



## 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.

## Epigenetics Modifications Introduction

Epigenetics is the study of heritable traits that do not involve changes to their underlying DNA sequence. Epigenetic signals within the cell modulate particular transcriptional states (epigenetic states) necessary for the cell to "remember" previous stimulus events, including developmental signals and environmental changes (1). The molecular foundation of epigenetic signalling is the remodeling of chromatin, which occurs through a combination of (a) post-translational modification of amino acids in histone proteins, and (b) DNA methylation. The interplay between chromatin remodeling and other signaling molecules such as transcription factors and non-coding RNA controls the level and pattern of transcription from chromatin necessary to generate a given epigenetic state in the cell (2). The classic examples of epigenetic processes are cellular differentiation and maintenance of cell identity through multiple cell divisions over the lifetime of a multi-cellular organism (3).

Epigenetic signals can be divided into two main categories, cis- and trans-. Trans-epigenetic states are primarily self-propagating transcriptional states maintained via feedback loops and transcription factor (TF) networks (4). For example, if an external stimulus causes a TF to activate its own transcription, the result is a (trans) epigenetic state that becomes self-sustaining when the stimulus is removed, and continues after cell division. Small RNAs (sRNAs) also sometimes function as trans-epigenetic signals (5). Trans-epigenetic states are often found in prokaryotes and single-celled eukaryotes, where they support cellular memory.

Fig 1 : Modification : dC Analogs

# Epigenetics Modifications **Design Protocols**

## Epigenetics Modifications Applications

Gene Link provides researchers with custom synthetic DNA or RNA oligonucleotides specifically designed, based on customer specifications, for use as research tools for in vitro or in vivo studies into the molecular basis of epigenetic phenomenon. Specifically:

(1) 5-methyl-dC-modified oligos. For DNA methylation studies, synthetic oligos with 5-methyl-dC substituted for dC at different CpG sites in promoters or enhancers can be used to ascertain the base positional effect of 5-methyl-dC on transcription.

(2) 5-hm-dC modified oligos. The role of DNA de-methylation in epigenesis is still not well understood. It is speculated that 5-hm-dC may play an important role as an intermediate in the conversion of 5-methyl-dC back to dC (20). TET1 is known to convert 5-methyl-dC to 5-hm-dC, but as yet there is no known cellular enzymatic pathway for subsequent conversion of 5-hm-dC to dC. Synthetic oligos modified with 5-hm-dC can be used as DNA templates to test possible enzyme pathways for such a conversion.

(4) Chromatin re-modeling studies. Either kind of methylated-dC oligo can be used in conjunction with histone proteins modified at various amino acid positions in biophysical studies to ascertain the nature of the binding interactions between modified histones and methylated DNA, and the effect of these interactions on transcription.

(3) sRNA and ncRNA oligos. sRNA and ncRNA oligonucleotides may play important roles in the establishment of cis-epigenetic states, particularly as bridging molecules capable of directing chromatin modifiers to target sites on a genome, either in a global or local manner. Gene Link is a leader in the synthesis of RNA oligos of unparalleled purity for use in specialized applications, including epigenetic studies. If the customer requires it, sRNA and ncRNA oligos made by Gene Link can be modified to enhance their stability for in vivo work.

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18. Penn, N.W. 'Modification of Brain Deoxyribonucleic Acid Base Content with Maturation in Normal and Malnourished Rats'. *Biochem. J.* (1976), 155: 709-712.
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## Modification Code List

<b>Modification</b>	<b>Code</b>	<b>Catalog Number</b>
5-Carboxy dC	[5-ca-dC]	26-6891
5-formyl dC	[5-for-dC]	26-6910
5-hm dC (5-Hydroxymethyl-dC)	[5hmdC]	26-6707
5-Hydroxymethyl-dU	[5hmdU]	26-6898
5-methyl deoxycytosine [5mdC]	[5mdC]	26-6413
5-methyl-Cytosine [5mrC]	[5mrC]	27-6945
N6-Methyl dA (m6dA)	[m6dA]	26-6601
N6-Methyl rA (m6A)	[m6A]	27-6601
rZebularine	[rZ]	27-6435
Zebularine- deoxy-5 methyl	[dZ-5me]	26-6547



