Click Chemistry Introduction

'Click chemistry' was defined by Sharpless and co-workers in 2001 (1) to describe a set of powerful, highly reliable and selective organic reactions which can be used for the rapid and facile synthesis of useful new compounds and combinatorial libraries. Each of these compounds is composed of small, modular sub-units stiched together through heteroatom linkages (C-X-C). Click chemistry reactions are simple, modular, stereospecific, very high yielding, wide in scope, can be conducted in benign/easily removable solvents, and generate side products which are easily removable by non-chromatographic methods. The primary driving force behind the development of click chemistry is the pharmaceutical industry's need to generate very large combinatorial libraries of small-molecule (< 500 Dalton) compounds that can be screened as drug candidates. Click chemistry has the potential to accelerate the drug discovery process, as it makes each reaction in the multi-step synthesis of a small molecule fast, efficient and predictable.

Although there are several types of organic reactions that fit the definition of click chemistry, for modification of oligonucleotides, the relevant one is the copper(I)-catalyzed [3+2] cycloaddition reaction between alkynes and azides (1,2). This reaction is extremely selective and regiospecific for conjugation reactions involving an oligo and a labeling moiety, as well as coupling reactions between two oligos. More detailed descriptions of specific click chemistry applications are provided in 'Click Chemistry Applications'.

Click Chemistry Design Protocols

This section contains an example of a Copper(I)-catalyzed click reaction. This protocol may be used as a starting point for optimization of your particular click chemistry procedures.

I. Preparation of the 'Click Solution'

1. NOTE: The 'click solution' (0.1 M CuBr / 0.1 M TBTA 1:2 (v/v) in DMSO/t-BuOH 3:1 (v/v)) must always be freshly prepared prior to use!
2.Dissolve 1 mg CuBr in 70 µl DMSO/t-BuOH 3:1 (v/v) to obtain a 0.1 M solution. This solution must be freshly prepared and cannot be stored.
3.Dissolve 54 mg TBTA in 1 ml DMSO/t-BuOH 3:1 (v/v) for a 0.1 M solution. This solution can be stored at -20C.
4.Add 1 volume of the 0.1 M CuBr solution quickly to 2 volumes of the 0.1 M TBTA solution to obtain the click solution, which is ready to use.

II. Click Procedure for Short DNA Oligos

Procedure using CuBr: To 5 µl of a 2 mM DNA solution (10 nmol) in water, 2 µl of an azide solution (50 mM, 50 nmol, 5 eq. in DMSO or in 3:1 (v/v) DMSO/t-BuOH), 3 µl of a freshly prepared solution containing 0.1 M CuBr and 0.1 M TBTA ligand in a 1:2 (v/v) ratio in 3:1 (v/v) DMSO/t-BuOH is added. The mixture is thoroughly mixed and shaken at 25C for 3 h. The reaction is subsequently diluted with 0.3 M NaOAc (100 µl) and the DNA is precipitated using 1 ml cold EtOH. The supernatant is then removed and the residue is washed twice with 1 ml cold EtOH. The washed residue is re-dissolved in pure water (20 µl) and can be used without further purification.

Click Chemistry Applications

Although the copper(I)-catalyzed alkyne-azide [3+2] cycloaddition reaction has many potential uses as a method for synthesis of unique oligo-based research tools, two specific applications currently dominate, (A) conjugation of anti-sense/siRNA oligonucleotides to cell-penetrating peptides (CPP), and (B) labeling of oligonucleotides with biotin and/or fluorescent dyes.

The efficacy of anti-sense and siRNA oligos in vivo is severely limited due to their inability to cross the cell membrane (3). One method for significantly increasing cell uptake of these oligos is to covalently conjugate cell penetrating peptides (CPP) to the oligo, most commonly through amide or disulfide linkages (4,5). While relatively straightforward to perform, such conjugations show wide variability in final yield, and often require complex and/or multiple purification. In addition, if the peptide is highly cationic, achieving a successful conjugation reaction can itself be problematic, due to non-specific binding between the cationic peptide and the anionic phosphate backbone of the oligo (6). Using click chemistry to form the CPP-oligo conjugate by linking an azidopeptide to an alkyne-modified oligo can provide an effective solution to these problems, as yield is essentially quantitative, and because the conjugation can be performed on a solid support, the need for expensive, multi-step purification is often eliminated (7-9). Please see the references provided for more details.

Similar advantages (quantitative yield, simple purification) favor the use of click chemistry for labeling oligos with such moieties as biotin, fluorescent dyes, and haptens (10-12). In all these cases, the oligo is alkyne-modified and the labels all contain an active azide group. A number of azide-modified labels are available, including biotin, desthiobiotin, 6-FAM, HEX and TET, among others. Additional labels will become available over time.

References

1. 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.
2. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
3. Akhtar, S., Hughes, M.D., Khan, A., Bibby, M., Hussain, M., Nawaz, Q., Double, J., Sayyed, P. The delivery of antisense therapeutics. *Adv. Drug. Deliv. Rev.* (2000), **44**: 3-21.
4. Lundin, P., Johansson, H., Guterstam, P., Holm, T., Hansen, M., Langel, A., El Andaloussi, S. Distinct uptake routes of cell-penetrating peptide conjugates. *Bioconjug. Chem.* (2008), **19**: 2535-2542.
5. Frederic, H., May Catherine, M., Gilles, D., Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. *Br. J. Pharmacol.* (2009), **157**: 195-206.
6. Lu, K., Duan, Q-P., Ma, L., Zhao, D-X. Chemical strategies for the synthesis of peptide-oligonucleotide conjugates. *Bioconjug. Chem.* (2010), **21**: 187-202.
7. Gogoi, K., Mane, M.V., Kunte, S.S., Vaijayanti, A.K. A versatile method for the preparation of conjugates of peptides with DNA/PNA/analog by employing chemo-selective click reaction in water. *Nucleic Acids Res.*, (2007), **35**: e139.
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9. Wenska, M., Alvira, M., Steunenberg, P., Stenberg, A., Murtola, M., Stromberg, R. An activated triple bond linker enables 'click' attachment of peptides to oligonucleotides on solid support. *Nucleic Acids Res.*, (2011), **39**: 9047-9059.
10. Seo, T.S., Li, Z., Ruparel, H., Ju, J. Click chemistry to construct fluorescent oligonucleotides for DNA sequencing. *J. Org. Chem.*, (2003), **68**: 609-612.
11. Hall, L.M., Gerowska, M., Brown, T. A highly fluorescent DNA toolkit: synthesis and properties of oligonucleotides containing new Cy3, Cy5 and Cy3B monomers. *Nucleic Acids Res.*, (2012), **40**: e108.
12. Wengel, J., Astakhova, I.K. Interfacing click chemistry with automated oligonucleotide synthesis for the preparation of fluorescent DNA probes containing internal xanthene and cyanine dyes. *Chemistry*, (2013), **19**: 1112-1122.

Modificaton Code List

Modification	Code	Catalog Number
3'-O-propargyl/Alkyne A 2'-5' linked	[PPG-3-O-A]	27-6919A
3'-O-propargyl C/Alkyne C 2'-5' linked	[PPG-3-O-C]	27-6919C
3'-O-propargyl/Alkyne G 2'-5' linked	[PPG-3-O-G]	27-6919G
3'-O-propargyl/Alkyne U 2'-5' linked	[PPG-3-O-U]	27-6919U
5-Ethynyl-dU (Alkyne 5EdU)	[5Ethynyl-dU]	26-6629
5-Ethynyl-dU TIPS (Alkyne 5EdU TIPS)	[5E-dU-TIPS]	26-6615
Alkyne C8 dC (5-Octadiynyl-dC)	[Alk-C8-dC]	26-6995
Alkyne C8 dT (5-Octadiynyl-dT)	[Alk-C8-dT]	26-6996
Alkyne Photocleavable NHS	[Alk-PC-N]	26-6753
Alkyne-Propargyl PEG4 N Oligo	[Alk-PEG4-N]	26-6752
Alkyne-Modifier Serinol	[Alk-Ser]	26-6925
Alkyne-C2-(Propargyl-PEG1) NHS	[Alk-C2-N]	26-6924
Alkyne-C3 (3')	[Alk-C3]	26-6739
Alkyne-PEG4-Maleimide	[Alk-PEG4-Mal]	26-6764
Azide C3 3'	[N3-C3-3]	26-6720
Azide C6 (5')	[N3-C6-5]	26-6718
Azide dA (8-Azide dA)	[N3-dA]	26-6765A
Azide dC (5-Azide dC)	[N3-dC]	26-6765C
Azide dG (8-Azide dG)	[N3-dG]	26-6765G
Azide dT (5')	[N3-dT]	26-6719

Azide dU (5-Azide dU)	[N3-dU]	26-6765U
Azide Photocleavable NHS	[N3-PC-N]	26-6755
Azide PEG3 Maleimide Oligo	[N3-PEG3-Mal]	26-6761
Azide PEG4 N Oligo	[N3-PEG4-N]	26-6754
Azide rA (8-Azide rA)	[N3-rA]	27-6765A
Azide rC (5-Azide rC)	[N3-rC]	27-6765C
Azide rG (5-Azide rG)	[N3-rG]	27-6765G
Azide rU (5-Azide rU)	[N3-rU]	27-6765U
Azide-C2 NHS	[N3-C2-N]	26-6741
Azide butyrate N	[N3-C4-N]	26-6922
Azide-C6 NHS	[N3-C6-N]	26-6740
Azide-Picolyl PEG4 N Oligo	[N3-PIC-PEG4-N]	26-6798
BCN Endo 5' (Bicyclononyne) 5'	[BCN-5]	26-6771
BCN Endo Internal	[BCN-Int]	26-6771I
BCN Endo N	[BCN-N]	26-6777
BCN Endo PEG2 N	[BCN-PEG2-N]	26-6778
BCN Endo PEG4 N	[BCN-PEG4-N]	26-6779
BCN-3' (Bicyclononyne) 3'	[BCN-3]	26-6743
BiotinTEG Azide	[Bio-TEG-N3]	26-6721
Coumarin Azide	[Cou-N3]	26-6726
DBCO Photocleavable NHS	[DBCO-PC-N]	26-6744

