Structural Studies Introduction

Investigational studies into nucleic acid structure, and protein-nucleic acid structure interactions and structure-activity relationships require oligonucleotides in which a variety of different modifications, or combinations of modifications, have been incorporated. The specific type, or types, of modifications required is highly dependent on the hypothesis being tested in the study. Gene Link has extensive experience synthesizing complex oligos for use in structural studies, and can provide the customer with knowledgeable technical support in designing oligos with appropriate modifications in this area.

Structural Studies Design Protocols

Oligos for Structural Studies—Design Considerations

Nucleic acid structural studies cover a variety of topics, and typically require both modified and unmodified oligonucleotides for use as research tools in the particular study under question. The specific type, or types, of modifications required is highly dependent on the hypothesis being tested in the study. 1. Structure-Activity Relationships

Modifications such as 7-deaza-dG, 7-deaza-8-aza-dG, etc.) are used to investigate the effect on the activity of an oligonucleotide by an enzyme (e.g., polymerases, reverse transcriptases, restriction enzymes, etc.) when key structural elements are changed. These modifications typically have properties (hydrogen bonding, base-stacking, etc) that are significantly different from those of the standard nucleotide bases, which can be used to probe structure-activity relationships between the oligo and an enzyme. 2. Crystallography/Cross-Linking

Halogenated nucleotides (brominated, iodinated) are useful both in crystallography studies of oligonucleotide structure and in cross-linking studies to probe protein-nucleic acid complex structure. 3. Fluorescent Nucleosides

Etheno-dA and Pyrrolo-dC are two modifications with fluorescent properties that make them useful as probes into DNA structure and dynamics. Etheno-dA is often used to observe transitions between DNA structural types. Pyrrolo-dC base pairs with G like the standard dC nucleotide, and its small size does not disturb the double-helix. It has markedly different fluorescent levels in single-stranded vs. double-stranded DNA, so it is particularly useful for studying regions of local duplex melting.

Structural Studies Applications

Halogenated bases, cross-linkers, and intercalators all can be used as modifications in oligos slated for use in nucleic acid and protein-nucleic acid structural studies. Details for these types of modifications are provided in the introduction and applications sections of the relevant modification category. Several other modifications can also be used in structural work. For example, selective incorporation of etheno-dA, a highly fluorescent derivative of dA, into DNA or RNA oligos can be particularly useful in structure-function studies of RNA, RNA-protein complexes, and the mechanism of base excision repair (BER) of alkylated DNA damage (1,2). Also, since exocyclic etheno dA adducts likely play an important role in carcinogenesis, etheno-dA-modified oligos can be used as research tools for the study of carcinogenesis in various tissues (3). Another example is N4-Et-dC-modified oligos, which have been used in structure-function studies of better understand how CpG-containing oligos stimulate the innate immune system, and which structural elements in cytosine and guanine bases are required for recognition of, and interaction with, protein/receptor factors responsible for immunostimulation (4).

References

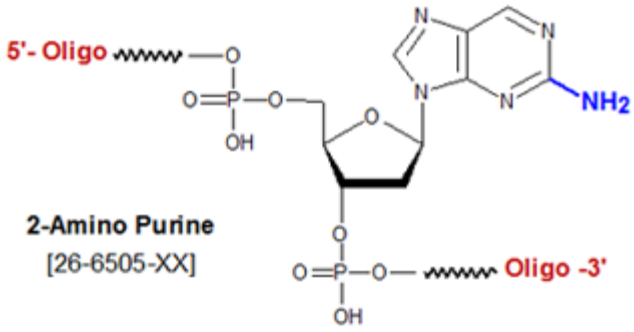
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(3) Chung, F-L., Chen, H-J.C., Nath, R.G. Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. Carcinogenesis (1996), 17: 2105-2111.
(4) Kandimalla, E.R., Yu, D., Zhao, Q., Agrawal, S. Effect of chemical modifications of cytosine and guanine in a CpG-motif of oligonucleotides: structure-immunostimulatory activity relationships. Bioorg. Med. Chem. (2001), 9: 807-813.

