



## Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## DNA Damage Repair Introduction

DNA in cells can be damaged by both environmental factors (for example, UV light, high-energy radiation, or mutagenic industrial chemicals) and normal metabolic processes (for example, production of reactive oxygen species (ROS) or replication errors) (1). While some lesions result from single-stranded or double-stranded breaks in the DNA sugar-phosphate backbone, in most cases, the nucleotide bases have been modified chemically. If such a lesion occurs within a gene, the structural distortion could alter or eliminate the ability of the cell to transcribe or replicate the gene, or possibly lead to a potentially harmful mutation that would deleteriously affect the survival of the daughter cells after the parent cell undergoes mitosis (2). The cell has corresponding DNA repair mechanisms that enable it to respond to the various types of DNA damage described above, mechanisms such as DNA base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), or direct reversal (3-6). The DNA Damage Repair category encompasses those modifications that can be incorporated into oligonucleotides for use as research tools in investigational studies into the various biochemical processes involved in the damage and repair of DNA or RNA in the cell.

# DNA Damage Repair **Design Protocols**

## DNA Damage Repair--Assay Considerations

When designing oligos incorporating modified bases suitable for DNA damage/repair studies, it is important to properly match the type of lesion under study and the analytical method chosen for detection. I. Nucleotide Excision Repair (NER)

The standard in vitro assay for NER is a reconstitution of this repair system in cell-free extract using six recombinantly expressed NER factors (RPA, XPA, XPC, TFIIH, XPG, and XPF)(9) and a synthetic oligo duplex as template, modified with an adduct known to induce NER (10). II. Base Excision Repair (BER)

The standard in vitro assay for BER is a reconstitution of this repair system in whole cell extracts using a synthetic oligo duplex as template, containing a modified base (e.g., 8-oxo-dG) known to induce BER (11). It is also possible to experimentally monitor BER in vivo (12). III. UV-Induced DNA Damage

For studying UV-light induced DNA damage (formation of cyclobutane pyrimidine dimers (CPDs) or 6,4-photoproducts) in particular genomic regions, PCR-based techniques typically are the analytical method of choice, most commonly ligation-mediated PCR (LMPCR) (13). However, PCR-based methods are not suitable for telomeric regions, because telomeres are composed of thousands of copies of the short tandem repeat 5'TTAGGG/5'CCCTAA, and thus have no unique PCR priming sites. So, for telomeric regions, immunoprecipitation of DNA damage (IPoD) is used (14).

## DNA Damage Repair Applications

Many DNA damage/repair studies are focused on the potential mutational or genotoxic consequences that could arise from specific single types of DNA lesions. However, more recently, attention has begun to be paid on the potential deleterious effects of clusters of lesions, located on either the same or complementary strands. Lesion cluster formation is particularly relevant when the damaging agent is ionizing radiation, and the relative reparability of such clusters compared with single-base lesions is an active research topic (7). Another recent area of interest is the relationship between DNA sequence context (for example, single vs. runs of Gs) and both the location and number of lesions caused by DNA damaging agents, oxidizers in particular (8).

A variety of modified nucleotide phosphoramidites, suitable for use in investigational studies of DNA damage/repair mechanisms, are commercially available that can be incorporated into oligonucleotides during solid-phase synthesis. In addition, Gene Link's extensive experience in synthesizing oligos with unusual, or challenging combinations of, modifications makes us an attractive choice for supplying modified oligos for use in (a) DNA damage and repair studies, (b) the development of assays for detecting specific types of DNA damage, or monitoring specific DNA repair processes, (c) the development of assays that utilize DNA damage and repair processes to detect mutagenic or genotoxic substances in the environment. See the relevant tech sheet for a particular modification for details.

## References

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- (14) Rochette, P.J., Brash, D.E. Human Telomeres Are Hypersensitive to UV-Induced DNA Damage and Refractory to Repair. *PLoS Genetics* (2010), 6(4): e1000926.

