Nuclease Resistance Introduction

Like cellular DNA and RNA, synthetic oligonucleotides are prone to degradation once introduced into the cell or body fluids. Such degradation is due to the ubiquitous presence of nuclease enzymes (both exonucleases and endonucleases), as well as chemical instability (particularly for RNA). Under normal cellular conditions, this leads to fast in vivo degradation of oligos and a short half-life (1). In addition, the ease with which RNAse, being very stable, can contaminate laboratory equipment and benchtop surfaces means that RNA is susceptible to degradation under normal laboratory conditions as well. To reduce or eliminate this susceptibility, nuclease-resistant modifications (for example, phosphorothiolation or 2'-OMethyl RNA bases) can be introduced into oligonucleotides slated for in vivo and/or regular benchtop work.
Nuclease Resistance Design Protocols

Nuclease Resistant Oligos for In vivo Applications and Design Considerations

While oligonucleotides are quickly degraded (typically within 15-30 minutes) by nuclease in both in vitro and in vivo contexts, the need to incorporate nuclease resistance into oligonucleotides is critically important for in vivo applications. Within serum or the cell, oligonucleotides can be degraded by both endo- and exonucleases. In serum, the 3'-exonucleases are of greatest concern (7), while within the cell, both 3'- and 5'-exonucleases are problematic (8). Endonucleases can also be an issue in those cases where the oligo contains a restriction site.

Based on the above, designing a nuclease-resistant oligonucleotide for in vivo applications primarily involves modifying it so as to protect it from exonucleases, while minimizing potential deleterious side-effects (such as reduced duplex stability, increased toxicity, or induction of off-target biological effects). The simplest and most cost-effective way to do this is to design the oligo as a ‘gapmer’, in which the linkages of the three end-most 5' and 3'-bases are phosphorothiolated, with the remaining bases in the middle having native phosphorodiester linkages. Such phosphorothiolated ‘gapmers’ are highly resistant to both 5'- and 3'-exonuclease degradation. In addition, because phosphorothiolation lowers the binding affinity of the oligo for its target (Tm of the oligo-target duplex is lowered between 0.5C and 1.5C per linkage, depending on sequence), use of only six such linkages often yields an acceptable balance between nuclease resistance and binding affinity (if increased binding affinity is required, other modifications can also be incorporated into the oligo, such as 2'-fluoro pyrimidines, 2'-O-methyl RNA bases, or C5-propyne pyrimidines). Finally, since large numbers of phosphorothiolate linkages can be toxic (due to the presence of sulfur), using only a small number of such linkages in an oligo minimizes cellular toxicity.

If phosphorothiolation is not desired, other modifications can be used. One option is to use methylphosphonates instead of phosphorothiolation for the 5'- and 3'-end positions of the ‘gapmer’. Methylphosphonates lower an oligo's binding affinity more than phosphorothiolation, however, so the use of additional modifications, such as 2'-fluoro-pyrimidines, is advisable to counteract this effect. More commonly, the substitution of 2'-O-methyl RNA bases at some or all positions of an oligo is used as an alternative to phosphorothiolation. Since the nuclease resistance conferred by 2'-O-methyl RNA lies between that of standard bases (no resistance) and phosphorothiolation (highly resistant), extensive/complete 2'-O-methylation is frequently chosen when a high level of nuclease resistance is required. 2'-O-methylation also confers higher binding affinity (that is, higher duplex Tm) to the oligo for its target, a desirable property, in many cases.
Nuclease Resistance Applications

For antisense or RNAi applications, incorporation of modifications conferring nuclease resistance is essentially indispensable and such modifications are used intensely. The most popular modification used for this purpose is phosphorothiolation, in which a phosphodiester backbone linkage is replaced by a phosphothiolate linkage. Such linkages are highly resistant to nuclease degradation, but they also lower duplex stability by about 0.5C per phosphorothioate linkage. However, judicious use of this modification (for example, placing them only at the three endmost bases of each end of the oligo to minimize exonuclease degradation) can produce excellent nuclease resistance while still maintaining reasonably good duplex stability (2). 2'-O Methyl (or other 2'-O-substituted) RNA bases also confer nuclease resistance to an oligo, and have the added benefit of increasing duplex stability as well (3,4). However, duplexes formed between oligos having 2'-OMethyl bases at all positions and RNA are incapable of activating RNase H activity (3), and this fact must be kept in mind if the user wishes to use such oligos for antisense applications. Recently, “mirror-image” (L)-nucleotide base phosphoramidites have become available as well. Oligonucleotides containing (L)-nucleotides are completely immune to nuclease attack at the incorporated positions (5). While (L)-nucleotides also do not base pair with natural (D)-nucleotides (6), they still could potentially be incorporated in, for example, the stems of molecular beacons to protect them from degradation by exonucleases.
References

