



## 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.

## 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

- (1) Srivastava, S.C., Raza, S.K., Misra, R. 1,N6-etheno deoxy and ribo adenoGine and 3,N4-etheno deoxy and ribo cytidine phosphoramidites. Strongly fluorescent structures for selective introduction in defined sequence DNA and RNA molecules. *Nucleic Acids Res.* (1994), 22: 1296-1304.
- (2) Dosanjh, M.K., Roy, R., Mitra, S., Singer, B. 1, N6-ethenoadenine is preferred over 3-methyladenine as substrate by a cloned human N-methylpurine-DNA glycosylase (3-methyladenine-DNA glycosylase). *Biochemistry* (1994), 33: 61624-1628.
- (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.

## Modification Code List

Modification	Code	Catalog Number
2-Amino Purine deoxyribose	[2-AP]	26-6505
5-bromo deoxycytosine (Br dC)	[5-Br-dC]	26-6411
5-bromo deoxyuridine (Br-dU)	[5Br-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
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



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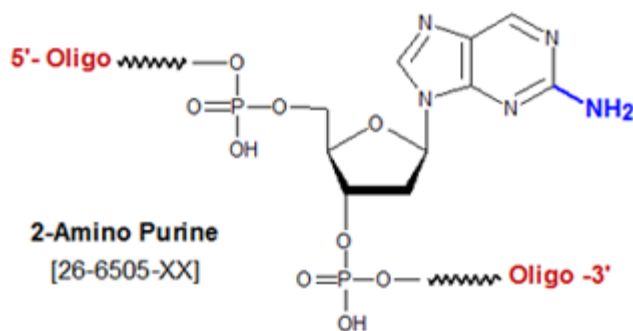
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### 2-Amino Purine

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



2-Amino Purine (2-AP) is a fluorescent molecule that is classified as an adenine and guanine analog, and thus can pair with both thymine and cytosine bases (1,2). It is an attractive choice for use as a probe in nucleic acid secondary structural studies, both because its fluorescence is highly sensitive to the nature of the local environment, and because it usually does not significantly affect duplex stability (3). Examples include the hairpin-loop structure of the (CAG)<sub>8</sub> repeat, involved in several neurodegenerative disorders 2-AP substituted for A (4), the G-quadruplex telomeric structure [AGGG(TTAGGG)<sub>3</sub>] 2-AP substitute for A (5). 2-AP also has been used to characterize the effects of DNA mismatch repair on mutagenesis induced by several different nucleoside analogs (6). **References**

1. Jean JM, Hall KB (2001). "2-Aminopurine fluorescence quenching and lifetimes: role of base stacking". *Proc. Natl. Acad. Sci. U.S.A.* 98 (1): 37-41. doi:10.1073/pnas.011442198.
2. Negishi, K.; Bessho, T.; Hayatsu, H. Nucleoside and nucleobase analog mutagens. *Mutat. Res.* (1994), **318**: 227-238.
3. Ballin, J.D., et al. Local RNA Conformational Dynamics Revealed by 2-Aminopurine Solvent Accessibility. *Biochemistry* (2008), **47**: 7043-7052.
4. Degtyareva, N.N.; Reddish, M.J.; Sengupta, B.; Petty, J.T. Structural Studies of a Trinucleotide Repeat Sequence Using 2-Aminopurine. *Biochemistry* (2009), **48**: 2340-2346.
5. Kimura, T.; Kawai, K.; Fujitsuka, M.; Tetsuro, M. Monitoring G-quadruplex structures and G-quadruplex-ligand complex using 2-aminopurine modified oligonucleotides. *Tetrahedron* (2007), **63**: 3585-3590.
6. Negishi, K.; et al. Binding specificities of the mismatch binding protein, MutS, to oligonucleotides containing modified bases. *Nucleic Acids Res. Supplement No. 1* (2001), 221-222.



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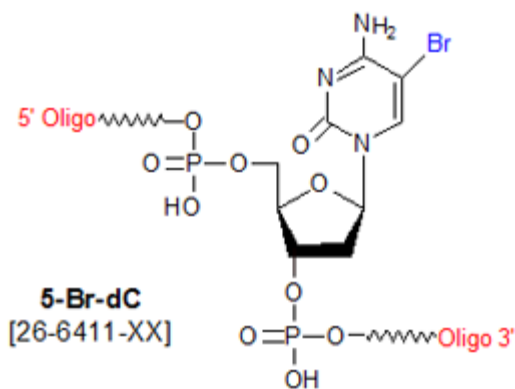
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### 5-Br dC

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 isomorphous 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 MgCl<sub>2</sub>. 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**

- Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.* (1997), **276**: 494-523.
- Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
- 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.
- 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).