DBCO PEG13 NHS	[DBCO-PEG13-N]	26-6746
DBCO PEG4 NHS	[DBCO-PEG4-N]	26-6745
DBCO Serinol	[DBCO-Ser]	26-6736
DBCO-C2 NHS	[DBCO-C2-N]	26-6742
DBCO-C6 NHS	[DBCO-C6-N]	26-6929
DBCO-dT	[DBCO-dT]	26-6927
DBCO-Maleimide	[DBCO-Mal]	26-6760
DBCO-TEG (5')	[DBCO-TEG]	26-6928
DesthiobiotinTEG Azide	[DesBioTEG-N3]	26-6725
Ethynyl-dSpacer (Alkyne, 1- Ethynyl dSpacer)	[Ethynyl-dABS]	26-6737
Fam-TEG Azide	[Fam-TEG-N3]	26-6722
Hex-Azide-6	[Hex-N3]	26-6723
Iodo-dT-5'	[I-dT]	26-6926
Methylene Blue (MB2-Azide)	[MB-N3]	26-6988
Propargyl/Alkyne-3'-O-5-Me-dC	[PPG-3-O-5me-dC]	26-6946
TCO NHS Oligo	[TCO-N]	26-6756
TCO-C6	[TCO-C6]	26-6797
TCO-PEG12 5' Oligo	[TCO-PEG12-5]	26-6759F
TCO-PEG12 N Oligo	[TCO-PEG12-N]	26-6759
TCO-PEG3 Maleimide Oligo	[TCO-PEG3-Mal]	26-6763
TCO-PEG4 Oligo	[TCO-PEG4-N]	26-6757

Tet Azide	[Tet-N3]	26-6724
Tetrazine methyl Oligo	[meTz-N]	26-6758
Tetrazine methyl Photocleavable Oligo	[meTz-PC-N]	26-6750
Tetrazine methyl PEG12 NHS Oligo	[meTz-PEG12-N]	26-6794
Tetrazine Methyl PEG4 Maleimide Oligo	[meTzPEG4-Mal]	26-6762
Tetrazine methyl PEG4 Oligo	[meTz-PEG4-N]	26-6749
Tetrazine methyl Sulfo Oligo	[me-Tz-Sulfo-N]	26-6796
Tetrazine-PEG5 Oligo	[Tz-PEG5-N]	26-6748
Tetrazine-Sulfo Oligo	[Tz-Sulfo-N]	26-6747

3'-O-propargyl A 2'-5' linked

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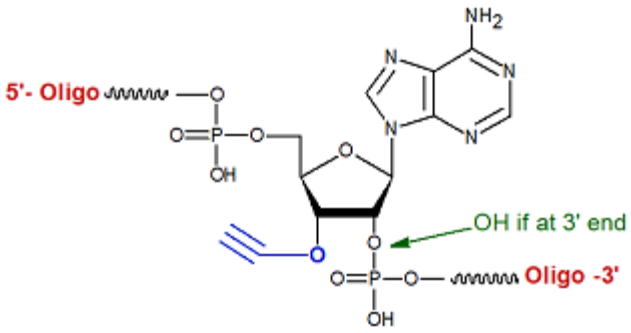

3'-O Propargyl A 2'-5 linked
[27-6919A-XX]

3'-O-propargyl C 2'-5' linked

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Reference Catalog Number	27-6919C
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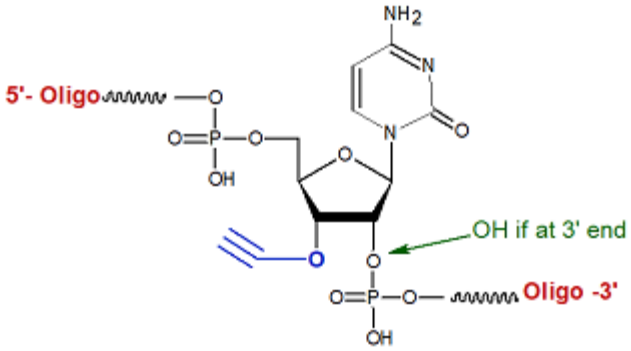
Internal	Y
Molecular Weight(mw)	330.2



3'-O Propargyl C 2'-5 linked
[27-6919C-XX]

3'-O-propargyl G 2'-5' linked

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Internal	Y
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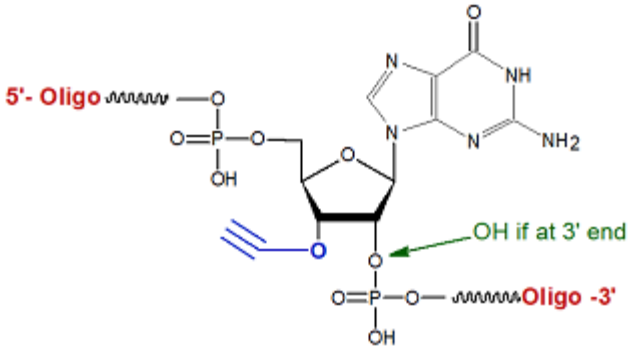

3'-O Propargyl G 2'-5 linked
[27-6919G-XX]

3'-O-propargyl U 2'-5' linked

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Internal	Y
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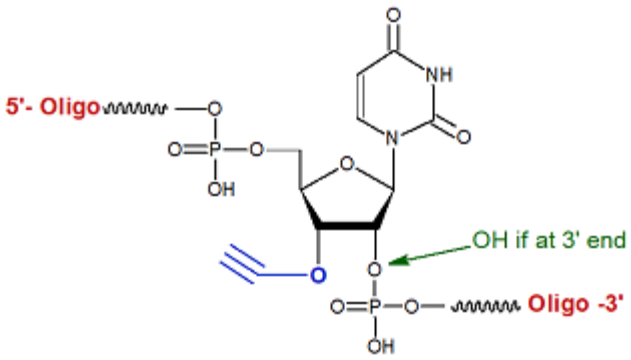

3'-O Propargyl U 2'-5 linked
[27-6919U-XX]

5-Ethynyl-dU (Alkyne 5EdU)

Category	Click Chemistry
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Reference Catalog Number	26-6629
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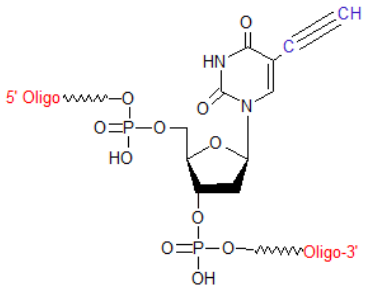
3 Prime	Y
Internal	Y
Molecular Weight(mw)	314.19



5-Ethynyl-dU (5EdU)
[26-6629-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

5-Ethynyl-dU offers convenient click conjugation with an azide to generate a label rigidly attached to one of the oligonucleotide bases. The alkyne group is separated from the oligo by an 11-atom spacer arm, 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.

Intellectual Property. baseclick GmbH has been granted the following patents (1-3) besides its further patent applications (4-5). |1. WO 2006/117161 (New labelling strategies for the sensitive detection of analytes)|2. WO 2008/952775 (Click chemistry for the production of reporter molecules)|3. WO 2010/115957 (Click Chemistry on heterogeneous catalysts)|4. PCT/EP 2013/064610 (Anandamide-modified nucleic molecules)|5. PCT/EP 2015/056007 (Self-assembly of DNA Origami: a diagnostic tool)|baseclick GmbH holds a worldwide exclusive license for granted patent application|WO 03/101972 (Copper-catalysed ligation of azides and acetylenes for the nucleic acid field) in the area of diagnostics and research. As Glen Research and baseclick are partners, Glen Research is now able to help in sublicensing this outstanding technology. Gene Link purchases this product from Glen Research for custm oligo synthesis.

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.

5-Ethynyl-dU TIPS (Alkyne 5EdU TIPS)

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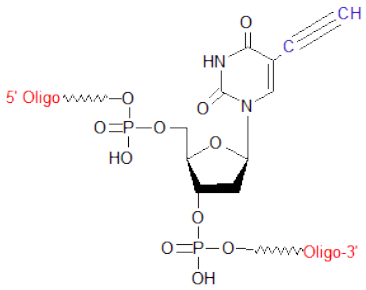
3 Prime	Y
Internal	Y
Molecular Weight(mw)	314.19



5-Ethynyl-dU (5EdU)
[26-6615-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

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References

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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.

Alkyne C8 dC

Category	Click Chemistry
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Reference Catalog Number	26-6995
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3 Prime	Y
Internal	Y
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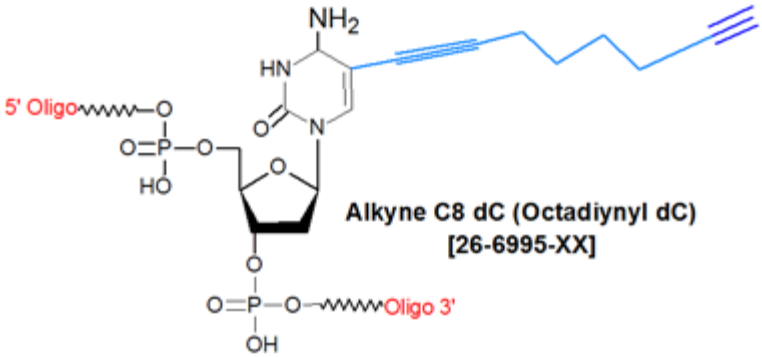

Alkyne C8 dT

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3 Prime	Y
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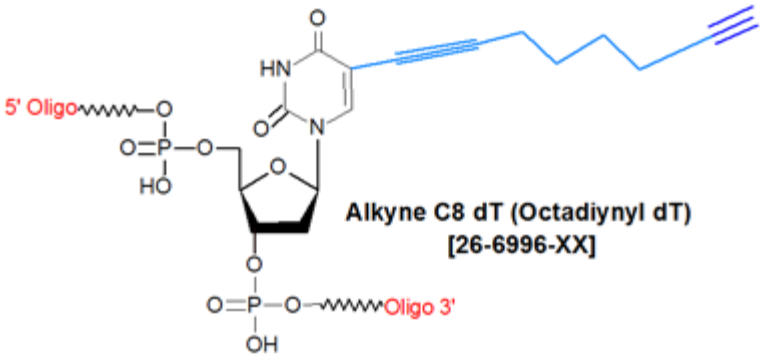

Alkyne C8 dT (Octadiynyl dT)
[26-6996-XX]

Alkyne PC NHS

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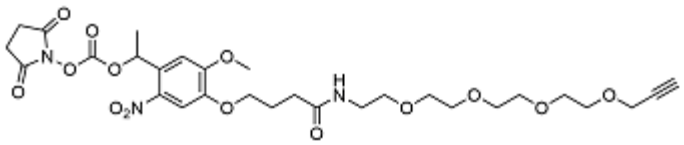
3 Prime	Y
Internal	Y
Molecular Weight(mw)	302.11



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.

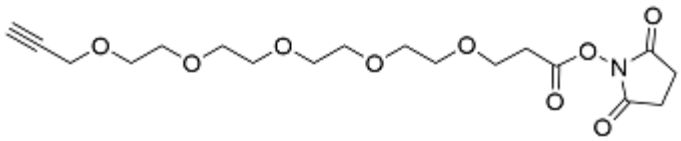
Photo Cleavage Protocol Cleavage occurs by irradiation with near-UV light (300-350 nm, >90% cleavage occurs within 5-25 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).

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.

