



## 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.

## Cross-Linkers Introduction

The ability to utilize UV light to cross-link bases in double-stranded and triple-stranded DNA, or cross-link bases in oligonucleotides with other types of molecules (such as proteins), is often a necessary for successful performance of structural studies, for example the probing of nucleic acid secondary structure, or the structure of protein-nucleic acid complexes. Cross-linker modifications generally fall into two categories, nucleic acid intercalators (for example, psoralen) and halogenated bases (for example, 5-Br-dC). Incorporation of a nucleic acid intercalator into an oligo permits site-specific targeting of the cross-link into double-stranded and triple-stranded DNA. Incorporation of a modified base, capable of forming cross-links, into an oligonucleotide is often the method of choice when an intra-strand cross-link is needed, or a direct cross-link to a protein or other molecule is desired.

# Cross-Linkers Design Protocols

## Oligonucleotides Incorporating Cross-Linkers--Design Considerations

Oligonucleotides containing photo-crosslinker modifications can be valuable research tools for probing the structure of DNA-protein complexes. In particular, it is those amino acids and bases in contact with each other that can be cross-linked, and thus identified as the specific units involved in binding DNA to protein. Cross-linking can be generated either by steady-state UV irradiation or pulsed lasers. For such studies, 5-halogenated uracils/cytosines are commonly used as cross-linker modifications in the DNA template. (7,8), and several excellent protocols are publicly available (9). Positioning of the halogenated bases within the DNA can be done systematically by progressive substitution along the DNA until cross-linking with an amino acid is achieved, or other structural information can be used to guide the choice of where to place the modified base(s).

When inter- or intra-strand cross-linking between duplex or triplex DNA at a thymidine position is desired, the cross-linking intercalator psoralen is typically chosen. The amount of cross-linking achieved can be tightly controlled by varying the dose of 360 nm UV light applied. Although the use of psoralen-modified oligos is primarily considered with respect to their ability to cross-link duplex or triplex DNA, cross-linking to mRNA is also possible. A 5'-psoralen-modified DNA oligo containing puromycin can be cross-linked to the 3'-end of a long mRNA template. The resulting photo-crosslinked product efficiently forms mRNA-protein fusion products (10).

## Cross-Linkers Applications

One of the most commonly used intercalator cross-linkers is psoralen, which is used to probe nucleic acid secondary structure at specific points in both duplex and triplex DNA. Specifically, psoralen forms cross-links with thymidine. In duplex DNA, after intercalation, psoralen can form either monoadducts with one adjacent thymidine, or diadducts with two thymidines adjacent to it, depending on the particular UV wavelength it's exposed to (1). These adducts can occur on the same or complementary strands. For triplex DNA, psoralen C6-modified homopyrimidine oligos are used to bind to a complementary homopurine-homopyrimidine duplex, thereby forming a triplex that can be cross-linked together at the triplex-duplex junction point (2). Demonstration of the existence of triple-helix-directed gene modification and the involvement of nucleotide excision repair mechanism in DNA interstrand cross-link repair are two examples of the use of psoralen-modified oligos as research tools (3-4). Halogenated bases are a second class of UV cross-linker used for probing biomolecular structure, particularly the structure of protein-DNA complexes. 5-Br-dC and 5-Br-dG have been incorporated into dG-dC oligos capable of easily changing into the Z-conformation. This property allowed such oligos to function as probes for detecting and studying Z-DNA binding proteins (5). Substituting 5-Br-dU at several thymine positions of oligos allowed them to be used to characterize the binding of Nuclear Factor BA1 with DNA (6). See the relevant tech sheets of the different halogenated bases offered by Gene Link for additional examples.

