Halogenated Bases Introduction

Halogenated nucleotides, for example, 5-Br-dC, 5-I-dU, are modified with a halogen on the base (NOT the ribose). Halogenated nucleotides are incorporated into oligonucleotides for determining DNA/RNA structural characteristics by X-ray crystallography (1). In addition, because they are photo-labile, oligonucleotides modified with halogenated bases can be UV cross-linked with other molecules, for example proteins, in order to investigate the structure and function of complexes between DNA and the other molecules (2). This property of photo-lability can also be taken advantage of in aptamer development, where halogenated bases can be incorporated into an aptamer, post-SELEX, to create a photo-aptamer. Such photo-aptamers still retain essentially the same binding affinity for their target, but are now capable of being UV cross-linked to those targets (3).

Halogenated Bases Design Protocols

Incorporation of Halogenated Bases into Oligos—Design Considerations
I. Use in X-ray Crystallographic Structural Studies of Nucleic Acid or Protein-DNA Complexes

Incorporating halogenated bases into oligonucleotides slated for X-ray crystallography-based structural studies is a convenient method for dealing with the “phase problem” in crystal studies. Light waves (such as X-rays) have both an amplitude (related to intensity) and a phase. When light is physically measured by a suitable detector (photomultiplier tube, CCD camera, etc), the intensity of the light is measured, but the phase information is lost. However, because X-ray crystallography is a diffraction-based method, important information related to the position of the atoms in the crystal is lost. Phase information must be restored for correct structure determination. The classical method for obtaining this phase information is by Multiple Isomorphous Replacement (MIR). For this method, a set of multiple-heavy-atom isomorphic derivatives of the original molecule (ex: a tRNA molecule) are synthesized. Synthesis typically involves soaking a crystal of the native molecule in a heavy atom solution, or co-crystallization with the heavy atom. Each derivative must then be separately crystallized, and X-ray diffraction data collected on it, as is corresponding data from the native molecule. A Patterson difference map is then constructed from all the data in order to reveal the location of the heavy atom(s) in the unit cell. Knowing this location allows both the amplitude and phase of the atoms in the molecule to be determined, which leads to an accurate structure.

While MIR works well, its chief drawback is that many heavy-atom derivatives have to be synthesized, purified and analyzed, making it a complex, time-consuming, trial-by-error method. For nucleic acids, the incorporation of halogenated bases into DNA or RNA (either by chemical or enzymatic synthesis) allows for the use of the alternative method known as Multiwavelength Anomalous Dispersion (MAD). For this method, only one heavy-atom-derivitized DNA/RNA molecule needs to be synthesized, here by targeted substitution of specific bases with their halogenated counterpart (ex: dU with 5-I-dU). All of the diffraction data necessary for correct structural determination is collected from this one sample. The simplicity and speed of this approach makes it the preferred method for structural studies involving DNA, RNA, or protein-nucleic acid complexes.

An excellent introduction to X-ray crystallography of macromolecules is found here. II. Use as UV Crosslinkers for Investigation of Protein-DNA Complexes

Halogenated nucleotides are UV-photo-labile molecules. Oligonucleotides containing a halogenated base at a specific position can be UV-crosslinked with another molecule at that location. Halogen-base-modified oligos (especially those with 5-halogenated uracil or 5-halogenated cytosine) are particularly useful in investigational studies into the points of contact (binding locations) between a protein and a nucleic acid in a complex formed between the two (see DNA Protein Crosslinks).

Halogenated Bases Applications

Currently, the primary use of bromo- and iodo-halogenated bases (for example, 5-iodo-dU, 5-bromo-dG) is to facilitate DNA, or protein-DNA, structure determination in either X-ray crystallography or UV-crosslinking studies. For X-ray crystallography, incorporation of such halogenated bases into DNA permits the use of the multi-wavelength anomalous dispersion (MAD) technique to obtain the phase information necessary to calculate the electron density of the molecule's unit cell. The chief advantage of the MAD technique compared to the traditional multiple isomorphous replacement (MIR) method is simplicity. MAD allows for the measurement of all the diffraction data with the same sample, whereas MIR requires synthesis of, and collection of diffraction data from, multiple heavy-atom isomorphic derivatives of the original molecule (4). For UV-crosslinking, the photo-lability of halogenated nucleotides makes their incorporation into DNA useful for investigational studies into protein-DNA complexes, for example, to help determine the binding location and characteristics of a DNA binding protein for its target.