Alkyne PEG4 NHS

Category	Click Chemistry
Modification Code	Alk-PEG4-N
Reference Catalog Number	26-6752
5 Prime	Y
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.
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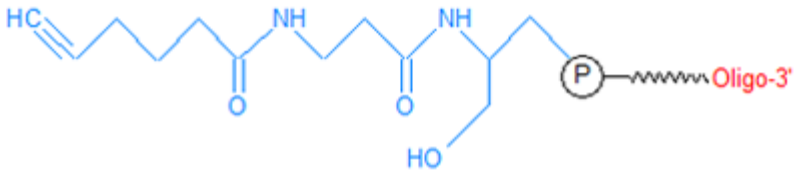
Alkyne Serinol

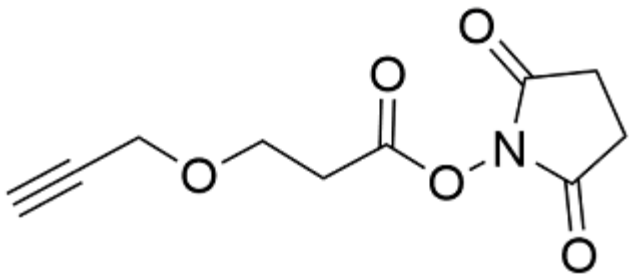
Category	Click Chemistry
Modification Code	Alk-Ser
Reference Catalog Number	26-6925
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	334.26



Alkyne-Modifier Serinol
[26-6925-XX]

Alkyne-C2 N

Category	Click Chemistry
Modification Code	Alk-C2-N
Reference Catalog Number	26-6924
5 Prime	Y
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.

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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.

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References

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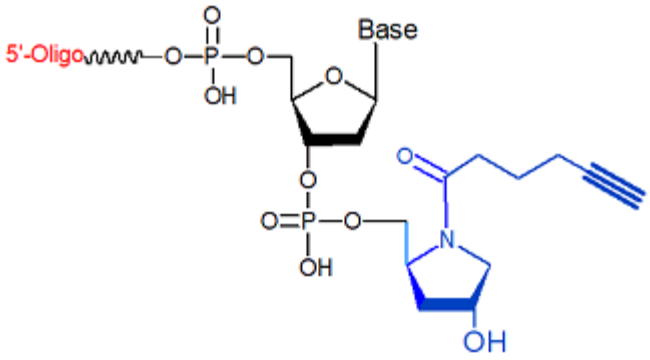
Alkyne-C3

Category	Click Chemistry
Modification Code	Alk-C3
Reference Catalog Number	26-6739
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	273.1

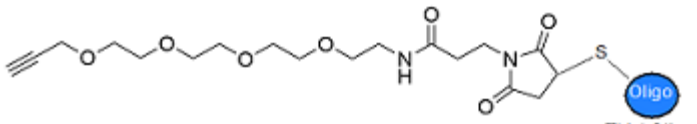


Alkyne C3 (for 3')

[26-6739-XX]

Alkyne-PEG4-Maleimide

Category	Click Chemistry
Modification Code	Alk-PEG4-Mal
Reference Catalog Number	26-6764
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	382.41



Alkyne PEG4 Maleimide Oligo

[26-6764-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

This modification is a post synthesis maleimide conjugation to a reduced thiol amino group thus an additional modification with thiol group is required. A C3 or C6 thiol group can be placed at the 5' or for internal positions Thiol C6 dT modified base is used.
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 PEG4 Maleimide 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 a thiol group. 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

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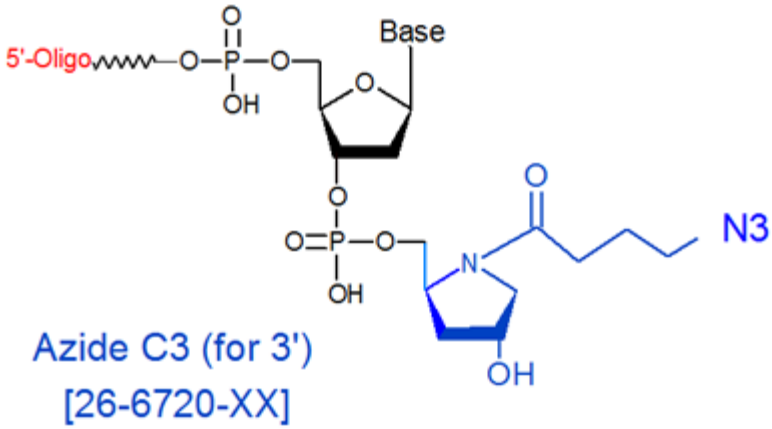
Azide C3 3'

Category	Click Chemistry
Modification Code	N3-C3-3
Reference Catalog Number	26-6720
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	202.25



Click here for a complete list of Click Chemistry Oligo Modifications

Copper-free Click Chemistry Modifications

Use azide modified oligos with DBCO Cyclooctyne-based modifications for ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

Azide C3 is available to introduce a stable azide group at the 3' of an oligo. Use Azide butyrate NHS [26-6922] for introduction of azide at internal or 5' position by conjugating 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.

png">
References

1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2]**: 565-568.
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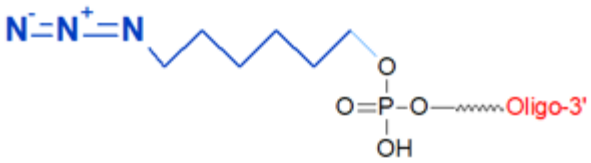
Azide C6 (5')

Category	Click Chemistry
Modification Code	N3-C6-5
Reference Catalog Number	26-6718
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	205.15



Azide C6 (5')
[26-6718-XX]

Copper-free Click Chemistry Modifications

Use azide modified oligos with DBCO Cyclooctyne-based modifications for ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

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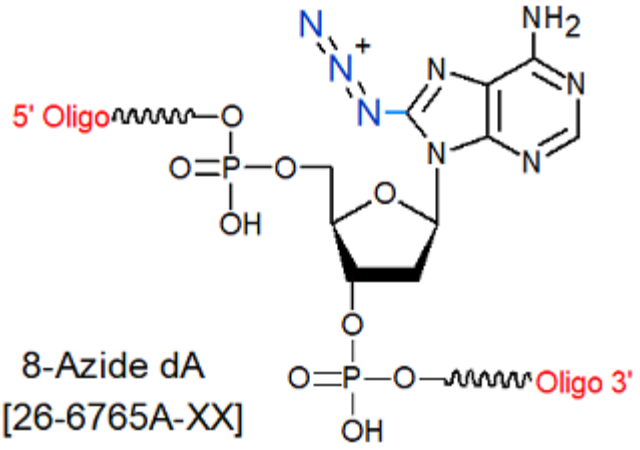
Azide dA

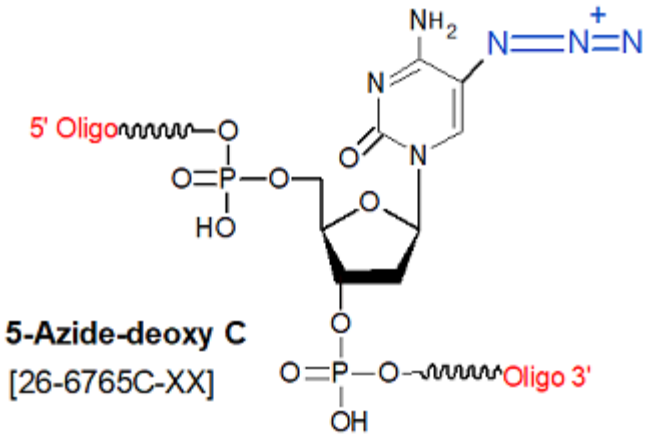
Category	Click Chemistry
Modification Code	N3-dA
Reference Catalog Number	26-6765A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	354.23



8-Azide dA
[26-6765A-XX]

Azide dC

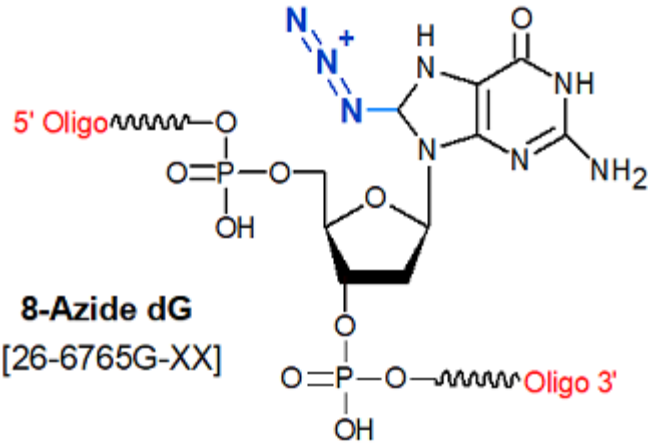
Category	Click Chemistry
Modification Code	N3-dC
Reference Catalog Number	26-6765C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	330.2



5-Azide-deoxy C
[26-6765C-XX]

Azide dG

Category	Click Chemistry
Modification Code	N3-dG
Reference Catalog Number	26-6765G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	370.22

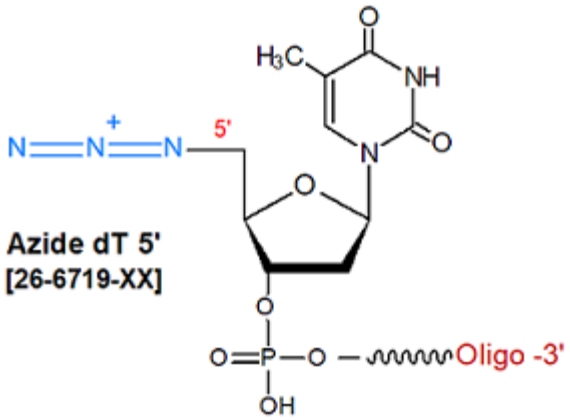


8-Azide dG

[26-6765G-XX]

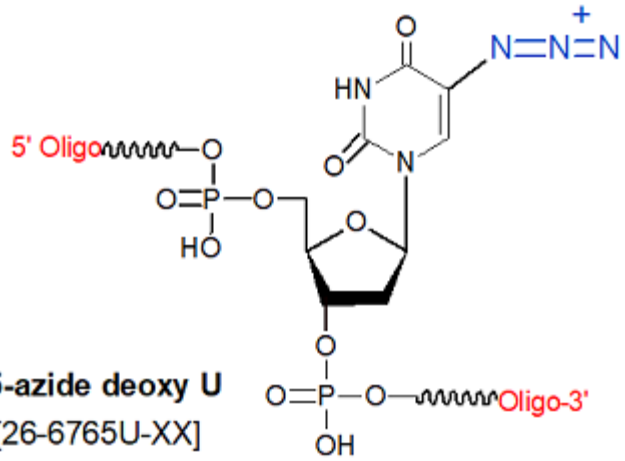
Azide dT (5')

Category	Click Chemistry
Modification Code	N3-dT
Reference Catalog Number	26-6719
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	329.21



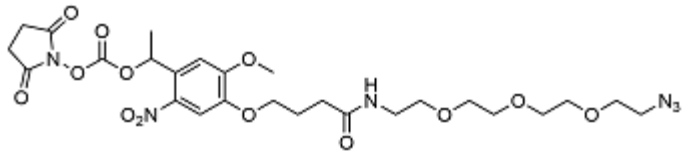
Azide dU

Category	Click Chemistry
Modification Code	N3-dU
Reference Catalog Number	26-6765U
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	331.19



Azide PC NHS

Category	Click Chemistry
Modification Code	N3-PC-N
Reference Catalog Number	26-6755
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	85



Click here for a complete list of Click Chemistry Oligo Modifications

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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.