## References

- (1) Pieles, U., Englisch, U. Psoralen covalently linked to oligodeoxyribonucleotides: synthesis, sequence specific recognition of DNA and photo-cross-linking to pyrimidine residues of DNA. *Nucleic Acids Res.* (1989), 17: 285-299.
- (2) Takasugi, M., Guendouz, A., Chassignol, M., Decout, J.L., Lhomme, J., Thuong, N.T., Helene, C. Sequence-specific photo-induced cross-linking of the two strands of double-helical DNA by a psoralen covalently linked to a triple-helix-forming oligonucleotide. *Proc. Natl. Acad. Sci. USA* (1991), 88: 5602-5606.
- (3) Barre, F-X., Ait-Si-Ali, S., Giovannangeli, C., Luis, R., et al. Unambiguous demonstration of triple-helix-directed gene modification. *Proc. Natl. Acad. Sci. USA* (2000), 97: 3084-3088.
- (4) Wang, X., Peterson, C.A., Zheng, H., Nairn, R.S., Legerski, R.J., Lei, L. Involvement of Nucleotide Excision Repair in a Recombination-Independent and Error-Prone Pathway of DNA Interstrand Cross-Link Repair. *Mol. Cell. Biol.* (2001), 21: 713-720.
- (5) 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.
- (6) Kardassis, D.; Zannis, V.I.; Cladaras, C. Purification and Characterization of the Nuclear Factor BA1. *J. Biol. Chem.* (1990), 265: 21733-21740.
- (7) Meisenheimer, K.M., Koch, T.H. Photocross-linking of nucleic acids to associated proteins. *Crit. Rev. Biochem. Mol. Biol.* (1997), 32: 101-140.
- (8) Steen, H., Jensen, O.N. Analysis of protein-nucleic acid interactions by photochemical cross-linking and mass spectrometry. *Mass Spectrometry Reviews* (2002), 21: 163-182.
- (9) Chodosh, L.A. UV Crosslinking of Proteins to Nucleic Acids. *Curr. Prot. Mol. Bio.* (2001), 12.5.1-12.5.8.
- (10) Kurz, M., Gu, K., Lohse, P.A. Psoralen photo-crosslinked mRNA-puromycin conjugates: a novel template for the rapid and facile preparation of mRNA-protein fusions. *Nucleic Acids Res.* (2000), 28: E83.

## Modification Code List

<b>Modification</b>	<b>Code</b>	<b>Catalog Number</b>
5-bromo dC (5-Br dC)	[5-Br-dC]	26-6411
5-bromo dU (5-Br-dU)	[5-Br-dU]	26-6412
5-Iodo deoxycytosine dC	[5-I dC]	26-6414
5-iodo deoxyuridine dU	[5-I-dU]	26-6415
CNVK Photo Cross Linker	[cnvK]	26-6539
Convertible dG (2-Fluoro deoxy inosine)	[2-FdI]	26-6671
Psoralen C6-5'	[Pso-C6-5']	26-6686



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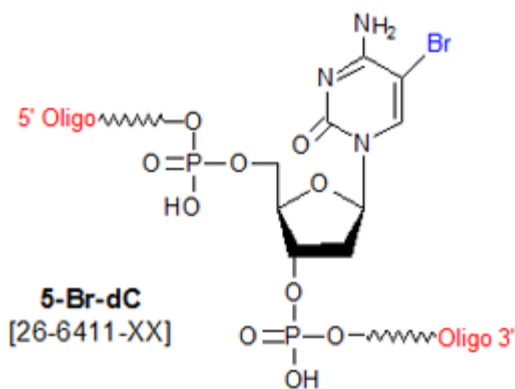
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## Oligo Modifications

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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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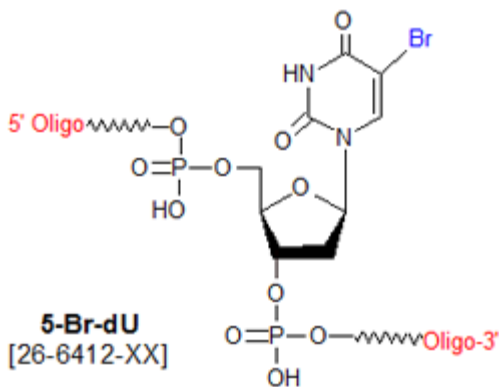
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### 5-Br dU

Category	Minor Bases
Modification Code	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 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**

- 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.
- 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.

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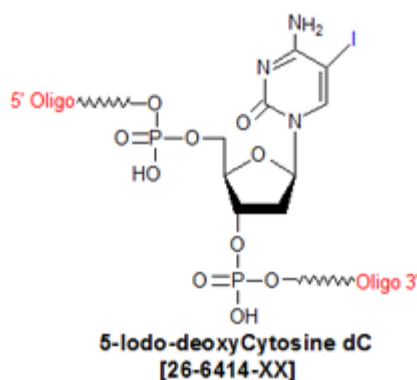
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### 5-I dC

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**

1. Hendrickson, W.; Ogata, C. Phase determination from multiwavelength anomalous diffraction measurements. *Meth. Enzymol.* (1997), **276**: 494-523.
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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.

