gene fragments in the serum of patients who had suffered aneurysmal subarachnoid hemorrhage, a type of stroke.[4] This study investigated the relationship of these gene fragment concentrations with post hemorrhage complications and clinical outcomes. In a third recent publication, these probes were used to detect a mutation of epidermal growth factor receptor (EGFR) associated with lung cancer.[5] The MGB Eclipse® hydrolysis probe was used in a 6-color digital PCR assay for the monitoring of EGFR mutations. In the first two studies, MGB Eclipse® was paired with FAM, and in the third study, MGB Eclipse® was paired with ROX.

In GR29.1, we introduced 3'-CDPI$_3$ MGB™ CPG that allowed researchers to take advantage of the unique properties of this modification. When MGB at the 3'-position is used with Eclipse® Quencher Phosphoramidite at the penultimate position, a functional MGB Eclipse® for probe synthesis is created. This is an approach that some customers have been employing. However, having an already assembled MGB Eclipse® support would be more straightforward and match the standard MGB Eclipse® structure used in qPCR probes. Glen Research is excited to share that an MGB Eclipse® CPG is now available to our customers.

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See technical snippets on back cover

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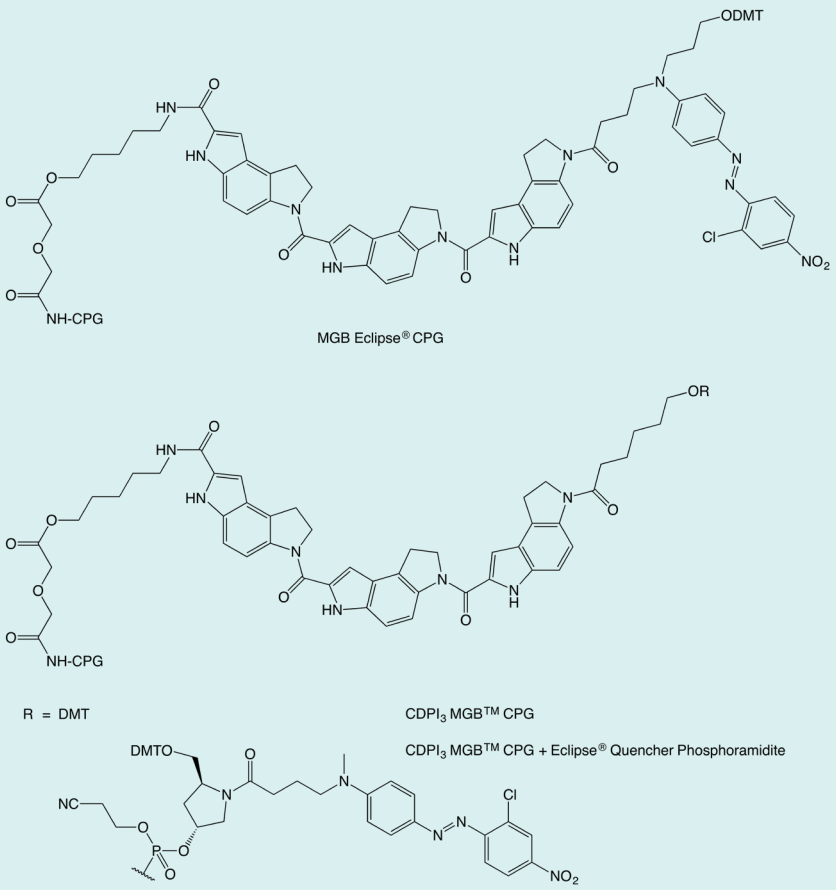

Figure 1. MGB Eclipse® structures

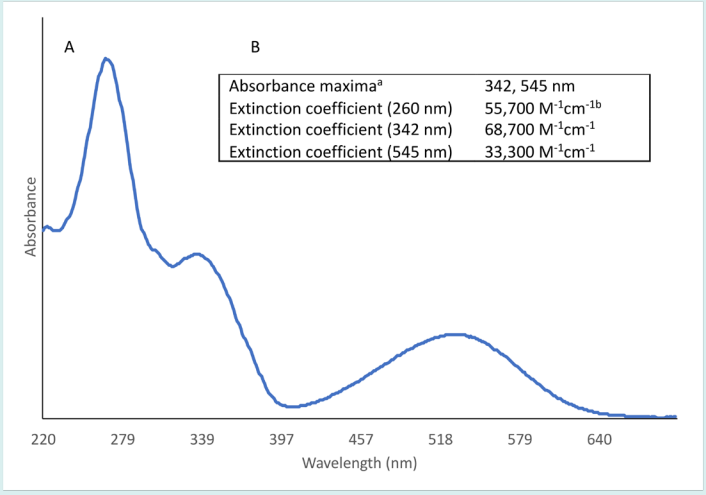

Figure 2. Absorbance of MGB Eclipse®. (A) Absorbance spectrum of DMT-T_{10}-MGB Eclipse®. (B) Spectral properties of MGB Eclipse®. [a]Measured for T_8-MGB Eclipse®. [b]MGB Eclipse® contribution only.

The structure of the MGB Eclipse® CPG is very close to that of the 3'-$CDPI_3$ MGB™ CPG (Figure 1). The only difference is that the linker between the tripeptide and the DMT is increased in length by 2 atoms to accommodate a branched position for the quencher. As a combination of two modifications, MGB Eclipse® exhibits spectral properties that retain the absorbances of both of its two components (Figure 2). The absorbance maximum of the MGB is red-shifted from 340 to 342 nm, while the absorbance maximum of the Eclipse is red-shifted from 530 to 545 nm. The Eclipse absorbs in the approximate range of 400 to 640 nm, but it should be noted that MGB Eclipse will work with far red-emitting chromophores as well.

Since the Eclipse® quencher is highly stable and safe to all standard deprotection methods, the MGB portion dictates the conditions of synthesis and deprotection. Similar to the use of 3'-$CDPI_3$ CPG, UltraMild synthesis and CSO oxidation are recommended in order to achieve the best results. If standard monomers and iodine oxidation are used, acceptable results can be obtained when deprotection is performed in NH_4OH/EtOH 3:1 (v/v) for 17 hr at 55 °C.