<table>
<thead>
<tr>
<th>Modification</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2'-Fluoro deoxyadenosine (2'-F-A)</td>
<td>26-6692</td>
</tr>
<tr>
<td>2'-Fluoro deoxycytosine (2'-F-C)</td>
<td>26-6463</td>
</tr>
<tr>
<td>2'-Fluoro deoxyguanosine (2'-F-G)</td>
<td>26-6693</td>
</tr>
<tr>
<td>2'-Fluoro deoxyuridine (2'-F-U)</td>
<td>26-6462</td>
</tr>
<tr>
<td>2'-O methyl adenosine A</td>
<td>27-6410A</td>
</tr>
<tr>
<td>2'-O methyl cytosine C</td>
<td>27-6410C</td>
</tr>
<tr>
<td>2'-O methyl guanosine G</td>
<td>27-6410G</td>
</tr>
<tr>
<td>2'-O methyl uridine U</td>
<td>27-6410U</td>
</tr>
<tr>
<td>3'-dA (2'-5' linked)</td>
<td>26-6490</td>
</tr>
<tr>
<td>3'-dC (2'-5' linked)</td>
<td>26-6491</td>
</tr>
<tr>
<td>3'-dG (2'-5' linked)</td>
<td>26-6492</td>
</tr>
<tr>
<td>3'-dT (2'-5' linked)</td>
<td>26-6493</td>
</tr>
<tr>
<td>3'-O methyl bases (2'-5' linked)</td>
<td>27-6458N</td>
</tr>
<tr>
<td>3'-O methyl rA (2'-5' linked)</td>
<td>27-6458A</td>
</tr>
<tr>
<td>3'-O methyl rC (2'-5' linked)</td>
<td>27-6458C</td>
</tr>
<tr>
<td>3'-O methyl rG (2'-5' linked)</td>
<td>27-6458G</td>
</tr>
<tr>
<td>3'-O methyl rU (2'-5' linked)</td>
<td>27-6458U</td>
</tr>
<tr>
<td>3'-rA (2'-5' linked)</td>
<td>26-6682</td>
</tr>
<tr>
<td>3'-rC (2'-5' linked)</td>
<td>26-6911</td>
</tr>
<tr>
<td>3'-rG (2'-5' linked)</td>
<td>26-6912</td>
</tr>
<tr>
<td>Chemical</td>
<td>Code</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>3'-rU (2'-5' linked)</td>
<td>26-6913</td>
</tr>
<tr>
<td>L-DNA dA</td>
<td>26-6941</td>
</tr>
<tr>
<td>L-DNA dC</td>
<td>26-6942</td>
</tr>
<tr>
<td>L-DNA dG</td>
<td>26-6943</td>
</tr>
<tr>
<td>L-DNA dT</td>
<td>26-6944</td>
</tr>
<tr>
<td>L-RNA rA</td>
<td>27-6941</td>
</tr>
<tr>
<td>L-RNA rC</td>
<td>27-6942</td>
</tr>
<tr>
<td>L-RNA rG</td>
<td>27-6943</td>
</tr>
<tr>
<td>L-RNA rU</td>
<td>27-6944</td>
</tr>
<tr>
<td>Phosphorothioate (SOX)</td>
<td>26-6401</td>
</tr>
<tr>
<td>propyne dC</td>
<td>26-6501</td>
</tr>
<tr>
<td>propyne dU</td>
<td>26-6502</td>
</tr>
</tbody>
</table>
2'-F-A

<table>
<thead>
<tr>
<th>Category</th>
<th>Nuclease Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>fA</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6692</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>331.2</td>
</tr>
</tbody>
</table>

2'-Fluoro-deoxyadenosine (2'-F-A) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-'OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target (1).

2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) **Anti-sense oligos & Nuclease Resistance**: When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the Tm of its duplex with RNA increases relative to that formed by an unmodified anti-sense DNA oligo by 1.8°C per 2'-F RNA residue added (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.

(b) **Aptamers**: The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) **siRNA & Nuclease Resistance**: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and in vivo using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) **LNA Alternative**: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.
REFERENCES:
2'-F-C

2'-Fluoro-cytosine (2'-F-C) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target.

2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) Anti-sense oligos & Nuclease Resistance: When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the Tm of its duplex with RNA increases relative to that formed by an unmodified anti-sense DNA oligo by 1.8°C per 2'-F RNA residue added (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.

(b) Aptamers: The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) siRNA & Nuclease Resistance: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and in vivo using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) LNA Alternative: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.
REFERENCES:
2'-F-G

Category | Nuclease Resistance
---|---
Modification Code | fG
Reference Catalog Number | 26-6693
5 Prime | Y
3 Prime | Y
Internal | Y
Molecular Weight(mw) | 347.19

2'-Fluoro-deoxyguanosine (2'-F-G) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target (1).

2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) **Anti-sense oligos & Nuclease Resistance**: When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the Tm of its duplex with RNA increases relative to that formed by an unmodified anti-sense DNA oligo by 1.8°C per 2'-F RNA residue added (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.