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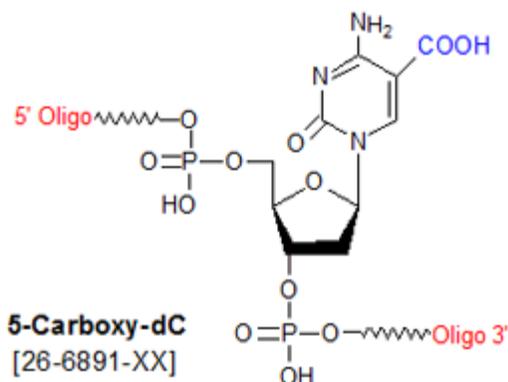
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### 5-ca dC

Category	Epigenetics
Modification Code	5-ca-dC
Reference Catalog Number	26-6891
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	333.19



5-carboxy deoxycytosine (5-carboxy-dC) was first synthesized in 2007 by Sumino et al (1) as an anion carrier that, when incorporated into oligonucleotides, would result in increased duplex and triplex stabilities. Initial studies with 5-carboxy-dC modified oligos 13mer and 14mer in length showed that this modified base does indeed increase duplex stability over the corresponding unmodified oligos, but forms relatively unstable triplexes with their target dsDNA (1). 5-carboxy-dC thus potentially could serve as a duplex stabilizing moiety for oligonucleotides slated for any application requiring formation of duplexes with higher  $T_m$ .

However, current research interest in 5-carboxy-dC is focused on its potential role 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 (2), and also in certain autoimmune disorders and cancer (3). Recent observations of the presence of 5-hydroxymethyl-dC (5hm-dC) in a variety of tissues, most notably neuronal cells (4,5), and the discovery of an enzymatic pathway for conversion of 5-Me-dC to 5hm-dC, mediated by the enzyme Tet1 (6), has spurred efforts to determine whether or not 5-hm-dC is then subsequently converted to dC through a 5-carboxy-dC intermediate. In 2011, Ito and co-workers showed that Tet enzymes are able to convert 5hm-dC to 5-carboxyl-dC, and also observed the presence of 5-carboxy-dC in mouse embryonic stem cells and various mouse organ tissues. Genomic content of both 5hm-dC and 5-carboxy-dC can be modulated through overexpression or depletion of Tet proteins in these tissues (7). These experiments provide strong supporting evidence for DNA demethylation occurring via a Tet-mediated enzymatic pathway involving 5-carboxy-dC as a key intermediate. 5-carboxy-dC modified oligos can serve as important research tools for probing the DNA demethylation process.

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7. Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C., Zhang, Y. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science* (2011), **333**: 1300-1303.



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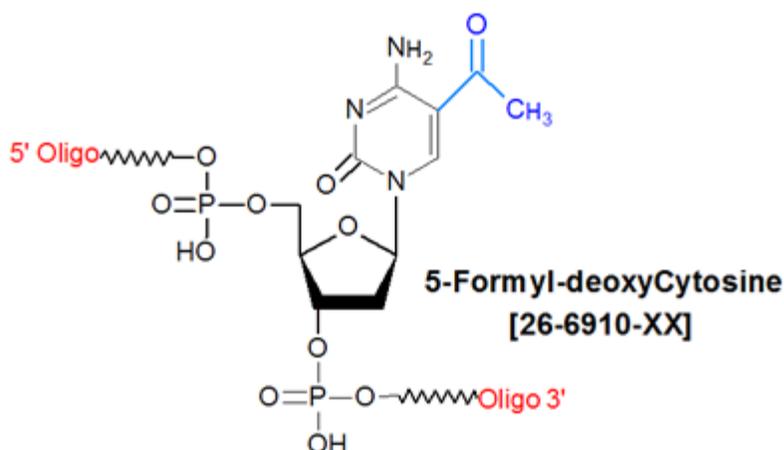
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### 5-formyl dC

Category	Epigenetics
Modification Code	5-for-dC
Reference Catalog Number	26-6910
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	317.19