To better understand the behavior of MGB Eclipse® under deprotection conditions, additional tests were performed. A poly-T sequence was synthesized on the MGB Eclipse® CPG using CSO oxidation, and the deprotection was carried out with ammonium hydroxide in three different environments: room temperature for 2 hours (UltraMild), room temperature for 17 hours, and 55 °C for 17 hours (Figure 3). These conditions simulate what would be required to deprotect iPr-Pac-dG, dmf-dG and ibu-dG, respectively. As expected, UltraMild deprotection looked relatively good. When the deprotection was extended overnight, oligonucleotide quality was not reduced significantly. However, this did not hold true when the reaction was incubated at 55 °C overnight. Prolonged heating in ammonium hydroxide clearly damages the MGB

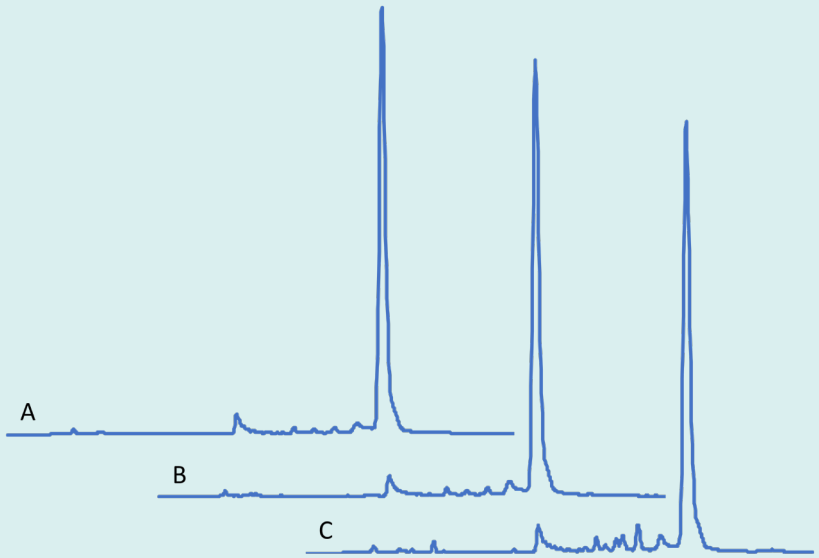

Figure 3. MGB Eclipse® oligonucleotide chromatograms. DMT-T_{10}-MGB Eclipse® on CPG was deprotected with ammonium hydroxide at room temperature for 2 hours (A), room temperature for 17 hours (B) and 55 °C for 17 hours (C). After deprotection, the oligonucleotides were analyzed by RP-HPLC.

Eclipse® and should be avoided. Based on these results, the use of dmf-dG and CSO oxidation is another viable alternative for producing MGB Eclipse® oligonucleotides of good quality.

MGB Eclipse® is an excellent modification for qPCR probes. By providing the CPG for this modification, our customers will now have a more standard and readily accessible tool in designing qPCR assays for existing and future needs.

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Eclipse® is a registered trademark of ELITechGroup® Inc.

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Item	Pack Size	Catalog No.
MGB Eclipse® CPG	0.1 g	20-5927-01
	1.0 g	20-5927-10
1 µmol columns	Pack of 4	20-5927-41
0.2 µmol columns	Pack of 4	20-5927-42
10 µmol column (ABI)	Pack of 1	20-5927-13
15 µmol column (Expedite)	Pack of 1	20-5927-14

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New Products — Dye NHS Esters

Glen Research offers a wide range of dyes for the labeling of oligonucleotides, including but not limited to fluorescein, rhodamine and cyanine dyes. Of these, a large majority are available as either phosphoramidite or support-bound reagents that are used during solid phase oligonucleotide synthesis. This synthesis method is typically the most convenient way of preparing fluorescently-labeled oligonucleotides, but there are circumstances in which alternative methods are desirable, particularly for fluorophores that are more sensitive to basic oligonucleotide deprotection conditions.

If the fluorophore is not added during solid phase synthesis, it would need to be conjugated to the oligonucleotide by post-synthetic modification. This is commonly performed with one of two methods: NHS esters[1] and click chemistry.[2] The former involves reacting an excess of activated esters with amine-labeled oligonucleotides to form amide bonds, while the latter employs a cycloaddition of azido- and alkynyl-modified entities. Both methods can be very effective and high yielding, and Glen Research offers several reagents for each approach.

In the previous issue of the Glen Report, the utility of oligonucleotide NHS ester labeling was highlighted (GR32.26). To give our customers more options for fluorophore-labeling, we are adding four dyes to our NHS ester offerings: Fluorescein, ROX, sulfoCyanine 3 and sulfoCyanine 5 (Figure 1). Each of these dyes fluoresces in different regions of the visible spectrum (Table 1) and has been used extensively for the labeling of oligonucleotides, peptides, proteins and other amine-containing molecules/biomolecules. While fluorescein phosphoramidites are widely available, the other three can only be attached to oligonucleotides by post-synthetic modification.

Fluorescein NHS Ester

Fluorescein is a very important fluorophore. The carboxy form (FAM) is utilized for applications ranging from immunofluorescence to quantitative PCR, and it is especially popular for oligonucleotides, as evidenced by the twelve fluorescein products that we carry. Red-shifted versions of FAM, such as TET, HEX and JOE, can also be found in our catalog. Users of our fluorescein phosphoramidites and supports will know that methylamine can react with fluorescein to form a non-fluorescent impurity during oligonucleotide deprotection, as described in GR25.13. Although there is a workaround for this, the NHS ester approach (using the 6-FAM isomer) bypasses this issue altogether. When paired with a 5'-amino-modifier C6 (10-1906, 10-1907, 10-1916 or 10-1947), the resulting product would be identical to that generated with 5'-Fluorescein Phosphoramidite (10-5901). Likewise, the NHS ester paired with amino-modifier C6 dT (10-1039) will give exactly the same product as the one obtained with Fluorescein-dT Phosphoramidite (10-1056).

