Oligo Modifications

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 repairability 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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(7) Shikazono, N., Pearson, C., O'Neill, P., Thacker, J. (2006) "The roles of specific glycosylases in determining the mutagenic consequences of clustered DNA", Nucleic Acids Res. 34: 3722-3730.
(8) Margolin, Y., Shafirovich, V., Geacintov, N.E., DeMott, M.S., Dedon, P.C. (2008) "DNA Sequence Context as a Determinant of the Quantity and Chemistry of Guanine Oxidation Produced by Hydroxyl Radicals and One-Electron Oxidants", J. Biol. Chem. 283: 35569-35578.
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(10) Prakash, S., Prakash, L. Nucleotide excision repair in yeast. Mutat. Res. (2000), 451: 13-24.
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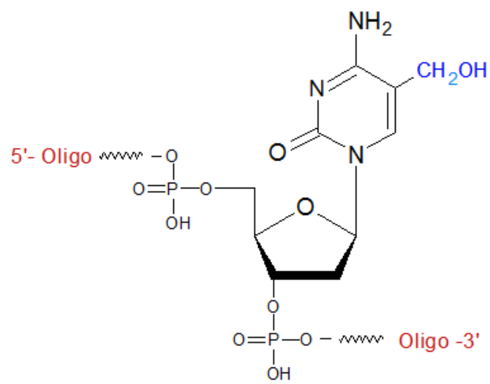
(12) Sundaresakumar, P. Use of novel assays to measure in vivo base excision DNA repair. M.S. Thesis, San Jose State Univ, 2009, 105 pages, Pub # 1470953. (13) Tornaletti, S., Pfeifer G.P. Ligation-Mediated PCR for Analysis of UV damage. In: Pfeifer, G.P., ed., Technologies for detection of DNA damage and mutations. New York: Plenum Press, 1996, pp. 199-209.
(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.

Modificaton 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

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 β-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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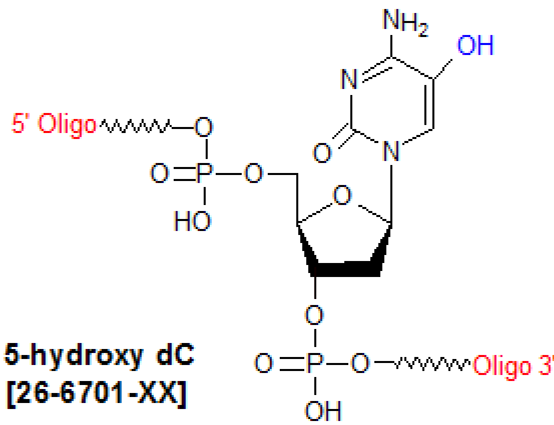
5-OH dC

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



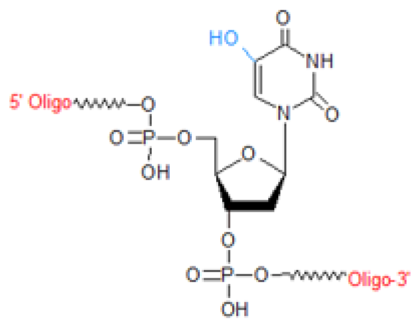
5-hydroxy dC
[26-6701-XX]

5-hydroxy deoxycytosine (5-OH-dC) is classified as an oxidized nucleotide, and is primarily used in studies of oxidative DNA damage and associated repair mechanisms. In the cell, 5-OH-dC DNA lesions are formed by reaction of cytosine with reactive oxygen species (ROS) generated either via normal oxidative metabolic processes or by UV ionizing radiation. 5-OH-dC can potentially mispair with both A and C (leading to C-to-T transitions or C-to-G transversions) (1). 5-OH-dC lesions can deaminate to form a second lesion, 5-hydroxy-deoxyuridine (5-OH-dU). As a single-base lesion, 5-OH-dC is removed by the base excision repair (BER) mechanism and the native cytosine base restored (2). However, the observation of 5-OH-dC in cellular DNA from liver, kidney and brain tissue at levels that remain relatively constant and high over time, suggests that the BER system is not completely effective at removing this lesion, and its presence in DNA may be a significant factor in both tumorigenesis and the aging process (3). **References**

1. Feig, D.I., Sowers, L.C., Loeb, L.A. Reverse chemical mutagenesis: Identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl. Acad. Sci. USA.* (1994), **91**: 6609-6613.
2. Nilsen, H., Krokan, H.E. Base excision repair in a network of defence and tolerance.*Carcinogenesis* (2001), **22**: 987-998.
3. Wagner, J.R., Hu, C-C., Ames, B.N. Endogenous oxidative damage of deoxycytidine in DNA. *Proc. Natl. Acad. Sci. USA.* (1992), **89**: 3380-3384.