(b) **Aptamers**: The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) **siRNA & Nuclease Resistance**: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and in vivo using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) **LNA Alternative**: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.
REFERENCES:
**2'-F-U**

<table>
<thead>
<tr>
<th>Category</th>
<th>Nuclease Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>fU</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6462</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>308.16</td>
</tr>
</tbody>
</table>

2'-Fluoro-deoxyuridine (2'-F-U) is classified as a 2'-Fluoro RNA monomer. The substitution of a highly electronegative fluorine atom for the 2'-OH group of the ribose results in the ring of a 2'-F-ribonucleoside having a C3'-endo (i.e., RNA-type) conformation. Consequently, an RNA oligonucleotide containing a 2'-F RNA monomer adopts the more thermodynamically stable A-form helix on hybridization to a target (1).

2'-F RNA modifications have been shown to be particularly useful in the following oligo-based applications:

(a) **Anti-sense oligos & Nuclease Resistance**: When 2'-F RNA is incorporated into an anti-sense DNA oligo (resulting in a 2'-F RNA/DNA chimeric), the Tm of its duplex with RNA increases relative to that formed by an unmodified anti-sense DNA oligo by 1.8°C per 2'-F RNA residue added (1). The DNA/RNA duplex formed between a chimeric 2'-F-RNA/DNA anti-sense oligo and its RNA target also is a substrate for RNase H. With respect to nuclease resistance, while 2'-F RNA nucleotides do provide some nuclease resistance when incorporated into DNA, phosphorothiolation of the 2'-F RNA phosphodiester linkages is recommended, because it strongly enhances such resistance. This effect becomes particularly important if the 2'-F RNA nucleotide is to be incorporated at one or more of the first three positions of the 5'- or 3'-ends of the oligo.

(b) **Aptamers**: The addition of 2'-F pyrimidines (C, U) into RNA aptamers has been shown to provide them with both considerably increased nuclease resistance and equal or higher binding affinity for their ligands, compared to the corresponding unmodified aptamers (2,3). The incorporation of 2'-F RNA nucleotides into RNA aptamers as an optimization strategy is becoming an increasingly common practice.

(c) **siRNA & Nuclease Resistance**: siRNA synthesized with 2'-F pyrimidines have been shown to have greatly increased stability in plasma compared to 2'-OH siRNA (4,5). In one study, levels of inhibition for 2'-F siRNA and 2'-OH siRNA, in cell culture and *in vivo* using BALB/c mice transfected with pGL3 luciferase, were similar over time (4). In another study, siRNA fully substituted with both 2'-F RNA and 2'-O-Methyl RNA nucleotides displayed not only enhanced stability in plasma, but also >500-fold increase in capability to down-regulate gene expression compared with the corresponding unmodified siRNA (5).

(d) **LNA Alternative**: The increased thermal stability and nuclease resistance provided by 2'-F RNA residues make them attractive as lower-cost substitutes in any application involving LNA-modified oligos.
REFERENCES:
**2'-O methyl A**

<table>
<thead>
<tr>
<th>Category</th>
<th>Nuclease Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>mA</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>27-6410A</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight (mw)</td>
<td>343.24</td>
</tr>
</tbody>
</table>

2'-O-Methyl adenosine A is classified as a 2'-O-Methyl RNA monomer. 2'-O-Methyl nucleotides are most commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

The hydrogen bonding behavior of a 2'-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2'-O-Methyl nucleotides improves duplex stability. Indeed, incorporation of a 2'-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2'-O-Methyl RNA/DNA chimeric), lead to an increase in the Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, of 1.3°C per 2'-O-Methyl RNA residue added (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2'-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos.

**References**


2'-O methyl C

Category: Nuclease Resistance
Modification Code: mC
Reference Catalog Number: 27-6410C
5 Prime: Y
3 Prime: Y
Internal: Y
Molecular Weight (mw): 319.21

2'-O-Methyl cytosine C is classified as a 2'-O-Methyl RNA monomer. 2'-O-Methyl nucleotides are most commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

The hydrogen bonding behavior of a 2'-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2'-O-Methyl nucleotides improves duplex stability. Indeed, incorporation of a 2'-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2'-O-Methyl RNA/DNA chimeric), lead to an increase in the Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, of 1.3°C per 2'-O-Methyl RNA residue added (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2'-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos. References
2′-O methyl G

Category

<table>
<thead>
<tr>
<th>Modification Code</th>
<th>Nuclease Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>mG</td>
<td></td>
</tr>
</tbody>
</table>

Reference Catalog Number 27-6410G

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight (mw) 359.24

2′-O-Methyl guanosine G is classified as a 2′-O-Methyl RNA monomer. 2′-O-Methyl nucleotides are most commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2′-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

The hydrogen bonding behavior of a 2′-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2′-O-Methyl nucleotides improves duplex stability. Indeed, incorporation of a 2′-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2′-O-Methyl RNA/DNA chimeric), lead to an increase in the Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, of 1.3°C per 2′-O-Methyl RNA residue added (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2′-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos. References


2'-O methyl U

Category          Nuclease Resistance
Modification Code mU
Reference Catalog Number 27-6410U
5 Prime           Y
3 Prime           Y
Internal          Y
Molecular Weight(mw) 320.2

2'-O-Methyl uracil U is classified as a 2'-O-Methyl RNA monomer. 2'-O-Methyl nucleotides are most commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate internucleotide linkages within the oligo.