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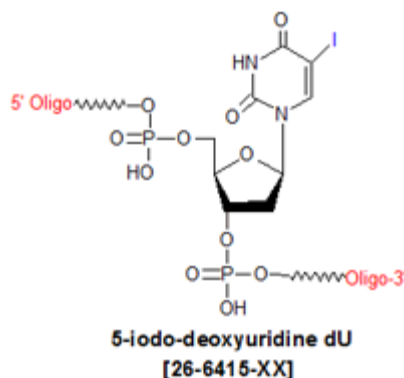
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### 5-I dU

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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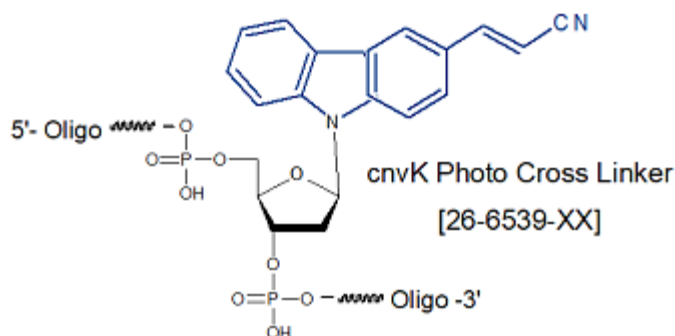
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### cnvK Photo Cross Linker

Category	Photo Cleavable
Modification Code	cnvK
Reference Catalog Number	26-6539
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	396.33



#### Nature Methods: Application Note: Light-Seq: light-directed in situ barcoding of biomolecules in fixed cells and tissues for spatially indexed sequencing.

Jocelyn Y. Kishi., Ninning Liu, Emma R. West, Kuanwei Sheng, Jack J. Jordanides, Matthew Serrata<sup>1</sup>, Constance L. Cepko, Sinem K. Saka, and Peng Yin.

Nature Methods | VOL 19 | 1394 November 2022 | 1393-1402 | [www.nature.com/naturemethods](http://www.nature.com/naturemethods)  
<https://doi.org/10.1038/s41592-022-01604-1>

Oligonucleotide incorporated with a 3-cyanovinylcarbazole nucleoside ( $\text{CNV}_K$ ) can be induced to undergo rapid photo cross-linking to the complementary strand at one wavelength and rapid reversal of the cross-link is possible at a second wavelength. Neither wavelength has the potential to cause significant DNA damage. Irradiation of a duplex containing a single incorporation of  $\text{CNV}_K$  at 366nm led to 100% cross-linking to thymine base in 1 second, although complete cross-linking to cytosine takes 25 seconds (1) A 30 second irradiation time should cover all situations. In addition, it was demonstrated that the purine bases were unreactive to cross-linking, allowing differentiation between pyrimidines and purines at the target site. The authors also determined the effect of sequence contexts around the  $\text{CNV}_K$  site and demonstrated that the identity of bases on either side of the cross-linking site has little effect on the reaction. Once cross-linked, the UV melting temperature of the duplex was raised by around 30 degree C relative to the duplex before irradiation. Complete reversal of the cross-link takes place at 312nm in 3 minutes. This facile reversal reaction is, therefore, accomplished with no damage to normal DNA.

In a later publication, a further application of this cross-linking technique was investigated (2); when  $\text{CNV}_K$  was cross-linked with a dC residue in duplex DNA, heating at 90 degree C for 3.5 hours led to deamination of the cytosine base to form uracil in the complementary strand. Reversal of the cross-link at 312nm led to a DNA strand in which dC had been converted to dU. The authors showed that this transformation is specific for the dC residue opposite the  $\text{CNV}_K$  and any further adjacent dC residues are unaffected.

Similarly, the authors have shown that <sup>CNV</sup>K can be cross-linked to an adjacent RNA strand (3).

#### **Recommended Further Reading**

Glen Report 30.21: CNVK and CNVD-Ultrafast Reversible Photo-Crosslinkers for DNA or RNA.

#### **References**

- (1) Y. Yoshimura, and K. Fujimoto, Org Lett, 2008, 10, 3227-30.
- (2) K. Fujimoto, K. Konishi-Hiratsuka, T. Sakamoto, and Y. Yoshimura, ChemBioChem, 2010, 11, 1661-4.
- (3) Y. Yoshimura, T. Ohtake, H. Okada, and K. Fujimoto, ChemBioChem, 2009, 10, 1473-6.



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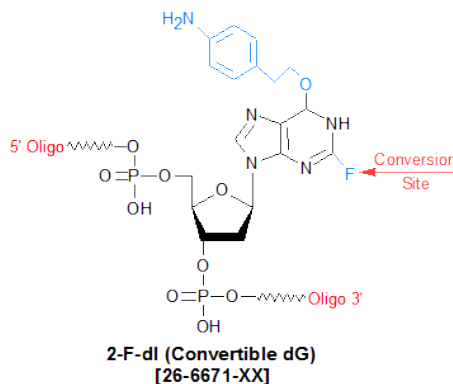
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### Convertible dG (2-F-dI)

Category	Minor Bases
Modification Code	2-FdI
Reference Catalog Number	26-6671
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	332.18



Gene Link supplies Convertible modified oligos protected with oligo bound to CPG solid support or can conjugate the convertible site to user specified ligand.