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



5-hydroxy deoxyuridine (OH dU)
[26-6695-XX]

5-hydroxy deoxyuracil (5-OH-dU) is classified as an oxidized nucleotide, and is primarily used in studies of oxidative DNA damage and associated repair mechanisms. In the cell, 5-OH-dU DNA lesions are formed by spontaneous deamination of the lesion 5-OH-dC (itself formed by reaction with reactive oxygen species (ROS) generated either via normal oxidative metabolic processes or by UV ionizing radiation). 5-OH-dU can potentially mispair with A (leading to C-to-T transitions) (1). In fact, 5-OH-dU is now known to be the primary chemical precursor for such transitions in cellular DNA (2). As a single-base lesion, 5-OH-dU is removed by the base excision repair (BER) mechanism and the native cytosine base restored (3). However, the observation of 5-OH-dU in cellular DNA from liver, kidney and brain tissue at levels that remain relatively constant over time, suggests that the BER system is not completely effective at removing this lesion, and its presence in DNA may be a significant factor in both tumorigenesis and the aging process (4). **References**

1. Feig, D.I., Sowers, L.C., Loeb, L.A. Reverse chemical mutagenesis: Identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl. Acad. Sci. USA.* (1994), **91**: 6609-6613.
2. Thiviyanathan, V., Somasunderam, A., Volk, D.E., Hazra, T.K., Mitra, S., Gorenstein, D.G. Base-pairing Properties of the Oxidized Cytosine Derivative, 5-Hyroxy Uracil. *Biochem. Biophys. Res. Commun.* (2008), **366**: 752-757.
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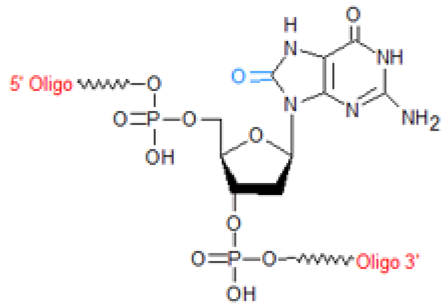
4. Wagner, J.R., Hu, C-C., Ames, B.N. Endogenous oxidative damage of deoxycytidine in DNA. *Proc. Natl. Acad. Sci. USA.* (1992), **89**: 3380-3384.

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



8-Oxo deoxyguanosine (8-Oxo dG)
[26-6434-XX]

8-Oxo-deoxyguanosine (8-Oxo-dG) is classified as an oxidized nucleotide, and is primarily used in studies of oxidative DNA damage and associated repair mechanisms. In the cell, 8-Oxo-dG DNA lesions are formed by reaction with reactive oxygen species (ROS) generated either via normal oxidative metabolic processes, UV ionizing radiation, or 2-nitropropane (an industrial solvent and component of tobacco smoke) (1). 8-Oxo-dG can potentially mispair with A (leading to G-to-T transversions) (2). As a single-base lesion, 8-Oxo-dG is removed by the base excision repair (BER) mechanism and the native guanine base restored (3). In the cell, 8-Oxo-dG does not appear to be strongly mutagenic (4). **References**