Modificaton Code List

Modification	Code	Catalog Number
2-Amino Purine deoxyribose	[2-AP]	26-6505
5-bromo dC (5-Br dC)	[5-Br-dC]	26-6411
5-bromo dU (5-Br-dU)	[5-Br-dU]	26-6412
5-Fluoro deoxyuridine dU	[5-F-dU]	26-6416
5-Iodo deoxycytosine dC	[5-I dC]	26-6414
5-iodo deoxyuridine dU	[5-I-dU]	26-6415
8-methyl-dG	[8-me-dG]	26-6466
etheno dexoyadenosine dA	[Eth-dA]	26-6506
N4-Ethyl dC [N4-Et-dC]	[N4-Et-dC]	26-6685
pseudoUridine-2'deoxy (psi-dU)	[psi-dU]	26-6531
pseudoUridine ribo (psi rU)	[psi-rU]	27-6531
5'-Pyrene Cap (5')	[Pyr-Cap]	26-6949
Pyrrolidine (Pyr)	[Pyr]	26-6465
Pyrrolo-dC	[Pyr-dC]	26-6892
rZebularine	[rZ]	27-6435
Zebularine- deoxy-5 methyl	[dZ-5me]	26-6547

2-Amino Purine

Category	Others
Modification Code	2-AP
Reference Catalog Number	26-6505
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	313.21

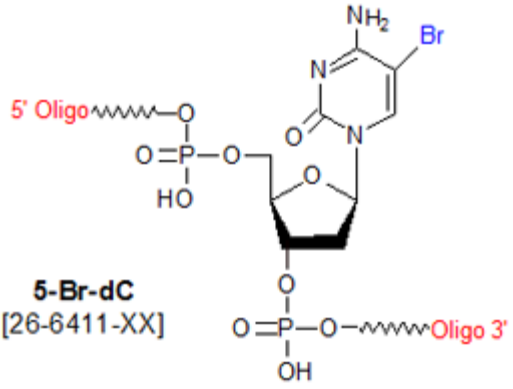


Oligo Modifications

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

5-Br dC

Category	Minor Bases
Modification Code	5-Br-dC
Reference Catalog Number	26-6411
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	368.08



5-Bromo deoxycytosine (5-Br-dC) is classified as a halogenated nucleotide, and is primarily used to facilitate the determination of DNA structure by X-ray crystallography (1). When incorporated into a DNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

Halogenated nucleotides are also photo-labile, and can be used in UV-crosslinking experiments to investigate the structure of protein-DNA complexes. For example, incorporation of 5-Br-dC (and 5-Br-dG) into a 22-base dC-dG oligo resulted in the oligo being able to readily flip into the Z-DNA conformation in 10 mM MgCl2. This oligo was used as a probe to detect Z-DNA binding proteins (3).

An intriguing use of 5-Br-dC is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (4). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC nucleotides is then replaced by photo-labile 5-Br-dC to generate the corresponding photo-aptamer. Because substitution of bromine for methyl at the 5-position of the base does not significantly change the steric properties of the oligo, the photo-aptamer typically has nearly the same binding affinity for the target as that of the (non-photo-reactive) original. **References**

1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
3. Herbert, A.G.; Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. *Nucleic Acids Res.* (1993), **21**: 2669-2672.
4. Schneider, D.J.; Wilcox, S.K.; Zichi, D.; Nieuwlandt, D.; Carter, J.; Gold, L. Improved SELEX and Photo-SELEX.

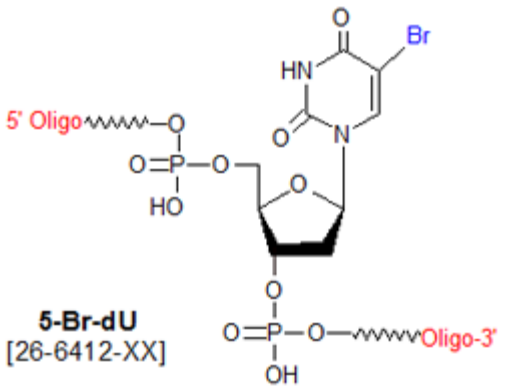
(2008), PCT/US2008/070371 (WO/2009/012410).