ROX NHS Ester

Alongside FAM, JOE and TAMRA, ROX is a rhodamine dye that has played a significant role in dideoxy Sanger sequencing. The dye itself is also commonly used as a passive reference dye for qPCR applications. ROX is not available as a phosphoramidite, and as such, the use of this NHS ester is the standard method of incorporating ROX into oligonucleotides. This NHS ester is being offered as the 6-isomer.

sulfoCyanine 5/3 NHS Esters

Cyanine 5 and 3 are popular indocyanine fluorophores that exhibit low, non-specific binding and relatively pH-independent fluorescence. For many years, Glen Research has been offering phosphoramidites and supports for these two dyes. With the addition of the NHS esters, customers will have another approach at their disposal. This can be particularly useful for cyanine 5, as it is one of the more deprotection sensitive dyes we offer. Unlike FAM NHS, these cyanine NHS esters do not match the structure of their phosphoramidite counterparts. The linker is different and each reagent contains 2 sulfonate substitutions directly on the indocyanine nuclei. The sulfonates make the dyes less susceptible to aggregation and do not significantly change their fluorescent properties. In the NHS ester form, sulfoCyanine dyes are highly soluble in water, giving users the option of not using DMF or DMSO to first dissolve the NHS ester.

Labeling Process & Results

Using our standard NHS ester coupling procedure (GR32.26), very good conjugation results were obtained. For each reaction, RP-HPLC results showed one product peak corresponding to the desired 5'-fluorophore-labeled product and some unlabeled 5'-amino oligonucleotide. Initial coupling efficiencies of 85, 99, 98 and 98 % were observed for FAM, ROX, sulfoCyanine 3 and sulfoCyanine 5, respectively.

Based on these results, the FAM NHS ester appeared to be relatively more difficult to couple. This could be because the NHS ester couples slower, is hydrolyzed faster or a combination of both. Regardless of the underlying cause, an NHS ester coupling can be and should be much closer to quantitative yield. As such, a subsequent set of FAM NHS ester labeling reactions was performed. The first was carried out as before, a second was carried out with 3-fold less volume of buffer and a third was reacted with 3-fold more equivalents of NHS ester. The results are summarized (Table 2).

Reaction 1 was a repeat of the original evaluation, and the 87 % figure is consistent with the 85 % figure obtained earlier. As expected, using more NHS ester in reaction 3 helped to drive the label yield higher, but reducing the reaction volume in reaction 2 helped even more, giving quantitative labeling. By reducing the reaction volume 3-fold, the concentrations of both the NHS ester and the oligonucleotide were increased 3-fold. For a second order reaction, the reaction rate was effectively increased 9-fold. It should be pointed out that at higher concentrations, NHS esters will also hydrolyze more rapidly, but using smaller volumes is effective because the hydrolysis is essentially a first order reaction.

We are excited to provide our customers with additional options for obtaining fluorophore-

labeled oligonucleotides. These NHS esters are very effective, and if any issues arise in terms of labeling yield for these or any other NHS esters, please consider reducing the reaction volume.

References

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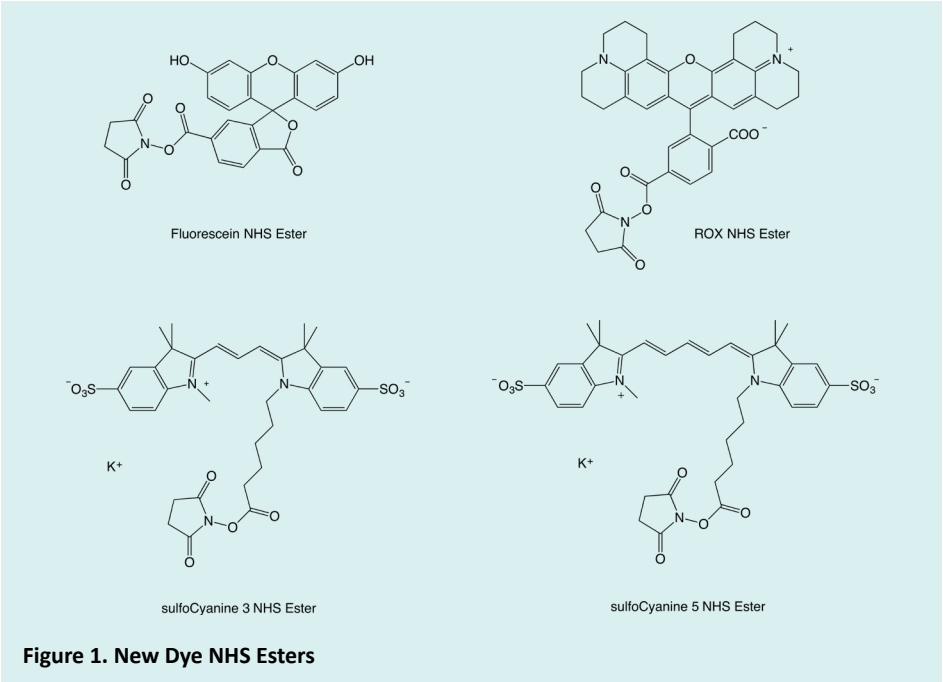

Figure 1. New Dye NHS Esters

Table 1. Spectral properties of the four fluorophores

	FAM	ROX	sulfoCyanine 3	sulfoCyanine 5
Absorbance maximum (nm)	495	588	547	646
Emission maximum (nm)	521	608	563	662
Extinction Coefficient ($M^{-1}cm^{-1}$, 260 nm)	20,900	22,600	6,000	7,500
Extinction Coefficient ($M^{-1}cm^{-1}$, A_{max})	75,000	82,000	150,000	250,000

Table 2. FAM NHS ester conjugation condition investigation. Each reaction used 6 % of a 1 µmol (~60 nmol) scale synthesis of 5'-amino-oligonucleotide. The buffer was sodium bicarbonate, pH 9, and the reaction volume is the sum of the oligo solution in buffer and the NHS ester in DMSO.

	Amount of NHS ester (µmol)	Buffer Concentration (M)	Final Reaction Volume (µL)	Coupling Efficiency (%)
Rxn 1	0.3	0.1	157	87
Rxn 2	0.3	0.3	57	100
Rxn 3	0.9	0.1	170	94

Item	Pack Size	Catalog No.
Fluorescein NHS Ester	2.4 mg	50-5901-22
	9.5 mg	50-5901-25
ROX NHS Ester	3.2 mg	50-5911-22
sulfoCyanine 3 NHS Ester	3.8 mg	50-5913-22
sulfoCyanine 5 NHS Ester	3.9 mg	50-5915-22

Application Note — Protein Labeling with NHS Esters

The use of NHS esters is an effective and selective method for labeling primary aliphatic amines. The chemistry works very well for amine-labeled oligonucleotides, as demonstrated in the previous article, but it perhaps works even better for proteins that naturally contain reactive amine residues (courtesy of the N-terminus) and lysine side chains. The addition of biotin, small organic fluorophores or other small molecule labels to proteins is very common, and labeled proteins have been routinely used for applications ranging from immunofluorescence[1] to proteomics.[2]

Although the chemistry is the same, there are notable differences between oligonucleotide and protein NHS ester labeling. On an oligonucleotide, there is typically one single reactive amine and the goal is to achieve quantitative conjugation yield, while for a protein, there are many reactive amines. According to the UniProt Database, lysines make up 5.6 % of the amino acids found in proteins.[3] If the average human protein is 500 amino acids in length, that would equate to 28 lysines. Typically, to minimize the possibility of disrupting the protein function or structure, only a fraction of the amines is converted. As such, the location of conjugation is random rather than fixed. A pure single protein, upon labeling, could be converted to a mixture of differentially labeled versions of the same protein. If a specific lysine was absolutely critical to the protein's function, random lysine labeling ensures that the protein sample as a whole is not significantly affected.