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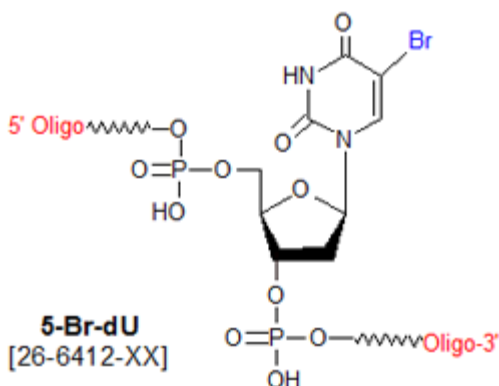
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### 5-Br dU

Category	Minor Bases
Modification Code	5Br-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 isomorphous 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 Specificity (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.

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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).



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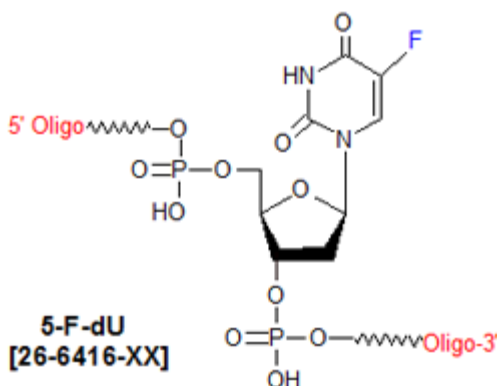
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### 5-F dU

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**

1. Habener, J.F.; Vo, C.D.; Le, D.B.; Gryan, G.P.; Ercolani, L.; Wang, A.H.-J.. 5-Fluorodeoxyuridine as an alternative to the synthesis of mixed hybridization probes for the detection of specific gene sequences. *Proc. Natl. Acad. Sci. USA* (1988), **85**: 1735-1739.
2. Puffer, B.; Kreutz, C.; Rieder, U.; Ebert, M.-O.; Konrat, R.; Micura, R. 5-Fluoro pyrimidines: labels to probe DNA and RNA secondary structures by 1D 19F NMR Spectroscopy. *Nucleic Acids Res.* (2009), **37**: 7728-7740.
3. Durland, R.H.; Rao, T.S.; Revankar, G.R.; Tinsley, J.H.

- ; Myrick, M.A.; Seth, D.M.; Rayford, J.; Singh, P.; Jayaraman, K. Binding of T and T analogs to CG pairs in antiparallel triplexes. *Nucleic Acids Res.* (1994), **22**: 3233-3240.
4. Gowers, D.M.; Fox, K.R. Towards mixed sequence recognition by triple-helix formation. *Nucleic Acids Res.* (1999), **27**: 1569-1577.
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).



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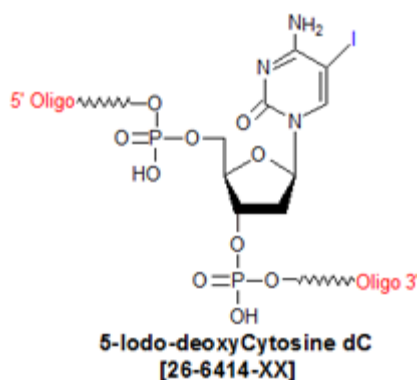
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### 5-I dC

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 (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 isomorphous 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**

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- 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).



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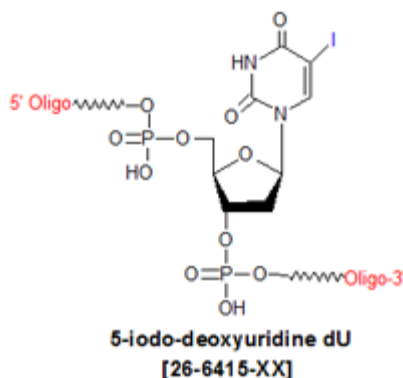
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### 5-I dU

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 (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 isomorphous 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**