Oligo Modifications

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

5-Br dU

Category	Minor Bases
Modification Code	5-Br-dU
Reference Catalog Number	26-6412
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	369.07



5-Bromo deoxyuridine (5-Br-dU) is classified as a halogenated nucleotide, and is primarily used to facilitate the determination of DNA structure by X-ray crystallography (1). When incorporated into a DNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

Halogenated nucleotides are also photo-labile, and can be used in UV-crosslinking experiments to investigate the structure of protein-DNA complexes. For example, substitution of 5-Br-dU for thymine into a 25-bp DNA duplex containing the EcoK1 restriction site AAC(N6) enabled UV-crosslinking of the duplex to the Specifity (S) sub-unit of the EcoK1 enzyme. The observation of crosslinking only between the 5-Br-dU complementary to the first adenine in the restriction site demonstrated close contact between the major groove at this sequence and the S subunit (3). In another structural study, single-stranded oligonucleotides in which 5-Br-dU was substituted for thymine at several positions was used to characterize the binding of Nuclear Factor BA1 with DNA (4).

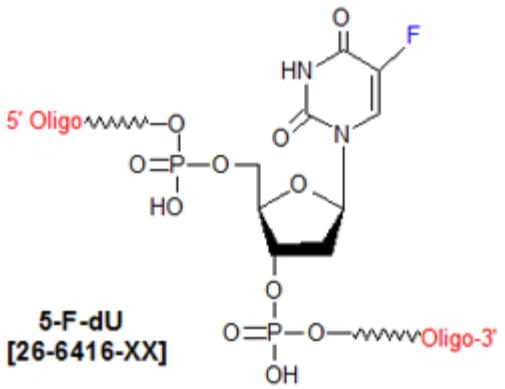
5-Br-dU can also be used in conjugation with the photo-SELEX technique to generate photo-aptamers capable of cross-linking to their target (5). For example, photo-aptamers selected from a candidate nucleic acid mixture containing 5-Br-dU instead of thymine could subsequently be optimized by retaining only those 5-Br-dU capable of being photo-crosslinked to the target, replacing the rest with thymine. **References**

1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
3. Chen, A.; Powell, L.M.; Dryden, D.T.F.; Murray, N.E.; Brown, T. Tyrosine 27 of the specificity polypeptide of EcoK1 can be UV crosslinked to a bromodeoxyuridine-substituted DNA target sequence.

Nucleic Acids Res. (1995), **23**: 1177-1183.
4. Kardassis, D.; Zannis, V.I.; Cladaras, C. Purification and Characterization of the Nuclear Factor BA1. *J. Biol. Chem..* (1990), **265**: 21733-21740.
5. Gold, L.; Zichi, D.; Wilcox, S.K.; Schneider, D.J.; Nieuwlandt, D.; Carter, J. SELEX and PHOTOSELEX. (2009), (US2009/0098549).

5-F dU

Category	Duplex Stability
Modification Code	5-F-dU
Reference Catalog Number	26-6416
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	308.16



5-Fluoro deoxyuridine (5-F-dU) is classified as a halogenated nucleotide. 5-F-dU is able to pair with both A and G purines, and A:(5-F-dU)and G:(5-F-dU) base pairs are more stable than A:T and G:T (mismatch) base pairs, respectively. This property has been used to synthesize single, unique hybridization probes for use in cDNA library screening. Such individual probes are more selective for particular gene sequences, particularly low abundance sequences, than sets of mixed hybridization probes, which use often leads to spurious hybridization (1).

5-F-dU can be incorporated into oligos as labels to enable probing of DNA/RNA secondary structure by 19F NMR (2). Oligos in which 5-F-dU was substituted for T have also been used to probe the structure of T-CG inversions in anti-parallel triple helices (3). In that study, 5-F-dU was found to have higher binding affinity for the CG base pair than thymine, and much higher affinity than other halogenated derivatives. Thus, 5-F-dU may have the potential to enhance the ability of a triple-helix forming oligo (TFO) to recognize this motif within a target DNA or RNA molecule (4).

