310 Locked nucleic acid (LNA)- The therapeutic potential of chemically synthesized oligonucleotides

Asset Overview

Product Type	Small molecules (Synthesized oligonucleotides)
Indication	Various diseases including cancer
Current Stage	Hit discovery
Target(MoA)	Conformation of Watson-Crick binding into oligonucleotides
Brief Description	Researchers at the University of Oxford have developed a chemical synthesis strategy for producing DNA incorporating non-natural backbone structures and locked nucleic acid (LNA) sugars which convey desirable properties such as more selective and robust binding to complementary nucleic acids and greater resistance to enzymatic degradation.
Organization	University of Oxford

Differentiation

□ **Locked nucleic acids (LNA)**

- A class of high-affinity RNA analogs in which the ribose ring is "locked" in the ideal conformation for Watson-Crick binding
- LNA oligonucleotides exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand
- LNA oligonucleotides can be designed to have a similar affinity towards all types of sequences regardless of the GC-content

□ **Triazole-linked LNAs**

- Triazole linkage which significantly enhances the thermal stability of the modified duplex
- Triazole linkage LNAs to yield oligonucleotides which display higher target binding affinities and greater resistance to enzymatic degradation
- Reagents have been developed which allow for facile incorporation of this functionality by standard automated solid-phase synthesis methods

□ **The main benefits of the Triazole-LNA approach**

- Significant increase in DNA:RNA duplex stability compared to use of triazole or LNA alone
- Less susceptibility to enzymatic degradation than native DNA/RNA
- Synthesis by rapid, efficient and scalable solid-phase techniques
- Ability to modulate or eliminate anionic charge on DNA/RNA analogue

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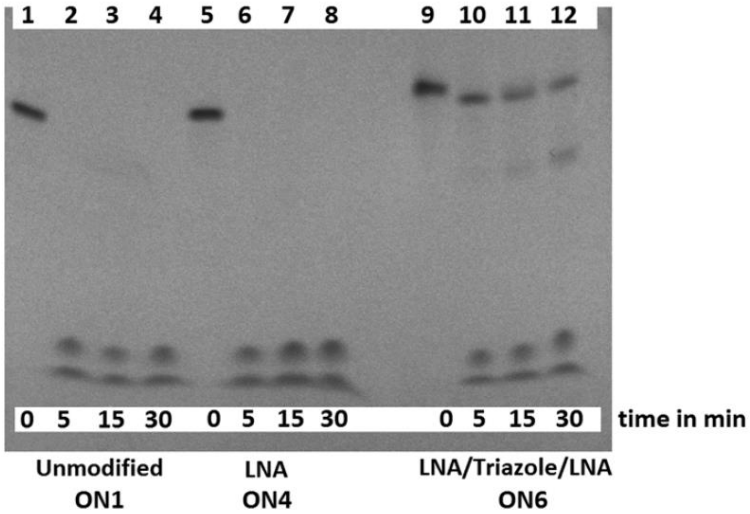
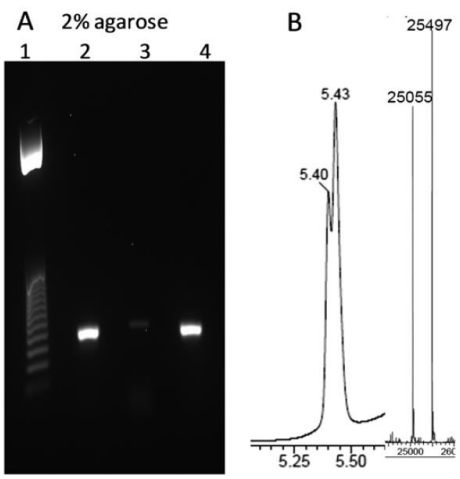
Key Data

LNA triazole stabilises ON's to 30-exonuclease digestion



The unmodified ON (lanes 2–4) and LNA ON (lanes 6–8) were fully digested within 5 min whereas the LNA–triazole–LNA ON was still visible after 30min (lane 12). It suggests that ON's containing multiple triazole–LNA linkages will have significant in vivo stability.