## Modification Code List

Modification	Code	Catalog Number
5-hm dC (5-Hydroxymethyl-dC)	[5hm-dC]	26-6707
5-hydroxy dC	[5-OH-dC]	26-6701
5-hydroxy deoxyuridine (OH dU)	[5-OH-dU]	26-6695
8-Oxo deoxyguanosine (8-Oxo dG)	[8-Oxo-dG]	26-6434
Abasic Site (dSpacer abasic furan-THF)	[dABS]	26-6435
dihydro dT (5-6 DHT)	[5-6-DHT]	26-6890
dihydro dUracil (5-6 DHdU)	[5-6-DHdU]	26-6683
deoxyuridine dU	[dU]	26-6408
etheno dexoyadenosine dA	[Eth-dA]	26-6506
N3-methyl-dC [m3dC]	[m3dC]	26-6903
N6-Methyl dA (m6dA)	[m6dA]	26-6601
O6 Methyl dG	[O6-Me-dG]	26-6409
rAbasic Site (rSpacer abasic furan)	[rABS]	26-6442
Thymidine Glycol	[Tg-Thy-Glycol]	26-6487
Cis-syn Thymine Dimer Cyclobutane Pyrimidine Dimer (CPD) [Cis-TT]		26-6680



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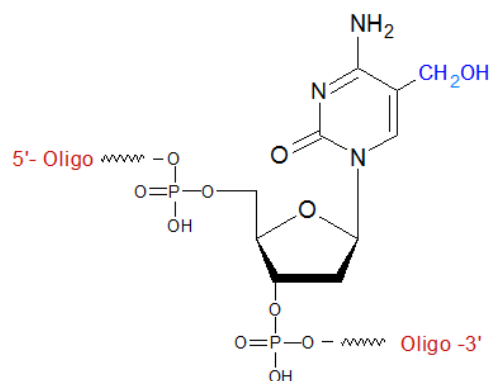
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### 5-hm dC

Category	Epigenetics
Modification Code	5hm-dC
Reference Catalog Number	26-6707
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



5-Hydroxymethyl-dC

[26-6707-XX]

5-Hydroxymethyl cytosine (5-hm-dC) is a minor DNA base; its presence in DNA strands was first observed in T-even bacteriophages (1). In such viruses, 5-hm-dC is often glycosylated, and this modified base protects phage DNA from cleavage by host restriction endonucleases after infection (2), and thus serves a **direct epigenetic role** in T-even phages. 5-hm-dC was first reported in mammalian systems in 1972, by Penn *et al.*, who found relatively high levels of this modified base in DNA extracted from the brains of adult rats, mice and frogs (~ 15% of total cytosines) (3). In a follow-up study, Penn reported the observation of a highly statistically significant increase in 5-hm-dC in rat brain tissue as rats grew from newborn (~ 8% of total cytosines) to adult (~ 18% of total cytosines), and speculated that 5-hm-dC-containing DNA, or the base itself, might be implicated in the maintenance of steady-state neuronal activity, and possibly associated with synaptosomal mitochondria (4).

However, because the presence of 5-hm-dC in mammalian brain tissue could not be confirmed in other studies conducted around the same time, the topic languished for the next 30 years. Then, in 2009, Kriaucionis and Heintz (5) reported the presence of high levels of 5-hm-dC in Purkinje neurons from mouse brain tissue, with the 5-hm-dC specifically localized to CpG regions, thus both confirming the results of Penn *et al.*'s 1972 paper and expanding on it by definitively localizing 5-hm-dC to CpG regions of DNA, suggesting that this modified base plays an important epigenetic regulatory role in the central nervous system of mammals. Shortly thereafter, Tahiliani *et al.* (6) reported that the enzyme TET1 catalyzes the conversion of 5-methyl-dC to 5-hm-dC, both *in vitro* and *in vivo*, further strengthening the case for such a role.

However, it is possible that the role of 5-hm-dC is as an intermediate in a putative (active) oxidative demethylation pathway for conversion of 5-Me-dC to dC. Demethylation of 5-Me-dC is necessary for epigenetic control of gene expression in the cell, and plays a key role in cellular reprogramming, embryogenesis, establishment of maternal and paternal methylation patterns in the genome (7), and also in certain autoimmune disorders and cancer (8). The discovery of an enzymatic pathway for conversion of 5-Me-dC to 5hm-dC, mediated by the enzyme Tet1 has spurred efforts to determine whether or not 5-hm-dC is then subsequently converted to dC through a 5-formyl-dC or 5-carboxy-dC intermediate.