General Labeling Procedure

1. Prepare a 5-20 mg/mL protein solution in 0.1 M bicarbonate buffer (pH 8-9) or phosphate buffered saline (PBS).
2. Dissolve NHS ester in a small/minimal amount of DMF or DMSO.
3. Add NHS ester solution to protein solution

4. Agitate the mixture by pipetting and incubate at room temperature for 1-4 hr.

5. Separate the protein-conjugate from salts and excess label by size exclusion on a Glen Gel-Pak™ desalting column or equivalent.

In planning these experiments, the first step is to understand how much NHS ester is required. This will be dependent on the protein, protein concentration and the degree of labeling desired. For the latter, some applications require minimal labeling, whereas others may require multiple labels per protein molecule. For a more predictable outcome, one may choose to perform a series of small-scale labeling experiments prior to scaling up. As with any NHS ester coupling, a non-amine-based buffer needs to be used. This would typically be a bicarbonate buffer (pH 8-9), but for very pH sensitive proteins, PBS, which mimics physiological conditions, might be a better choice. At pH 7.4, the NHS ester coupling reaction is much slower, but NHS ester hydrolysis is slower as well. As a result, reactions in PBS will also work well but will require longer periods of incubation time.

To further illustrate the similarities and differences of labeling between oligonucleotides and proteins, we performed a couple of bovine serum albumin (BSA) labeling experiments with Fluorescein NHS Ester. BSA is one of the most commonly used proteins in science. The mature protein has a length of 583 amino acids, a molecular weight of 66,463 Da and a total of 59 lysine residues. Of these lysines, some are glycosylated or succinylated and others may simply be inaccessible, but there are still many available for conjugation. 6.5 equivalents of FAM NHS ester in DMSO was added to a 10 mg/mL solution of BSA in 0.1 M sodium bicarbonate (pH 9.0). After incubating the reaction for 1 hour at room temperature, the reaction was desalted on a Glen Gel-Pak™ column. UV/VIS absorbance analysis of the labeled BSA revealed a degree of labeling of 1.1. The same exact reaction was also performed in PBS. This second labeling experiment was incubated for 4 hours at room temperature and gave a somewhat lower degree of labeling of 0.9.

For perspective, these BSA labeling reactions were performed at approximately 2.5-fold less concentration than the initial amino oligonucleotide reactions in the previous article, and we know how important reagent concentrations can be in terms of reaction rates. BSA is clearly more readily labeled than 5'-amino oligonucleotide, but BSA does not appear to be anywhere close to 60-fold (59 lysine residues and the N-terminus) more reactive. This is partly due to the fact that lysine side chains have notably higher pKa's than a typical aliphatic alkyl amine, and partly due to the fact that, as mentioned earlier, some of the lysines may not be accessible.

Traditionally, Glen Research has been providing high quality reagents for the oligonucleotide synthesis community, but as we have discussed here, a subset of the products can also be applied to other areas, such as protein labeling. All the NHS ester and azide (not discussed) labels we offer will work with proteins, and our Glen Gel-Pak columns will desalt proteins just as well as oligonucleotides.

References

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Application Note — Cleavable Linkages

Oligonucleotides with selectively cleavable linkages are desirable for many applications. These linkages have been used for mass spectrometry-facilitated transcriptome analysis,[1] release of therapeutic oligonucleotides,[2] massive structural rearrangement of DNA nanostructures,[3] blocked cleavable primers[4] and biomarker discovery.[5] Glen Research offers several products that can be used in these applications, and they fall into four categories based on the mechanism of cleavage.

- Photo-cleavable Linkers
- Hydrolysis-cleavable Linkers
- Enzymatic-cleavable Linkers
- Disulfide-cleavable Linkers

Among these, the most popular would likely be photocleavage. Photocleavage is attractive because it requires no external factors other than light and can therefore be precisely controlled. If one synthesizes an oligonucleotide with PC-Spacer (Figure 1A), one would be able to cleave the 2-(nitrophenyl)ethyl group rapidly and quantitatively using near-UV light at 300-350 nm to yield a capped 3'-phosphate and an uncapped 5'-phosphate. The latter fragment would be compatible with ligation. In addition to PC Spacer, PC Linker, PC Biotin and PC Amino-Modifier are also available. In the same manner as PC spacer, PC Linker allows researchers to attach anything desired, while the remaining two products act as photocleavable labels. PC cleavable linkages have been previously highlighted for biomarker screening (GR27.14) and purification (GR30.16). 3-Cyanovinylcarbazole (^{CNV}K), a photo-crosslinker, can also be considered as a photocleavable linkage. ^{CNV}K will crosslink with a hybridized DNA or RNA strand with 366 nm irradiation, and the formation of the resulting ^{CNV}K-C/T/U photo-adduct can be reversed with 312 nm irradiation (Figure 1B).

For those working with DNA and other base-stable backbones, another option is the single insertion of an RNA nucleotide as a hydrolyzable linkage. Any RNA nucleotide should be compatible. The resulting chimeric oligonucleotide can be quantitatively cleaved in 1 M NaOH at 65 °C for 30 min. After cleavage, a 5'-OH and a 2'/3'-phosphate are released (Figure 1C). In practice, this works very well, and the TBDMS or TOM group does not even need to be removed beforehand. As an alternative, enzymatic cleavage can be used. For instance, the commonly used enzyme ribonuclease A will rapidly cleave rC and rU insertions without silyl protection in a wide variety of reaction conditions.