1. Feig, D.I., Sowers, L.C., Loeb, L.A. Reverse chemical mutagenesis: Identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl. Acad. Sci. USA.* (1994), **91**: 6609-6613.
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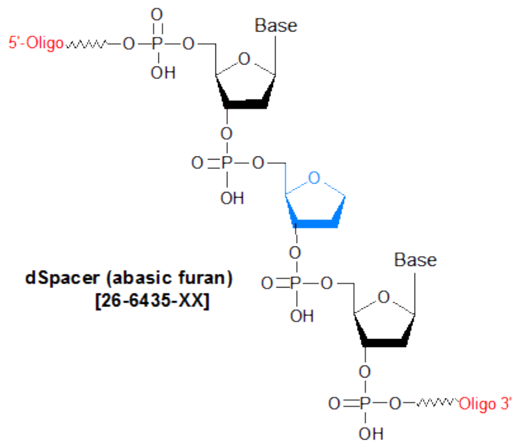
Abasic Site (dSpacer tetrahydrofuran-THF)

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



dSpacer (abasic furan) is a tetrahydrofuran derivative, in which a methylene group occupies the 1 position of 2'-deoxyribose. dSpacer is commonly used to mimic an abasic site in an oligonucleotide. In DNA, abasic sites are generated by hydrolysis of the glycosidic linkage to the nucleotide base, leaving just the sugar-phosphate backbone at that position. In the cell, abasic site formation occurs after a spontaneous depurination/depyrmidination event, by UV ionizing radiation, or as a Base Excision Repair (BER) intermediate (1, 2). Because such sites are fragile, they are easily susceptible to single-stranded/double-stranded breakage, and if not repaired by the BER mechanism, abasic lesions often lead to mutation by translesion synthesis during replication. The particular base incorporated opposite the lesion varies depending on organism and environmental conditions (3).

dSpacer is used as an abasic site mimic in synthetic oligonucleotides because it not only is structurally very similiar to the natural site, but it is considerably more stable, and thus can tolerate the chemical conditions used in oligo synthesis and purification (4).One or more consecutive dSpacer modifications can also be used simply to provide varying amounts of separation between different parts of an oligo sequence. **References**

1. Lindahl, T. Instability and decay of the primary structure of DNA. *Nature.* (1993), **362**: 709-715.
2. Nilsen, H., Krokan, H.E. Base excision repair in a network of defence and tolerance.*Carcinogenesis* (2001), **22**: 987-998.
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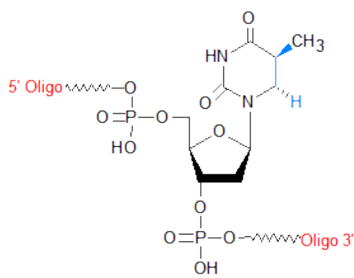
dihydro dT (5-6 DHT)

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



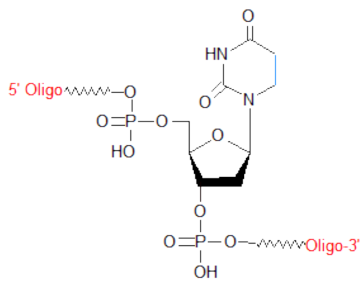
dihydro dT (5-6 DHT)
[26-6890-XX]

Dihydro dT (5,6-DHT) is primarily used in studies of irradiative DNA damage and associated repair mechanisms. In the cell, DHT DNA lesions are formed by gamma irradiation of deoxythymine under anoxic conditions, resulting in the addition of hydrogen at C5 and C6 of the thymine ring. DHT, unlike DHU (see its technical sheet), by itself appears to be neither mutagenic nor a replication block. However, when clustered with another lesion, e.g., 8-oxo-dG, there is evidence that the presence of DHT significantly enhances the mutagenicity of the other lesion (1). Because DHT is recognized and removed by endonuclease III and other eukaryotic endo III homologs, DHT-modified oligos are used in model systems for studying DNA damage and repair mechanisms. **References**

1. Shikazono, N., Pearson, C., O'Neill, P., Thacker, J. The roles of specific glycosylases in determining the mutagenic consequences of clustered DNA. *Nucleic Acids Res.* (2006), **34**: 3722-3730.

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



dihydro dU (5-6 DHU)
[26-6683-XX]

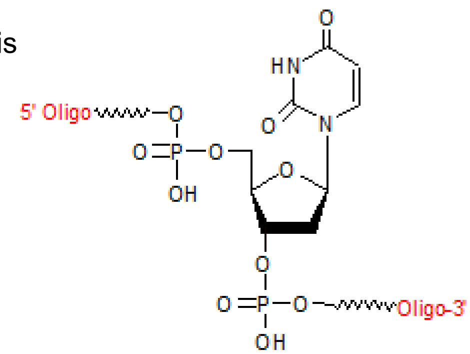
dihydro dUracil (5-6 DHdU) modification requires the use of mild reagents for synthesis and there is an additional charge of $250 charge per order.