5-formyl deoxycytosine (5-f-dC) pairs with dG, but also is capable of mispairing with both dA and dT; in the latter case, at a level that is 3-4 times higher than either unmodified dC or 5-Me-dC (1). Consequently, 5-formyl-dC is highly mutagenic, capable of driving both C-to-T transitions and C-to-A transversions (1). However, current research interest in 5-formyl-dC is focused, not on its mutagenic properties, but rather on its potential role 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 (2), and also in certain autoimmune disorders and cancer (3). Recent observations of the presence of 5-hydroxymethyl-dC (5hm-dC) in a variety of tissues, most notably neuronal cells (4,5), and the discovery of an enzymatic pathway for conversion of 5-Me-dC to 5hm-dC, mediated by the enzyme Tet1 (6), has spurred efforts to determine whether or not 5-hm-dC is then subsequently converted to dC through a 5-formyl-dC intermediate. In 2011, Ito and co-workers showed that Tet enzymes are able to convert 5hm-dC to 5-formyl-dC, and also observed the presence of 5-formyl-dC in mouse embryonic stem cells and various mouse organ tissues. Genomic content of both 5hm-dC and 5-formyl-dC can be modulated through overexpression or depletion of Tet proteins in these tissues (7). These experiments provide strong supporting evidence for DNA demethylation occurring via a Tet-mediated enzymatic pathway involving 5-formyl-dC as a key intermediate. 5-formyl-dC modified oligos can serve as important research tools for probing the DNA demethylation process. **References**

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3. Richardson, B.C. Role of DNA methylation in the regulation of cell function: autoimmunity, aging and cancer. *J. Nutr.* (2002), **132(8 Suppl)**: 2401S-2405S.
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7. Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C., Zhang, Y. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science* (2011), **333**: 1300-1303.



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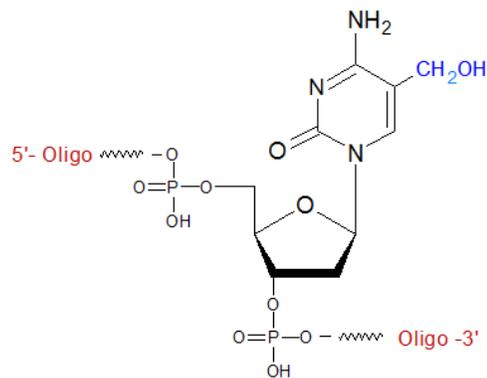
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### 5-hm dC

Category	Epigenetics
Modification Code	5hmdC
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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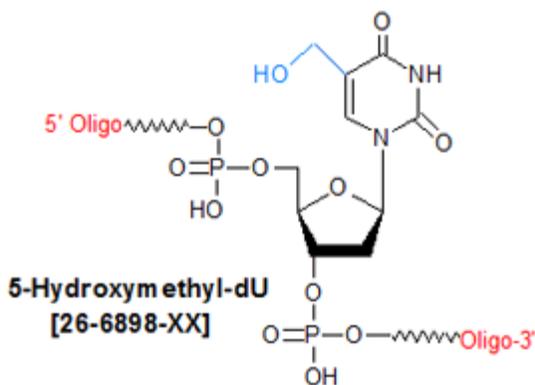
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### 5-hm dU

Category	Epigenetics
Modification Code	5hmdU
Reference Catalog Number	26-6898
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.19





# Product Specifications

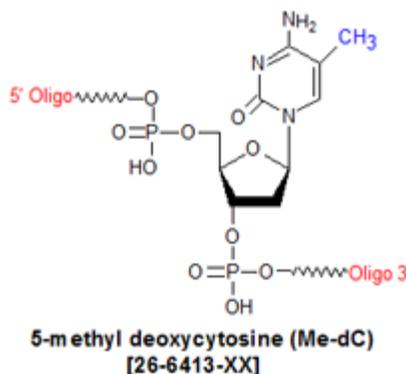
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### 5-Me dC

Category	Epigenetics
Modification Code	5mdC
Reference Catalog Number	26-6413
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	303.21



5-methyl deoxycytosine (5-Me-dC) pairs with dG, and when substituted for dC in an oligonucleotide, increases the stability of the resulting duplex relative to the comparable unmodified form, raising the  $T_m$  by 1.3degC per 5-Me-dC residue added (1,2). 5-Me-dC thus can be used to **improve the ability of an oligo to hybridize to its target**. The presence of the hydrophobic 5-methyl group presumably acts to exclude water molecules from the duplex.  
Modifications Increasing Duplex Stability and Nuclease Resistance

#### Modification

#### Duplex Stability [ $T_m$ Increase]

Nuclease Resistance Locked Analog Bases Increased [2- 4C per substitution] Increased 2-Amino-dA Increased [3.0C per substitution] Similar to DNA C-5 propynyl-C Increased [2.8C per substitution] Increased C-5 propynyl-U Increased [1.7C per substitution] Increased 2'-Fluoro Increased [1.8C per substitution] Increased 5-Methyl-dC Increased [1.3C per substitution] Similar to DNA 2'-O Methyl Increased Increased Phosphorothioate Slightly decreased Increased [Click here for complete list of duplex stability modifications](#)

5-Me-dC is particularly useful in the following applications:

(a)Strong-binding PCR primers: 5-Me-dC-modified PCR primers have been shown to prime far better than their unmodified counterparts in PCR reactions, consistently yielding more product per cycle, permitting amplification at very high annealing temperatures (as high as 72degC), and interestingly, allowing excellent priming from within palindromic sequences (1). The improvement in priming efficiency could significantly reduce the number of amplification-related mutations in PCR products.