Azide PC 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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.

Photo Cleavage Protocol Cleavage occurs by irradiation with near-UV light (300-350 nm, >90% cleavage occurs within 5-25 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).

References

1. Huisgen, R. *Angew. Chem. Int. Ed.* (1963), **2**: 565-568.
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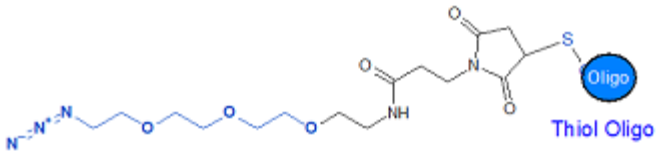
Azide PEG3 Maleimide

Category	Click Chemistry
Modification Code	N3-PEG3-Mal
Reference Catalog Number	26-6761
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	369.37



Azide PEG3 Maleimide Oligo
[26-6761-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

This modification is a post synthesis maleimide conjugation to a reduced thiol amino group thus an additional modification with thiol group is required. A C3 or C6 thiol group can be placed at the 5' or for internal positions Thiol C6 dT modified base is used.
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.

Azide PEG3 Maleimide can be used to introduce an active azide group to a thiol-modified oligonucleotide. The Azide C2 PEG3 Maleimide is then manually attached to the oligo through the thiol 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.

Azide PEG4 NHS

Category	Click Chemistry
Modification Code	N3-PEG4-N
Reference Catalog Number	26-6754
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	274.3

Azide PEG4 N Oligo
[26-6754-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.

Azide PEG4 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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

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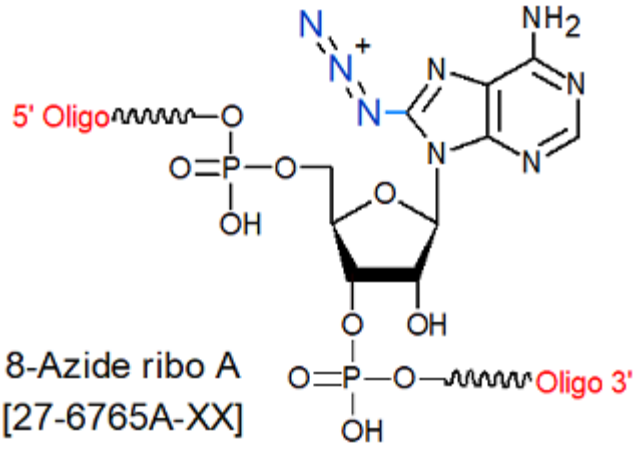
Azide rA

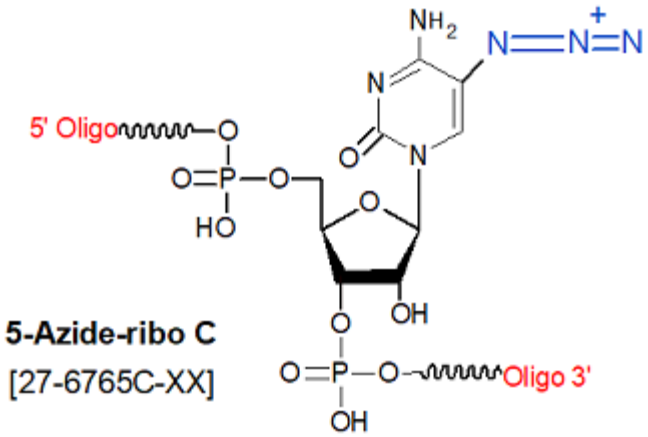
Category	Click Chemistry
Modification Code	N3-rA
Reference Catalog Number	27-6765A
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	370.23



8-Azide ribo A
[27-6765A-XX]

Azide rC

Category	Click Chemistry
Modification Code	N3-rC
Reference Catalog Number	27-6765C
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	346.2

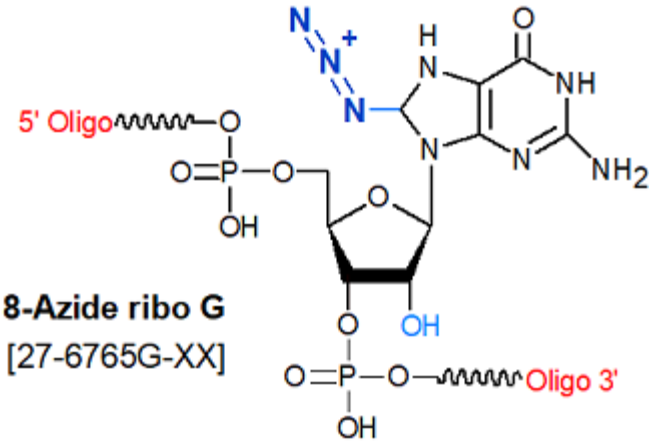


5-Azide-ribo C

[27-6765C-XX]

Azide rG

Category	Click Chemistry
Modification Code	N3-rG
Reference Catalog Number	27-6765G
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	386.23



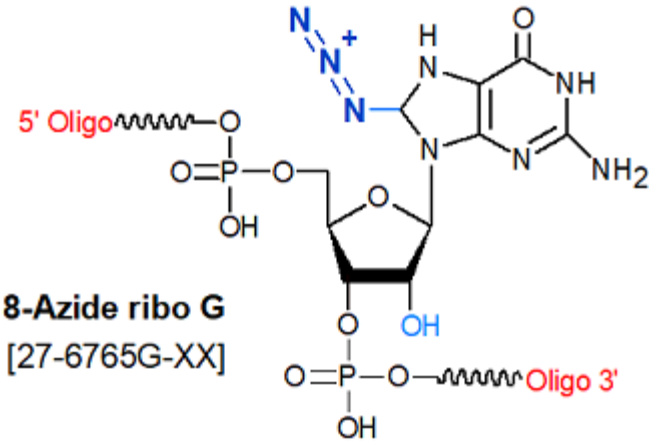
8-Azide ribo G

[27-6765G-XX]

Oligo Modifications

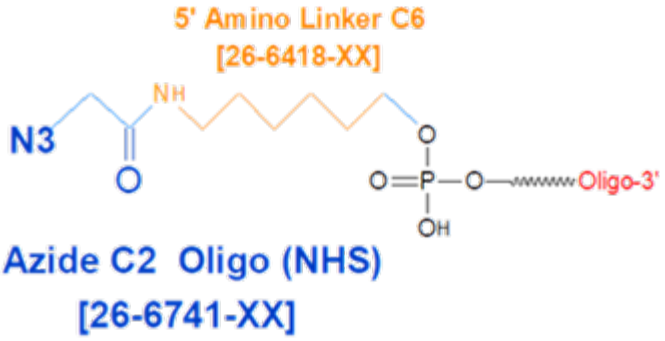
Azide rU

Category	Click Chemistry
Modification Code	N3-rU
Reference Catalog Number	27-6765U
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	361.22



Azide-C2 NHS

Category	Click Chemistry
Modification Code	N3-C2-N
Reference Catalog Number	26-6741
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	84.93



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.

Azide C2 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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

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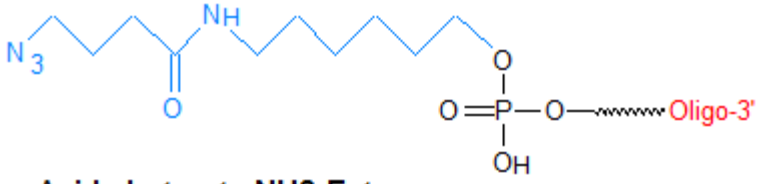
Azide-C4 NHS (butyrate)

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

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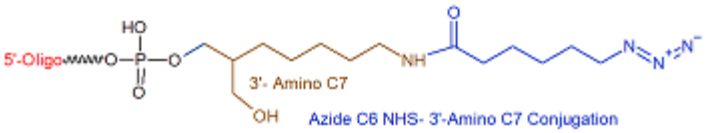
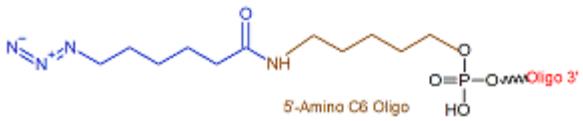
Azide-C6 NHS

Category	Click Chemistry
Modification Code	N3-C6-N
Reference Catalog Number	26-6740
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	140.14





Azide C6 NHS
[26-6740-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.

Azide C6 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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

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Azide-Picolyl PEG4 NHS

Category	Click Chemistry
Modification Code	N3-PIC-PEG4-N
Reference Catalog Number	26-6798
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	408.48

Azide Picolyl PEG4 NHS Oligo
[26-6798-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.

Picolyl azide-PEG4-NHS ester is a bifunctional PEG linker comprised of a picolyl moiety that assists with a copper-chelating system within its structure and an NHS ester that directly and efficiently reacts with amine-bearing moieties. Compared with conventional azides, picolyl azide will allow to use at least 10-fold less copper catalyst to yield the same click reaction rate as regular azide, because picolyl moiety can chelate Cu to raise the effective concentration of Cu(I) at the reaction site. The PEG4 chain improves the modification's water solubility.

Picolyl Azide PEG4 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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.