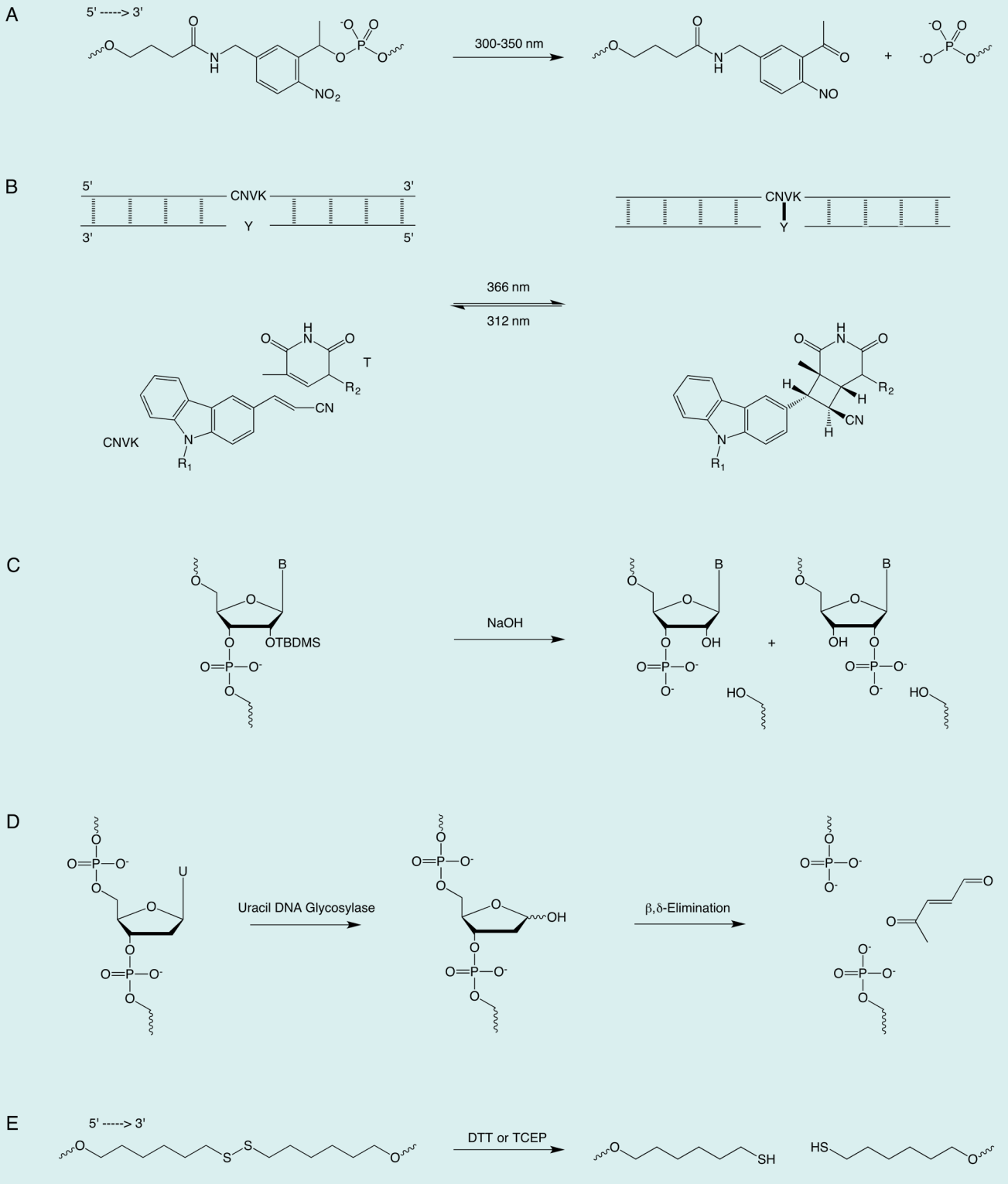

Figure 1. Cleavage Reactions. (A) PC Spacer; (B) ^{CNV}K; (C) 2'-OTBDMS RNA; (D) 2'-deoxyuridine; (E) Thiol-Modifier C6 S-S. Y = standard pyrimidine nucleotide; B = standard nucleobase; U = uracil.

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Item	Pack Size	Catalog No.
PC Spacer Phosphoramidite	100 µmol	10-4913-90
	0.25 g	10-4913-02
PC Linker Phosphoramidite	100 µmol	10-4920-90
	0.25 g	10-4920-02
PC Biotin Phosphoramidite	100 µmol	10-4950-90
	0.25 g	10-4950-02
PC Amino-Modifier Phosphoramidite	100 µmol	10-4906-90
	0.25 g	10-4906-02
3-Cyanovinylcarbazole Phosphoramidite (CNVK)	50 µmol	10-4960-95
	100 µmol	10-4960-90
	0.25 g	10-4960-02
Ac-C-CE Phosphoramidite	0.25 g	10-3015-02
	0.5 g	10-3015-05
	1.0 g	10-3015-10
U-CE Phosphoramidite	0.25 g	10-3030-02
	0.5 g	10-3030-05
	1.0 g	10-3030-10
dU-CE Phosphoramidite	100 µmol	10-1050-90
	0.25 g	10-1050-02
Thiol-Modifier C6 S-S	100 µmol	10-1936-90
	0.25 g	10-1936-02

On the topic of enzymatic cleavage, 2'-deoxyuridine can also act as a cleavable linkage. The repair enzyme, uracil DNA glycosylase, will remove uracil bases from single- and double-stranded DNA. The resulting abasic site is not very stable and readily undergoes elimination. In the presence of amines, the deoxyribose will undergo β- and subsequently δ-elimination to give 4-oxopentanal, while leaving a phosphate on both of the released oligonucleotide fragments (Figure 1D).

The final category for cleavable linkers is disulfides. Disulfides are readily cleaved by reducing agents such as dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) to release free sulfhydryl groups (Figure 1E). This would typically be accomplished with Thiol-Modifier C6 S-S, but all other disulfide linkages would also be applicable.

With all these different cleavable linkages, researchers can choose their preferred method based on compatibility with downstream applications. If the linker needs to retain Watson-Crick base pairing, the RNA strategy would be perfect. Likewise, if a ligation is desired after cleavage, then one of the PC products would be a better fit.