In 2011, Ito and co-workers showed that Tet enzymes are able to convert 5hm-dC to both 5-formyl-dC and 5-carboxy-dC, and also observed their presence in mouse embryonic stem cells and various mouse organ tissues. Genomic content of 5hm-dC, 5-formyl-dC and 5-carboxy-dC can be modulated through overexpression or depletion of Tet proteins in these tissues (9). These experiments provide strong supporting evidence for DNA demethylation occurring via a Tet-mediated enzymatic pathway involving 5-hm-dC as a key intermediate. 5-hm-dC modified oligos can serve as important research tools for probing the DNA demethylation process.

The availability of 5-hm-dC as a phosphoramidite enables the incorporation of this modified base into synthetic oligonucleotides for use as research tools to help researchers definitively determine the role of this minor base in the biochemistry of brain and other tissues.

### 5-hydroxymethylated dC oligos

Oligos modified with 5-OH me dC (5-hmc) are totally resistant to cleavage by Hpa II restriction enzyme. Msp I and Msp JI restriction enzymes will digest these oligos to almost completion. Usually there is 25-30% resistant species remaining due to resistant protecting groups leftover during synthesis. Higher quantities of enzyme and longer incubation times (18-20 hrs) tends to increase digestion to greater than 90%. Oligos containing 5-hmc can be glucosylated by using T4  $\beta$ -glucosyltransferase and thus resistant to Msp I digestion to discern between 5-mc and 5-hmc. The 5-OH group of 5-hmc is glucosylated and becomes completely resistant to Msp I digestion.

### References

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## Product Specifications

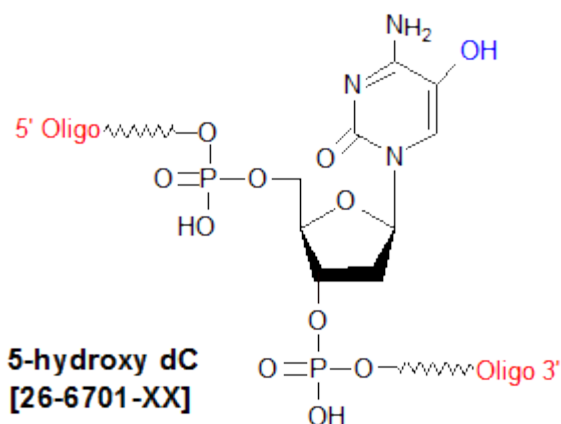
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### 5-OH dC

Category	Epigenetics
Modification Code	5-OH-dC
Reference Catalog Number	26-6701
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	305.18







## Product Specifications

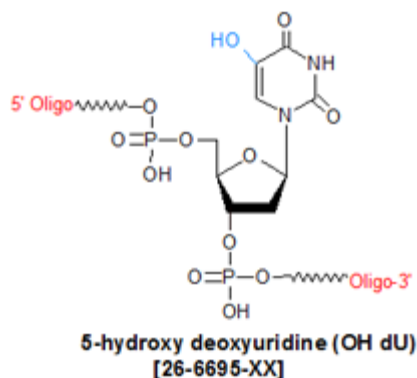
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### 5-OH dU

Category	Minor Bases
Modification Code	5-OH-dU
Reference Catalog Number	26-6695
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17





## Product Specifications

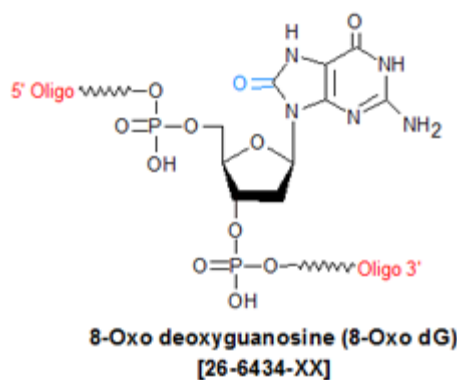
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### 8-Oxo dG

