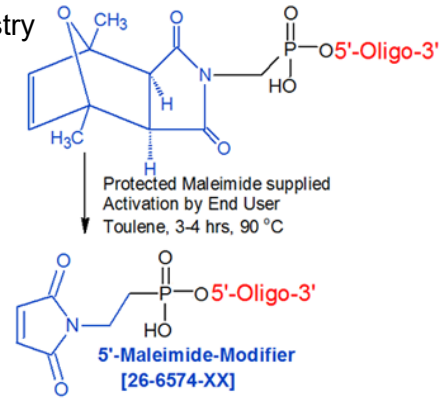
Maleimide (5')

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



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

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

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

Deprotection by heating in Toluene

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

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

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