Because the dipole moment of the C-F bond in 5-F-dU is similar to that of the C-Br bond in both 5-Br-dU and 5-Br-dC, 5-F-dU can function as a non-photoreactive “polarity placeholder” during conversion of a SELEX-identified aptamer into a photo-aptamer (5). For example, aptamers selected from a candidate nucleic acid mixture containing 5-F-dU instead of thymine could subsequently be optimized post-SELEX by replacing 5-F-dU with either 5-Br-dU or 5-Br-dC, both of which are highly photo-reactive. The similarity in the relevant dipole moments of these halogenated nucleotides helps ensure that the binding affinity of the post-SELEX-optimized photo-aptamer for its target is the same, or nearly the same, as that of the original aptamer. **References**

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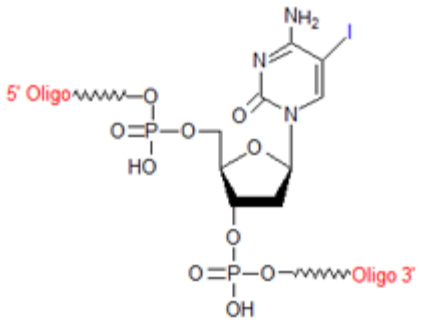
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Oligo Modifications

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5-I dC

Category	Minor Bases
Modification Code	5-I dC
Reference Catalog Number	26-6414
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	415.08



5-Iodo-deoxyCytosine dC
[26-6414-XX]

5-Iodo deoxycytosine (5-I-dC) is classified as a halogenated nucleotide, and is primarily used to facilitate the determination of DNA structure by X-ray crystallography (1). When incorporated into a DNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

Halogenated nucleotides are also photo-labile, and can be used in UV-crosslinking experiments to investigate the structure of protein-DNA complexes. For example, 5-I-dC (or 5-I-dU) was incorporated into a set of 14-base oligos for cross-linking studies of these oligo sets with the Ku protein, a DNA repair protein that binds to broken DNA ends and thus triggers a double-strand DNA break repair pathway (3). The researchers in this case took advantage of the fact that iodopyrimidines cross-link with amino acid residues in close contact with the C5 position of thymine or cytosine in the major groove of DNA (4).

An intriguing use of 5-I-dC is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (5). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC nucleotides is then replaced by photo-labile 5-I-dC to generate the corresponding photo-aptamer. Because substitution of iodine for methyl at the 5-position of the base does not significantly change the steric properties of the oligo, the photo-aptamer typically has nearly the same binding affinity for the target as that of the (non-photo-reactive) original. **References**

1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
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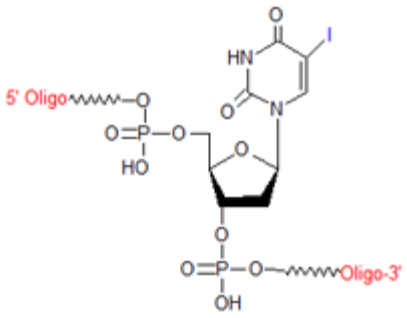
Ku Bound at a Single DNA End.*J. Biol. Chem.* (1999), **274**: 20034-20039.
4. Meisenheimer, K.M.; Koch, T.H. Photocross-linking of nucleic acids to associated proteins.*Crit. Rev. Biochem. Mol. Biol.* (1997), **32**: 101-140.
5. Schneider, D.J.; Wilcox, S.K.; Zichi, D.; Nieuwlandt, D.; Carter, J.; Gold, L. Improved SELEX and Photo-SELEX. (2008), PCT/US2008/070371 (WO/2009/012410).

Oligo Modifications

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5-I dU

Category	Minor Bases
Modification Code	5-I-dU
Reference Catalog Number	26-6415
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	416.07



5-iodo-deoxyuridine dU
[26-6415-XX]

5-Iodo deoxyuridine (5-I-dU) is classified as a halogenated nucleotide, and is primarily used to facilitate the determination of DNA structure by X-ray crystallography (1). When incorporated into a DNA molecule, the multi-wavelength anomalous dispersion (MAD) technique can be applied to obtain the phase information necessary to correctly calculate the electron density for the unit cell of the molecule under study. Because the MAD technique allows for the measurement of all the diffraction data with the same sample, is a much simpler to use than the traditional multiple isomorphous replacement (MIR) method for phase determination, which requires the synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (2).

Halogenated nucleotides are also photo-labile, and can be used in UV-crosslinking experiments to investigate the structure of protein-DNA complexes. For example, 5-I-dU (or 5-I-dC) was incorporated into a set of 14-base oligos for cross-linking studies of these oligo sets with the Ku protein, a DNA repair protein that binds to broken DNA ends and thus triggers a double-strand DNA break repair pathway (3). The researchers in this case took advantage of the fact that iodopyrimidines cross-link with amino acid residues in close contact with the C5 position of thymine or cytosine in the major groove of DNA (4).