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Technical Note – DNA and RNA Nucleoside Numbering System

DNA and RNA nucleotide building blocks contain three components: a heterocyclic base, a pentose sugar, and a phosphate. For the first two components, a precise numbering and nomenclature system is needed to communicate a universally distinct chemical structure and name. This article is devoted to facilitating the understanding of the atom numbering system and associated nomenclature in DNA and RNA nucleotide building blocks, both of which are governed by the International Union of Pure and Applied Chemistry (IUPAC) rules and guidelines.

The numbering system for the heterocyclic base is arguably the most interesting, as it covers the two types of heterocyclic bases: purine bases (A, & G), and pyrimidine bases (C, T, & U). In this numbering system, all nitrogen atoms have odd numbers, **1,3,7,** and **9** (the last two only apply to purine bases). It should be noted that all structures in our catalog and website depict nucleosides in their *syn*-conformation for convenience, as opposed to the anti-conformation found in Watson-Crick base pairs. The *syn*- and *anti*-conformations differ only in the rotation of the N-glycosidic bond (Figure 1).

While the numbering may appear arbitrary, IUPAC developed a defined numbering system to reduce ambiguity. Key to the numbering system is where to start, and for a ring system, numbering starts on the largest ring and at the first substitution of carbon. Numbering continues in a way that enables the lowest numbers for substitutions.

The pyrimidine ring numbering system starts by assigning number **"1"** to the Nitrogen atom bonded to the pentose sugar (N-glycosidic bond), before counting clockwise to complete the numbering assignments (Figure 1-A). For purine, there are two fused rings: a six-membered ring (pyrimidine), and a five-membered ring (imidazole). In adenine, the numbering system for the pyrimidine follows a counterclockwise direction, starting from the Nitrogen atom **"1"** to the carbon atom **"6"** bonded to the exocyclic amine. The imidazole ring numbering system follows a clockwise direction, starting with the Nitrogen atom **"7"** and ending with the Nitrogen atom at the N-glycosidic bond position, "Nitrogen number **9**" (Figure 1-B).

The numbering of the pentose sugar is more straightforward than that of the nucleobases. IUPAC assigns number **"1"** to the carbon atom in the Carbon-Nitrogen linkage "glycosidic bond." Carbon **"1"** in the pentose sugar bonds to Nitrogen **"1"** in the pyrimidine ring and Nitrogen **"9"** in the purine ring. From Carbon **"1,"** the numbering follows the carbon chain sequentially in a clockwise direction to Carbon **"5"** (Figure 1-C).

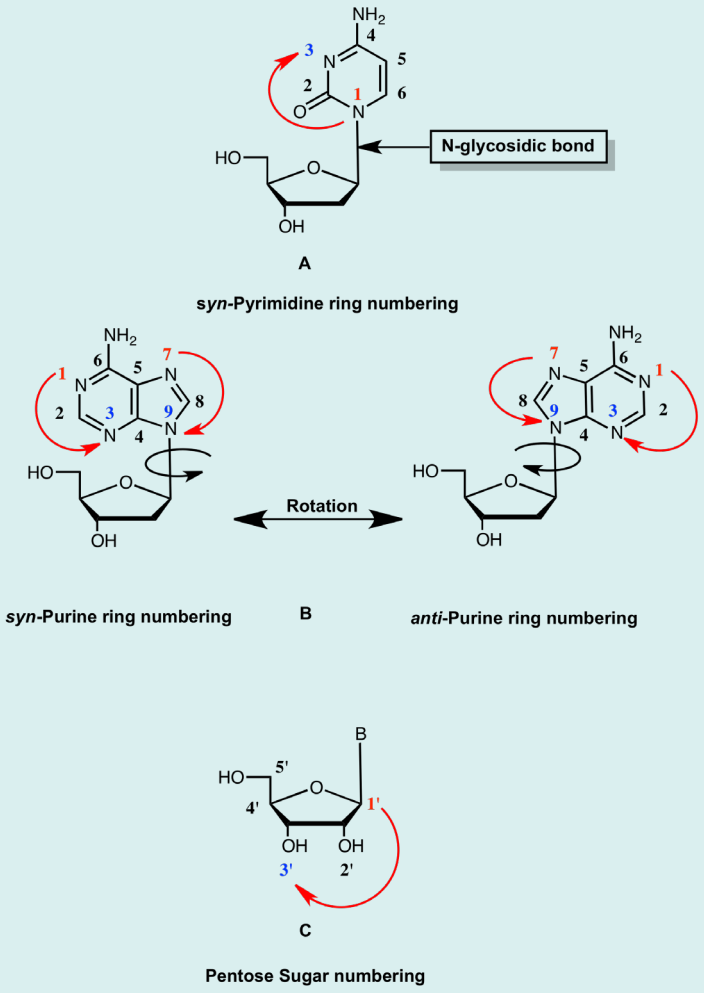

Figure 1. The Numbering System for the Purine and Pyrimidine rings

Glen Research offers a large catalog of phosphoramidites, and of these, a significant portion have modified nucleobases. More than 70 modified nucleobases are available. This number is for DNA, and would be notably higher if RNA, 2'-OMe, and 2'-F versions were included. The 5-position in pyrimidines is by far the most popular attachment point for modifications. This is because 1) there are a lot of natural modifications attached to this location, 2) it is convenient to modify at a position that does not interfere with hybridization, and 3) modifications from this position are generally tolerated by a lot of nucleic acid-modifying enzymes. There are 5-halide modifications for crosslinking (Figure 2-A), alkynes for click chemistry, amino-modifiers for sequence modification and oxidized modifications for epigenetic study. In addition to the 5-position, modifications can also be found at the 2-position and 4-position. For instance, 2-thio-dT is useful in examining protein-DNA interaction by acting as a photosensitizing probe, and 4-thio-dT is useful for photo cross-linking and photo affinity labeling experiments (Figure 2-B). Finally, several modifications involve multiple positions, including tricyclic cytosines (several of which are fluorescent) via the 4- and 5-positions, and 5,6 dihydropyrimidines, naturally occurring modifications that are formed as damage products due to exposure of DNA to ionizing radiation.