Category	Minor Bases
Modification Code	8-Oxo-dG
Reference Catalog Number	26-6434
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.21





## Product Specifications

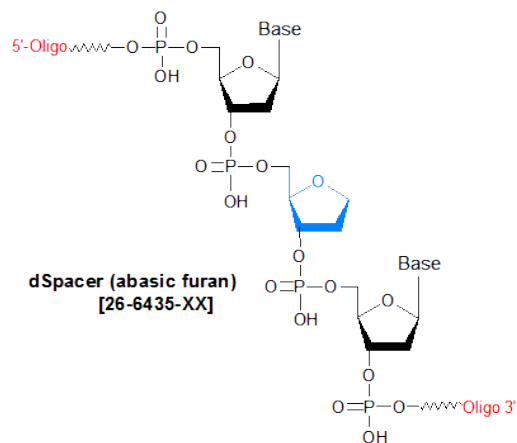
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## Oligo Modifications

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### Abasic Site (dSpacer tetrahydrofuran-THF)

Category	Spacers
Modification Code	dABS
Reference Catalog Number	26-6435
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	180.1





## Product Specifications

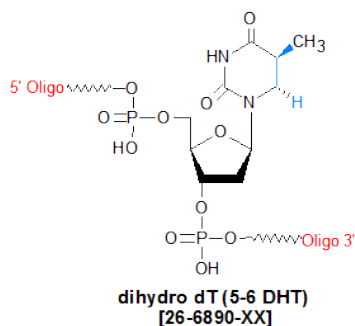
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## Oligo Modifications

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### dihydro dT (5-6 DHT)

Category	Minor Bases
Modification Code	5-6-DHT
Reference Catalog Number	26-6890
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.21





## Product Specifications

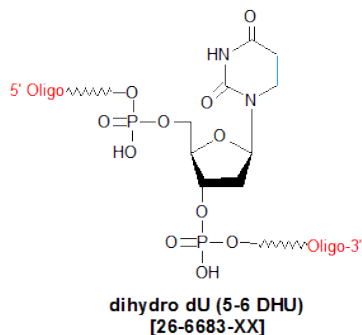
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## Oligo Modifications

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### dihydro dU (5-6 DHdU)

Category	Minor Bases
Modification Code	5-6-DHdU
Reference Catalog Number	26-6683
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	292.19





## Product Specifications

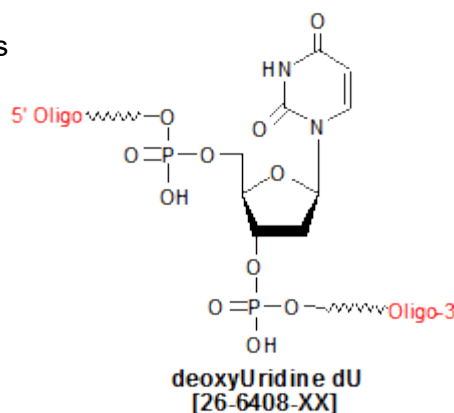
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### dU

Category	DNA Oligo Synthesis
Modification Code	dU
Reference Catalog Number	26-6408
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



Deoxyuridine (dU) is a pyrimidine deoxyribonucleoside, and a derivative of the nucleoside uridine, with the only difference being that, in dU, a hydrogen (-H) group is substituted for uridine's -OH group located at the 2'-position of the ribose. dU is generated in cellular DNA as a deamination product of dC (deoxycytidine), with the deamination process catalyzed by the enzyme AID (activation-induced cytidine deaminase) (1). AID is a B cell-specific gene that is necessary for antibody gene diversification via class-switch recombination and somatic hypermutation (2, 3). The dC-to-dU conversion(s) by AID occurs in the IgG locus, with various gene diversification pathways arising from the different DNA repair mechanisms used by B-cells to repair the dU lesion (1).