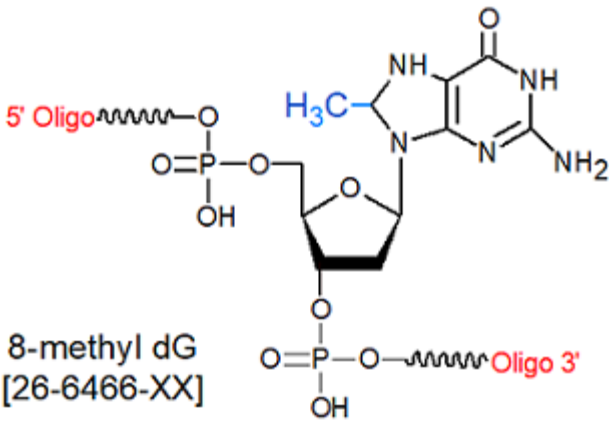
An intriguing use of 5-I-dU is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (5). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC nucleotides is then replaced by photo-labile 5-I-dU to generate the corresponding photo-aptamer. Because substitution of iodine for methyl at the 5-position of the base does not significantly change the steric properties of the oligo, the photo-aptamer typically has nearly the same binding affinity for the target as that of the (non-photo-reactive) original. **References**

1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
3. Yoo, S.; Kimzey, A.; Dynan, W.S. Photocross-linking of an Oriented DNA Repair Complex.

Ku Bound at a Single DNA End.*J. Biol. Chem.* (1999), **274**: 20034-20039.
4. Meisenheimer, K.M.; Koch, T.H. Photocross-linking of nucleic acids to associated proteins.*Crit. Rev. Biochem. Mol. Biol.* (1997), **32**: 101-140.
5. Schneider, D.J.; Wilcox, S.K.; Zichi, D.; Nieuwlandt, D.; Carter, J.; Gold, L. Improved SELEX and Photo-SELEX. (2008), PCT/US2008/070371 (WO/2009/012410).

8-methyl-dG

Category	Duplex Stability
Modification Code	8-me-dG
Reference Catalog Number	26-6466
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	343.21



The structural plasticity of DNA allows it to switch from its canonical right-handed B-form into non-canonical alternative arrangements such as left-handed Z-DNA and multi-stranded G-quadruplexes. Modifying specific bases within synthetic oligonucleotides is a powerful approach for rationally engineering and controlling these spatial shifts.
8-Methyl-2'-deoxyguanosine (8-Me-dG) is a crucial tool in this field. Replacing the hydrogen atom at the C8 position of guanine with a bulky methyl group introduces a sterile clash with the sugar ring. This structural constraint shifts the glycosidic torsion angle away from the anti conformation and locks the base into a preferred syn glycosidic conformation.

1. Z-DNA Conformation Stabilization and B-Z Transitions

Right-handed B-DNA requires nucleosides to adopt an anti conformation, whereas left-handed Z-DNA alternates between anti and syn orientations. Because 8-Me-dG is pre-organized into the syn conformation, its incorporation drastically reduces the energetic threshold needed for B-to-Z DNA transitions. [1, 2] • Low Salt Transition: Unmodified oligonucleotides require unphysiologically high salt environments or chemical additives to form Z-DNA. Oligonucleotides synthesized with 8-Me-dG can form stable Z-DNA under physiological salt conditions, or even in the absence of salt. [1, 2, 3] • Positional Dependency: Circular Dichroism (CD) studies have shown that position matters. Placing the modification toward the center of alternating purine-pyrimidine sequences maximizes Z-form stability. This application provides researchers with robust tools to evaluate the molecular pathways of Z-DNA-specific binding proteins like ADAR1. [1]

2. Rational Structural Tuning of G-Quadruplexes (G4)

G-quadruplexes are four-stranded nucleic acid structures formed by guanine-rich regions layered into G-tetrads. The overall topology of a quadruplex (parallel, antiparallel, or hybrid) relies strictly on the precise coordination of syn and anti orientations across its guanosine strands. [1, 2] • Topology Stabilization: Substituting standard dG with 8-Me-dG at specific syn positions significantly stabilizes alternative topologies. For example, in the Thrombin Binding Aptamer (TBA), targeted replacement improves structural stability and enhances thrombin inhibitory activity Accelerating Quadruplex Assembly: Incorporating 8-Me-dG at the 5'-end of tetramolecular parallel G-quadruplex sequences accelerates folding kinetics by 15-fold or more.

