Conjugation & Surface Attachment Introduction

Incorporation of reactive organic functional groups, particularly primary amine, thiol (sulfhydryl), or carboxylate groups, at specific sites within an oligonucleotide allows for subsequent conjugation of the oligo with a number of different affinity, reporter or protein labels, depending on the application. Such labels need to be appropriately reactive to the incorporated functional group, for example, NHS esters or isothiocyanates in the case of primary amines, and iodoacetamides or maleimides in the case of primary thiols. Examples of labels includes fluorescent dyes/quenchers, digoxigenin, biotin, and enzymes. Functionally-derivitized oligos can also be covalently attached to surfaces such as glass slides or gold microspheres for use in various microarray or nanoelectronic applications.

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The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

Conjugation & Surface Attachment Design Protocols

Conjugation/Surface Attachment--Design Considerations

Over the past 20 years, a wide variety of robust, publicly available protocols have been developed either to conjugate oligonucleotides to chemical moieties (e.g., to haptens, enzymes, fluorescent dyes) for use as probes, or to covalently attach oligonucleotides to a solid surface (e.g., glass slides) for use in DNA microarray applications. Many oligonucleotide-based assays actually combine both of these aspects in one package. Optimal design of such combination assays requires consideration of several different parameters. **I. DNA Microarrays (2-D)**

DNA microarrays are excellent platforms for high-density screening applications, as a large number of different sequences can be immobilized to a planar surface for interrogation of a sample. Either amino- or thiol-end-modified oligonucleotides can be covalently attached to glass slides or silicon wafers that have been suitably modified chemically for that purpose. In addition, it is critical that the microarray be designed so that it has the appropriate oligo surface density to ensure sufficient hybridization between immobilized oligo probe and target occurs to obtain a good signal. In particular, optimal surface coverage decreases with increasing oligo length dependent, presumably due to steric hindrance (6). **II. Microspheres (3-D)**

Oligonucleotides attached to microspheres are used in a variety of assays requiring oligos that are immobilized on a solid support that can be freely suspended in solution. The use of magnetic microspheres with oligo-dT to capture mRNA from cell lysates is one well-known application. Another particularly interesting such application is termed “liquid assays”. Here a probe oligo is covalently attached to a polystyrene microsphere containing a fluorescent dye inside them. By using microspheres with different dyes, or dye combinations, and a unique oligo probe for each color microsphere, highly multiplexed, solution-based hybridization assays can be designed in convenient, microtiter-plate format. Such assays exhibit rapid hybridization times, which is an important advantage. It is important to remember, however, that careful optimization of hybridization conditions may need to be done to ensure robust, reproducible detection of all the sample targets being probed for. The targets may be an oligo, cDNA, PCR product, or even a protein, and are fluorescently labeled (with the label different from that of the fluorescent microsphere). After hybridization, the microspheres are typically assayed by flow cytometry, with the fluorescence of the microsphere identifying the probe and the simultaneous fluorescence of the target indicating hybridization (7,8). **III. Conjugation to Amino-Modified Oligos**

While many chemical moieties are available as phosphoramidites, and so can be directly incorporated into an oligo during synthesis, others are not. The latter often have properties that are particularly useful for oligos slated for use as probes. For example, Alexa dyes (highly fluorescent) and digoxigenin (DIG--low background/high sensitivity in situ probes) are available only as NHS esters. Their incorporation into an oligonucleotide requires the presence of an primary amino group on the oligo for conjugation.

Generally speaking, conjugating an NHS-ester to an amine-modified oligo is an excellent way to generate a modified oligo. The resulting amide linkage between the modification and the oligo is very stable, and the modified oligo can be stored long term at -20C.

Conjugation & Surface Attachment Applications

One of the most common applications for amino-modified oligos is for conjugation to fluorescent dyes through an NHS ester. Many dyes with desirable properties (for example, absorption/emission wavelengths or high fluorescence intensity) are not available as phosphoramidites, but only as NHS esters. Rhodamine-based dyes and Alexa dyes are two common examples. Certain haptens such as digoxigenin also require conjugation to amino-labeled oligos via an NHS ester, due to lack of a phosphoramidite for it. Amino-labeled oligos also widely used in the manufacture of DNA microarrays used in gene expression studies, with the oligos covalently immobilized to a glass or other silicon-based flat surface through the amine (1). Thiol-modified oligos can be conjugated to a variety of fluorescent or non-fluorescent molecules; the conjugation chemistry here is typically either maleimide or iodoacetamide-based (2). The orthogonality of the corresponding conjugation chemistries for amine and thiol groups allows for the synthesis of oligos with novel combinations of modifications. Thiol-modified oligos can also be immobilized to glass slides, gold flat surfaces or microspheres for use in DNA microarray, nanoelectronic and DNA sensor-based applications (3, 4). However, because the chemical linkage between a single thiol group and gold is somewhat labile, the DTPA (dithiolphosphoramidite) modification permits multiple tethering of an oligo to a gold surface. Incorporation of three units of DTPA has been shown to provide maximum stability (5).

References

(1) Immobilization Chemistry. in Immobilization of DNA on Chips II, C. Whittman (Vol. Ed.). Springer-Verlag, Berlin, Heidelberg (2005): 51-53.
(2) Connolly, B.A., Rider, P. Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specfic probes. Nucleic Acids Res. (1985), 13: 4485-4502.
(3) Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. Anal. Biohem. (1999), 266: 23-30.
(4) Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. Proc. Natl. Acad. Sci. USA (2005), 102: 13383-13385.
(5) Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates Nucleic Acids Res. (2002), 30: 1558-1562.
(6) Guo, Z., Guilfoyle, R.A., Thiel, A.J., Wang, R., Smith, L.M. Direct fluorescence analysis of genetic polymorphisms by hybridization with oligonucleotide arrays on glass supports. Nucleic Acids Res. (1994), 22: 5456-5465.
(7) Yang, L., Tran, D.K., Wang, X. BADGE, Beads Array for the Detection of Gene Expression, a high-throughput diagnostic bioassay. Genome Res. (2001), 11: 1888-1898.
(8) Defoort, J.P. Simulataneous detection of multiplex-amplified human immunodeficiency virus type 1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA using a flow cytometer microsphere-based hybridization assay. J. Clin. Microbiol. (2000), 39: 131-140.