LNA triazole DNA template is correctly amplified by PCR



PCR amplification of this modified template was achieved using Gotaq DNA polymerase. The PCR reaction requires a long extension time for first few cycles (5 min), in agreement with a previous report of LNA-modified templates being amplified by PCR. The amplicon was shown by agarose gel electrophoresis and mass spectrometry to be the fully extended product.

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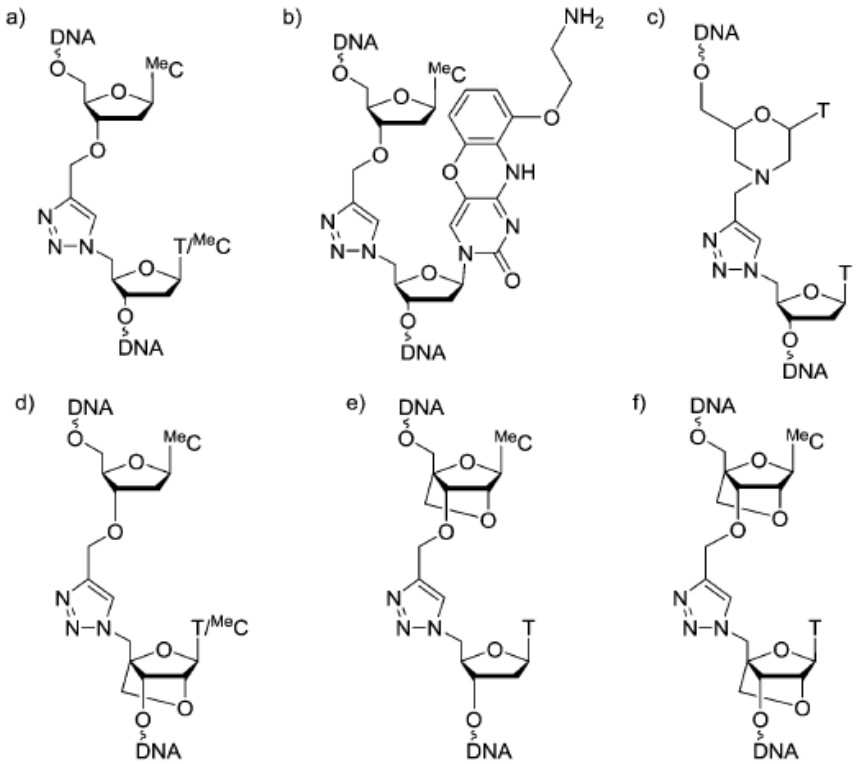
Modified DNA backbones



(a) Biocompatible triazole. (b) Triazole G-clamp. (c) Triazole-linked morpholino. (d) Triazole 3′-LNA. (e) Triazole 5′-LNA. (f) Triazole 3′,5′-LNA. T = thymin-1-yl, MeC = 5-methylcytosin-1-yl.

Thermal melting data for duplexes containing a single triazole linkage

		DNA target		RNA target	
ON code	ON sequence (5′–3′)	T_m [a]	ΔT_m [b]	T_m [a]	ΔT_m [b]
ON1	CGACG^{Me}CTTGCAGC	64.2		62.8	
ON2	CGACG^{Me}CtT^{L}TGCAGC	58.2	−6.0	62.0	−0.8
ON3	CGACG^{Me}CtTTG CAGC	55.3	−8.9	56.6	−6.2
ON4	CGACG^{Me}CT^{L}TGCAGC	67.5	+3.3	68.9	+6.1
ON5	CGACG^{Me}C^{L}tTTGCAGC	52.7	−11.5	55.5	−7.2
ON6	CGACG^{Me}C^{L}tT^{L}TGCAGC	58.4	−5.8	62.9	+0.1

Melting temperatures (Tm) were obtained from the maxima of the first derivatives of the melting curves (A260 vs. temperature) recorded in a buffer containing 10 mM phosphate and 200 mM NaCl at pH 7.0 using 3.0 mM concentrations of each strand. b DTm = change in Tm for a modified duplex relative to the unmodified duplex. TL is LNA thymidine, MeC is 5-methylcytosine and t is a triazole linkage. DNA target: 50-dGCT GCA AGC GTC G. RNA target: 50-rGCU GCA AGC GUC G.

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Intellectual Property

Patent No.	
Application Date	
Status	
Country	

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