For the purines, the modifications are more spread out, partly because there are more positions available. From the 8-position, there are 8-Oxo-purines that allow for investigation of the structure and activity of oligonucleotides containing an 8-Oxo mutation, which is formed naturally when DNA is subjected to oxidative conditions or ionizing radiation. In addition, there are 8-Bromo-purines that can be used in crystallography studies of oligonucleotide structure and cross-linking studies of protein-DNA complex structure. Finally, there are 8-Amino-purines that can be very useful in triplex formation. It should be noted that, like 8-Oxo-dG, 8-Amino-dG can also be a mutagenic lesion. Another set of modifications for modulating the base

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pairing properties of the purine involve the 2- and 6-positions. Some of these will base pair with both dC and dT (dI, dK), while others form an additional interaction with dT (2-Amino-dA) or an artificial base pair with isodC (isodG).

In addition to direct substitutions on the purines and pyrimidines, there are a couple of other categories of modifications. The first are modifications directly on the rings. This would be a carbon in place of a nitrogen (deaza), or vice versa (aza). For instance, 7-deaza-G has a carbon at position 7, while 5-aza-dC contains a nitrogen at position 5 (Figure 2-C&D). These substitutions would of course affect the hydrogen bonding interactions of the resulting nucleobases. The second category would include modifications on amines or hydroxyls attached to the purine and/or pyrimidine rings. In these cases, the modifications are designated "NX" or "OX," where "X" is the number of the ring's atom. For example, N6-methyl-dA has a methylated exocyclic amine at the 6-position (Figure 2-E).

See Product Table on Page 11

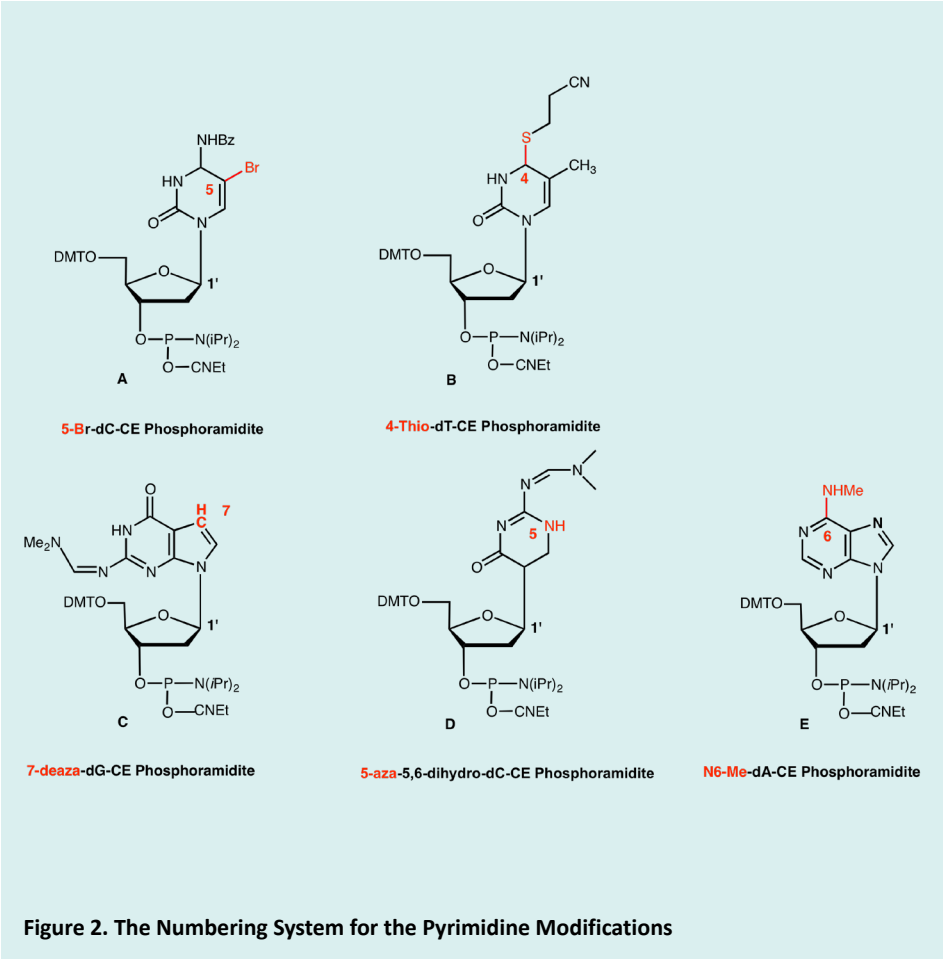

Figure 2. The Numbering System for the Pyrimidine Modifications

Product Offerings Update – K&A Synthesizer Reagents

K&A offers a line of closed column synthesizers for oligonucleotide synthesis. With 4 to 32 column positions, these relatively compact systems are capable of synthesis scales of up to 10 µmol. Many of our customers use these instruments, and to better support them, we have assembled a list of compatible reagents: https://www.glenresearch.com/browse/k-a.

This is essentially the Expedite list with additional larger bottle sizes to match some of the higher throughput configurations of the K&A instruments. Standard DNA phosphoramidites are available in 20-400 neck and 1 and 2 oz Boston Round bottles, as well as larger 28-400 neck and 200 mL Boston Round bottles. For all other phosphoramidite products, please look for the Expedite (E) format/packaging corresponding to either a 1 or 2 oz Boston Round bottle. These phosphoramidites are supplied in a standard ABI style slider vial, and a clean Boston Round bottle is also included. After solvation of the phosphoramidite in appropriate diluent, the resulting solution will need to be transferred to the Boston Round bottle. For the columns, both Expedite style columns, 40 nmol/200 nmol and 1 µmol scale, are compatible. For larger scales, the Twist 10 µmol/15 µmol column is also compatible, but due to how close the column positions are on the instrument, only every other position can be occupied by these larger diameter columns. Finally, we offer all of our standard liquid reagents in 28-400 (200/450 mL) and 38-400 (2 L) neck sizes for these instruments.

K&A Synthesizer Reagent Compatible Columns

Item	Pack Size	Catalog No.
Empty Synthesis Columns, 40nm, 0.2um Expedite Style	Package of 10	20-0021-02
Empty Synthesis Columns, 1um Expedite Style	Package of 10	20-0021-01
Empty Synthesis Columns – TWIST 10um/15um	Package of 10	20-0040-00

The list below includes all the pyrimidine and purine modifications discussed and many more. These are the DNA versions only. The list is not exhaustive, and very closely related products, like those that differ by only a protecting group, are not shown. Some products are applicable to multiple applications but are only listed once.