Modificaton Code List

Modification	Code	Catalog Number
Alkyne-Modifier Serinol	[Alk-Ser]	26-6925
Alkyne-C2-(Propargyl-PEG1) NHS	[Alk-C2-N]	26-6924
Amino Allyl	[AmAllyl-5]	26-6731
Aminoallyl rU	[AmAll-rU]	27-6548
Amino Mod C12-5'	[AmC12]	26-6420
Amino Mod C3-5	[AmC3-5]	26-6405F
Amino Mod C6	[AmC6]	26-6418
Amino C6 U (RNA)	[AmC6U]	27-6422
Amino deoxyadenosine dA C6	[Am-dA-C6]	26-6666
Amino dC N4 PEG3 amino linker	[Am-dC-C6]	26-6670
Amino deoxyguanosine dG C6	[Am-dG-C6]	26-6669
5'-Amino dT	[5-Am-dT]	26-6657T
Amino deoxythymidine dT C6	[Am-dT-C6]	26-6438
Amino modifier serinol	[Am-Ser]	26-6715
Amino Spacer 7 C6 Internal	[AmSp7-C6-Int]	26-6560
Amino Spacer 7-C6 (3')	[AmSp7-C6]	26-6425
Azide butyrate N	[N3-C4-N]	26-6922
5-Carboxy-C10	[CO-C10]	26-6717
Carboxy-dT	[CO-dT]	26-6697
Maleimide-Modifier (5')	[Mal]	26-6574

PC Amino C6 (Photocleavable)	[PCAmC6]	26-6690
6-Thio-dG (S6-dG)	[S6-dG]	26-6533
4-Thio dU (s4dU)	[s4dU]	26-6445
4-Thio-dT (S4dT)	[S4dT]	26-6538
4-Thio-Uridine (s4U)	[s4U]	27-6445
Thiol C6 dT	[S-C6-dT]	26-6483
Thiol SS Serinol Dipod (DTSPA)	[DTSPA]	26-6573
Thiol SS-C3 3'	[SS-C3-3]	26-6482
Thiol SS-C6	[SS-C6]	26-6419

Alkyne Serinol

Category	Click Chemistry
Modification Code	Alk-Ser
Reference Catalog Number	26-6925
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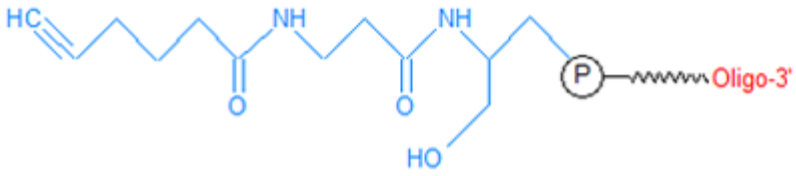
3 Prime	Y
Internal	Y
Molecular Weight(mw)	334.26



Alkyne-Modifier Serinol
[26-6925-XX]

Alkyne-C2 N

Category	Click Chemistry
Modification Code	Alk-C2-N
Reference Catalog Number	26-6924
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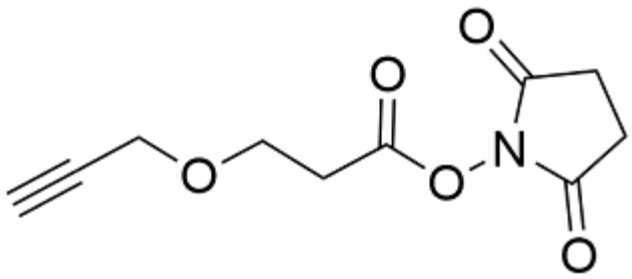
3 Prime	Y
Internal	Y
Molecular Weight(mw)	302.11



Click here for a complete list of Click Chemistry Oligo Modifications

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.

Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol * The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. * Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide, BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

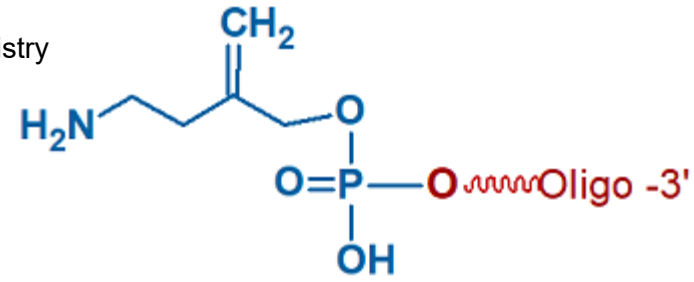
Alkyne NHS ester can be used to incorporate an active alkyne onto the 5'or 3' end of an oligonucleotide, as well as at an internal position. Incorporation of this modification to the oligo is done via conjugation to an active primary amine (such as Amino Linker C6). As a result, the alkyne group is separated from the oligo by a spacer arm of varying length, which serves to reduce steric interaction between the reactive group and the oligo. The presence of the alkyne allows the user to use Click Chemistry (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate it to a variety of azide-containing labels/tags (e.g., fluorescent dyes, biotin, or oligos, with extremely high regioselectivity and efficiency (1,2). When conjugation to an azide-oligo is desired, preparation of the azide-oligo can be achieved using either an Azidobutyrate NHS Ester or the 5' Bromohexyl modifier (see their respective tech sheets for details). Click chemistry can be used to form short, cyclic oligos that can be used as research tools in various biophysical and biological studies (3). In particular, they have considerable potential for in vivo work, as cyclic oligos are known to be very stable in serum for up to several days.

References

1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.
3. Kumar, R., El-Sagheer, A., Tumpane, J., Lincoln, P., Wilhelmsson, L.M., Brown, T. Template-Directed Oligonucleotide Strand Ligation, Covalent Intramolecular DNA Circularization and Catenation Using Click Chemistry. *J. Am. Chem. Soc.* (2007), **129**: 6859-6864.

Amino Allyl

Category	Conjugation Chemistry
Modification Code	AmAllyl-5
Reference Catalog Number	26-6731
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	101.14



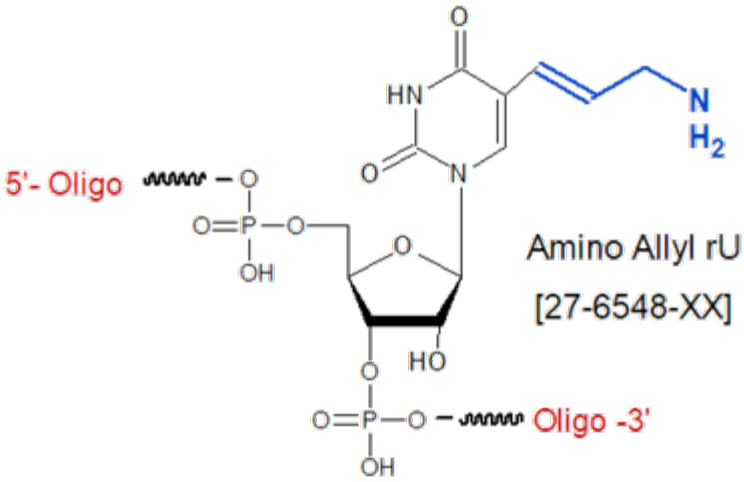
Amino Allyl

26-6418-XX

Oligo Modifications

Amino Allyl rU

Category	Others
Modification Code	AmAll-rU
Reference Catalog Number	27-6548
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	363.79



Amino C12-5'