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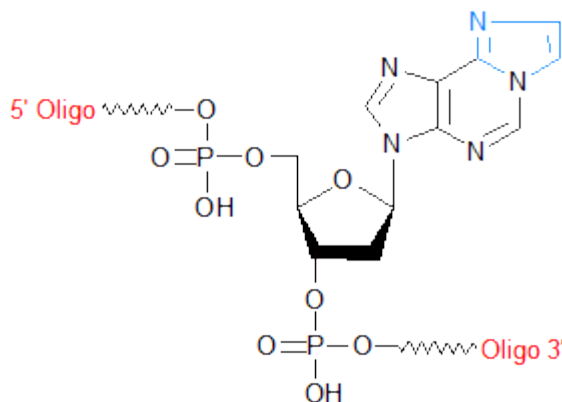
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### etheno dA

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



**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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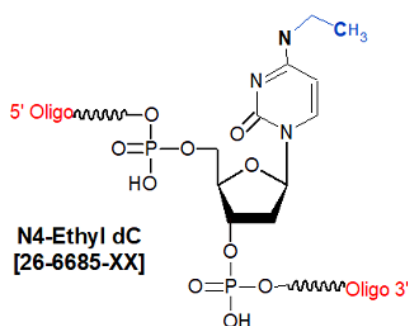
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## Oligo Modifications

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  $T_m$  of the duplexes measured. For these unmodified probes, the  $T_m$  range was 39degC and 52degC, respectively. When N4-Et-dC was substituted for dC in these probes, the  $T_m$  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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## Product Specifications

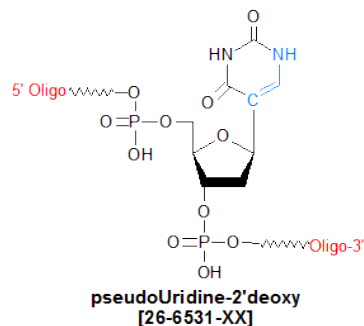
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## Oligo Modifications

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

### 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 ("psi") is a C-glycoside isomer of uridine, and is the most common modified nucleoside found in structural RNA, such as tRNA, rRNA, snRNA, and snoRNA (1,2). Psi-modified RNA can be used as research tools for studies into the roles of this residue in RNA structure and function in the cell. Currently, the role of psi in RNA is a subject of active research, with some things now known. Psi can coordinate a water molecule through its free N1 hydrogen, thereby inducing a modest increase in rigidity on the nearby sugar-phosphate backbone. The presence of psi also enhances base-stacking. Such effects have been proposed as explanations for the deleterious functional effects observed in mutant bacterial strains that lack certain psi residues in tRNA or rRNA (2). Also, based on recent studies, it has been proposed that psi may offer RNA molecules protection from radiation (3). **References**

1. Hamma, T., Ferre-D-Amare, A.R. Pseudouridine synthases. *Chem. Biol.* (2006), **13**: 1125-1135.
2. Charette, M., Gray, M.W. Pseudouridine in RNA: what, where, how, and why. *IUBMB Life* (2000), **49**: 341-351.
3. Monobe, M., Arimoto-Kobayashi, S., Ando, K. beta-Pseudouridine, a beer component, reduces radiation-induced chromosome aberrations in human lymphocytes. *Mut. Res-Genet. Toxicol. and Env. Mutagen.* (2003), **538**: 93-99.



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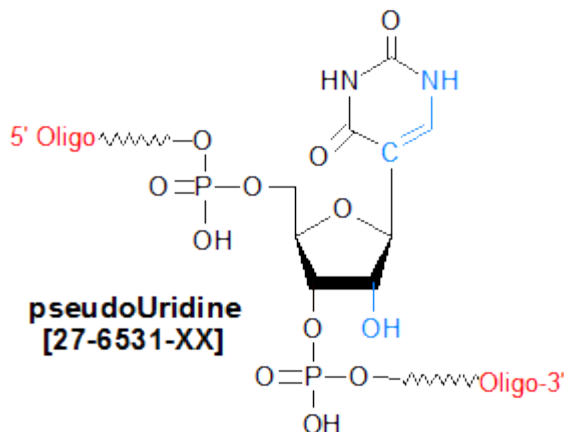
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## Oligo Modifications

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

### 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



Pseudouridine-("psi") is a C-glycoside isomer of uridine, and is the most common modified nucleoside found in structural RNA, such as tRNA, rRNA, snRNA, and snoRNA (1,2). Psi-modified RNA can be used as research tools for studies into the roles of this residue in RNA structure and function in the cell. Currently, the role of psi in RNA is a subject of active research, with some things now known. Psi can coordinate a water molecule through its free N1 hydrogen, thereby inducing a modest increase in rigidity on the nearby sugar-phosphate backbone. The presence of psi also enhances base-stacking. Such effects have been proposed as explanations for the deleterious functional effects observed in mutant bacterial strains that lack certain psi residues in tRNA or rRNA (2). Also, based on recent studies, it has been proposed that psi may offer RNA molecules protection from radiation (3). **References**