2-Fluoro-deoxyinosine (2-F-dI) is classified as a convertible dG nucleotide. After incorporation into an oligo, reaction of the 2-fluorine on the inosine base with a primary amine displaces the fluorine atom, and converts the nucleotide into a N2-substituted dG. Oligos containing 2-F-dI modifications are useful precursors in studies requiring cross-linking, at G position(s), between oligos, or between an oligo and an enzyme. For example, 2-F-dI modified oligos have been reacted with disulfide-containing diamines (1) or thiopropylamines (2) in order to subsequently form disulfide-crosslinked DNA duplexes. Such oligos have also been reacted with bis-(3-aminopropyl)disulfide dihydrochloride, and the disulfide-containing oligo intermediate coupled to a short-lived HIV-1 reverse transcriptase kinetic intermediate to form stable enzyme-oligo complexes. The ability to synthesize such complexes have enabled deeper study of the DNA translocation mechanism of HIV-1 RT (3).

In order to minimize the possibility of unwanted side reactions with the exocyclic amines of other bases of the oligo, it must be fully protected and still attached to the synthesis solid support when reacted with the primary amine. Consequently, for customers ordering 2-F-dI-modified oligonucleotides, Gene Link supplies the oligo attached to a solid support for subsequent conversion to the appropriate N2-modified dG by the enduser.

See examples below of Convertible dG (2-Fluoro deoxy inosine) to various amino derivatives.

Protocol for conversion of 2-FI (convertible G) to the appropriate N2-modified dG.

#### References

1. Erlanson, D.A.; Chen, L.; Verdine, G.L. DNA Methylation through a Locally Unpaired Intermediate. *J. Am. Chem. Soc.* (1993), **115**: 12583-12584.
2. Erlanson, D.A.; Glover, J.N.M.; Verdine, G.L. Disulfide Cross-linking as a Mechanistic Probe for the B<-->Z Transition in DNA. *J. Am. Chem. Soc.* (1997), **119**: 6927-6928.

3. Sarafianos, S.G.; et al. Trapping HIV-1 Reverse Transcriptase Before and After Translocation on DNA. *J. Biol. Chem.* (2003), **278**: 16280-16288.



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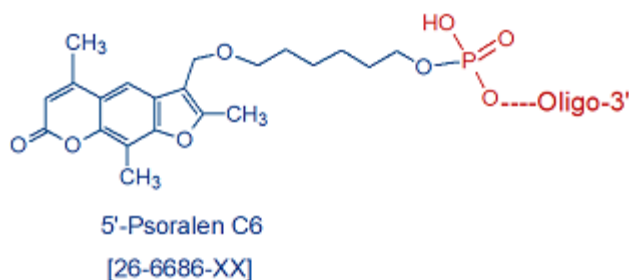
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### Psoralen C6-5'

Category	Intercalators
Modification Code	Pso-C6-5
Reference Catalog Number	26-6686
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	420.4



Psoralen C6 is a heterocyclic compound capable of intercalating between bases, and cross-link bases, in both double-stranded and triple-stranded DNA. It is attached to a C6 linker in order to facilitate a psoralen-modified oligonucleotide's ability to intercalate and cross-link with triple-stranded DNA. Psoralen is typically used as a ss/ds DNA intercalating or cross-linking reagent, for the purpose of probing nucleic acid secondary structure (1). Upon exposure to long wavelength UV light (350 nm), psoralen forms covalent cyclobutane linkages to thymidine. Psoralen can form two different types of adducts with thymidine. The first is a monoadduct, in which the psoralen moiety binds to one adjacent thymidine on the same or complimentary strand. The second is a diadduct, in which psoralen binds to two thymidines adjacent to it, either on the same or complimentary strand (2). Diadducts formed between adjacent thymidines are photo-reversible with short wavelength UV light (254 nm). In addition to cross-linking duplex DNA, Psoralen-C6 homopyrimidine oligos can be used to bind to a complementary homopurine-homopyrimidine duplex, to form a triple-helix that can then be cross-linked together at the triplex-duplex junction point (3). Psoralen-modified oligonucleotides are widely used as research tools; representative examples of such use are shown in these references (4,5). **References**

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