Biotin-NHS is an N-hydroxysuccinimide ester (NHS ester) of biotin. Biotin-NHS can be used to internally label an oligonucleotide with biotin at a non-T position (that is, at a G, C, or A position). To accomplish this, amino dG-C6/dC-C6/dA-C6 is first incorporated into the oligonucleotide, thereby placing an active primary amino group at the desired position. Biotin-NHS is then conjugated to the amino group in a separate reaction to form the final biotin-labeled product.

Biotin is an affinity label that can be incorporated at either the 5'- or 3'-end of an oligonucleotide, or at an internal position. Biotin has a high affinity for the bacterial protein, streptavidin, which can be conjugated to a solid support (such as magnetic beads) for use as a capture and immobilization medium for a biotinylated oligo. In the biotin phosphoramidite, the biotin is attached to a long spacer arm, which acts to minimize steric hindrance between the biotin moiety and the oligo, thereby providing streptavidin easy access to the biotin. Biotinylated oligos are most commonly used as probes or primers in a variety of in vitro and in vivo applications.

Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the purification of DNA binding proteins. In this context, the biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography. For isolation of DNA binding proteins, the streptavidin-biotin-oligonucleotide complex is incubated with a crude cell extract containing nuclear proteins. Following appropriate washes, the proteins that bind selectively to the oligonucleotide sequence can be eluted under conditions that disrupt the protein:DNA complex. Because the binding of biotin to streptavidin is essentially irreversible and is resistant to chaotropic agents and extremes of pH and ionic strength, the elution conditions can be relatively stringent.

Biotin-NHS can also be used to biotinylate a large amount of oligonucleotide aminated at the 5'- or 3'-end, in aqueous solution and at relatively low cost (1). References