References

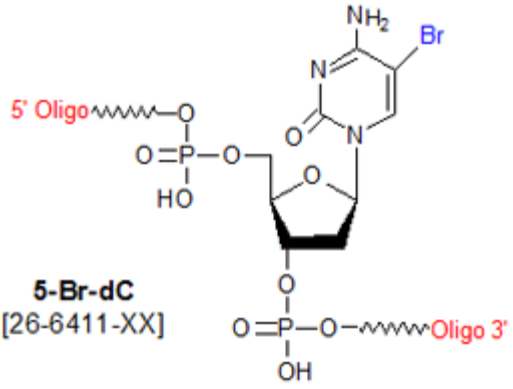
(1) Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. Meth. Enzymol.. (1997), 276: 494-523.
(2) Herbert, A.G.; Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. Nucleic Acids Res. (1993), 21: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

Modificaton Code List

Modification	Code	Catalog Number
5-bromo dC (5-Br dC)	[5-Br-dC]	26-6411
5-bromo dU (5-Br-dU)	[5-Br-dU]	26-6412
5-bromo rC (5-Br rC)	[5-Br-rC]	27-6551
5-bromo rU (5-Br rU)	[5-Br-rU]	27-6552
5-Fluoro deoxyuridine dU	[5-F-dU]	26-6416
5-Iodo ribocytosine (5-I C)	[5-I-rC]	27-6553
5-Iodo deoxycytosine dC	[5-I dC]	26-6414
5-iodo deoxyuridine dU	[5-I-dU]	26-6415
5-Iodo ribouridine (5-I U)	[5-I-rU]	27-6554

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).

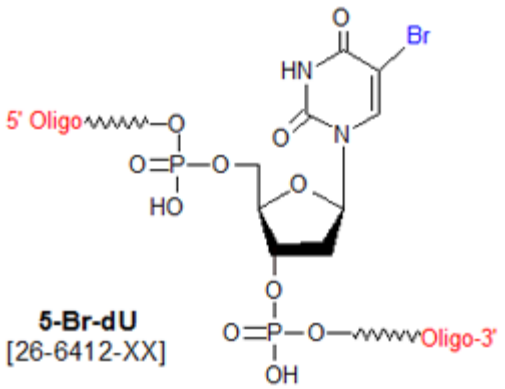
An intriguing use of 5-Br-dC is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (4). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC 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).

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-Br-dU
[26-6412-XX]

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.
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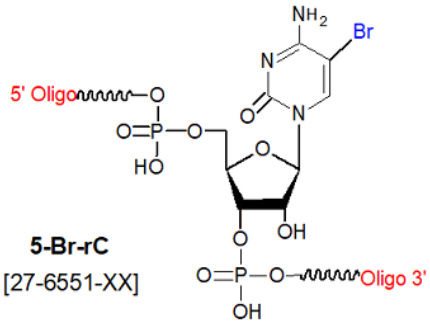
5. Gold, L.; Zichi, D.; Wilcox, S.K.; Schneider, D.J.; Nieuwlandt, D.; Carter, J. SELEX and PHOTOSELEX. (2009), (US2009/0098549).

5-Br rC

Category	RNA Oligo Synthesis
Modification Code	5-Br-rC
Reference Catalog Number	27-6551
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	384.08



5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA 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).

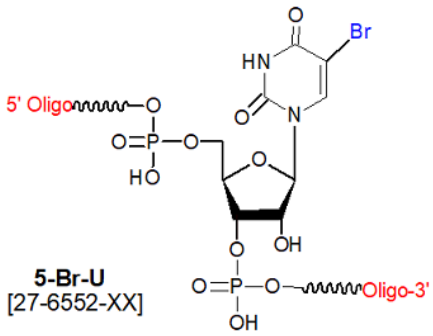
An intriguing use of 5-Br-dC is as a post-SELEX modification to convert a SELEX-identified aptamer into a photo-aptamer (4). In this case, 5-methyl-dC serves as a non-photoreactive “placeholder” in the candidate nucleotide mixture used for aptamer selection during SELEX. One or more of the 5-methyl-dC nucleotides is then replaced by photo-labile 5-Br-dC to generate the corresponding photo-aptamer. As 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

5-Br rU

Category	RNA Oligo Synthesis
Modification Code	5-Br-rU
Reference Catalog Number	27-6552
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	385.07



5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA 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).