This rapid assembly happens because the modification correctly pre-aligns the required syn conformations, which minimizes entropic penalties during structural initiation.

3. Enhancement of Aptamers and Nucleic Acid Therapeutics.

For therapeutic oligonucleotides like Antisense Oligonucleotides (ASOs), siRNAs, and aptamers to remain active in vivo, they must resist biochemical breakdown. [1] • Nuclease Resistance: The bulky C8-methyl group physically shelters the adjacent phosphodiester backbone from cellular nucleases without disrupting essential Watson-Crick pairing capabilities. This modification enhances metabolic stability and prolongs serum half-life. • Conformational Control: By pre-arranging active configurations, 8-Me-dG aids in developing highly target-specific aptamers that remain rigid and bound to target proteins under physiological conditions. [1, 2]

4. Probing Biomarker Damage and Repair Pathways

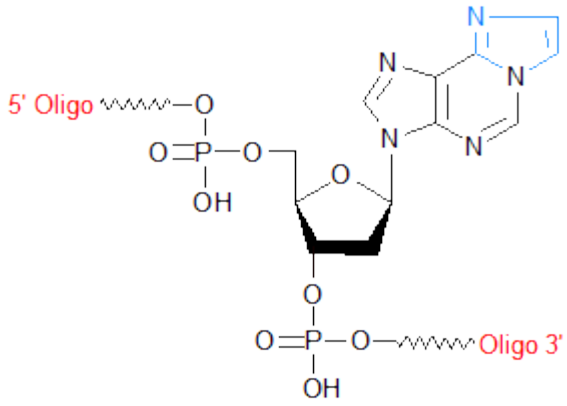
Endogenous cellular stress and environmental alkylating toxins naturally modify purines at the C8 position. [1] • Synthetic Lesion Model: Oligonucleotides synthesized with 8-Me-dG act as defined controls to simulate structural cellular damage. This allows researchers to track how error-prone DNA polymerases bypass lesions during replication, as well as how base excision repair (BER) systems identify and replace damaged nucleotides.

References

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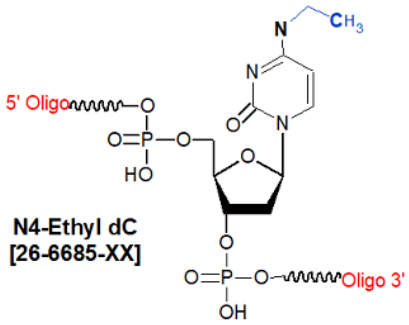
Carcinogenesis (1996), **17**: 2105-2111.

Oligo Modifications

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

N4-Ethyl dC [N4-Et-dC]

Category	Duplex Stability
Modification Code	N4-Et-dC
Reference Catalog Number	26-6685
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	317.42



N4-Ethyl-deoxycytidine (N4-Et-dC) is typically used to minimize the deleterious effect of large variations in GC content in target/probe sequences on the results produced by techniques involving simultaneous hybridization of many sequences, for example, DNA chip or reverse hybridization protocols (1). Due to the higher thermal stability of C:C base pairs, high-GC content sequences may contain mis-matches yet still stably hybridize to a probe or target (resulting in false positives), while low-GC content sequences may perfectly match to probe or target but the strands may dissociate upon washing (resulting in false negatives). This problem can be particularly acute in cases where the probes are short oligos (octamers, nonamers, etc.) A clever solution to this problem is to modify oligonucleotide probes to equalize (normalize) the thermal stability of G:C and A:T base pairs formed upon hybridization to the target, thereby making hybridization dependent only on oligo length and not on base composition. N4-Et-dC base pairs with dG, but the N4-Et-dC : dG base pair has a thermal stability similar to an A:T base pair instead of a C:G base pair. The dramatic effect on thermal stability was shown in two hybridization studies in which different sets of probes having GC content ranging from 0% to 100% were hybridized to their respective natural targets, and the Tm of the duplexes measured. For these unmodified probes, the Tm range was 39degC and 52degC, respectively. When N4-Et-dC was substituted for dC in these probes, the Tm range of the duplexes was only 7degC and 16degC, respectively (2,3).