dC-to-dU conversion via cytidine deamination is also implicated in innate immunity to retroviruses. Here deamination of dC is mediated by the enzyme APOBEC3G, which is present in T cells, acting on the first (minus) strand cDNA of retroviruses. Generation of dU produces a dU /dG mismatch in the retroviral cDNA duplex, resulting in a dC-to-dT transition mutation on the minus-strand cDNA, and a dG-to-dA transition on the plus-strand (4). The presence of dU in the minus-strand cDNA could lead to innate immunity by one or more of the following: (a) hypermutation capable of disabling viral functions, (b) degradation by BER (base excision repair), (c) plus-strand cDNA mis-replication (5). dU can be used to modify oligos for use in studies of DNA damage and associated repair mechanisms.

Oligos modified with dU can serve as effective research tools for mechanistic studies of both adaptive and innate immunity in animal systems. 1. Neuberger, M.S., Harris, R.S., Di Noia, J., Petersen-Mahrt, S.K. Immunity through DNA deamination. *Trends Biochem. Sci.* (2003), **28**: 305-312.

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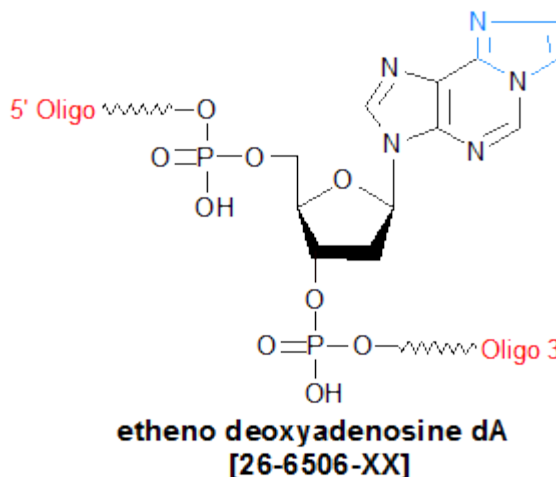
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### etheno dA

Category	Minor Bases
Modification Code	Eth-dA
Reference Catalog Number	26-6506
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	337.23



1,N-6 etheno deoxyadenosine (Etheno-dA) is a highly fluorescent derivative of dA, and can be incorporated at any position(s) within a DNA or RNA oligonucleotide. Etheno-dA has excitation maxima at 270 nm and 300 nm, and an emission maximum at 410 nm. Selective introduction of etheno-dA into DNA or RNA oligonucleotides is particularly useful in various structure-function studies of RNA, protein-RNA complexes, and DNA-RNA based diagnostics applications (1). However, because etheno-dA does not base-pair with dT or dU, oligos containing etheno-dA at either the 3'-end or in the middle will not function as either a sequencing or PCR primer. Etheno-dA-modified primers must have the modification(s) located either at or close to the 5'-end in order to so function (1).

Etheno-dA-modified oligonucleotides have proven particularly useful in the study of the repair of alkylated DNA damage by the base-excision-repair (BER) mechanism. For example, such modified oligos were used to elucidate the function of N-methylpurine DNA glycosylase (2), as well as providing insights into how this BER enzyme facilitates resistance of astrocyte brain tumors (malignant astrocytomas) to DNA-alkylation-based chemotherapy agents (such as nitrosoureas) (3). Exocyclic etheno DNA adducts likely play an important role in carcinogenesis in both rodents and humans (4), and etheno-dA-modified oligonucleotides can be used as research tools for the study of carcinogenesis in various tissues.

#### References

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*Carcinogenesis* (1996), **17**: 2105-2111.