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Modification Code	AmC12
Reference Catalog Number	26-6420
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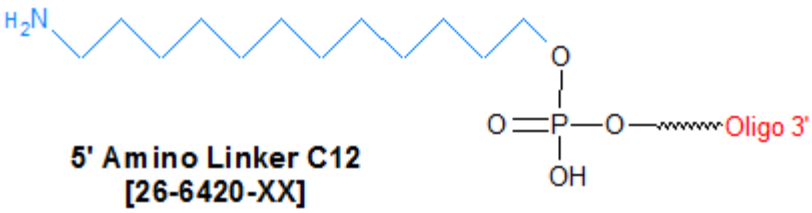
3 Prime	N
Internal	Y
Molecular Weight(mw)	263.32



5' Amino Linker C12
[26-6420-XX]

Amino C3-5'

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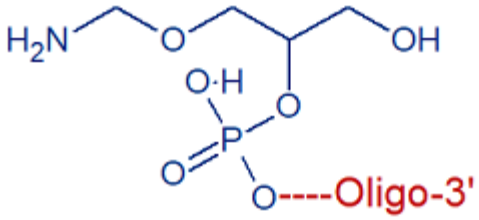
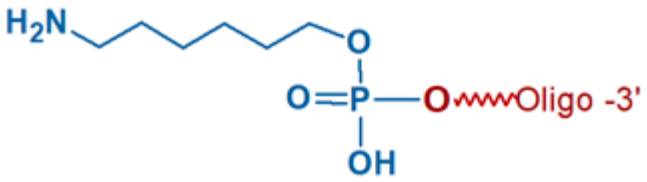
Reference Catalog Number	26-6405F
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	137.08



Amino C3 5'
[26-6405F-XX]

Amino C6

Category	Conjugation Chemistry
Modification Code	AmC6
Reference Catalog Number	26-6418
5 Prime	Y
3 Prime	N
Internal	Y
Molecular Weight(mw)	179.16

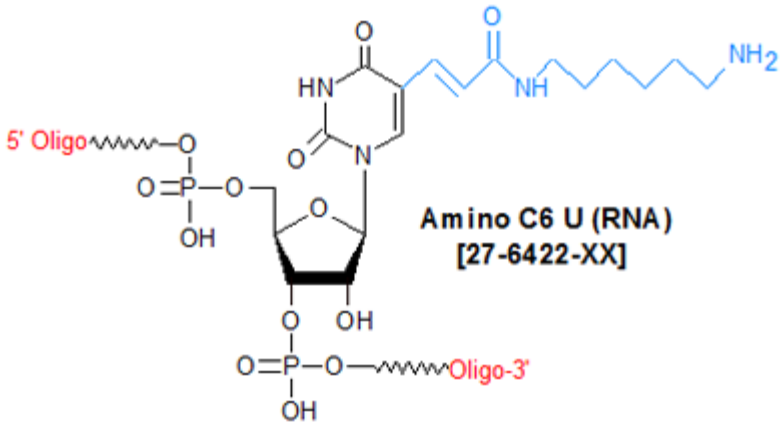


5' Amino C6 Linker

26-6418-XX

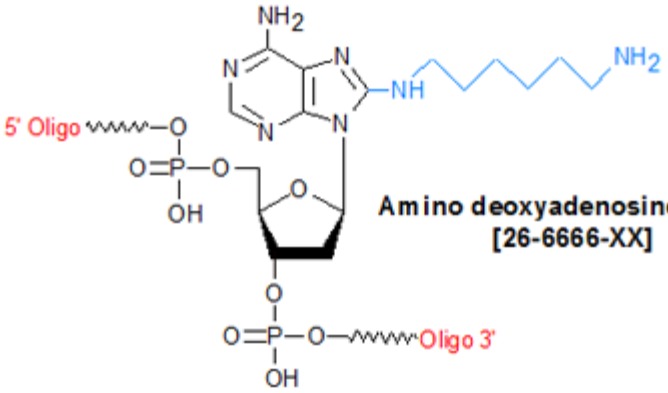
Amino C6 U

Category	Minor Bases
Modification Code	AmC6U
Reference Catalog Number	27-6422
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	474.4



Amino dA C6

Category	Minor Bases
Modification Code	Am-dA-C6
Reference Catalog Number	26-6666
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	427.4



Amino deoxyadenosine dA C6
[26-6666-XX]

Amino dC C6

Category	Minor Bases
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Reference Catalog Number	26-6670
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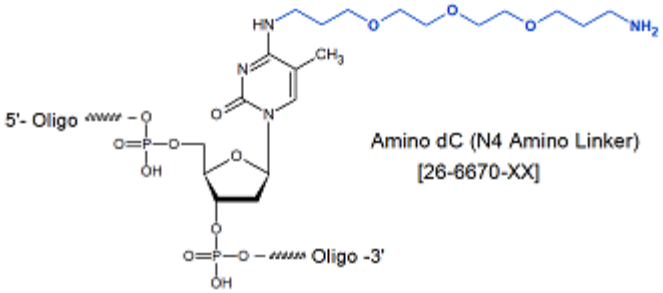
3 Prime	Y
Internal	Y
Molecular Weight(mw)	507.51



Amino dG C6

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Reference Catalog Number	26-6669
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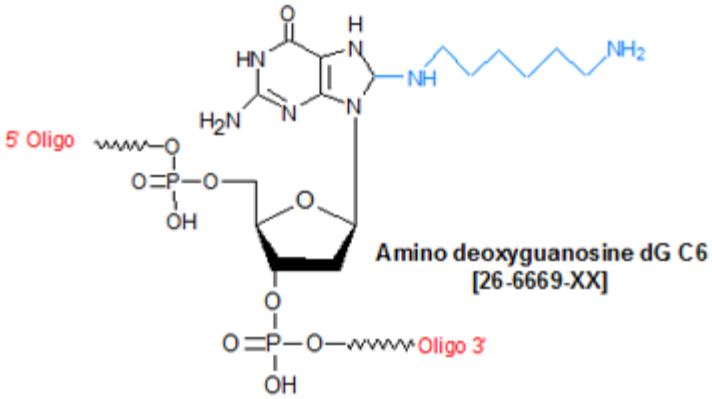
3 Prime	Y
Internal	Y
Molecular Weight(mw)	428.38



Amino dT 5'

Category	Conjugation Chemistry
Modification Code	5-Am-dT
Reference Catalog Number	26-6657T
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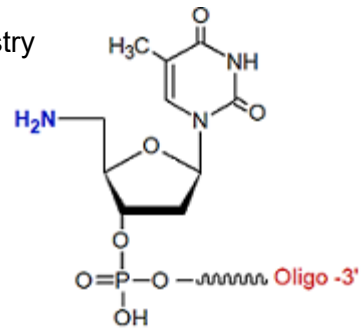
3 Prime	N
Internal	N
Molecular Weight(mw)	303.21



5'-Amino dT
[26-6657T-XX]