The hydrogen bonding behavior of a 2'-O-Methyl RNA/RNA base pair is closer to that of an RNA/RNA base pair than a DNA/RNA base pair. Consequently, the presence of 2'-O-Methyl nucleotides improves duplex stability. Indeed, incorporation of a 2'-O-Methyl nucleotide into an anti-sense oligo (resulting in a 2'-O-Methyl RNA/DNA chimeric), lead to a increase in the Tm of its duplex with RNA, relative to that formed by an unmodified anti-sense DNA oligo, of 1.3°C per 2'-O-Methyl RNA residue added (2). Moreover, from a synthesis standpoint, the coupling efficiency of 2'-O-Methyl phosphoramidites are higher than those of RNA monomers, resulting in higher yield of full-length oligos. References
3'-deoxyadenosine (3'-dA)–(2'-5' linked) is deoxy at the 3'-position of the ribose, instead of at the usual 2'-position. 3'-deoxynucleotides of A, C, and T are also available from Gene Link. 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan et al. (2) studied the potential for 2',5'-linked DNA oligos as anti-sense molecules. High selectivity for 3',5' RNA over 3',5' DNA was observed, presumably due to the 2',5'-linkages destabilizing duplexes formed with 3',5' DNA more than those formed with 3',5'-RNA (for 2',5' DNA:3',5' RNA duplexes, DeltaTm is only about – 0.5 degC per 2',5' linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2'-5' linkages lowers the Tm of 2',5' DNA:3',5' RNA duplexes even less, ~ – 0.2 degC per phosphorothiolated 2',5'-linkage substitution. (by contrast, phosphorothiolation of a 3',5' linkage lowers the Tm of 3',5' DNA:RNA duplexes by ~ 0.5 to ~ 2.0 degC). Thus, 2',5'-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2',5'-linked DNA oligos, whether phosphorothiolated or not, do not support RNAse H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3',5' phosphorothiolate linkages into a 2',5' phosphorothiolated oligo at an appropriate place (that is, making a 2',5'/3',5' phosphorothiolated chimera) restores the oligo’s ability to support RNAse H activity. Furthermore, 2',5'-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3',5' DNA oligos show considerable levels of such binding.

In summary, this research suggests that 2',5'/3',5' phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3',5' to ensure that it can support RNAse H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2',5'-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).
The authors showed several polymerases, and HIV reverse transcriptase, can successfully use a string of 2-4 2',5'-linked DNA nucleotides as a template to synthesize its complementary strand with high fidelity, and speculated that the polymerases were serving as a “template for the template”, i.e., compensating for structural deficiencies in the 2',5'-linked DNA that, in non-enzymatic contexts, would preclude genetic information transfer for 2',5'-linked DNA. **References**


3'-dC (2'-5' linked)

<table>
<thead>
<tr>
<th>Category</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>3dC(2'-5')</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6491</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>289.18</td>
</tr>
</tbody>
</table>

3'-deoxycytidine (3'-dC)–(2'-5' linked) is deoxy at the 3'-position of the ribose, instead of at the usual 2'-position. 3'-deoxynucleotides of A, C, and T are also available from Gene Link. 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3,5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan et al. (2) studied the potential for 2',5'-linked DNA oligos as anti-sense molecules. High selectivity for 3',5' RNA over 3',5' DNA was observed, presumably due to the 2',5'-linkages destabilizing duplexes formed with 3',5' DNA more than those formed with 3',5'-RNA (for 2',5' DNA:3',5' RNA duplexes, DeltaTm is only about – 0.5 degC per 2',5' linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2'-5' linkages lowers the Tm of 2',5' DNA:3',5' RNA duplexes even less, ~ – 0.2 degC per phosphorothiolated 2',5' linkage substitution. (by contrast, phosphorothiolation of a 3',5' linkage lowers the Tm of 3',5' DNA:RNA duplexes by ~ 0.5 to ~ 2.0 degC). Thus, 2',5'-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2',5'-linked DNA oligos, whether phosphorothiolated or not, do not support RNase H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3',5' phosphorothiolate linkages into a 2',5' phosphorothiolated oligo at an appropriate place (that is, making a 2',5'/3',5' phosphorothiolated chimera restores the oligo’s ability to support RNase H activity. Furthermore, 2',5'-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3',5' DNA oligos show considerable levels of such binding.

In summary, this research suggests that 2',5'/3',5' phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3',5' to ensure that it can support RNase H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2',5'-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).
The authors showed several polymerases, and HIV reverse transcriptase, can successfully use a string of 2-4 2',5'-linked DNA nucleotides as a template to synthesize its complementary strand with high fidelity, and speculated that the polymerases were serving as a “template for the template”, i.e., compensating for structural deficiencies in the 2',5'-linked DNA that, in non-enzymatic contexts, would preclude genetic information transfer for 2',5'-linked DNA. References
### 3’-dG (2’-5’ linked)

<table>
<thead>
<tr>
<th>Category</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>dG(2’-5’)</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6492</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>329.21</td>
</tr>
</tbody>
</table>