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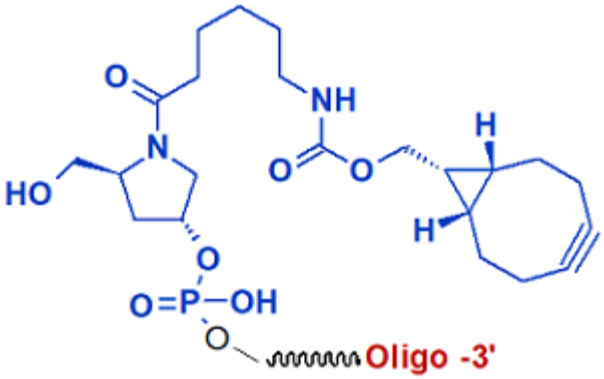
BCN Endo 5' (Bicyclononyne) 5'

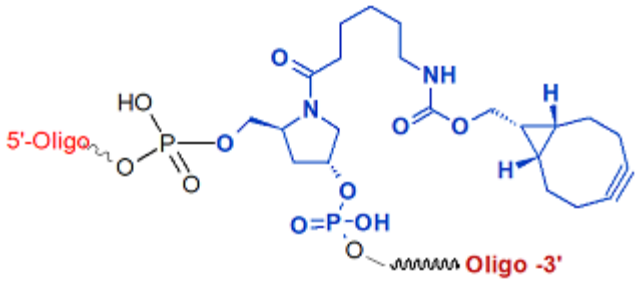
Category	Click Chemistry
Modification Code	BCN-5
Reference Catalog Number	26-6771
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	468.49



BCN Endo 5'
26-6771-XX

BCN Endo Internal

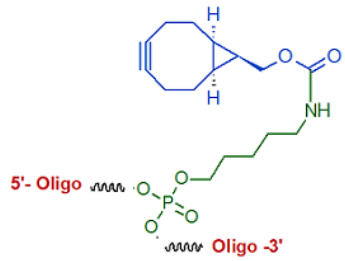
Category	Click Chemistry
Modification Code	BCN-Int
Reference Catalog Number	26-6771I
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	468.49



BCN Endo Internal
26-6771I-XX

BCN Endo NHS

Category	Click Chemistry
Modification Code	BCN-N
Reference Catalog Number	26-6777
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	177.03



Amino C6 linker Internal
Amino C3 and C12 also available for 3' and 5'

BCN Endo NHS [26-6777-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

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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.

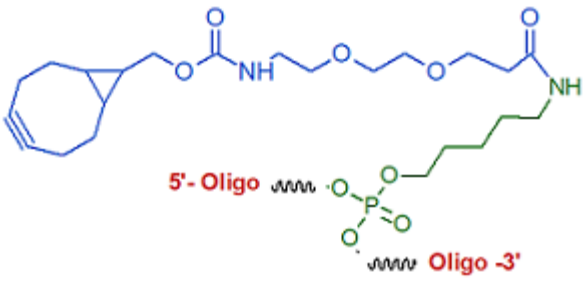
Bicyclononyne (BCN) is stable and one of the most reactive cyclooctynes for copper-free click chemistry. Unlike dibenzocyclooctyne (DBCO), BCN is reactive both to azides (strain-promoted azide-alkyne cycloaddition, SPAAC) and tetrazines (inverse electron demand Diels-Alder reaction, IEDDA).
BCN-labeled oligonucleotides may be used for the conjugation to azide- or tetrazine-containing solid surfaces, polymers, and large proteins.

DBCO conjugation chemistry is based on the reaction of a dibenzylcyclooctyne (DBCO) linker with an azide linker to form a stable triazole. The dibenzocyclooctyne group (DBCO) allows Copper-free Click Chemistry to be done with live cells, whole organisms, and non-living samples. DBCO groups will preferentially and spontaneously label molecules containing azide groups (-N3). Within physiological temperature and pH ranges, the DBCO group does not react with amines or hydroxyls, which are naturally present in many biomolecules. Reaction of the DBCO group with the azide group is significantly faster than with the sulfhydryl group (-SH, thiol).

Cyclooctyne-based modifications offers the ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers DBCO NHS modification with various length of Carbon and PEG for preparing oligos inserting a DBCO group at any position within the oligonucleotide. DBCO NHS are post synthesis conjugation and requires a primary amino group. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqueous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

BCN Endo PEG2 NHS

Category	Click Chemistry
Modification Code	BCN-PEG2-N
Reference Catalog Number	26-6778
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	336.43



Amino C6 linker Internal
Amino C3 and C12 also available for 3' and 5'

BCN Endo PEG2 NHS [26-6778-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.

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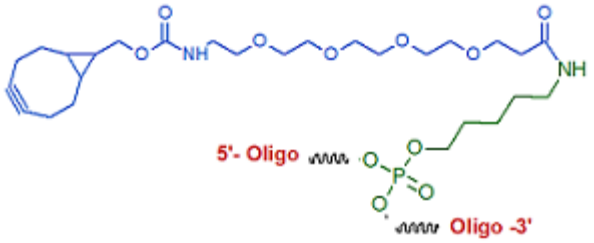
BCN Endo PEG4 NHS

Category	Click Chemistry
Modification Code	BCN-PEG4-N
Reference Catalog Number	26-6779
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	424.53



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,
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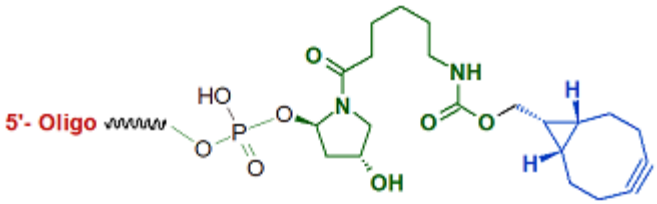
BCN-3' (Bicyclononyne) 3'

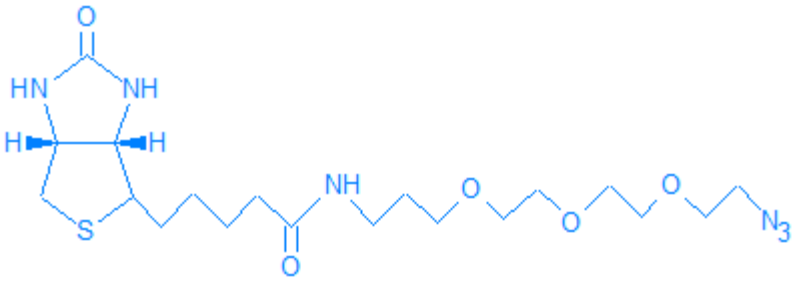
Category	Click Chemistry
Modification Code	BCN-3
Reference Catalog Number	26-6743
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	468.49



BCN-3
[26-6743-XX]

BiotinTEG Azide

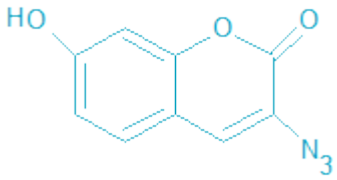
Category	Click Chemistry
Modification Code	Bio-TEG-N3
Reference Catalog Number	26-6721
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	444.55



Biotin-TEG Azide
[26-6721-XX]

Coumarin Azide

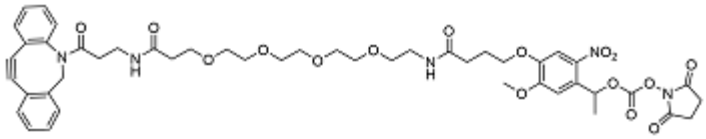
Category	Click Chemistry
Modification Code	Cou-N3
Reference Catalog Number	26-6726
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	203.15



Coumarin Azide
[26-6726-XX]

DBCO PC NHS

Category	Click Chemistry
Modification Code	DBCO-PC-N
Reference Catalog Number	26-6744
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	847.96



Click here for a complete list of Click Chemistry Oligo Modifications

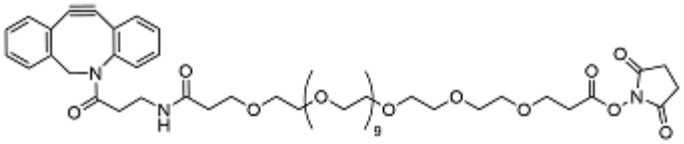
This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6.
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.
Photocleavable DBCO-NHS ester contains a spacer arm containing a photocleavable moiety, this can be efficiently photoreleased, typically >90% in 5-25 minutes using an near-UV low intensity lamp (e.g. 365 nm lamp at 1-5 mW/cm2). Cyclooctyne-based (dibenzocyclooctynes, DBCO) modifications offers the ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

Photo Cleavage Protocol Cleavage occurs by irradiation with near-UV light (300-350 nm, >90% cleavage occurs within 5-25 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).

DBCO PEG13 NHS

Category	Click Chemistry
Modification Code	DBCO-PEG13-N
Reference Catalog Number	26-6746
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	1046



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.

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Click here for a complete list of Click Chemistry Oligo Modifications
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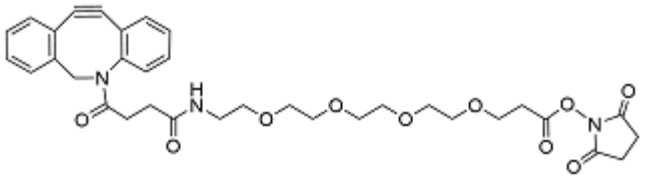
DBCO PEG4 NHS

Category	Click Chemistry
Modification Code	DBCO-PEG4-N
Reference Catalog Number	26-6745
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	551.68



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,
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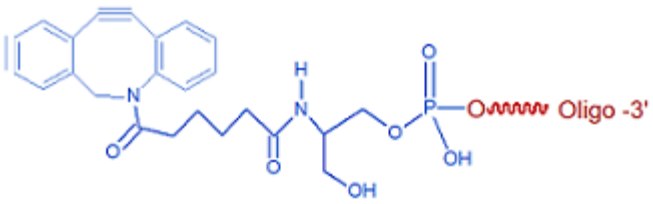
Cyclooctyne-based (dibenzocyclooctynes, DBCO) modifications offers the ease of copper-free click reagents. These are simple to use and has excellenet click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

DBCO Serinol

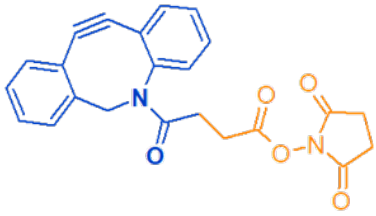
Category	Click Chemistry
Modification Code	DBCO-Ser
Reference Catalog Number	26-6736
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	468.45



5'-DBCO-Serinol
[26-6736-XX]

DBCO-C2 NHS

Category	Click Chemistry
Modification Code	DBCO-C2-N
Reference Catalog Number	26-6742
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	289.23



DBCO-C2 NHS
26-6742-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.
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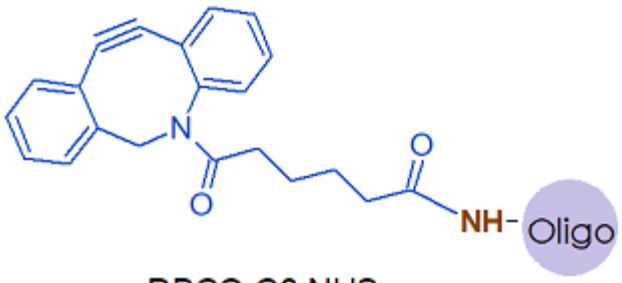
DBCO-C6 NHS

Category	Click Chemistry
Modification Code	DBCO-C6-N
Reference Catalog Number	26-6929
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	315.1



DBCO C6 NHS
[26-6929-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.