Amino dT C6

Category	Minor Bases
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Reference Catalog Number	26-6438
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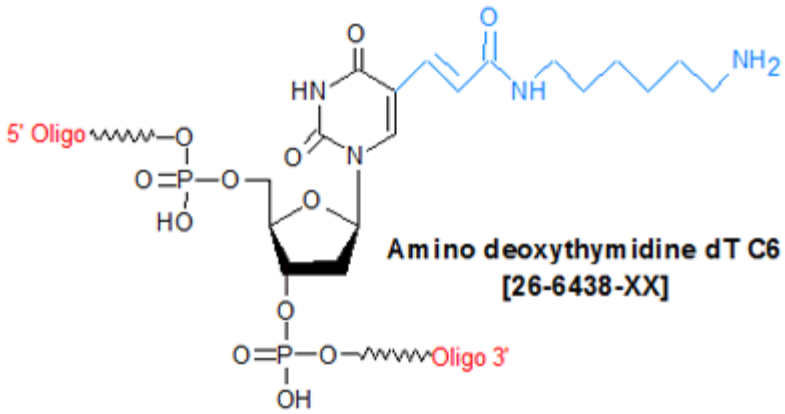
3 Prime	Y
Internal	Y
Molecular Weight(mw)	458.41



Amino deoxythymidine dT C6
[26-6438-XX]

Amino Serinol

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Reference Catalog Number	26-6715
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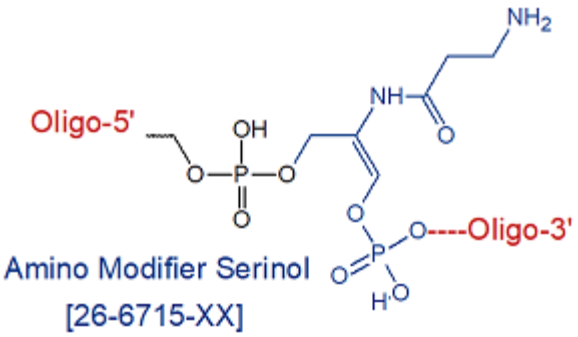
3 Prime	Y
Internal	Y
Molecular Weight(mw)	224.15



Amino Spacer 7 C6 Internal

Category	Conjugation Chemistry
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Reference Catalog Number	26-6560
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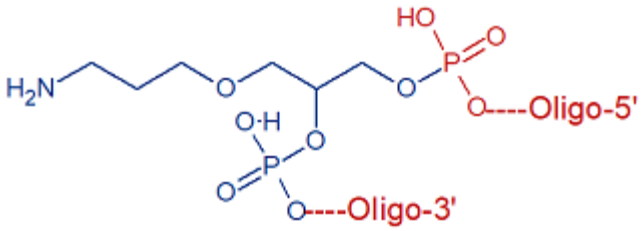
3 Prime	Y
Internal	Y
Molecular Weight(mw)	211.2



Amino Spacer7 C6 Internal
[26-6560]

Amino Spacer 7-C6 (3')

Category	Conjugation Chemistry
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Reference Catalog Number	26-6425
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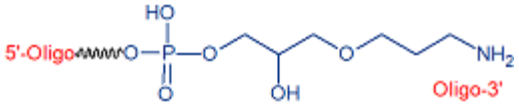
3 Prime	Y
Internal	Y
Molecular Weight(mw)	211.2



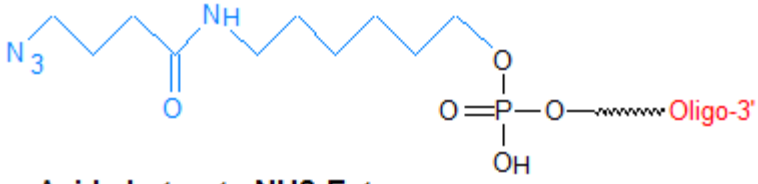
Amino Spacer 7 C6

[26-6425-XX]

Azide-C4 NHS (butyrate)

Category	Click Chemistry
Modification Code	N3-C4-N
Reference Catalog Number	26-6922
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	113.12

5' Amino Linker C6
[26-6418-XX]



Azide butyrate NHS Ester
[26-6922-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.
Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol * The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. * Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.
Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.

Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,
BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.

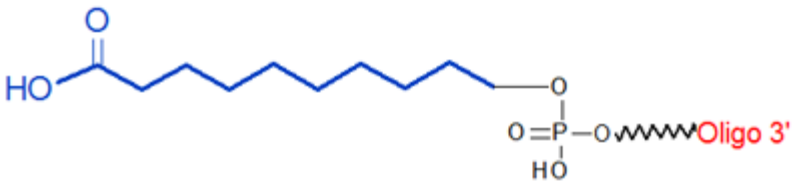
Azidobutyrate NHS ester can be used to introduce an active azide group to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C6 for the 5' end or amino C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azidobutyrate NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The presence of the azide allows the user to use "Click Chemistry" (a [3+2] cycloaddition reaction between alkynes and azides, using copper (I) iodide as a catalyst) to conjugate the azide-modified oligo to a terminal alkyne-modified oligo with extremely high regioselectivity and efficiency (1,2). Preparation of the alkyne-modified oligo can be achieved using the 5'-Hexynyl modifier (see its respective tech sheet for details). Click chemistry can be used to form short, cyclic oligos that can be used as research tools in various biophysical and biological studies (3). In particular, they have considerable potential for in vivo work, as cyclic oligos are known to be very stable in serum for up to several days.

References

1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
2. Rostovtsev, V.V., Green, L.G., Fokin, V.V., Sharpless, K.B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* (2002), **41**: 2596-2599.
3. Kumar, R., El-Sagheer, A., Tumpane, J., Lincoln, P., Wilhelmsson, L.M., Brown, T. Template-Directed Oligonucleotide Strand Ligation, Covalent Intramolecular DNA Circularization and Catenation Using Click Chemistry. *J. Am. Chem. Soc.* (2007), **129**: 6859-6864.

Carboxy-C10

Category	Conjugation Chemistry
Modification Code	CO-C10
Reference Catalog Number	26-6717
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	250.23

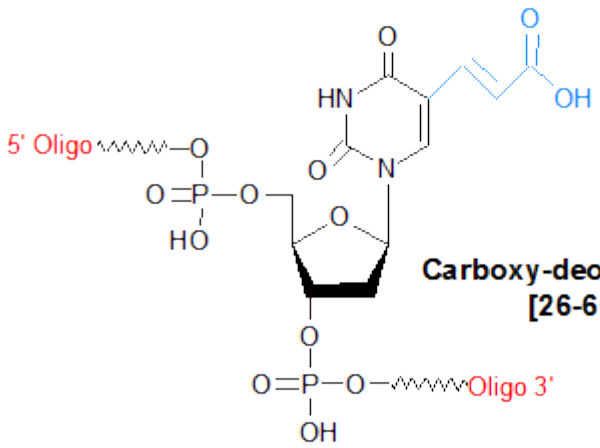


Carboxy C10 5'

[26-6717-XX]

Carboxy-dT

Category	Minor Bases
Modification Code	CO-dT
Reference Catalog Number	26-6697
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	360.22