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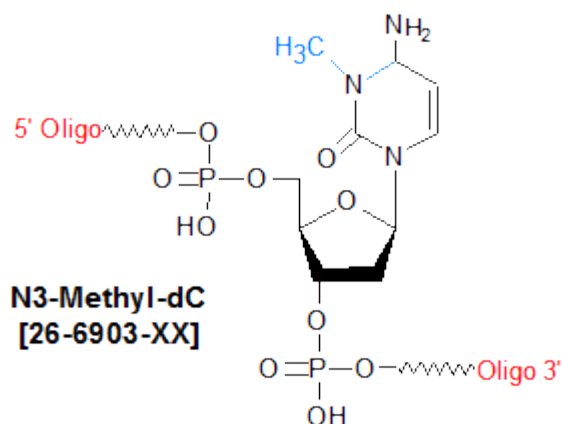
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### N3-methyl-dC [m3dC]

Category	Others
Modification Code	m3dC
Reference Catalog Number	26-6903
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	847.93





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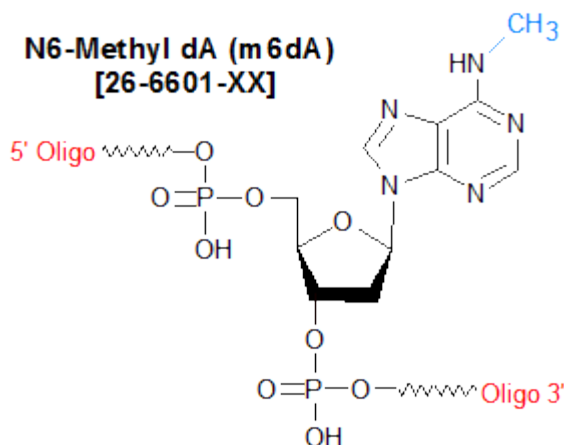
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### N6-Methyl dA (m6dA)

Category	Epigenetics
Modification Code	m6dA
Reference Catalog Number	26-6601
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	327.24





## Product Specifications

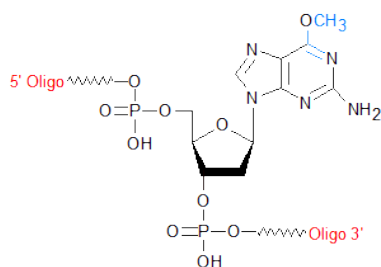
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### O6 Methyl dG

Category	Minor Bases
Modification Code	O6-Me-dG
Reference Catalog Number	26-6409
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.24



**O6 Methyl deoxyguanosine dG**  
[26-6409-XX]



## Product Specifications

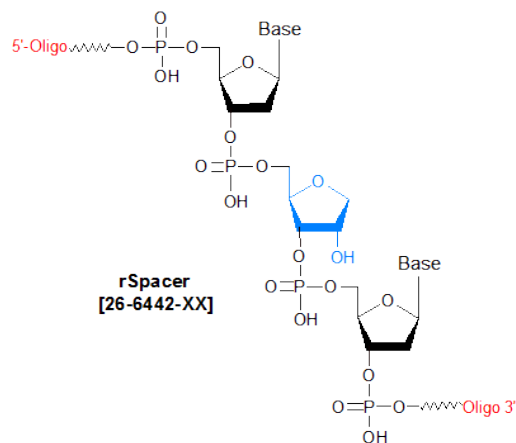
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### rSpacer

Category	Spacers
Modification Code	rABS
Reference Catalog Number	26-6442
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	196.09





## Product Specifications

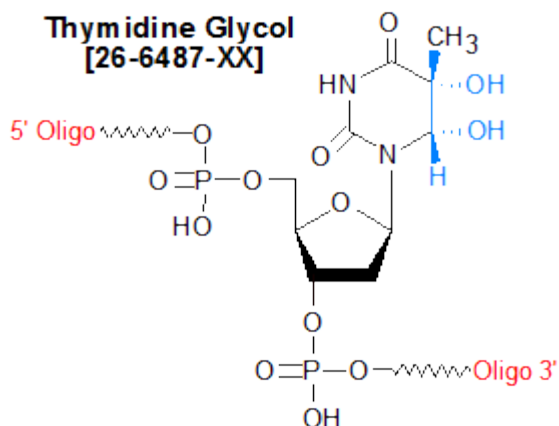
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Thymidine Glycol

Category	Minor Bases
Modification Code	Tg-Thy-Glycol
Reference Catalog Number	26-6487
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	338.21





## Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### Thymine Dimer Cis-syn

Category	Others
Modification Code	Cis-TT
Reference Catalog Number	26-6680
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	608.39