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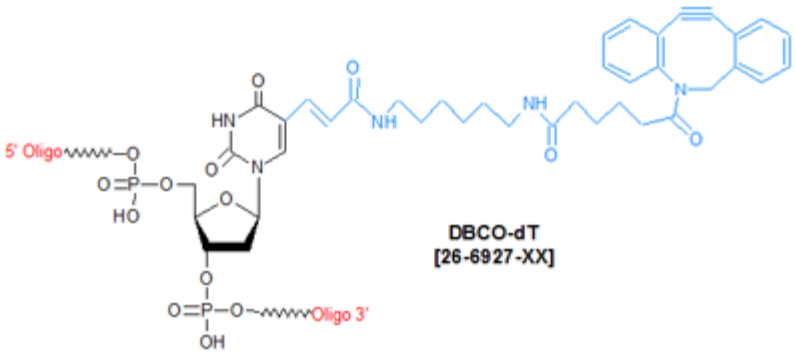
Addition of DBCO-Sulfo-NHS is post synthesis and requires synthesis of oligo with primary amino group.

DBCO-dT

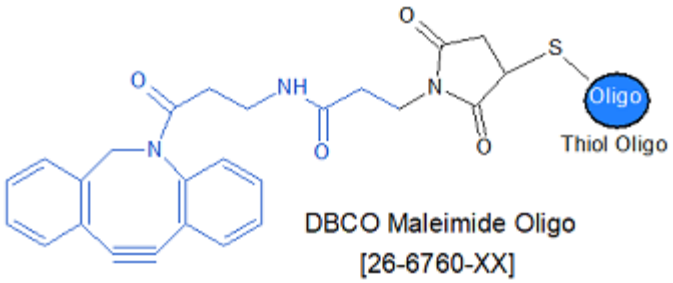
Category	Click Chemistry
Modification Code	DBCO-dT
Reference Catalog Number	26-6927
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	773.77



DBCO-dT
[26-6927-XX]

DBCO-Maleimide

Category	Click Chemistry
Modification Code	DBCO-Mal
Reference Catalog Number	26-6760
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	427.4



DBCO Maleimide Oligo
[26-6760-XX]

Click here for a complete list of Click Chemistry Oligo Modifications

This modification is a post synthesis maleimide conjugation to a reduced thiol amino group thus an additional modification with thiol group is required. A C3 or C6 thiol group can be placed at the 5' or for internal positions Thiol C6 dT modified base is used.
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.
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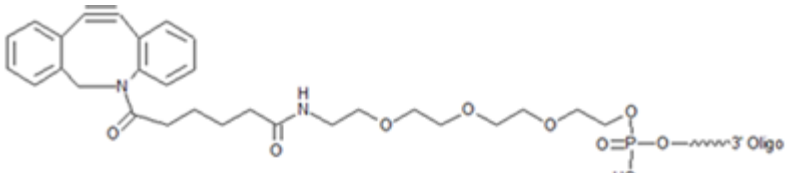
Click here for a list of conjugation chemistry modifications. **Click Chemistry Ligand conjugation** requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,
BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.
Cyclooctyne-based (dibenzocyclooctynes, DBCO) modifications offers the ease of copper-free click reagents. These are simple to use and has excellenet click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

DBCO-TEG (5')

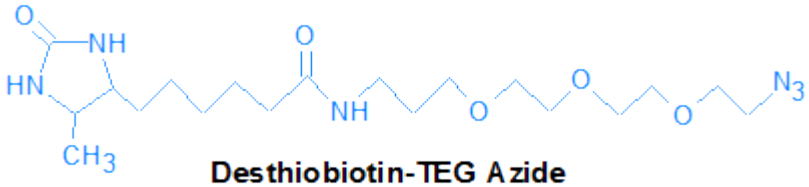
Category	Click Chemistry
Modification Code	DBCO-TEG
Reference Catalog Number	26-6928
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	570.57



5'-DBCO-TEG
[26-6828-XX]

DesthiobiotinTEG Azide

Category	Click Chemistry
Modification Code	DesBioTEG-N3
Reference Catalog Number	26-6725
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	414.5



Desthiobiotin-TEG Azide
[26-6725-XX]

Click here for a list of other Affinity Ligand Modifications.
This modification is a post synthesis conjugation to BCN, alkyne or DBCO modification at the appropriate site for click conjugation. Gene Link offers post synthesis click free conjugation to oligos labelled with BCN at the 5' or 3' end. The azide group of Methylene Blue is linked to BCN group on the oligo. BCN group is required on the oligo. Additional charges applies for BCN

BCN-3
BCN-5
YIELD
Post synthesis conjugation modifications yields are lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis.

Desthiobiotin-TEG Azide is a desthiobiotin attached to a 15-atom mixed polarity triethylene glycol spacer with an azide group at the end. 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 Desthiobiotin-TEG Azide 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). The spacer acts to minimize steric hindrance between the desthiobiotin moiety and the oligo.

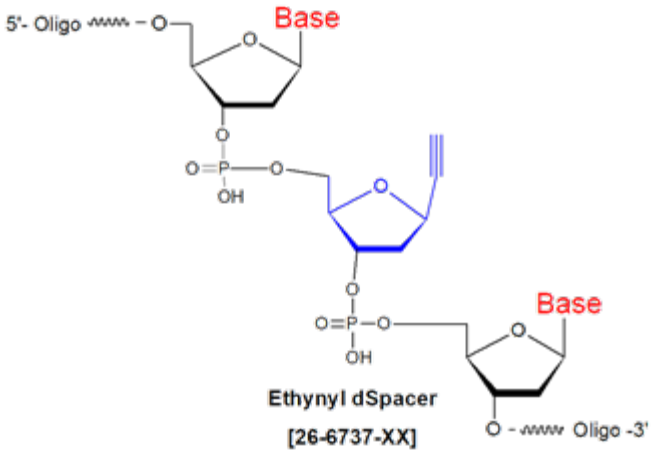
Like biotin, desthiobiotin binds to streptavidin, but its binding affinity is considerably less (2x10E-9 M) than that of biotin (4.0x10E-14 M) (3). Consequently, oligonucleotides labeled with desthiobiotin can be easily displaced from streptavidin by biotin, thereby making recovery of the labeled oligo (for example, in affinity purification protocols) from a streptavidin-coated support a relatively simple process (4). Desthiobiotin-labeled oligos can also be conveniently eluted from streptavidin-coated supports by incubation in distilled water at 95C for 10 minutes (5).

Gene Link recommends substitution of desthiobiotin for biotin for those cases where reversible capture of oligonucleotides is desirable. **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. Green, N.M. Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* (1970), **18A**: 418-424.
4. Hirsch, J.D., Eslamizar, L., Filanoski, B.J., Malekzadeh, N., Haugland, R.P., Beechem, J.M., Haugland, R.P. Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. *Anal. Biochem.* (2002), **308**: 343-357.
5. van Doom, R., Slawiak, M., Szemes, M., Dullemans, A.M., Bonants, P., Kowalchuk, G.A., Schoen, C.D. Robust Definition and Identification of Multiple Oomycetes and Fungi in Environmental Samples by Using a Novel Cleavable Padlock Probe-Based Ligation Detection Assay.*Appl. Environ. Microbiol.* (2009), **75**: 4185-4193.

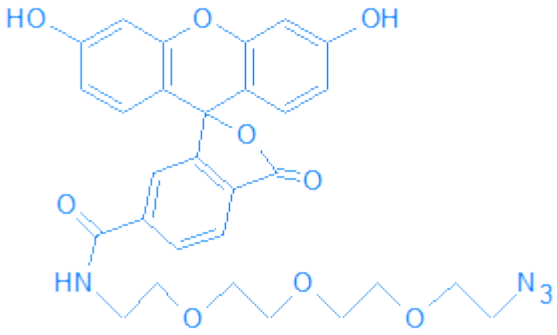
Ethynyl-dSpacer (Alkyne)

Category	Click Chemistry
Modification Code	Ethynyl-dABS
Reference Catalog Number	26-6737
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	20412



Fam-TEG Azide

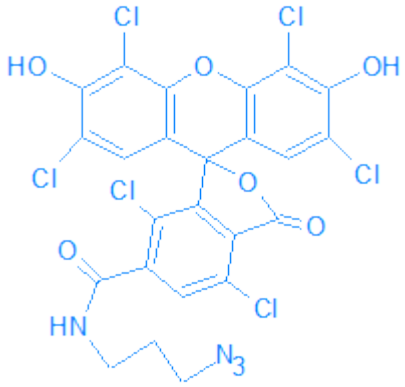
Category	Click Chemistry
Modification Code	Fam-TEG-N3
Reference Catalog Number	26-6722
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	576.55



6-FAM-TEG Azide
[26-6722-XX]

Hex-Azide-6

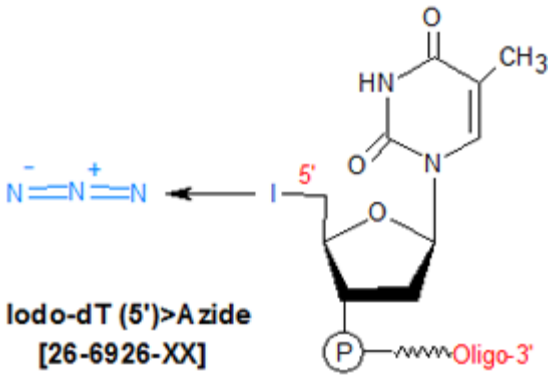
Category	Click Chemistry
Modification Code	Hex-N3
Reference Catalog Number	26-6723
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	665.09



6-HEX Azide
[26-6723-XX]

Iodo-dT-5'

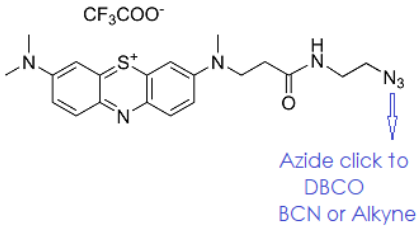
Category	Minor Bases
Modification Code	I-dT
Reference Catalog Number	26-6926
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	414.09



Iodo-dT (5')>Azide
[26-6926-XX]

Methylene Blue Azide

Category	Redox Electrochemical
Modification Code	MB-N3
Reference Catalog Number	26-6988
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	553



Methylene Blue Azide Oligo
[26-6988-XX]

This modification is a post synthesis conjugation to BCN, alkyne or DBCO modification at the appropriate site for click conjugation. Gene Link offers post synthesis click free conjugation to oligos labelled with BCN at the 5' or 3' end. The azide group of Methylene Blue is linked to BCN group on the oligo. BCN group is required on the oligo. Additional charges applies for BCN

BCN-3
BCN-5
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.

Methylene Blue Azide is a derivative of the well-known redox dye Methylene Blue. The azide derivative enables use in copper free click chemistry reactions with DBCO labelled reactants.
The dye can be reversibly reduced to the colorless leuko form. Upon oxidation (e.g. with oxygen) the dye recovers, and the absorption is fully restored.