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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. As 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**

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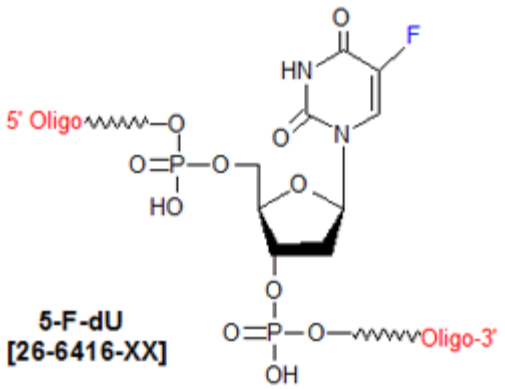
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.
3. Herbert, A.G.; Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. *Nucleic Acids Res.* (1993), **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).

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-F-dU
[26-6416-XX]

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.
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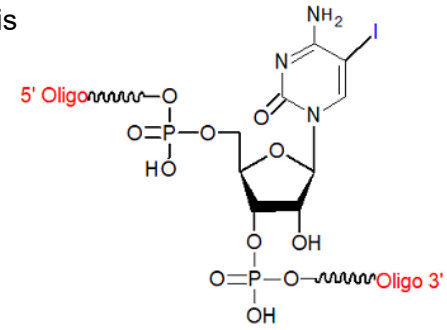
5. Schneider, D.J.; Wilcox, S.K.; Zichi, D.; Nieuwlandt, D.; Carter, J.; Gold, L. Improved SELEX and Photo-SELEX. (2008), PCT/US2008/070371 (WO/2009/012410).

Oligo Modifications

5-I C

Category	RNA Oligo Synthesis
Modification Code	5-I-rC
Reference Catalog Number	27-6553
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	431.08



5-Iodo-Cytosine (5-I-rC)
[27-6553-XX]

5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA 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. As 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

5-I dC

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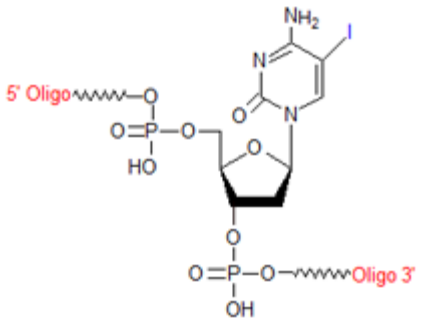
3 Prime	Y
Internal	Y
Molecular Weight(mw)	415.08



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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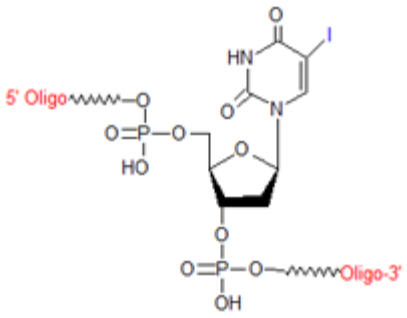
Oligo Modifications

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**

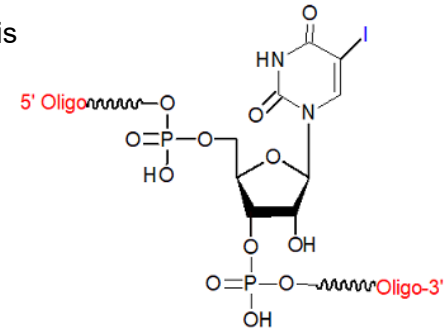
1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.*. (1997), **276**: 494-523.
2. Walsh M.A.; Evans G.; Sanishvili R.; Dementieva I.; Joachimiak, A. MAD data collection - current trends. *Acta Cryst.* (1999), **D55**: 1726-1732.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

Oligo Modifications

5-I U

Category	RNA Oligo Synthesis
Modification Code	5-I-rU
Reference Catalog Number	27-6554
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	432.07



5-iodo-uridine (5-I-rU)

[27-6554-XX]

5-halogenated rC, dC, rU and dU are primarily used to facilitate the determination of DNA and RNA structure by X-ray crystallography (1). When incorporated into a DNA or RNA 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. As 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.
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