5-Me-dC primers also could be useful in several PCR applications, *e.g.*, when short, specific primers are required, when only a limited quantity of template is available (*e.g.* ancient DNA), when DNA secondary structure in the primer binding site prevents binding of an unmodified primer, or when primer extension is blocked by downstream DNA secondary structure in the template.

(b) **Anti-sense:** Anti-sense oligonucleotides containing a CpG motif induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9). Substitution of 5-Me-dC for dC in these motifs can prevent or sharply reduce these undesirable immune responses (3).

(b) **DNA methylation studies:** Methylation of dC to 5-methyl-dC, when it occurs in CpG sites near promoters is associated with gene silencing, and is an important epigenetic mechanism in living organisms. Oligonucleotides incorporating 5-Me-dC have been used by a number of research groups as research tools to study the epigenetic effects of DNA methylation in such areas as tumorigenesis and the effects of cocaine on fetal heart development (4-6). **References**

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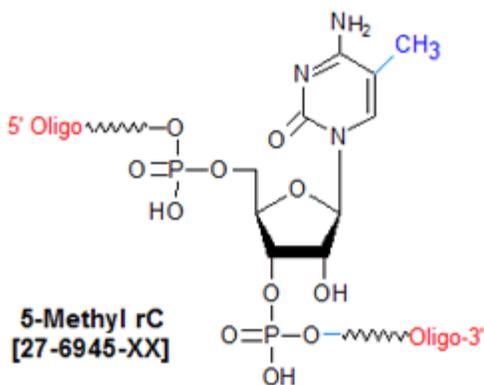
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

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

### 5-Me rC [5mrC]

Category	Epigenetics
Modification Code	5mrC
Reference Catalog Number	27-6945
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	319.21



5-methyl cytosine (5-Me-rC) is a modified ribonucleotide which pairs with rG in an RNA duplex. 5-Me-rC forms a Watson-Crick base pair with rG in a normal manner. The presence of 5-Me-rC in cellular RNA is widespread, but its function is not well understood. 5-Me-rC has been observed in several base positions of eukaryotic and archaeal tRNA, most notably at positions 48/49, at the junction between the variable region and TphiC stem (1), suggesting an important structural role for it. The location of 5-Me-rC in rRNA from many organisms (bacterial to human) also appears to be fairly well-conserved, again hinting at an important structural role (2). Archaeal rRNA is an exception, however, as the number and location of 5-Me-rC is highly variable, complicating the picture (3,4). 5-Me-rRNA is found in the 5'-cap structure of mRNA, as well as in tRNA-like structures within other RNA molecules, such as viral RNA and SINE elements (5).

While there is a recognition that 5-methyl-rC plays a structural role in stabilizing tRNA, and appropriate binding of Mg<sup>2+</sup> ions to it (6), little is known about how this modification's presence within tRNA and rRNA affects mRNA translation within the ribosome (2). Some evidence exists which suggests that the presence of 5-Me-rC, at least in yeast tRNA, is required to minimize translation errors, this is not definitive (7). In rRNA, its presence may assist with both tRNA recognition and peptidyl transfer (8).

One intriguing functional possibility for RNA methylation via 5-Me-rC is as a modulator of the innate human immune system. In one study, while a set of unmodified RNA strongly stimulated this system via Toll-like receptor activation, incorporation of 5-methyl-C into the oligos of this set dramatically reduced their stimulatory effect (9). These observations suggest that methylation interferes with the ability of the innate immune system to recognize RNA. Use of this principle may have therapeutic implications for a number of immune-system-related disorders.