Methylene Blue (e.g Atto MB2) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5' or 3'-end of an oligonucleotide, enables the modified oligo's use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For "signal-on" sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For "signal-off" sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.

Methylene blue is a pH indicator that changes color depending on the acidity or alkalinity of a solution. In acidic conditions (pH < 6), it appears blue, while in neutral to basic conditions (pH > 7), it can shift to a colorless or light blue form. This transition is due to changes in the molecular structure of methylene blue, which affects its light absorption properties.

References
1. Ricci, F., Lai, R.Y., Plaxco, K.W. Linear, redox modified DNA probes as electrochemical DNA sensors. *Chem. Comm.* (2007), **36**: 3768-3770.
2. Song, S., Wang, L., Li, J., Zhao, J., Fan, C. Aptamer-based biosensors. *Trends in Anal. Chem.* (2008), **27**: 108-117.
3. Ferapontova, E.E., Gothelf, K.V. Optimization of the Electrochemical RNA-Aptamer Based Biosensor for Theophylline by Using a Methylene Blue Redox Label. *Electroanalysis* (2009), **21**: 1261-1266.
4. Xiao, Y., Lubin, A.A., Heeger, A.J., Plaxco, K.W.. Label-free Electronic Detection of Thrombin in Blood Serum by Using an Aptamer-Based Sensor.*Angew. Chem. Int. Ed. Engl.* (2005), **44**: 5456-5459..

Copper-free Click Chemistry Modifications

Use azide modified oligos with DBCO Cyclooctyne-based modifications for ease of copper-free click reagents. These are simple to use and has excellent click performance in 17 hours or less at room temperature. Gene Link offers 5'-DBCO-TEG for preparing oligos with 5'-DBCO and a 15 tom triethylene glycol spacer arm, DBCO-dT for inserting a DBCO group at any position within the oligonucleotide and DBCO-sulfo-NHS Ester is also offered for post-synthesis conjugation reactions. DBCO-modified oligos may be conjugated with azides in organic solvents, such as DMSO, or aqeous buffers. Depending on the azide used, the reaction will go to completion in 4-17 hours at room temperature.

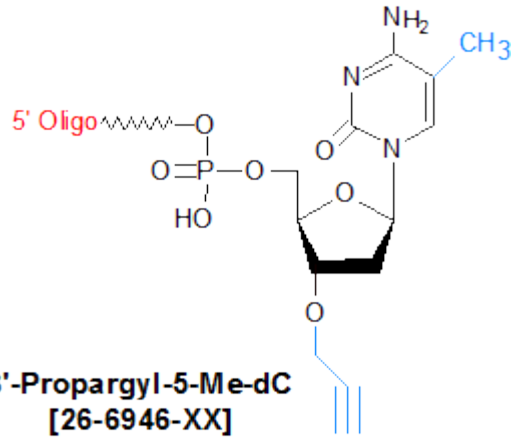
Azide C3 is available to introduce a stable azide group at the 3' of an oligo. Use Azide butyrate NHS [26-6922] for introduction of azide at internal or 5' position by conjugating to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the 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.

Propargyl-3'-5-Me-dC

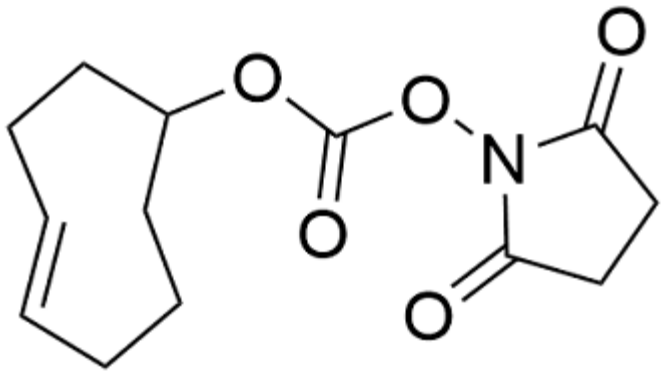
Category	Click Chemistry
Modification Code	PPG-3-O-5me-dC
Reference Catalog Number	26-6946
5 Prime	N
3 Prime	Y
Internal	N
Molecular Weight(mw)	341.26



3'-Propargyl-5-Me-dC
[26-6946-XX]

TCO NHS

Category	Click Chemistry
Modification Code	TCO-N
Reference Catalog Number	26-6756
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	153.21



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.

Click here for a complete list of Click Chemistry Oligo Modifications

TCO (trans-cyclooctene) 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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.

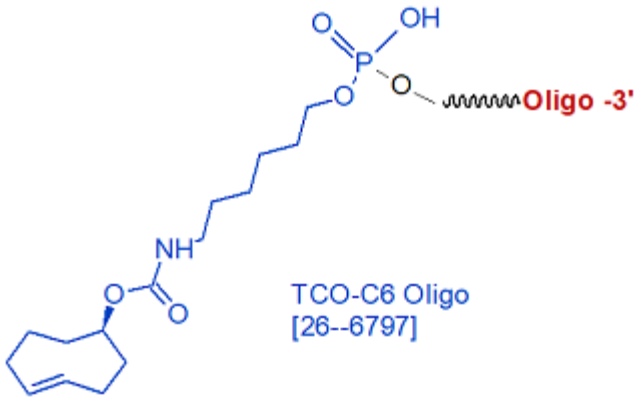
References

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.

Oligo Modifications

TCO-C6- 5'

Category	Click Chemistry
Modification Code	TCO-C6
Reference Catalog Number	26-6797
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	469.6



Click here for a complete list of Click Chemistry Oligo Modifications

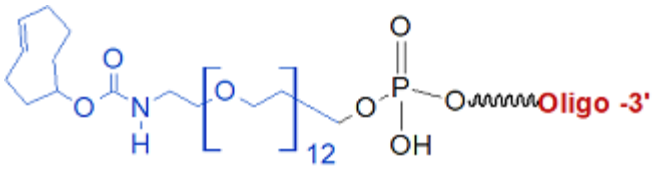
TCO-C6 (trans-cyclooctene) is incorporated at the 5' end of oligo using automated chemistry. Other TCO offered are post synthesis NHS ester to introduce an active TCO group to an amino-modified oligonucleotide. The ability to incorporate directly without NHS chemistry enables the incorporation of other ligands in the same oligo using NHS chemistry. 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 C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The TCO NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The presence of the TCO 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.

TCO-PEG12 5'

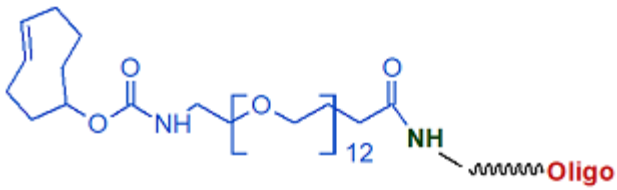
Category	Click Chemistry
Modification Code	TCO-PEG12-5
Reference Catalog Number	26-6759F
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	752.93



TCO PEG12 5'
26-6759F-XX

TCO-PEG12 NHS

Category	Click Chemistry
Modification Code	TCO-PEG12-N
Reference Catalog Number	26-6759
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	752.93



TCO PEG12 N Oligo
26-6759-XX

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.

Click here for a complete list of Click Chemistry Oligo Modifications

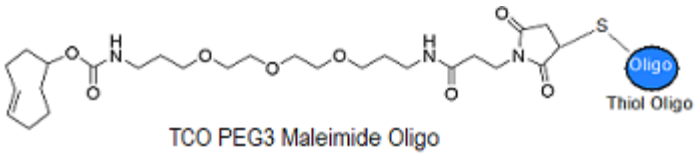
TCO (trans-cyclooctene) NHS ester can be used to introduce an active TCO group to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The TCO NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The presence of the TCO 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.

TCO-PEG3 Mal Oligo

Category	Click Chemistry
Modification Code	TCO-PEG3-Mal
Reference Catalog Number	26-6763
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	523.62



TCO PEG3 Maleimide Oligo
[26-6763-XX]

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.

Click here for a complete list of Click Chemistry Oligo Modifications

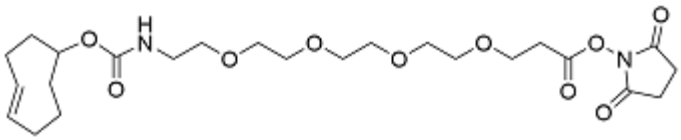
TCO (trans-cyclooctene) NHS ester can be used to introduce an active azide group to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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.

TCO-PEG4 NHS

Category	Click Chemistry
Modification Code	TCO-PEG4-N
Reference Catalog Number	26-6757
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	400.53



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.

Click here for a complete list of Click Chemistry Oligo Modifications

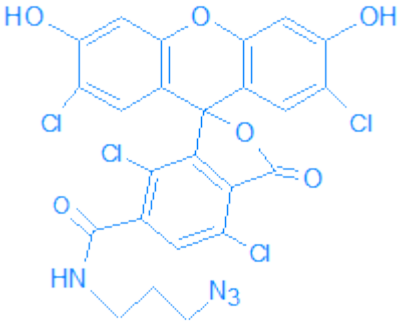
TCO (trans–cyclooctene) PEG4 NHS ester can be used to introduce an active azide group to an amino-modified oligonucleotide. Introduction can be done at either the 5'- or 3'-end, or internally. To do this, the oligo first must be synthesized with a primary amino functional group modification, e.g Amino C3, C6 or C12 for the 5' end or amino C3, C6 or C7 for the 3' end for the ends) or the amino C6 version of the base phosphoramidite (for internal labeling). The Azide C2 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.

Tet-Azide

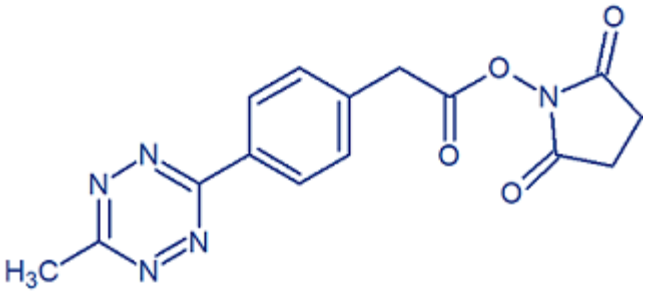
Category	Click Chemistry
Modification Code	Tet-N3
Reference Catalog Number	26-6724
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	596.2



6-TET Azide
[26-6724-XX]

Tetrazine methyl NHS

Category	Click Chemistry
Modification Code	meTz-N
Reference Catalog Number	26-6758
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	230.2



All NHS modifications are 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** NHS based modifications yields are lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis.

Click here for a complete list of Click Chemistry Oligo Modifications

Tetrazines are even more reactive than triazines toward nucleophiles and electron-rich dienophiles. This makes them attractive for click chemistry and they find application as conjugation tags for materials chemistry and, especially, for bio-orthogonal chemistry. In other applications they are attractive for high-energy materials, coordinating ligands, and as potent bioactive compounds.

