New Product - Locked Analog Phosphoramidites

Introduction

Locked nucleic acid (LNA) is a type of modified RNA that consists of nucleotides with a bicyclic sugar unit where the 2'-oxygen and the 4'-carbon atoms are connected with a methylene unit, as detailed in Figure 1. These structures were originally developed by Wengel and co-workers[1, 2] and since then, LNA has proven to be quite popular, particularly in the field of therapeutics where LNA has been evaluated in several clinical trials.[3-6] The most attractive aspect of LNA is that it exhibits unprecedented thermal stability towards complementary DNA and RNA. The additional ring conformationally restricts the sugar into an orientation that is particularly favorable for hybridization. A single insertion of an LNA nucleotide can enhance the melting temperature by more than 9 °C against RNA. This enhancement is sequence dependent and does decrease with increasing LNA content.[7] It should be pointed out that this stabilization does not come at the expense of specificity. In fact, LNA's exhibit mismatch specificity that is more often than not superior to that of DNA.

In addition to its ability to bind very tightly to DNA and RNA, LNA also has several other desirable traits. Like DNA and RNA, LNA is highly soluble in water and can be therapeutically delivered using standard DNA/RNA strategies. Also, the nucleoside phosphoramidites of LNA are commercially available and can be readily used with well-established solid phase synthesis methods to synthesize all-LNA or LNA/DNA/RNA chimeras. Not only does this make LNA widely available, but it also allows LNA nucleotides to be interspersed among DNA and RNA nucleotides, giving researchers a high degree of design control for their desired applications. Furthermore, LNA is well-tolerated in biological systems. A lot of this is because LNA oligonucleotides do not need to be as long to exhibit tight binding to their targets. Finally, as a backbone modification, LNA is highly nuclease resistant. Fully modified LNA is very stable in the presence of intra- and extracellular endo- and exonucleases while LNA/RNA and LNA/DNA chimeras show stabilities that are sequence dependent. For example, mixmers with alternating LNA and DNA residues will be much more stable than gapmers containing a stretch DNA flanked by LNA on the 5' and 3' termini.

Applications

The outstanding properties of LNA have been exploited in a wide range of applications. One of these areas involves the detection of single nucleotide polymorphisms (SNPs). A SNP is a variation in a single nucleotide that occurs at specific positions between members of the same species. These can occur in coding or regulatory regions and are associated with a wide range of human diseases. The use of LNA for SNP detection applications relies on the enhanced mismatch recognition relative to native DNA and have been carried out in various formats.

In allele-specific PCR, primers containing one single LNA residue across from the point of mutation allow PCR product to be generated only for the desired allele of interest. In one investigation, an allele specific LNA qPCR assay was designed to study mutations in oncogenes and had an analytical sensitivity of 0.1 %.[8]

In other experiments, LNA probes have also been used as capture probes for ELISA-like assays and fluorescence in situ hybridization (FISH) experiments.

Another area where the use of LNA has been investigated thoroughly is in the field of antisense to modulate gene expression by inactivating mRNA. This has been predominantly done in one of two ways. In the first, all-LNA or LNA/DNA mixmers are used as steric blocks to inhibit therapeutically interesting RNA targets, particularly those that have complex structures that make them inaccessible to typical antisense agents. In the second, DNA gapmers are used to facilitate RNase H cleavage of the RNA target. These sequences are usually composed of an internal DNA sequence of at least 7 nt that is sandwiched between two sections of LNA of 2-5 nt. The LNA termini provide affinity while the DNA region triggers cellular RNase H activity. As an example, Koch and coworkers have shown that a 13 nt gapmer containing an 8 nt DNA sequence was able to significantly lower non-high-density lipoprotein in both mice and non-human primates.[9]

Other areas where LNA has been used include the cleavage of RNA by DNAzymes, the detection of microRNA as well as the capture of RNA. LNA continues to be a highly desirable oligonucleotide modification.

In the past, Glen Research was able to provide LNA phosphoramidites for the research and development community, but, unfortunately, they had to be discontinued. Recent developments have allowed us to again provide the tools for this technology in the form of our LA phosphoramidites, as described below. As was the case in the past, the pyrimidine bases are thymine and 5-methylcytosine rather than uracil and cytosine, respectively, as shown in Figure 1.

Use of LA Phosphoramidites

The synthesis of LNA oligonucleotides is very similar to that of DNA oligonucleotides. The LA phosphoramidites can be dissolved in anhydrous acetonitrile to standard concentrations, except for 5-Me-C, which requires the use of a 1:1 mixture of methylene chloride and acetonitrile.

They are more sterically hindered compared to standard DNA phosphoramidites and therefore require a longer coupling time. Coupling times of 3 and 4 min are recommended for ABI and Expedite synthesizers, respectively, using tetrazole activator.

The oxidation of the phosphite after LA coupling is also slower compared to the similar DNA phosphite, and therefore a longer oxidation time is suggested. Using standard iodine oxidation procedures (0.02 M), 45 seconds has been found to be the optimal oxidation time on both ABI and Expedite instruments.

LA-containing oligonucleotides are deprotected following standard protocols. It is, however, advisable to avoid the use of methylamine when deprotecting oligos containing Me-Bz-C-LA, since this can result in introduction of an N4-methyl modification. LA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels.

LNA oligonucleotides are water soluble, can be separated by gel electrophoresis and precipitated by ethanol.

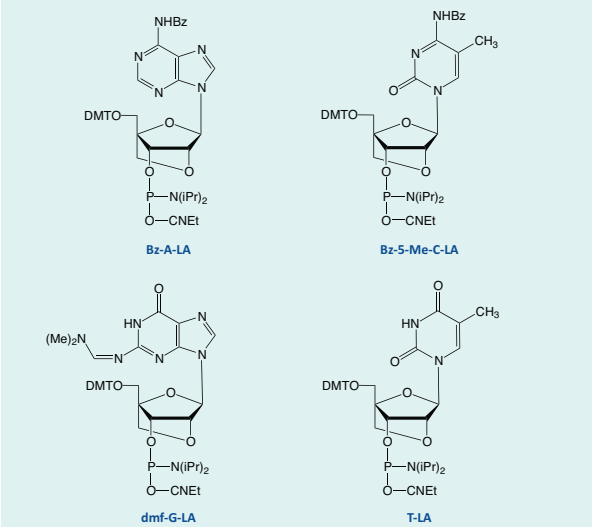

Figure 1: Structures of Locked Analog Phosphoramidites

References

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Item	Catalog No.	Pack	Price ($)
Bz-A-LA Phosphoramidite	10-2000-05	0.5g	75.00
	10-2000-10	1.0g	150.00
Bz-5-Me-C-LA Phosphoramidite	10-2011-05	0.5g	75.00
	10-2011-10	1.0g	150.00
dmf-G-LA Phosphoramidite	10-2029-05	0.5g	75.00
	10-2029-10	1.0g	150.00
T-LA Phosphoramidite	10-2030-05	0.5g	75.00
	10-2030-10	1.0g	150.00