The observation of RNA-dependent inheritance of certain phenotypes in mouse hints at a second possibility for RNA methylation: as a regulator of epigenetic inheritance patterns (10). The recent discovery that 5-Me-rC is widespread in both the coding and non-coding mRNA (esp. in the UTRs) of the human transcriptome supports this, and suggests that RNA methylation may play a much broader role in post-transcriptional control of cellular RNA than was previously believed (11), raising the possibility that RNA methylation may be critical to the ability of the cell to support various states of growth and differentiation.

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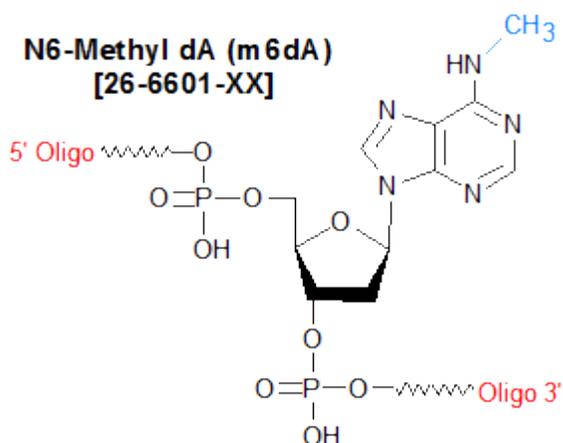
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## 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

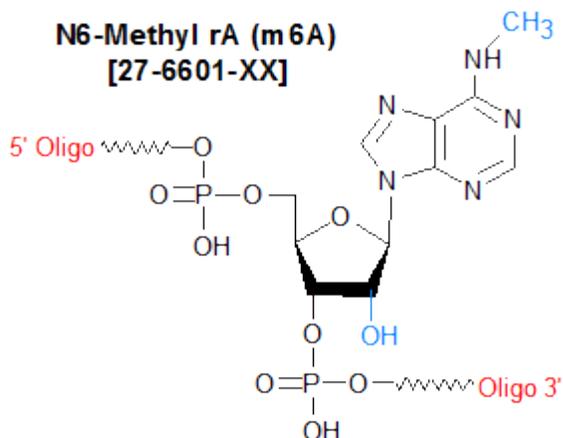
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## Oligo Modifications

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

### N6-Methyl rA (m6A)

Category	Epigenetics
Modification Code	m6A
Reference Catalog Number	27-6601
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.23



N6-methyl-riboadenosine (N6-methyl rA; m6A) is a common, fairly abundant RNA modification found in the mRNA of most eukaryotes (1,2); it has also been observed in tRNA, rRNA snRNA and in long non-coding RNA (3). While the biological importance of this modification remains poorly understood, results from a number of research studies suggest that regulation of m6A levels in mRNA may have significant effects on subsequent gene expression. The modification mainly appears in exons, 3'-UTRs and near stop codons. Within 3'-UTRs, N6-methyl-rA is associated with miRNA binding sites (4). The modification itself is catalyzed by a N6-methyl-rA methyltransferase complex that contains the METTL3 subunit (5). Silencing this methyltransferase dramatically affects N6-methyl-A cellular levels, gene expression and alternative RNA splicing patterns (6). The FTO and ALKBH5 genes, implicated in obesity risk, encode two different N6-methyl-rA demethylases; silencing of FTO with siRNA results in increased levels of N6-methyl-rA in poly(A) RNA (6), while FTO overexpression results in decreased levels (4). Moreover, modulation of the activities of these three enzymes can alter the expression of thousands of genes at the cellular level. This suggests that N6-methyl-rA plays an important role in RNA metabolism and as an epigenetic marker (7). **References**

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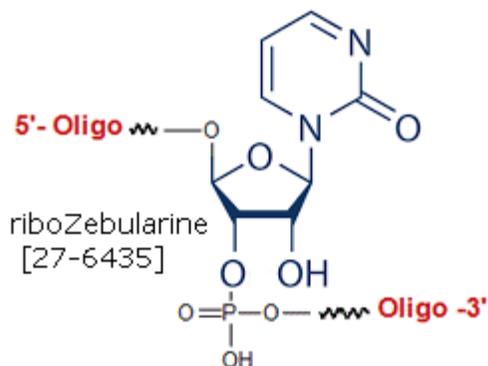
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## Oligo Modifications

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

### Zebularine ribo

Category	Structural Studies
Modification Code	rZ
Reference Catalog Number	27-6435
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17





## 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.

### Zebularine- deoxy-5 methyl

Category	Structural Studies
Modification Code	dZ-5me
Reference Catalog Number	26-6547
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	288.19

