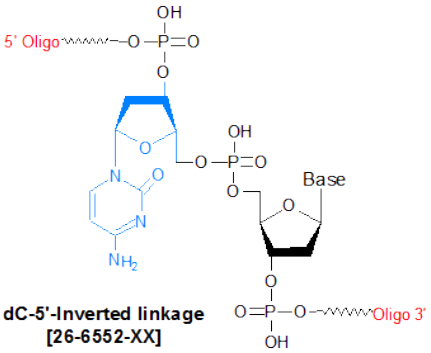
Inverted dC (reverse linkage)

Category	Minor Bases
Modification Code	Inv-dC
Reference Catalog Number	26-6552
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	289.18



dC-5'-Inverted linkage [26-6552-XX]

Reverse synthesis can be achieved by incorporation modifications where the synthesis orientation can be changed as desired. Oligo can be designed for the production of 5'-5' or 3'-3' linkages or a combination of these in the same oligo. These modified phosphodiester linkage modified oligos are useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for structural studies.

Construct Examples 5'-NNNNNNNN-3'-3'-NNNNNNNN-5'

The construct shown above starts at the right side in orange font 5' end with an inverted base, towards the left side is the 3' end. This orientation will continue with more sites of the inverted bases. Insertion of a standard bases shown in green font will have a 3'-3' phosphodiester linkage and to the left is the 5' end. 3'-NNNNNNNN-5'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The construct shown above is with a single [Inv-dT] to signify the orientation change point after the standard bases in green font; chemical synthesis starts from the 3' end. Note ALL bases shown in orange font after the first inverted bases towards the left will also be inverted bases to keep the reverse orientation.

The same construct is shown below but with standard orientation bases shown in green font inserted after the inverted base, this will reverse the polarity and thus the oligo will have a 5' and a 3' end. 5'-NNNNNNNN-3'--3'-[Inv-dT]-5'--5'-NNNNNNNN-3'

The CE phosphoramidite of 5'-3' reverse dC has the dimethoxytrityl (DMT) and phosphoramidite groups reversed from the normal case, that is, the DMT-group is attached to the 3'-OH, and the phosphoramidite attached to the 5'-OH, of the ribose. This reverse configuration allows for oligonucleotide synthesis in the 5' to 3' direction (instead of the standard 3' to 5' direction). Reverse synthesis is advisable in the following cases:

1. Formation of oligos containing hairpin loops with parallel strands. Oligos with hairpin loops are used for structural studies into duplex formation.

Typically the strands of the stem of the hairpin are anti-parallel. However, by switching to 5'-phosphoramidites for part of the synthesis of such an oligo (for example, initiating the switch during synthesis of the loop portion of the hairpin), the strands of the hairpin stem will be in parallel orientation (1).

2. Formation of nuclease resistant (5'-5', 3'-3') linkages. Anti-sense oligos containing terminal 5'-5' or 3'-3' linkages are highly resistant to exonuclease degradation. For the terminal 5'-5' linkage, the appropriate 5'-phosphoramidite is incorporated at the 5'-end in the final synthesis cycle. For the terminal 3'-3' linkage, the appropriate deoxynucleoside-5'-CPG is used as the solid support for the 3'-end, followed by synthesis of the oligo in the standard 3'-5' direction to make the terminal 3'-3' linkage (2).

Having a single inverted base at the 3' position with a 3'-3' linkage imparts the oligo exonuclease resistance and prevents extension by polymerases as there is no free 3' hydroxyl group to initiate synthesis.

3. 3'-terminal base/moiety cannot be attached to a CPG. Examples include 2',3'-ddT or ddI. **References**

1. van de Sande, J.H., Ramsing, N.B., Germann, M.W., Flhorstn, W., Kalisch, B.W., Clegg, R.C., Pon, R.T., Jovin, T.M. Parallel-Stranded DNA. *Science* (1988), **241**: 551-557.
2. Ortigao, J.F.R., Rosch, H., Selter, H., Frohlich, A., Lorenz, A., Montenarh, M., Seliger, H. Antisense effect of oligodeoxynucleotides with inverted terminal internucleotidic linkages: a minimal modification protecting against nucleolytic degradation.*Antisense Res. Dev.* (1992), **2**: 129-146.