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2. Charette, M., Gray, M.W. Pseudouridine in RNA: what, where, how, and why. *IUBMB Life* (2000), **49**: 341-351.
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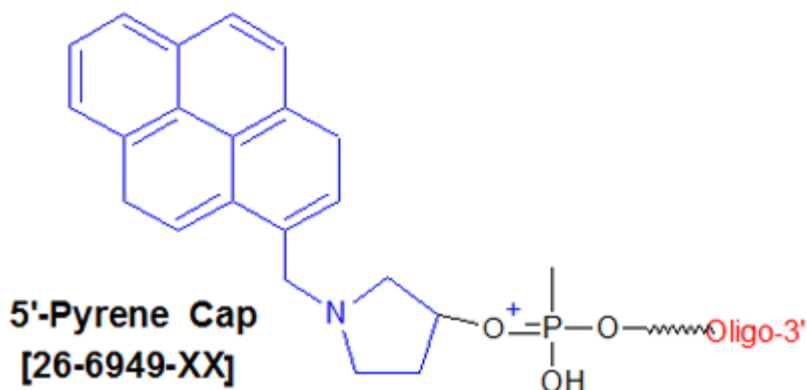
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## Oligo Modifications

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

### 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°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.<sup>2</sup> 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  $T_m$  is up to 18<sup>0</sup>C and the modification is effective in increasing the  $T_m$  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.

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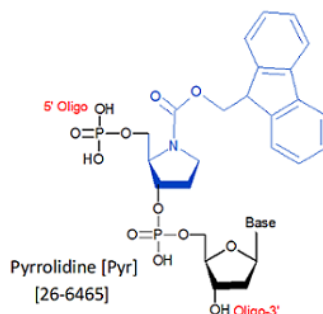
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## Oligo Modifications

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

### 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



#### Pyrrolidine: DNA Damage & Repair new base excision repair inhibitor

DNA is constantly under attack. Alkylating agents, ionizing radiation and oxidative stress can induce base modification or strand scission that, left unchecked, can lead to the development of cancer. Our cells are equipped with DNA damage-monitoring and repair enzymes to correct the damage via base excision repair (BER) or nucleotide excision repair (NER). Ironically, though, the up-regulation of these repair enzymes in cancerous cells frequently causes the development of drug resistance against chemotherapeutic reagents.

One of the most studied repair mechanisms is probably the base excision DNA repair pathway. In this pathway, DNA glycosylases recognize the damaged bases and catalyze their excision through hydrolysis of the N-glycosidic bond. Attempts to understand the structural basis for DNA damage recognition by DNA glycosylases have been hampered by the short-lived association of these enzymes with their DNA substrates. To overcome this problem, the design and synthesis of inhibitors that form stable complexes with DNA glycosylases are essential. Complexes can then be studied biochemically and structurally.

Toward this end, the Verdine group at Harvard synthesized a pyrrolidine analog that mimics the charged transition state of the enzyme-substrate complex. When incorporated into double-stranded DNA, they found the pyrrolidine analog (PYR), introduced through a synthetic oligo forms an extremely stable complex with the DNA glycosylase AlkA, exhibiting a dissociation constant in the pM range and potently inhibited the reaction catalyzed by the enzyme (1).

#### References

See Glen Research Report DNA Damage & Repair.

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2. O.D. Scharer, et al., J. Biol. Chem., 1998, 273, 8592-8597.
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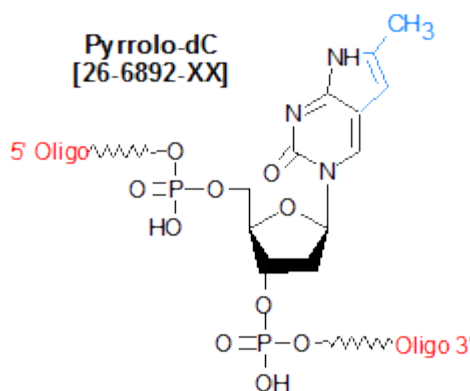
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## Oligo Modifications

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### 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