Dihydro dU (5,6-DHU) is primarily used in studies of irradiative DNA damage and associated repair mechanisms. In the cell, 5,6-DHU DNA lesions are formed by gamma irradiation of deoxycytosine under anoxic conditions, resulting in deamination followed by addition of hydrogen at C5 and C6 of the base. DHU is highly mutagenic, leading to C-to-T transitions at the mutation site (because DNA polymerase inserts A opposite the 5,6-DHU lesion) (1). Because DHU is recognized and removed by endonuclease III and other eukaryotic endo III homologs, DHU-modified oligos are used in model systems for studying DNA damage and repair mechanisms. **References**

1. Liu, J., Doetsch, P.W. Escherichia coli RNA and DNA polymerase bypasss of dihydrouracil: mutagenic potential via transcription and replication. *Nucleic Acids Res.* (1998), **26**: 1707-1712.

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
[26-6408-XX]

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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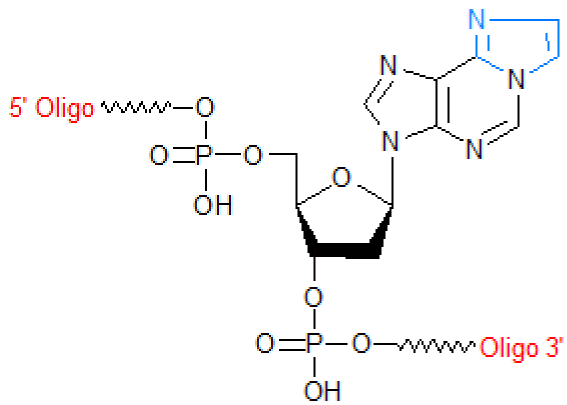
etheno dA

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



etheno deoxyadenosine dA
[26-6506-XX]

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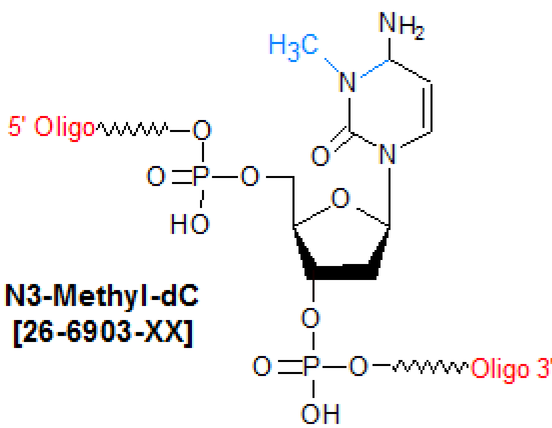
Carcinogenesis (1996), **17**: 2105-2111.

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



N3-Methyl-dC
[26-6903-XX]

N3-Methyl deoxycytosine (N3-Me-dC) is a methylated nucleoside base, and is primarily used in the study of DNA damage and repair mechanisms related to alkylation damage. N3-Me-dC lesions are highly toxic and mutagenic in all three domains of life (prokaryotes, eukaryotes, and archaea) (1).The N3-Me-dC lesion is primarily generated by SN2 alkylating reagents such as methyl methane sulfonate (MMS), dimethylsulfate and methyl halides, which react with the N3 position of cytosine (2,3). In cells, N3-methyl-dC acts as a lethal DNA replication block and is highly mutagenic, being 30% mutagenic in AlkB(-) E. coli (mostly C to T and C to A), and 70% mutagenic in E. coli that is both AlkB(-) and expresses SOS bypass enzymes (4,5). N3-Methyl-dC is restored to dC by a novel direct reversal repair mechanism. This mechanism removes the N3-methyl via oxidative demethylation catalyzed by the AlkB protein, and requiring AlkB-bound non-heme Fe(2+), molecular oxygen, and alpha-ketogluterate (6,7). **References**