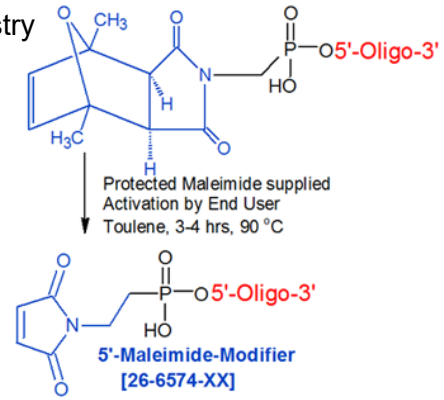


Carboxy-deoxythymidine dT
[26-6697-XX]

Oligo Modifications

Maleimide (5')

Category	Conjugation Chemistry
Modification Code	Mal
Reference Catalog Number	26-6574
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	203.09



MW Note: The molecular weight of 203.09 for maleimide is for the completely deprotected form after retro-Diels Alder reaction. Prior to retro-Diels Alder reaction the MW is 299.22. Gene Link provides maleimide modified oligos that requires retro-Diels Alder reaction by the user. See protocol below.

Maleimide Modifier (5') can be used to directly incorporate an active maleimide moiety onto the 5'-end of an oligonucleotide. The maleimide is separated from the 5'-end nucleotide base by a 2-carbon spacer arm to reduce steric interaction between it and the oligo. Maleimide-labeled oligos are typically used to form conjugates with thiol-labeled moieties, or Diels-Alder cycloaddition products.

As the maleimide moiety itself is not stable over the long-term, Gene Link provides lyophilized maleimido-oligos with all base protecting groups removed and as a maleimide-2,5-dimethylfuran cycloadduct.The cycloadduct serves to protect the maleimide from degradation. The customer then converts the cycloadduct to the active maleimide via a retro-Diels-Alder reaction using toluene and heat. The conversion protocol is provided with the modified oligo. If required the protected oligo should be aliquoted in smaller portions and stored dry.The retro-Diels-Alder reaction should be performed immediately prior to conjugation.

Deprotection by heating in Toluene

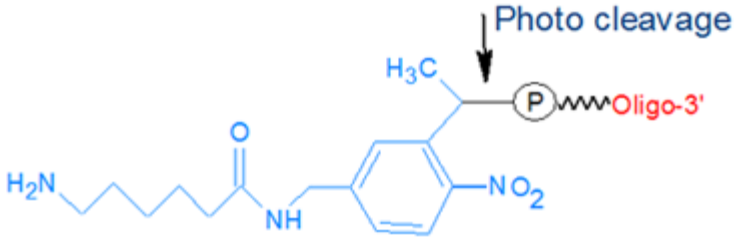
Gene Link provides the oligo in a dried lyophilized form. The maleimide is provided in a protected state and deprotection by Retro Diels-Alder reactions is required before use by the end user for conjugation.
The Retro Diels-Alder reactions involves dehydration by co-evaporation with anhydrous acetonitrile and anhydrous toluene. The Retro Diels-Alder reaction requires anhydrous conditions and any significant level of moisture can cause incomplete deprotection, hydrolysis, and/or addition of water to the maleimide. The evaporation of the toluene leaves a white residue ready for conjugation. A detailed procedure is shipped with the product. An abbreviated procedure is described below.

1. Suspend lyophilized oligo in 1 mL anhydrous Acetonitrile. It will be a suspension as oligo will NOT dissolve.
2. Evaporate using a speedvac.
3. Resuspend oligo in 1.5 mL Toluene.
4. Incubate for 4 hrs at 90^{O} C.
5. Cool to room temperature.
6. Evaporate toluene using a speedvac.

7. The oligonucleotide is now ready for conjugation.
Detailed deprotection Retro Diels-Alder reactions protocol Retro Diels-Alder Reaction protocol.

PC Amino C6 (Photocleavable)

Category	Photo Cleavable
Modification Code	PCAmC6
Reference Catalog Number	26-6690
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	371.32



PC Amino C6 (Photocleavable)
[26-6690-XX]

PC Amino C6 (photocleavable) is a non-nucleosidic moiety that can be used to incorporate a UV photo-cleavable primary amino group onto the 5' end of an oligonucleotide. The amino group is separated from the ' end nucleotide base by the photo-cleavable group and a 6-carbon spacer arm to minimize steric interaction between the amino group and the oligo (1). The presence of the amino group allows the user to end label the oligo with a variety of different affinity, reporter or protein moieties (as NHS esters or isothiocyanates), as well as immobilization on an activated solid support, depending on the application. Examples include biotin, digoxigenin, and fluorescent dyes or quenchers, enzymes (for example, alkaline phosphatase), and NHS-labeled beads. The photo-cleavable, located on the 5' phosphate can be selectively cleaved by illumination with UV light, thereby releasing the marker moiety or detachment of the oligo from the solid support (1). The released oligo will have a 5-phosphate.

An interesting application of PC Amino C6 is to use it to make a set of photo-cleavable peptide-DNA conjugates for use as photo-cleavable mass marker (PCMM) hybridization probes for detection of immobilized target DNA sequences by matrix-assisted laser desorption ionization (MALDI mass spectrometry (2). Here, a DNA oligonucleotide functions as the hybridization probe; the peptide is then released by UV photo-cleavage during the ionization/desorption phase of UV-MALDI, and thus serves as a specific marker (mass tag) that identifies the presence of the target sequence.

PC Amino C6 could also be used to create "caged" oligonucleotides, that is, oligonucleotides whose activity is suppressed until released by an external factor (such as UV light). Caging oligonucleotides (for example, tethering anti-sense or siRNA, via PC Amino C6, to a molecule that suppressed its activity) would provide new possibilities for controlling biological mechanisms (such as gene expression) in space and time (3).

Cleavage Protocol
Cleavage occurs by irradiation with near-UV light (300-350 nm, complete cleavage occurs within 5 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).
NHS ester-activated ligands react with primary amines to yield stable amide bonds. The reaction releases N-hydroxysuccinimide (NHS). NHS ester reaction scheme for chemical conjugation to a primary amine in an oligo is given below.

-

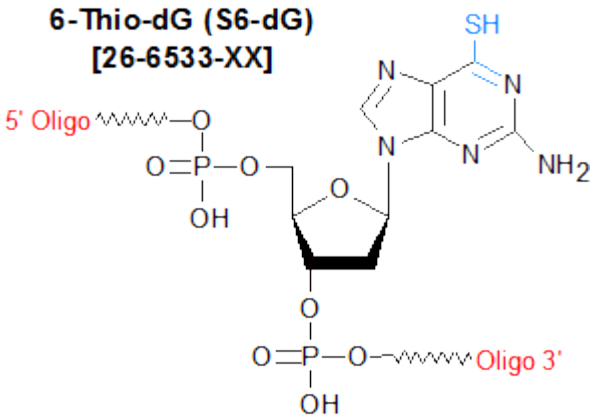
The primary amine labelled oligos can also be conjugated to carboxyl functional groups usually for solid supports applications using EDC mediated reaction as shown in the figure below.