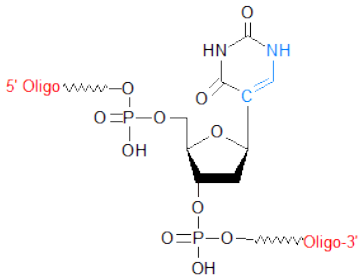
N4-Et-dC-modified oligos have also been used in structure-function studies to better understand how CpG-containing oligos stimulate the innate immune system, and which structural elements in cytosine and guanine bases are required for recognition of, and interaction with, protein/receptor factors responsible for immunostimulation (4). **References**

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pseudoU-2'deoxy

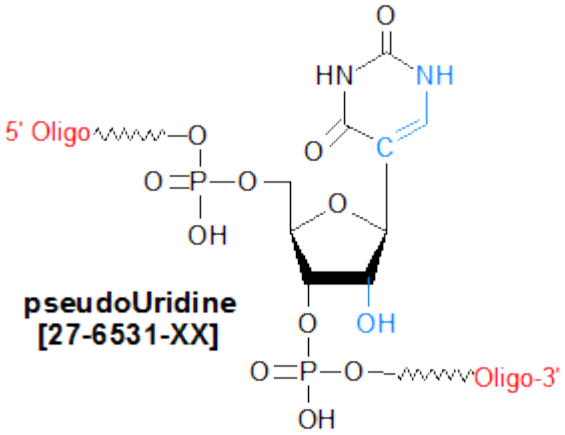
Category	Minor Bases
Modification Code	psi-dU
Reference Catalog Number	26-6531
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	290.17



pseudoUridine-2'deoxy
[26-6531-XX]

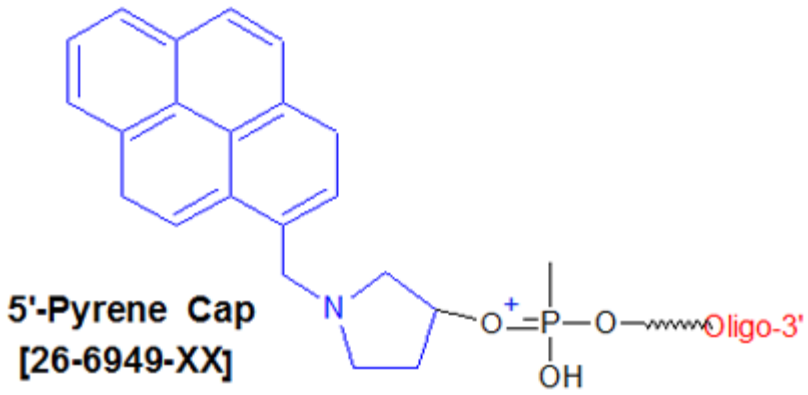
pseudoUridine (psi rU)

Category	Affinity Ligands
Modification Code	psi-rU
Reference Catalog Number	27-6531
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.17



Pyrene Cap (5')

Category	Duplex Stability
Modification Code	Pyr-Cap
Reference Catalog Number	26-6949
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	363.35



5' and 3' Caps for Increased Duplex Stability.
Pyrene, Stilbene and 3'-Uaq caps favor the formation of stable Watson-Crick duplexes by stacking on the terminal base pair.

Melting point increases of over 10^{o}C per modification can be realized for short duplexes
The caps fit canonical Watson-Crick base pairs and do not stack well on mismatched base pairs. This leads to increased base pairing selectivity at the terminal and the penultimate position of oligonucleotides featuring the caps. Base pairing fidelity is usually low at the termini, where fraying occurs frequently in the absence of caps. The beneficial effects of the caps are also realized when longer target strands are bound, so there is no need for blunt ends for the duplexes formed.2 The caps, when attached to the terminus of an oligonucleotide, also facilitate purification as their lipophilicity leads to prolonged retention on reversed phase columns or cartridges. Finally, capping of termini may discourage the degradation of oligonucleotides by exonucleases.