The tetrazine will react with strained alkenes such as trans-cyclooctene, norbornene and cyclopropene to yield a stable dihydropyridazine linkage. The extremely fast kinetics and selectivity enables the conjugation of two low abundance biopolymers in an aqueous and otherwise complex chemical environment. This bioorthogonal reaction possesses excellent selectivity and biocompatibility such that the complimentary partners can react with each other within richly functionalized biological systems, in some cases, living organisms. Thus, tetrazine-TCO ligation has found numerous applications in fluorescent imaging, drug delivery, PET and SPECT imaging, radionuclide therapy, radiochemistry or drug target identification among several others.

Biocompatible

Click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)

Chemoselective

Tetzines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency

Unprecedented kinetics

Inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available

Solubility

Easily dissolves in aqueous buffers

Methyltetrazine NHS Ester is one of the most stable tetrazines commercially available.

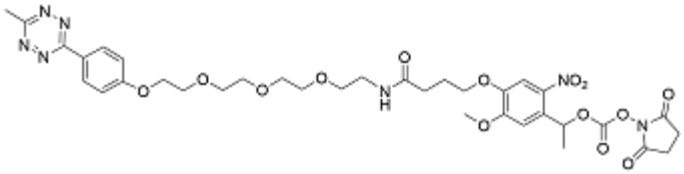
In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG4-NHS Ester. Methyltetrazine NHS is poorly soluble in aqueous solutions whereas methylterazine PEG4 NHS solubility is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine methyl PC NHS

Category	Click Chemistry
Modification Code	meTz-PC-N
Reference Catalog Number	26-6750
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	419.45



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Biocompatible – click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)
Chemoselective – tetzines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency
Unprecedented kinetics – inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available
Solubility – easily dissolves in aqueous buffers

Methyltetrazine-PEG4-NHS Ester is one of the most stable tetrazines commercially available.

In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG4-NHS Ester. The aqueous solubility of this reagent is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

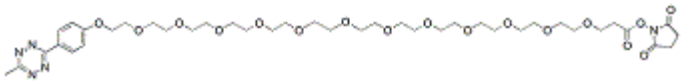
Photo Cleavage Protocol Cleavage occurs by irradiation with near-UV light (300-350 nm, >90% cleavage occurs within 5-25 minutes. Try using a Black Ray XX-15 UV lamp (Ultraviolet Products Inc., San Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, 300 nm cut-off, 1.1 mW intensity at~31 cm).

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine methyl PEG12 N

Category	Click Chemistry
Modification Code	meTz-PEG12-N
Reference Catalog Number	26-6794
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	771.93



All NHS modifications are 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** NHS based modifications yields are lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis.

Click here for a complete list of Click Chemistry Oligo Modifications

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Biocompatible
Click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)

Chemoselective
Tetzines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency

Unprecedented kinetics
Inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available

Solubility
Easily dissolves in aqueous buffers

Methyltetrazine-PEG-NHS Ester is one of the most stable tetrazines commercially available.

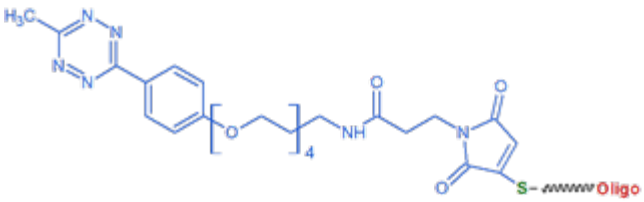
In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG-NHS Esters. The aqueous solubility of this reagent is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine Methyl PEG4 Maleimide

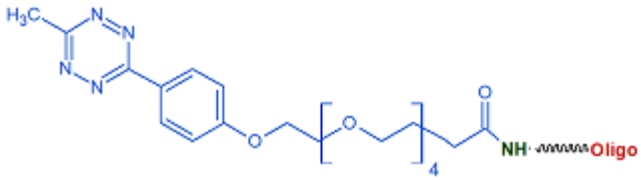
Category	Click Chemistry
Modification Code	meTzPEG4-Mal
Reference Catalog Number	26-6762
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	514.43



Tetrazine Methyl PEG4 Maleimide Oligo
[26-6762-XX]

Tetrazine methyl PEG4 NHS

Category	Click Chemistry
Modification Code	meTz-PEG4-N
Reference Catalog Number	26-6749
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	419.45



Tetrazine methyl PEG4 Oligo
[26-6749-XX]

All NHS modifications are 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** NHS based modifications yields are lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.
~2 nmol final yield for 50 nmol scale synthesis.
~5 nmol final yield for 200 nmol scale synthesis.
~16 nmol final yield for 1 umol scale synthesis.

Click here for a complete list of Click Chemistry Oligo Modifications

Tetrazines are even more reactive than triazines toward nucleophiles and electron-rich dienophiles. This makes them attractive for click chemistry and they find application as conjugation tags for materials chemistry and, especially, for bio-orthogonal chemistry. In other applications they are attractive for high-energy materials, coordinating ligands, and as potent bioactive compounds.

The tetrazine will react with strained alkenes such as trans-cyclooctene, norbornene and cyclopropene to yield a stable dihydropyridazine linkage. The extremely fast kinetics and selectivity enables the conjugation of two low abundance biopolymers in an aqueous and otherwise complex chemical environment. This bioorthogonal reaction possesses excellent selectivity and biocompatibility such that the complimentary partners can react with each other within richly functionalized biological systems, in some cases, living organisms. Thus, tetrazine-TCO ligation has found numerous applications in fluorescent imaging, drug delivery, PET and SPECT imaging, radionuclide therapy, radiochemistry or drug target identification among several others.

Biocompatible

Click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)

Chemoselective

Tetzines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency

Unprecedented kinetics

Inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available

Solubility

Easily dissolves in aqueous buffers

Methyltetrazine-PEG4-NHS Ester is one of the most stable tetrazines commercially available.

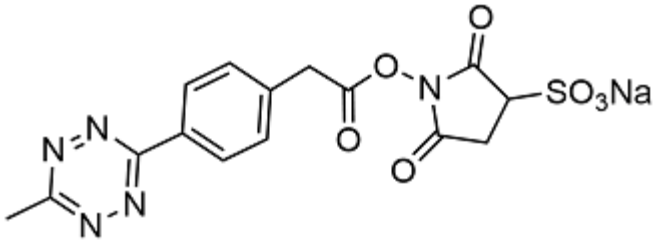
In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG4-NHS Ester. The aqueous solubility of this reagent is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine methyl Sulfo-NHS

Category	Click Chemistry
Modification Code	me-Tz-Sulfo-N
Reference Catalog Number	26-6796
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	315.27



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Methyl Tetrazine Sulfo is The tetrazine will react with strained alkenes such as trans-cyclooctene, norbornene and cyclopropene to yield a stable dihydropyridazine linkage. The extremely fast kinetics and selectivity enables the conjugation of two low abundance biopolymers in an aqueous and otherwise complex chemical environment. This bioorthogonal reaction possesses excellent selectivity and biocompatibility such that the complimentary partners can react with each other within richly functionalized biological systems, in some cases, living organisms. Thus, tetrazine-TCO ligation has found numerous applications in fluorescent imaging, drug delivery, PET and SPECT imaging, radionuclide therapy, radiochemistry or drug target identification among several others.
Biocompatible click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)
Chemoselective tetrazines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency
Unprecedented kinetics inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available
Solubility easily dissolves in aqueous buffers

Methyltetrazine-PEG4-NHS Ester is one of the most stable tetrazines commercially available.

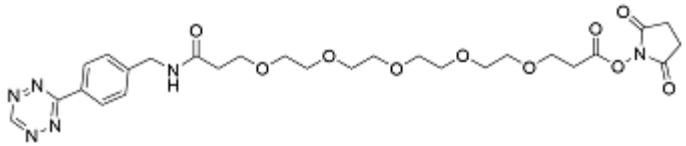
In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG4-NHS Ester. The aqueous solubility of this reagent is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine-PEG5-NHS

Category	Click Chemistry
Modification Code	Tz-PEG5-N
Reference Catalog Number	26-6748
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	419.45



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Biocompatible – click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)
Chemoselective – tetzines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency
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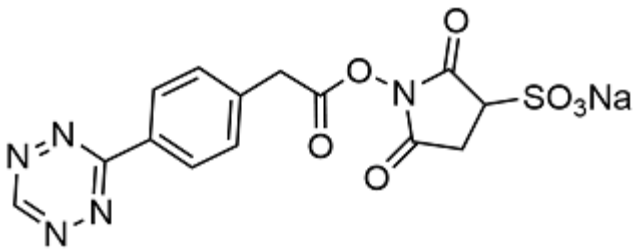
References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
.

Tetrazine-Sulfo-NHS

Category	Click Chemistry
Modification Code	Tz-Sulfo-N
Reference Catalog Number	26-6747
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	300.93



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Click here for a complete list of Click Chemistry Oligo Modifications

Tetrazines are even more reactive than triazines toward nucleophiles and electron-rich dienophiles. This makes them attractive for click chemistry and they find application as conjugation tags for materials chemistry and, especially, for bio-orthogonal chemistry. In other applications they are attractive for high-energy materials, coordinating ligands, and as potent bioactive compounds.
The tetrazine will react with strained alkenes such as trans-cyclooctene, norbornene and cyclopropene to yield a stable dihydropyridazine linkage. The extremely fast kinetics and selectivity enables the conjugation of two low abundance biopolymers in an aqueous and otherwise complex chemical environment. This bioorthogonal reaction possesses excellent selectivity and biocompatibility such that the complimentary partners can react with each other within richly functionalized biological systems, in some cases, living organisms. Thus, tetrazine-TCO ligation has found numerous applications in fluorescent imaging, drug delivery, PET and SPECT imaging, radionuclide therapy, radiochemistry or drug target identification among several others.
Biocompatible click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)
Chemoselective tetrazines and trans-cyclooctene groups do not react or interfere with other functional groups found in biological samples but conjugate to one another with high efficiency
Unprecedented kinetics inverse-electron demand Diels-Alder chemistry is the fastest bioorthogonal ligation available
Solubility easily dissolves in aqueous buffers

Methyltetrazine-PEG4-NHS Ester is one of the most stable tetrazines commercially available.

In addition to stabilization provided by the electron donating methyl group, the electron donating alkoxy substituent on the aromatic ring further improves the stability of Methyltetrazine-PEG4-NHS Ester. The aqueous solubility of this reagent is substantially enhanced by a hydrophilic polyethylene glycol (PEG) spacer arm.

References

1. Devaraj, N.K. and Weissleder, R. Biomedical Applications of Tetrazine Cycloadditions. *Acc Chem Res.*(2011) 44: 816–827
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