Technical Note - DNA and RNA Nucleoside Numbering System: Pyrimidine and Purine Modifications

Applications	Pyrimidine	Catalog No.	Purine	Catalog No.
Conjugation/Click Chemistry	TIPS-5-Ethynyl-dU-CE Phosphoramidite	10-1555		
	C8-Alkyne-dT-CE Phosphoramidite	10-1540		
	C8-Alkyne-dC-CE Phosphoramidite	10-1543		
Cross-Linking/Halogenated Nucleosides	5-Br-dC-CE Phosphoramidite	10-1080	8-Br-dA-CE Phosphoramidite	10-1007
	5-Br-dU-CE Phosphoramidite	10-1090	8-Br-dG-CE Phosphoramidite	10-1027
	5-I-dC-CE Phosphoramidite	10-1081		
	5-I-dU-CE Phosphoramidite	10-1091		
DNA Damage/Repair	5-OH-dC-CE Phosphoramidite	10-1063	1-Me-dA-CE Phosphoramidite	10-1501
	5-Hydroxymethyl-dU-CE Phosphoramidite	10-1093	8-Oxo-dA-CE Phosphoramidite	10-1008
	5-OH-dU-CE Phosphoramidite	10-1053	8-Oxo-dG-CE Phosphoramidite	10-1028
	5,6-Dihydro-dT-CE Phosphoramidite	10-1530	8-Amino-dG-CE Phosphoramidite	10-1079
	5,6-Dihydro-dU-CE Phosphoramidite	10-1550		
	Cis-syn Thymine Dimer Phosphoramidite	11-1330		
	Thymidine Glycol CE Phosphoramidite	10-1096		
Duplex Stability	5-Me-dC-CE Phosphoramidite	10-1060	2-Amino-dA-CE Phosphoramidite	10-1085
	AP-dC-CE Phosphoramidite	10-1097	N6-Me-dA-CE Phosphoramidite	10-1003
	dW-CE Phosphoramidite	10-1527	N6-Ac-N6-Me-dA-CE Phosphoramidite	10-1503
	N4-Et-dC-CE Phosphoramidite	10-1068	Pac-2-Amino-dA-CE Phosphoramidite	10-1585
	pdC-CE Phosphoramidite	10-1014		
	pdU-CE Phosphoramidite	10-1054		
Epigenetics/DNA Methylation	5-Carboxy-dC-CE Phosphoramidite	10-1066	1-Me-dA-CE Phosphoramidite	10-1501
	5-Formyl dC III CE Phosphoramidite	10-1564	O6-Me-dG-CE Phosphoramidite	10-1070
	5-Hydroxymethyl-dC-CE Phosphoramidite	10-1062		
PCR Sequencing/Duplex Effects	dmf-5-Me-isodC-CE Phosphoramidite	10-1065	dmf-isodG-CE Phosphoramidite	10-1078
	dP-CE Phosphoramidite	10-1047	dK-CE Phosphoramidite	10-1048
			dI-CE Phosphoramidite	10-1040
Sequence Modification/Amino-Modifiers	Amino-Modifier C2 dT	10-1037	Amino-Modifier C6 dA	10-1089
	Amino-Modifier C6 dT	10-1039	N2-Amino-Modifier C6 dG	10-1529
	Amino-Modifier C6 dC	10-1019	8-Amino-dA-CE Phosphoramidite	10-1086
Structural Studies/Activity Relationship	2'-deoxypseudoU-CE Phosphoramidite	10-1055	7-Deaza-dA-CE Phosphoramidite	10-1001
	2-Thio-dT-CE Phosphoramidite	10-1036	7-Deaza-dG-CE Phosphoramidite	10-1021
	4-Thio-dT-CE Phosphoramidite	10-1034	3-Deaza-dA-CE Phosphoramidite	10-1088
	4-Thio-dU-CE Phosphoramidite	10-1052	5-aza-5,6-dihydro-dC-CE Phosphoramidite	10-1511
Structural Studies/Fluorescent Nucleosides	tC-CE Phosphoramidite	10-1516	Etheno-dA-CE Phosphoramidite	10-1006
	tC°-CE Phosphoramidite	10-1517		

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Technical Snippets GR33.1

Why are phosphoramidites and synthesis supports with freezer storage conditions shipped at ambient temperature rather than with dry ice?

It is because cold shipping is not necessary when shipping these products. The bulk of these products are shipped at ambient temperature with priority shipping, no ice packs nor dry ice, and this is rarely an issue. These products are generally very stable and are not like proteins that will irreversibly denature beyond a certain temperature. The recommended storage temperatures are for long term storage, and a few days at ambient temperature do not impact product quality whatsoever. There is a small selection of products, less than 10, that are more susceptible to short term elevated temperatures, and those products are shipped with ice packs.

Products: All products that require freezer storage.

How does one synthesize an oligonucleotide with a 3'-inverted dT?

The synthesis is accomplished with a reverse synthesis dT support and standard phosphoramidites. An inverted dT modification at the 3'-end is a convenient way to reduce susceptibility to 3'-exonucleases as well as prevent DNA polymerase extension. By starting with a reverse dT support and then performing standard 3' to 5' synthesis, a 3'-3' linkage is created at the beginning (Figure 1).

Product: dT-5'-CPG (20-0302)

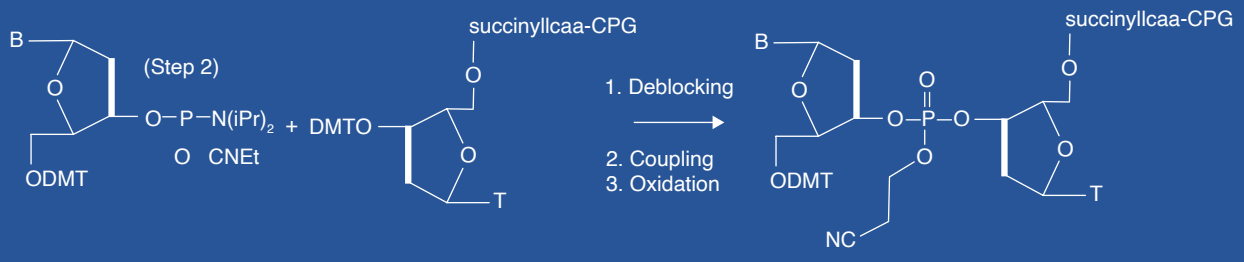

Figure 1. Formation of a 3'-inverted dT

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