Pyrrolo-dC is a fluorescent bicyclic analog of deoxycytidine that base pairs specifically with deoxyguanosine (but not A, C, or T). Its excitation and emission maxima are 350 nm and 450 nm, respectively. Because these values are significantly different from the UV absorption maxima of DNA (260 nm) and protein (280 nm), pyrrolo-dC is useful for probing the structures of protein-nucleic acid complexes. This modification also shows reduced fluorescence in duplex DNA compared to in single-stranded DNA, a property that can be used to monitor local DNA melting behavior. These properties were particularly useful in studies done to characterize the nature and size of the transcription bubble in T7 RNA polymerase elongation complexes (1), the structure and function of the polypurine tract in HIV-1 (2) and the binding interactions between DNA hairpins and the anti-tumor drug actinomycin D (3). An important feature of pyrrolo-dC is that its fluorescence allows it to report directly on its local environment with minimal perturbation of the system.

Pyrrolo-dC modified oligonucleotides have also been used as part of signal-on aptasensor systems for molecular recognition. In one such system, an unmodified aptamer specific for a target functions as the molecular recognition element, and a pyrrolo-dC oligo functions as the signal transduction element. The latter acts as a signaling probe that responds to structural changes in aptamers that occur when they bind to targets (4). **References**

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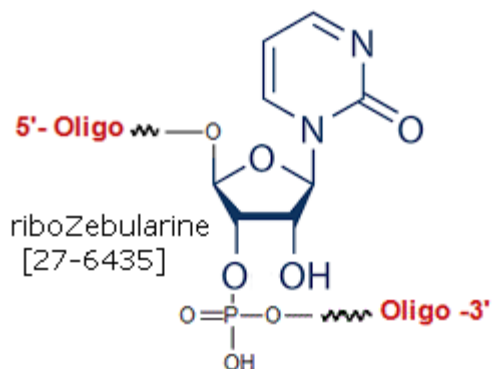
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## Oligo Modifications

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### 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 (pyrimidin-2-one ribonucleoside) may be regarded as a Cytidine derivative lacking the exocyclic amino group. Zebularine and Pyridin-2-one Ribonucleoside, the 3-deaza analogue of Zebularine, are prime candidates for use in evaluating ribozyme activity and function. It should be noted that Zebularine is mildly fluorescent, absorbing at 298nm and emitting at 367nm.

Cytosine Arabanoside (Ara-C) is an anti-viral drug which has achieved limited use. Its effect on DNA structure and activity can be investigated by incorporating it into synthetic oligonucleotides.

Zebularine (pyrimidin-2-one ribonucleoside) is a cytidine analogue that acts as a DNA demethylase inhibitor, as well as a cytidine deaminase inhibitor. This structure is very active biologically and Zebularine is now used as a potent anti-cancer drug. A 2'-deoxynucleoside analogue of Zebularine, 5-methyl-pyrimidin-2-one, 2'-deoxynucleoside, has been used to probe the initiation of the cellular DNA repair process by making use of its mildly fluorescent properties. This combination of biological activity and fluorescence properties would make 5-Me-2'-deoxyZebularine a strong addition to our array of nucleoside analogues.

#### References

1. Gildea, B.; McLaughlin, L. W. Nucleic Acids Res. 1989, 17(6), 2261-2281.
2. Singleton, S. F. et al., Organic Lett. 2001, 3, 3919-3922.
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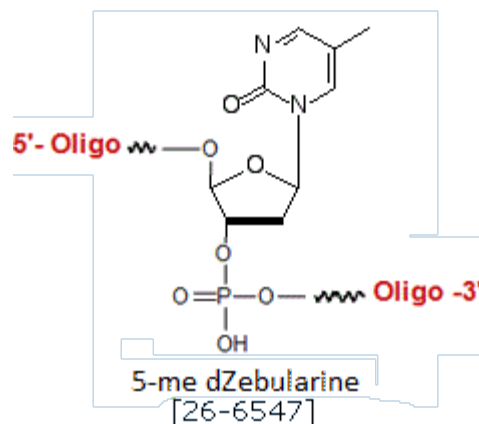
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## Oligo Modifications

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



Zebularine (pyrimidin-2-one ribonucleoside) may be regarded as a Cytidine derivative lacking the exocyclic amino group. Zebularine and Pyridin-2-one Ribonucleoside, the 3-deaza analogue of Zebularine, are prime candidates for use in evaluating ribozyme activity and function. It should be noted that Zebularine is mildly fluorescent, absorbing at 298nm and emitting at 367nm.

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