3'-deoxyguanosine (3’-dG)–(2’-5’ linked) is deoxy at the 3’-position of the ribose, instead of at the usual 2’-position (note: the 3’-deoxynucleotides of A, C, and T are also available from Gene Link). 3’-deoxynucleotide (2’,5’-linked) modifications are used to substitute 2’-5’ phosphodiester linkages for the usual 3’-5’ phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2’,5’-phosphodiester linkages selectively bind to complementary single-stranded 3’,5’-RNA over comparable 3,5’-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan et al. (2) studied the potential for 2’,5’-linked DNA oligos as anti-sense molecules. High selectivity for 3’,5’ RNA over 3’,5’ DNA was observed, presumably due to the 2’,5’-linkages destabilizing duplexes formed with 3’,5’ DNA more than those formed with 3’,5’-RNA (for 2’,5’ DNA:3’,5’ RNA duplexes, DeltaTm is only about −0.5 degC per 2’,5’ linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2’-5’ linkages lowers the Tm of 2’,5’ DNA:3’,5’ RNA duplexes even less, − 0.2 degC per phosphorothiolated 2’,5’-linkage substitution. (by contrast, phosphorothiolation of a 3’,5’ linkage lowers the Tm of 3’,5’ DNA:RNA duplexes by − 0.5 to − 2.0 degC). Thus, 2’,5’-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2’,5’-linked DNA oligos, whether phosphorothiolated or not, do not support RNAsE H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3’,5’ phosphorothiolate linkages into a 2’,5’ phosphorothiolated oligo at an appropriate place (that is, making a 2’,5’/3’,5’ phosphorothiolated chimera restores the oligo’s ability to support RNase H activity. Furthermore, 2’,5’-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3’,5’ DNA oligos show considerable levels of such binding.

In summary, this research suggests that 2’,5’/3’,5’ phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3’,5’ to ensure that it can support RNase H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2’,5’-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).
The authors showed several polymerases, and HIV reverse transcriptase, can successfully use a string of 2-4 2',5'-linked DNA nucleotides as a template to synthesize its complementary strand with high fidelity, and speculated that the polymerases were serving as a “template for the template”, i.e., compensating for structural deficiencies in the 2',5'-linked DNA that, in non-enzymatic contexts, would preclude genetic information transfer for 2',5'-linked DNA. References


3'-dT (2'-5' linked)

3'-deoxythymidine (3'-dT)–(2'-5' linked) is deoxy at the 3'-position of the ribose, instead of at the usual 2'-position (note: the 3'-deoxynucleotides of A, C, and T are also available from Gene Link). 3'-deoxynucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligo. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1,2). This property means that DNA oligos containing such linkages could be useful in either anti-sense applications or as ssRNA-specific probes.

Bhan et al. (2) studied the potential for 2',5'-linked DNA oligos as anti-sense molecules. High selectivity for 3',5' RNA over 3',5' DNA was observed, presumably due to the 2',5'-linkages destabilizing duplexes formed with 3',5' DNA more than those formed with 3',5'-RNA (for 2',5' DNA:3',5' RNA duplexes, DeltaTm is only about – 0.5 degC per 2',5' linkage substitution). Phosphorothiolation (which confers nuclease resistance) of 2'-5' linkages lowers the Tm of 2',5' DNA:3',5' RNA duplexes even less, ~ – 0.2 degC per phosphorothiolated 2',5'-linkage substitution. (by contrast, phosphorothiolation of a 3',5' linkage lowers the Tm of 3',5' DNA:RNA duplexes by ~ 0.5 to ~ 2.0 degC). Thus, 2',5'-linked DNA oligos show both high selectivity and good duplex stability for RNA target sequences. However, 2',5'-linked DNA oligos, whether phosphorothiolated or not, do not support RNAse H activity when bound to complementary RNA. But, substitution of six or seven contiguous 3',5' phosphorothiolate linkages into a 2',5' phosphorothiolated oligo at an appropriate place (that is, making a 2',5'/3',5' phosphorothiolated chimera) restores the oligo’s ability to support RNAse H activity. Furthermore, 2',5'-linked DNA oligos, whether phosphorothiolated or not, show little or no non-sequence specific binding to cellular proteins (by contrast, 3',5' DNA oligos show considerable levels of such binding.

In summary, this research suggests that 2',5'/3',5' phosphorothiolated chimeric oligos, in which 6-7 of the linkages are 3',5' to ensure that it can support RNAse H activity, have considerable potential as anti-sense reagents, due to their high selectivity for complementary RNA targets, and minimal non-sequence specific binding to cellular proteins.

In 2004, Sinha and co-workers showed that 2',5'-linked DNA has some capability to function as a template for polymerase-directed DNA synthesis of the complementary strand (3).
The authors showed several polymerases, and HIV reverse transcriptase, can successfully use a string of 2-4 2',5'-linked DNA nucleotides as a template to synthesize its complementary strand with high fidelity, and speculated that the polymerases were serving as a “template for the template”, i.e., compensating for structural deficiencies in the 2',5'-linked DNA that, in non-enzymatic contexts, would preclude genetic information transfer for 2',5'-linked DNA. References
3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-me base at the 3' end will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)–(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA. Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower T_m than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required.

Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

**Gapmers.** Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-O-MOE RNA) as “wings” at each terminus flanking a central 5- to 10-base “gap” of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

**Delivery.** The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane.
The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG
3'-O methyl rA

**Category**

**RNA Oligo Synthesis**

**Modification Code**

3OmA

**Reference Catalog Number**

27-6458A

**5 Prime**

Y

**3 Prime**

Y

**Internal**

Y

**Molecular Weight (mw)**

343.24

3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-me base at the 3' end will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)--(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA. Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required.

Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

**Gapmers.** Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-O-MOE RNA) as “wings” at each terminus flanking a central 5-to10-base “gap” of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

**Delivery.** The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane.
The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG
3'-O methyl rC

Category
Modification Code 3OmC
Reference Catalog Number 27-6458C
5 Prime Y
3 Prime Y
Internal Y
Molecular Weight(mw) 319.21

3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-me base at the 3' end will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)–(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA. Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'S5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'S5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required.

Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

Gapmers. Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-O-MOE RNA) as "wings" at each terminus flanking a central 5-to10-base “gap” of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane.
The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG
3'-O methyl rG

Category: RNA Oligo Synthesis
Modification Code: 3OmG
Reference Catalog Number: 27-6458G
5 Prime: Y
3 Prime: Y
Internal: Y
Molecular Weight (mw): 359.24

3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-Ome base at the 3' end will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)–(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA. Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-O-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

**Gapmers.** Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-O-MOE RNA) as "wings" at each terminus flanking a central 5- to 10-base "gap" of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

**Delivery.** The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane.
The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG
3'-O methyl rU

3'-O methyl bases (3'-Ome) form a 2'-5' phosphodiester linkage when placed internally. A single 3'-me base at the 3' end will prevent the oligo from extension by polymerases.

3'-O methyl bases (3'-Ome)--(2'-5' linked), are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA. Presumably this selectivity is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2',5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2',5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA. Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are both recognized therapeutic agents for the silencing of specific genes at the posttranscriptional level. Chemical modifications, particularly 2'-O-(2-Methoxyethyl)-oligoribonucleotides (2'-MOE bases) and 2'-O-Methyl bases are commonly used to confer nuclease resistance to an oligo designed for anti-sense, siRNA or aptamer-based research, diagnostic or therapeutic purposes, when specific 2'-OH is not required. Nuclease resistance can be further enhanced by phosphorothiolation of appropriate phosphodiester linkages within the oligo. These modifications confers nuclease resistance, high binding affinity towards complementary RNA, reduced unspecific protein binding and extended half-life in tissues.

Gapmers. Currently, the mainstream of the ASO is gapmer design ASOs. Gapmer design oligonucleotides, contain two to five chemically modified nucleotides (LNA, 2'-O methyl or 2'-MOE RNA) as “wings” at each terminus flanking a central 5-to10-base “gap” of DNA, enable cleavage of the target mRNA by RNase H, which recognizes DNA/RNA heteroduplexes. Usually all the phosphodiester linkages are converted to phosphorothioate.

Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane.
The following list of modifications are suitable for delivery system in addition to cell penetrating peptides.
Cholesterol
Tocopherol (alpha-tocopherol, a natural isomer of vitamin E)
PEG
3'-riboadenosine (3'-rA)–(2',5'-linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

A interesting application of the 3'-rA-(2',5'-linked) modification is as an activator for 2-5A dependent RNAse to direct it to cleave unique RNA sequences (2). In this approach, the 5'-phosphorylated, 2',5'-linked tetramer p5'A2'p5'A2'p5'A2'p5'A (abbreviated “2-5A” as covalently linked to anti-sense oligo, resulting in the chimera (2-5A:AS). The AS sequence of 2-5A:AS bound to a particular ssRNA target sequence, and he 2-5A activator sequence activated 2-5A-dependent RNA, causeing it to cleave the target after UpUp and UpA motifs. Selectively targeted destruction of ssRNA in vivo via this approach has potential applications for therapeutic control of gene expression in such diseases as cancer, viral infections, and certain genetic disorders. References

3'-rC (2'-5' linked)

<table>
<thead>
<tr>
<th>Category</th>
<th>Minor Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>3'-rC(2'5')</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6911</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>305.18</td>
</tr>
</tbody>
</table>

3'-ribocytidine (3'-rC)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA. References

### Oligo Modifications

3'-rG (2'-5' linked)

<table>
<thead>
<tr>
<th>Category</th>
<th>Minor Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>3'-rG(2'-5')</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6912</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>345.21</td>
</tr>
</tbody>
</table>

3'-riboguanosine (3'-rG)–(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectively is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

**References**

3'-ribouridine (3'-rU)-(2'-5' linked), and the other three 3'-ribonucleotide (2',5'-linked) modifications are used to substitute 2'-5' phosphodiester linkages for the usual 3'-5' phosphodiester linkages at some or all positions of an oligonucleotide. Oligonucleotides containing all, or primarily, 2',5'-phosphodiester linkages selectively bind to complementary single-stranded 3',5'-RNA over comparable 3',5'-DNA (1). Presumably this selectivity is a consequence of the 2',5'-linkages destabilizing duplexes formed with 3',5'-DNA more than those formed with 3',5'-RNA, leading to 2'5'-RNA:3',5'-DNA duplexes having much lower Tm than the corresponding 2'5'-RNA:3',5'-RNA duplexes. This property means that RNA oligos containing such linkages could be useful in anti-sense applications, as ssRNA-specific probes, or as ligands for affinity purification of cellular RNA.