5' Pyrene Cap
5'-Pyrene cap (Pyrenylmethylpyrrolindol)will produce a cap that is more lipophilic than the trimethoxystilbene. The aromatic stacking moiety is linked to the terminus of the DNA through a more rigid, cyclic linker than in the case of trimethoxystilbene. This feature may prove advantageous for researchers interested in exploiting the special photophysical properties of the pyrenyl substituent. The pyrrolindol linker is stereoregular, leading to a single product that can be readily purified by HPLC. The pyrenyl cap is the lead compound discovered in a recent combinatorial study that evaluated over 40 different caps. The cap proved particularly successful for hybridization probes with a 5'-terminal deoxyadenosine residue. Again, its duplex-stabilizing effect does not require blunt ends. The tertiary amino group can be expected to be protonated at physiological pH, producing a cationic functionality that may help to attract target strands electrostatically. The five membered ring presenting the pyrenyl stacking unit mimics the deoxyribose of natural nucleosides, making duplexes terminating in this cap more similar in shape to unmodified DNA than those capped with the trimethoxystilbene.

Trimethoxystilbene cap
Stilbenes have been successfully employed for covalently bridging the termini of oligonucleotide hairpins. The trimethoxystilbene cap that is the result of a recent study that focused on stilbenes that are covalently linked to only one of the two strands forming a duplex.

The three methoxy substituents interact with the 2'-methylene group of the nucleoside in the target strand. Together with the stacking on the terminal base pair, this leads to much-improved mismatch discrimination. The effect is observed for any of the four possible base pairs at the terminus.
Trimethoxystilbene cap increases the signal for the fully complementary target strand when used for hybridization probes immobilized on a glass surface in the form of a DNA microarray. This feature is particularly important for A/T-rich sequences that often cause false negatives. The selective stabilization of neighboring Watson-Crick base pairs helps to suppress cross hybridization that would otherwise lead to stronger false positive results.

3' Uaq Cap

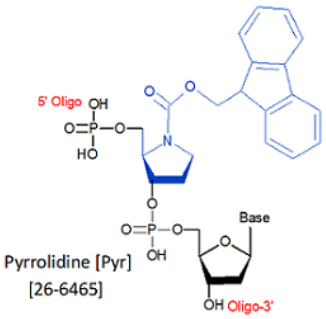
3'-Uaq Cap is available as a cap structure for the 3' of an oligo. It is a Uridine support modified with a 2'-anthraquinone residue and is the most effective oligonucleotide cap known to date. For short hybrid duplexes between DNA probes and RNA target strands, the increase in Tm is up to 18^0C and the modification is effective in increasing the Tm of DNA:DNA, RNA:RNA, and DNA:RNA hybrid duplexes. 3'-Uaq Cap also increases probe specificity by depressing the melting point of terminal mismatches.

References

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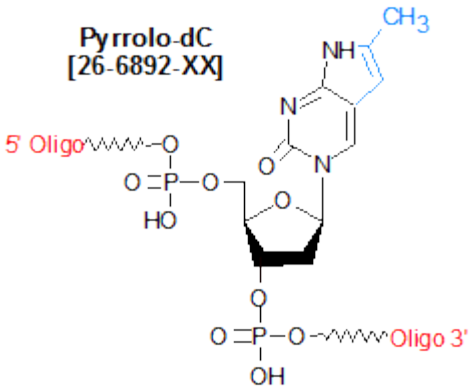
Pyrrolidine (Pyr)

Category	Structural Studies
Modification Code	Pyr
Reference Catalog Number	26-6465
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	178.1



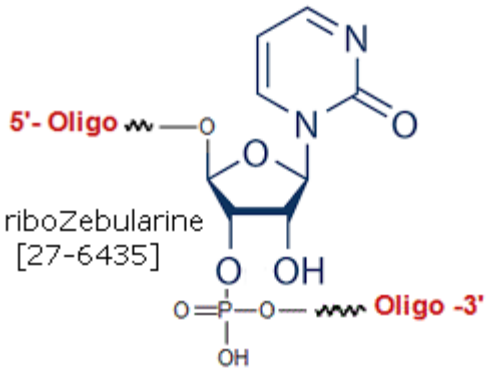
Pyrrolo-dC

Category	Structural Studies
Modification Code	Pyr-dC
Reference Catalog Number	26-6892
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	327.23



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



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