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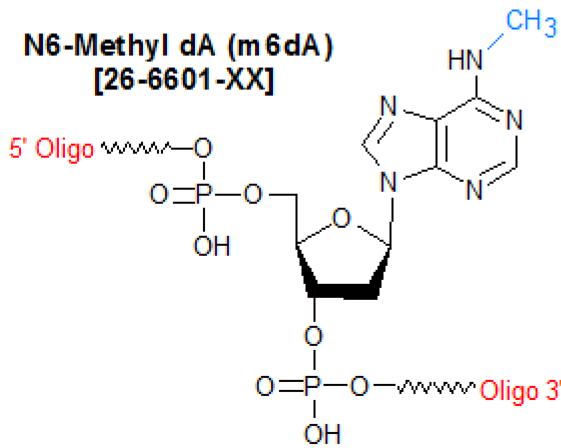
N6-Methyl dA (m6dA)

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



N6-Methyl-deoxyadenosine (N6-Me-dA) is a methylated nucleoside base that to date has only been found in bacterial and protist DNA (1). In these organisms, N6-Me-dA plays several roles, including post-replicative DNA mis-match repair, chromosome compaction and regulation of gene expression (2). Adenine methylation also is essential for either the viability or virulence of a number of pathological bacterial strains (3). Because of these properties, there is considerable interest in the bacterial enzyme N6-DNA methyltransferase (which methylates adenine) as a potential target for developing new anti-microbials (4), as well as the need to confirm whether or not this enzyme is present in mammals, including human (5). N6-Me-dA-modified oligonucleotides can serve as important research tools in such studies. **References**

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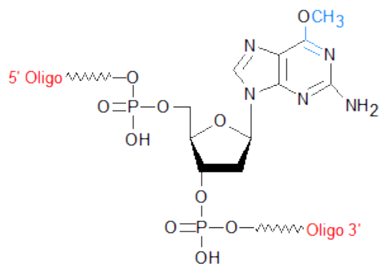
O6 Methyl dG

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]

O6-Methyl-deoxyguanosine (O6-Me-dG) is classified as an O-alkyl purine, and O6-Me-dG-modified oligonucleotides are primarily used in studies of the role of DNA alkylating agents in mutagenesis and carcinogenesis, and in studies into possible enzymatic mechanisms involved in repair of DNA alkylation damage. Both in vitro and in vivo, O6-Me-dG DNA lesions are formed by reaction with N-nitrosamines (known carcinogens), particularly those found in tobacco smoke (for example, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK)) (1,2). O6-Me-dG is highly mutagenic due to its strongly mis-pairing characteristics (DNA polymerases preferentially insert thymine opposite O6-Me-dG, leading to G-to-A transitions) (3). Generation of O6-Me-dG lesions in the K-ras proto-oncogene or p53 gene results in activation of the proto-oncogene or inactivation of p53, leading to loss of control over cell growth and cancer (4).O6-Me-dG lesions are repaired by the specialize repair protein O6-alkylguanine DNA alkyltransferase (AGT), which removes the methyl group (as well as other alkyl groups) from O6 via a "suicide" inactivation mechanism, thereby restoring the natural guanine base (5). **References**

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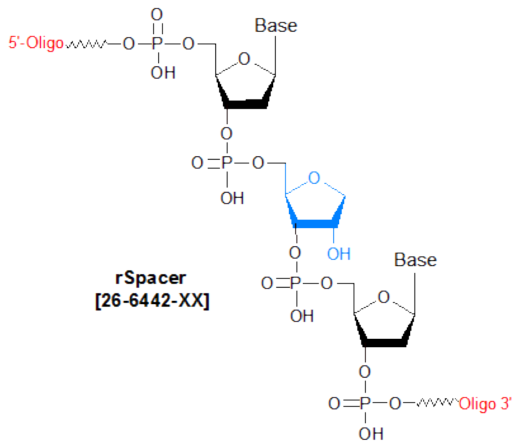
rSpacer