References

1. Olejnik, J., Krzymanska-Olejnik, E., Rothschild, K.J. Photocleavable aminotag phosphoramidites for 5'-termini DNA/RNA labeling. *Nucleic Acids Res.* (1998), **26**: 3572-3576.
2. Olejnik, J., Ludemann, H-C., Olejnik, E.K, Berkenkamp, S., Hillenkamp, F., Rothschild, K.J. Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS. *Nucleic Acids Res.* (1999), **27**: 4626-4631.
3. Tang, X., Su, M., Yu, LiLi, Lv, C., Wang, J., Li, Z. Photomodulating RNA cleavage using photolabile circular antisense oligodeoxynucleotides.*Nucleic Acids Res.* (2002), **38**: 3848-3855.

Thio 6-dG (s6dG)

Category	Structural Studies
Modification Code	S6-dG
Reference Catalog Number	26-6533
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	345.26



6-Thio-deoxyGuanosine (6-Thio-dG) is a nucleoside that, when incorporated into either DNA or RNA in the cell, exhibits potent cytotoxicity. Such cytotoxicity is most likely due to the 6-Thio-dG either inducing strand breakage or cross-linking to both DNA and proteins (1). The cytotoxic properties of 6-Thio-dG make it an effective cytotoxic agent for treating human leukemias. Its ability to photochemically cross-link to both nucleic acids and proteins also make 6-Thio-dG-modified oligonucleotides desirable reagents for use in studying binding interactions between DNA and DNA-binding proteins. In one study, 6-Thio-dG was shown to efficiently cross-link with EcoRV endonuclease and methyltransferase (2). Cross-linking was achieved with 340 nm UV light; because this wavelength is considerably removed from the UV absorbance maxima of the natural bases (260 nm), cross-linking can be achieved without additional UV damage to the DNA.

6-Thio-dG can also be used to study the properties of G-rich triple-helix forming oligonucleotides. For example, substitution of 6-Thio-dG for some or all dGs in such oligos results in inhibition of both oligo self-association and G-quartet formation, thereby favoring normal formation of triple helices (3).

In addition, because the thiol group of 6-Thio-dG is active, incorporation of this modified nucleoside into an oligo also incorporates a reactive thiol at that position, which can be utilized to selectively alkylate the sulfur at that position (4).

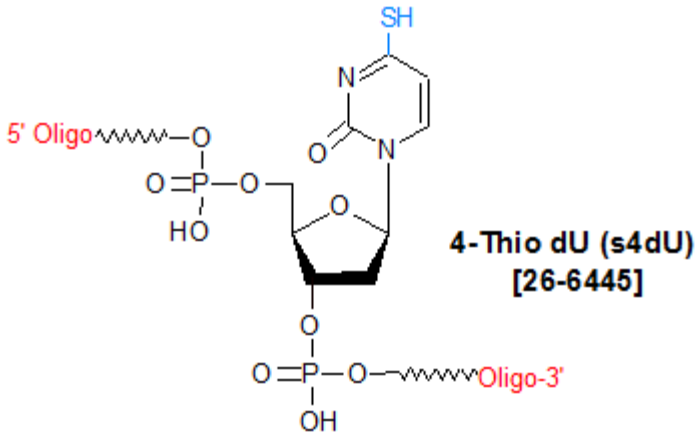
References

1. Christopherson, M.S., Broom, A.D. Synthesis of oligonucleotides containing 2'-deoxy-6-thioguanosine at a predetermined site. *Nucleic Acids Res.* (1991), **19**: 5719-5724.
2. Nikiforov, T.T., Connolly, B.A. Oligodeoxynucleotides containing 4-thiothymidine and 6-thiodeoxyguanosine as affinity labels for the Eco RV restriction endonuclease and modification methylase. *Nucleic Acids Res.* (1992), **20**: 1209-1214.
3. Rao, T.S., Durland, R.H., Seth, D.M., Myrick, M.A., Bodepudi, V., Revankar, G.R. Incorporation of 2'-Deoxy-6-thioguanosine into G-Rich Oligodeoxyribonucleotides Inhibits G-Tetrad Formation and Facilitates Triplex Formation.*Biochemistry* (1995), **34**: 765-772.
4. Coleman, R.S., Pires, R.M. Covalent cross-linking of duplex DNA using 4-thio-2'-deoxyuridine as a readily modifiable platform for introduction of reactive functionality into oligonucleotides.

Nucleic Acids Res. (1997), **25**: 4771-4777.

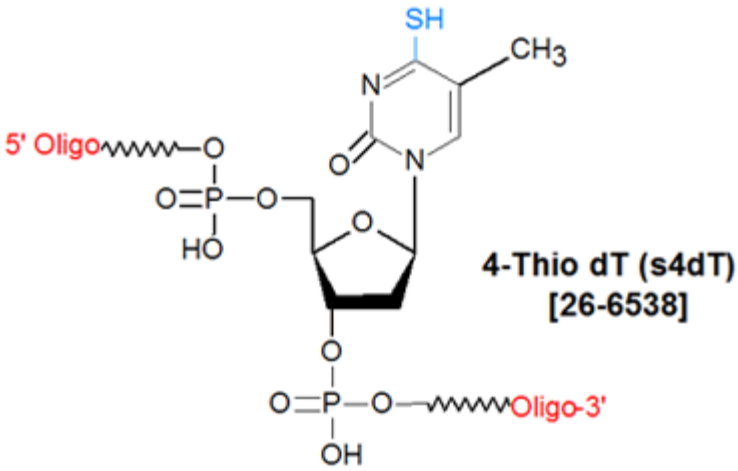
Thio-4 dU (s4dU)

Category	Others
Modification Code	s4dU
Reference Catalog Number	26-6445
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	306.23



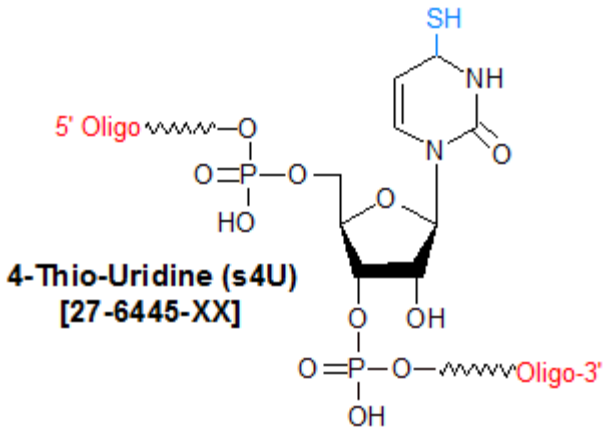
Thio-4-dT (S4dT)

Category	Structural Studies
Modification Code	S4dT
Reference Catalog Number	26-6538
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	320.26



Thio-4-rU (s4U)