References
L-DNA is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

L-DNA Applications References
2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J"rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671
5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
L-DNA is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

L-DNA Applications References
2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671
5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Young-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
L-DNA is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

L-DNA Applications References
2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671
5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
L-DNA is the left-turning and mirror image version of natural DNA, as opposed to the naturally occurring right-turning version called D-DNA. L-DNA is more stable than D-DNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-DNA but form a left-helical double-helix. Because of its chiral difference, L-DNA does not bind to its naturally occurring D-DNA counterpart.

One important aspect of L-DNA is that it is poor at hybridizing to D-DNA (2). This confers multiple uses, one being that the incorporation of L-DNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-DNA oligos with any combination of D-DNA bases including fluorescent dyes and all other available modifications.

L-DNA Applications References
2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671<br>
5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
L-RNA is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

L-DNA Applications

- References
  2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:y10.1093/nar/gkl671
  5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
**L-RNA rC**

<table>
<thead>
<tr>
<th>Category</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>L-rC</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>27-6942</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>305.19</td>
</tr>
</tbody>
</table>

L-RNA is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

**L-DNA Applications**

- References
  2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:y10.1093/nar/gkl671
  5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
L-RNA is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

L-DNA Applications

- References
2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matysiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671
5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
**L-RNA rU**

<table>
<thead>
<tr>
<th>Category</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>L-rU</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>27-6944</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>320.2</td>
</tr>
</tbody>
</table>

L-RNA is the left-turning and mirror image version of natural RNA, as opposed to the naturally occurring right-turning version called D-RNA. L-RNA is more stable than D-RNA to enzymatic degradation by certain nucleases (1). Since the two enantiomers are identical in structure other than their chiral differences, their intrinsic physical properties are generally equal to each other. This includes duplex stability, solubility, and selectivity as D-RNA but form a left-helical double-helix. Because of its chiral difference, L-RNA does not bind to its naturally occurring D-RNA counterpart.

One important aspect of L-RNA is that it is poor at hybridizing to D-RNA (2). This confers multiple uses, one being that the incorporation of L-RNA into the stem of a molecular beacon as it allows stem invasion to be avoided (3). Other areas that it can play an important role in would be zip-code microarrays (2) and as molecular tags for PCR (4). When used in nanocarriers, L-DNA has greater cellular uptake as well as greater serum stability. It is good for also reducing interaction between aptamers and nanocarrier skeletons (5).

Gene Link synthesizes L-RNA oligos with any combination of D-RNA bases including fluorescent dyes and all other available modifications and chimeric bases.

**L-DNA Applications**

- **References**
  2) Utilising the left-helical conformation of L-DNA for analysing different marker types on a single universal microarray platform Nicole C. Hauser, Rafael Martinez, Anette Jacob, Steffen Rupp, J’rg D. Hoheisel, Stefan Matsyiak Nucleic Acids Res. 2006 October; 34(18): 5101-5111. Published online 2006 September 20. doi:10.1093/nar/gkl671
  5) Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos Kyoung-Ran Kim, Taemin Lee, Byeong-Su Kim and Dae-Ro Ahn.
**Phosphorothioate (SOX)**

**Category**  
Antisense

**Modification Code**  
* * 

**Reference Catalog Number**  
26-6401

**5 Prime**  
Y

**3 Prime**  
Y

**Internal**  
Y

**Molecular Weight (mw)**  
16

Click here for more information on antisense design & applications

**Minimum Pricing**  
Note that the above pricing for phosphorothioate linkages is for one site only. Minimum charges apply for 15 sites per oligo.

**Phosphorothioate**  
Phosphorothioate modification is to the backbone linkage modifying the phosphodiester linkage to phosphorothioate. This imparts considerable nuclease resistance and is used widely in the design of antisense oligonucleotides (ODN).

An antisense oligonucleotide refers to a short, synthetic DNA or RNA strand that is complementary in sequence to a short target sequence on a particular mRNA strand, which upon specific hybridization to its target induces inhibition of gene expression. The mechanism of inhibition is based on two properties: first, the physical blocking of the translation process by the presence of the short double-stranded region, and second, in the case of antisense DNA, the resulting DNA-RNA duplex is susceptible to cleavage by cellular RNase H activity, which degrades the mRNA and prevents proper translation. The latter property is the classic mode of action for antisense oligos. The former property can be used when it is necessary to design an antisense oligo with certain modifications that result in it not supporting RNase-H activity (1,2).

**Phosphorothioate References**  

**Methyl phosphonate (mp)**  
Methyl phosphonate (mp) modification makes the phosphodiester linkage neutral charged.
The solubility of the oligo in aqueous solutions slowly decreases with increasing mp linkages; consider incorporating as many standard phosphodiester linkages as well in the oligo. Increasing percentage of DMSO from 0.5 to 10% may be used to solubilize the oligo.