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



Ribo rAbasic Site (rSpacer abasic furan) RiboSpacer (rSpacer) is a tetrahydrofuran derivative, in which a methylene group occupies the 1 position of 2'-ribose. rSpacer is commonly used to mimic an abasic site in an RNA oligonucleotide. Naturally-occurring abasic sites in RNA are less common than in DNA, due to RNA being less susceptible to depurination (1). However, once generated, either spontaneously or via an enzymatic pathway, RNA abasic sites are about 15-fold more stable than DNA abasic sites; this fairly high level of stability could have important biological consequences for long-lived RNAs (for example, tRNAs or rRNA) (2). While such biological consequences have been largely unexplored thus far, abasic site effects on RNA structure and activity has been observed for the case of the hammerhead ribozyme, which catalyzes phosphodiester bond cleavage (3). Introduction of abasic sites at different positions of this ribozyme's core significantly reduced ribozyme activity. Interestingly, the activity was partially rescued for some abasic positions by exogenous addition of the missing base. rSpacer-modified oligonucleotides could serve as important research tools for elucidating the effects of abasic sites on the structure and function of long-lived RNAs and ribozymes. **References**

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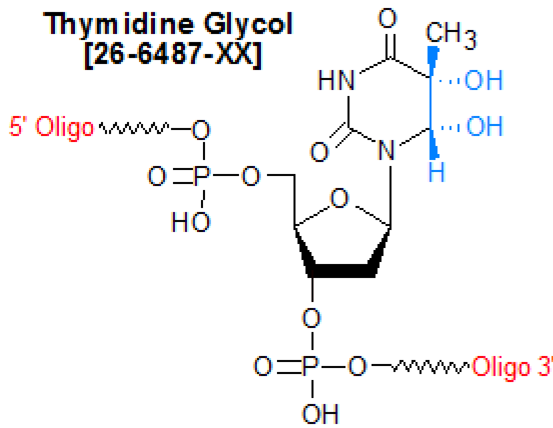
Thymidine Glycol

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



Thymidine Glycol is classified as an oxidized nucleotide, and is primarily used in studies of oxidative DNA damage and associated repair mechanisms. In the cell, thymidine glycol DNA lesions are formed when the 5,6-double bond of thymidine is oxidized by oxidative metabolic processes, ionizing radiation, or industrial chemical oxidizers like potassium permanganate and osmium tetroxide. Although it does not appear to be mutagenic, it generates more structural distortion to the double helix than any other oxidatively-damaged base. Possibly as a result of this, thymidine glycol effectively blocks DNA polymerases, resulting in stalled replication forks, making it a potentially lethal lesion. However, despite the more extensive structural distortion, thymidine glycol lesions are most commonly repaired by the BER, rather than the NER mechanism (1,2).

References

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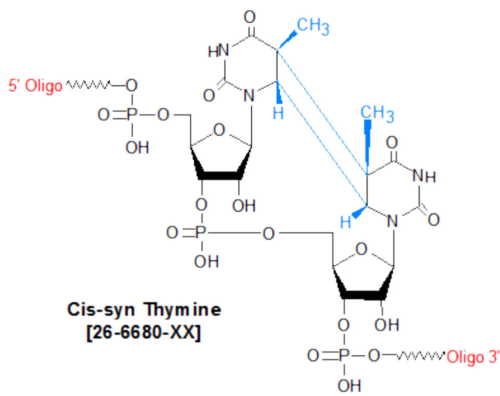
Thymine Dimer Cis-syn

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



Cis-syn thymine is classified as a cis-syn Cyclobutane Pyrimidine Dimer (CPD) of two thymine bases, and is primarily used in studies of UV-induced DNA damage and associated repair mechanisms. In the cell, cis-syn thymine dimer DNA lesions are primarily formed when two adjacent thymidine bases are irradiated by UV light (most commonly from sunlight). The result is the generation of a dimer in the form of a cyclobutane (1). This bulky adduct lesion causes large structural distortion in the double helix. While not mutagenic, they act as effective replication blocks; as such, they are potentially lethal to the cell (2). This lesion is repaired via one of two repair DNA repair mechanisms: direct reversal with the enzyme photolyase (which cleaves the dimer) (3) or by the nucleotide excision repair (NER) mechanism (4).
Oligos synthesized with cis-syn thymine dimer are stable for greater than 6 month when stored frozen, protected from light and preferably in an ethanol precipitated dried state. Reconstituted oligos should be preferably stored frozen in aliquots to avoid multiple freeze thaw cycles.

References

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