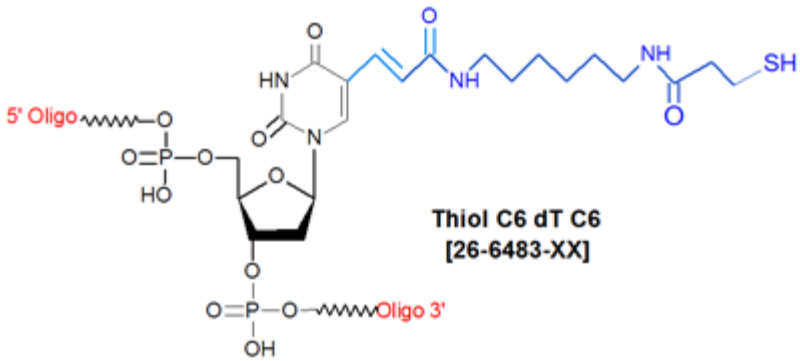
Category	Minor Bases
Modification Code	s4U
Reference Catalog Number	27-6445
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	322.22



Oligo Modifications

Thiol C6 dT

Category	Others
Modification Code	S-C6-dT
Reference Catalog Number	26-6483
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	546.53



Thiol-C6 dT is a thiol modifier designed to functionalize an oligonucleotide at an internal T position for conjugation to the desired solid support e.g gold. The resulting thiol group is separated from the oligo by a six-carbon spacer arm, to reduce steric interactions with the end of the oligo.

Thiolated oligonucleotides can be labeled with thiol-reactive dye / happen iodoacetamides or maleimides (for example, lucifer yellow iodoacetamide or fluorescein maleimide) for use as hybridization or PCR-based detection probes (1). They also can be conjugated to enzymes (for example, alkaline phosphatase or horseradish peroxidase), through bifunctional linkers (2). Finally, thiolated oligos can be attached to glass slides or gold surfaces for use in various microarray or nanoelectronic applications (3,4). However, because oligos labeled with only one thiol slowly dissociate from a gold surface at the temperatures (60C to 90C) and high salt concentrations commonly used to denature DNA duplexes (5), Gene Link recommends that researchers who plan to use such conditions to repeatedly strip and re-probe oligo arrays based on thiol-gold surface conjugation modify the oligos with DTPA, which incorporates two thiol groups into the oligo, thereby allowing for a more stable attachment to gold. For further information on DTPA, please see its technical sheet.

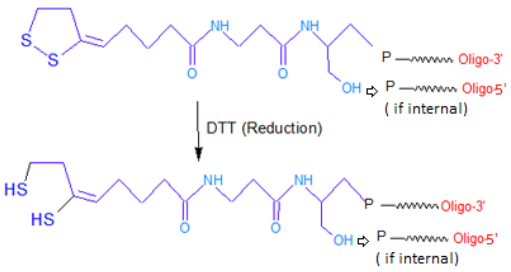
References

1. Connolly, B.A., Rider, P. Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specfic probes. *Nucleic Acids Res.* (1985), **13**: 4485-4502.
2. Ghosh, S.S, Kao, P.M., McCue, A.W., Chappelle, H.L. Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonuclotide-enzyme conjugate hybridization probes. *Bioconjugate Chem.* (1990), **1**: 71-76.
3. Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. *Anal. Biohem.* (1999), **266**: 23-30.
4. Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. *Proc. Natl. Acad. Sci. USA* (2005), **102**: 13383-13385.
5. Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates *Nucleic Acids Res.*

(2002), **30**: 1558-1562.

Thiol SS Dipod (DTSPA)

Category	Others
Modification Code	DTSPA
Reference Catalog Number	26-6573
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	412.46



Dithiol Serinol (Dipod Thiol; DTSPA)
[26-6573-XX]

Thiol SS Serinol Dipod DTSPA) is a disulfide-containing modifier designed to functionalize synthetic DNA or RNA with multiple thiol groups and can be incorporated at any position of the oligonucleotide. Each DTPA addition leads to two thiol groups. This modifier was designed for optimal tethering of oligonucleotides to a gold surface but it can also be used for multiple reactions with maleimides and other thiol-specific derivatives.
See Gene Link Manual for Gold Surface Conjugation for details Gold Surface Thiol Conjugation
After synthesis, Gene Link supplies the oligo to the customer in the **oxidized (disulfide)** form. The disulfide bond can then be reduced with TCEP or dithiothreitol (DTT) to generate the fully active thiolated oligo by the customer in his/her own laboratory.

SPECIAL NOTE. Prior to use, reduce any disulfide formation using 100 mM TCEP or DTT for 30 minutes at room temperature.TCEP 0.5M solutions is available from Gene Link, Catalog Number: 40-5116-10. TCEP use is recommended for reduction as conjugation efficiency are 2-3% higher if TCEP is used.

Thiol Reduction Protocol

Thiolated oligonucleotides can be labeled with thiol-reactive dye / happen iodoacetamides or maleimides (for example, lucifer yellow iodoacetamide or fluorescein maleimide) for use as hybridization or PCR-based detection probes (1). They also can be conjugated to enzymes (for example, alkaline phosphatase or horseradish peroxidase), through bifunctional linkers (2). Finally, thiolated oligos can be attached to glass slides or gold surfaces for use in various microarray or nanoelectronic applications (3,4). However, because oligos labeled with only one thiol slowly dissociate from a gold surface at the temperatures (60C to 90C) and high salt concentrations commonly used to denature DNA duplexes (5), Gene Link recommends that researchers who plan to use such conditions to repeatedly strip and re-probe oligo arrays based on thiol-gold surface conjugation modify the oligos with DTPA, which incorporates two thiol groups into the oligo, thereby allowing for a more stable attachment to gold. For further information on DTPA, please see its technical sheet.

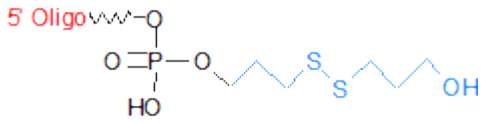
genelink.com/newsite/products/images/modificationimages/Thiol_Oligo_maleimide_Ligand_Conjugation.jpg">

References

1. Connolly, B.A., Rider, P. Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specfic probes. *Nucleic Acids Res.* (1985), **13**: 4485-4502.
2. Ghosh, S.S, Kao, P.M., McCue, A.W., Chappelle, H.L. Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonuclotide-enzyme conjugate hybridization probes. *Bioconjugate Chem.* (1990), **1**: 71-76.
3. Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. *Anal. Biohem.* (1999), **266**: 23-30.
4. Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. *Proc. Natl. Acad. Sci. USA* (2005), **102**: 13383-13385.
5. Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates *Nucleic Acids Res.* (2002), **30**: 1558-1562.