Methyl phosphonoamidites are deoxynucleoside amidites modified such that, when incorporated into an oligonucleotide, that base position will have a (electrically neutral) methyl phosphonate backbone linkage instead of the standard (negatively charged) phosphodiester linkage. Oligos containing one or more methyl phosphonate linkages will be resistant to nuclease degradation at those positions, and the lack of charge improves intracellular transport. Because of these properties, methyl phosphonolated oligos have been explored as anti-sense reagents (1). However, since methyl phosphonate linkages lower the oligo’s cellular uptake (2) as well as the Tm of the duplex formed with its RNA target (3), and, most importantly, also interfere with activation of RNase H activity (4), considerable care must be taken in choosing which, and how many, methyl phosphonate linkages to incorporate into a putative anti-sense oligo. In that regard, we note that 2'-O-Methyl RNA oligos containing a single 3'-end methyl phosphate "cap" (to eliminate 3'-exonuclease degradation) have been successfully used as anti-sense reagents (5). In addition, DNA extension primers containing such a "cap" have been used to characterize the nuclease activity of the yeast telomerase complex (6). Methylphosphonolated anti-sense oligos have also been used successfully to "mask" sites in U1 and U2 snRNPs required for spliceosome formation, and thus interfere with mRNA splicing (7). Many of the unique properties of methylphosphonolated oligos are due to the introduction of chirality into the phosphodiester backbone by the methyl group (8).

Methyl phosphonate (mp) References

Mesyl Phosphoramidate (Ms, u) Forty years of research have shown that antisense oligonucleotides have great potential to target mRNAs of disease-associated genes and noncoding RNAs. Among the vast number of oligonucleotide backbone modifications, phosphorothioate modification is the most widely used in research and the clinic. However, along with their merits are notable drawbacks of phosphorothioate oligonucleotides, including decreased binding affinity to RNA, reduced specificity, and increased toxicity. Here we report the synthesis and in vitro evaluation of the DNA analog mesyl phosphoramidate oligonucleotide. This oligonucleotide type recruits RNase H and shows significant advantages over phosphorothioate in RNA affinity, nuclease stability, and specificity in inhibiting key processes of carcinogenesis. Thus, mesyl phosphoramidate oligonucleotides may be an attractive alternative to phosphorothioates (1).
DNA analog in which the mesyl (methanesulfonyl) phosphoramidate group is substituted for the natural phosphodiester group at each internucleotidic position (2-5), the oligomers show significant advantages over the often-used DNA phosphorothioates in RNA binding affinity, nuclease stability, and specificity of their antisense action, which involves activation of cellular RNase H enzyme for hybridization-directed RNA cleavage. Biological activity of the oligonucleotide analog was demonstrated with respect to pro-oncogenic miR-21. A 22-nt anti-miR-21 mesyl phosphoramidate oligodeoxynucleotide specifically decreased the miR-21 level in melanoma B16 cells, induced apoptosis, reduced proliferation, and impeded migration of tumor cells, showing superiority over isosequential phosphorothioate oligodeoxynucleotide in the specificity of its biological effect. Lower overall toxicity compared with phosphorothioate and more efficient activation of RNase H are the key advantages of mesyl phosphoramidate oligonucleotides, which may represent a promising group of antisense therapeutic agents (1).


ASO's and siRNA Delivery. The development of effective delivery systems for antisense oligonucleotides is essential for their clinical therapeutic application. The most common delivery system involves a relatively hydrophobic molecule that can cross the lipid membrane. Cholesterol TEG, alpha-Tocopherol TEG (a natural isomer of vitamin E), stearyl and GalNAc modifications have been shown to effective for delivery of ASO’s and siRNA in addition to cell penetrating peptides. Click this link to view these modifications.
propyne dC

<table>
<thead>
<tr>
<th>Category</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>pdC</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6501</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>327.23</td>
</tr>
</tbody>
</table>

It has been shown that C-5 propyne analogs of dC and dT when substituted in phosphorothioate oligonucleotide imparts greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability. Based on the above information antisense oligonucleotide could either be Phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU. The use of propyne analogs is covered by patents and licensing agreements. The sale of propyne-modified oligos is for research use only. See license agreement*
propyne dU

<table>
<thead>
<tr>
<th>Category</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification Code</td>
<td>pdU</td>
</tr>
<tr>
<td>Reference Catalog Number</td>
<td>26-6502</td>
</tr>
<tr>
<td>5 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>3 Prime</td>
<td>Y</td>
</tr>
<tr>
<td>Internal</td>
<td>Y</td>
</tr>
<tr>
<td>Molecular Weight(mw)</td>
<td>328.22</td>
</tr>
</tbody>
</table>

It has been shown that C-5 propyne analogs of dC and dT when substituted in phosphorothioate oligonucleotide imparts greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability. Based on the above information antisense oligonucleotide could either be Phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU. The use of propyne analogs is covered by patents and licensing agreements. The sale of propyne-modified oligos is for research use only. See license agreement.