Thiol SS-C3 3'

Category	Conjugation Chemistry
Modification Code	SS-C3-3
Reference Catalog Number	26-6482
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	154.12



3' Thiol C3 SS
[26-6482-XX]

Note: Prior to use, reduce any disulfide formation using 100 mM TCEP or DTT for 30 minutes at room temperature.TCEP 0.5M solutions is available from Gene Link, Catalog Number: 40-5116-10. TCEP use is recommended for reduction as conjugation efficiency are 2-3% higher if TCEP is used. TCEP 0.5M solution
Thiol Reduction Protocol

Thiol-SS-C3 is a disulfide-containing modifier designed to functionalize an oligonucleotide with a reactive thiol (sulfhydryl) group at the 5' or 3'-end. This modification is incorporated into the oligonucleotide as a disulfide during oligonucleotide synthesis, in order to protect the thiol group from undesired side reactions. The oligo is supplied as a disulfide and the customer has to reduce it with TCEP or DTT or other reducing agent to generate the reduced thiol group. The thiol group is separated from the oligo by a three-carbon spacer arm, to reduce steric interactions with the end of the oligo.

Thiolated oligonucleotides can be labeled with thiol-reactive dye / happen iodoacetamides or maleimides (for example, lucifer yellow iodoacetamide or fluorescein maleimide) for use as hybridization or PCR-based detection probes (1). They also can be conjugated to enzymes (for example, alkaline phosphatase or horseradish peroxidase), through bifunctional linkers (2). Finally, thiolated oligos can be attached to glass slides or gold surfaces for use in various microarray or nanoelectronic applications (3,4). However, because oligos labeled with only one thiol slowly dissociate from a gold surface at the temperatures (60C to 90C) and high salt concentrations commonly used to denature DNA duplexes (5), Gene Link recommends that researchers who plan to use such conditions to repeatedly strip and re-probe oligo arrays based on thiol-gold surface conjugation modify the oligos with DTPA, which incorporates two thiol groups into the oligo, thereby allowing for a more stable attachment to gold. For further information on DTPA, please see its technical sheet.

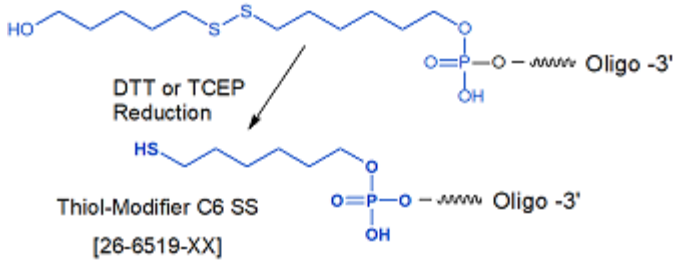
References

1. Connolly, B.A., Rider, P.

Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specfic probes. *Nucleic Acids Res.* (1985), **13**: 4485-4502.
2. Ghosh, S.S, Kao, P.M., McCue, A.W., Chappelle, H.L. Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonuclotide-enzyme conjugate hybridization probes. *Bioconjugate Chem.* (1990), **1**: 71-76.
3. Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. *Anal. Biohem.* (1999), **266**: 23-30.
4. Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. *Proc. Natl. Acad. Sci. USA* (2005), **102**: 13383-13385.
5. Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates *Nucleic Acids Res.* (2002), **30**: 1558-1562.

Thiol SS-C6

Category	Conjugation Chemistry
Modification Code	SS-C6
Reference Catalog Number	26-6419
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	196.2



Note 1: Thiol-SS-C6 when incorporated internally will cleave the oligo upon reduction at the S-S site.
Note 2: The molecular weight added to the oligo mw calculation for thiol modification is 196.2 for the reduced form. The oligo is supplied in the unreduced for with disulfide thiol mw of 328.4. The oligo with the unreduced form of disulfide is for the end user to perform the reduction before use.
Prior to use, reduce any disulfide formation using 100 mM TCEP or DTT for 30 minutes at room temperature.TCEP 0.5M solutions is available from Gene Link, Catalog Number: 40-5116-10. TCEP use is recommended for reduction as conjugation efficiency are 2-3% higher if TCEP is used. TCEP 0.5M solution
Thiol Reduction Protocol

Thiol-SS-C6 is a disulfide-containing modifier designed to functionalize an oligonucleotide with a reactive thiol (sulfhydryl) group at the 5'- or 3'-end, or an internal disulfide linkage. This modification is incorporated into the oligonucleotde as a disulfide during oligonucleotide synthesis, in order to protect the thiol group from undesired side reactions. After synthesis, Gene Link normally supplies the oligo to the customer in the **oxidized (disulfide)** form. The disulfide bond can then be reduced with TCEP or dithiothreitol (DTT) to generate the fully active thiolated oligo by the customer in his/her own laboratory. The resulting thiol group is separated from the oligo by a six-carbon spacer arm, to reduce steric interactions with the end of the oligo. Note that if this modification is incorporated internally, it can effectively serve as a C12 spacer between two oligos, with a disulfide linkage in the middle. **Reduction of the disulfide bond will generate two separate 3'- and 5'-thiolated oligos, respectively.**

Thiolated oligonucleotides can be labeled with thiol-reactive dye / happen iodoacetamides or maleimides (for example, lucifer yellow iodoacetamide or fluorescein maleimide) for use as hybridization or PCR-based detection probes (1). They also can be conjugated to enzymes (for example, alkaline phosphatase or horseradish peroxidase), through bifunctional linkers (2).

Finally, thiolated oligos can be attached to glass slides or gold surfaces for use in various microarray or nanoelectronic applications (3,4). However, because oligos labeled with only one thiol slowly dissociate from a gold surface at the temperatures (60C to 90C) and high salt concentrations commonly used to denature DNA duplexes (5), Gene Link recommends that researchers who plan to use such conditions to repeatedly strip and re-probe oligo arrays based on thiol-gold surface conjugation modify the oligos with DTPA, which incorporates two thiol groups into the oligo, thereby allowing for a more stable attachment to gold. For further information on DTPA, please see its technical sheet.

References

1. Connolly, B.A., Rider, P. Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specfic probes. *Nucleic Acids Res.* (1985), **13**: 4485-4502.
2. Ghosh, S.S, Kao, P.M., McCue, A.W., Chappelle, H.L. Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonuclotide-enzyme conjugate hybridization probes. *Bioconjugate Chem.* (1990), **1**: 71-76.
3. Roger, Y-H., Jiang-Baucom, P., Huang, Z-J., Bogdanov, V., Anderson, S., Boyce-Jacino, M.T. Immobilization of oligonucleotides onto a glass support via disulfide bonds: A method for preparation of DNA microarrays. *Anal. Biohem.* (1999), **266**: 23-30.
4. Ackerson, C.J., Sykes, M.T., Kornberg, R.D. Defined DNA/nanoparticle conjugates. *Proc. Natl. Acad. Sci. USA* (2005), **102**: 13383-13385.
5. Li, Z., Jin, R., Mirkin, C.A., Letsinger, R.L. Multiple thiol-anchor capped DNA-gold nanoparticle conjugates *Nucleic Acids Res.* (2002), **30**: 1558-1562.