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Synthesis and biological properties of triazole-linked locked nucleic acid†

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We have synthesized and studied the biological and biophysical properties of triazole-linked *ribo* and *xylo* locked nucleic acid (LNA). The combination of LNA with the Isobe triazole linkage gave high binding affinity when incorporated at the 3′ or 5′ termini of oligonucleotides, but low binding affinity at internal positions. Antisense oligonucleotides (ASOs) and siRNAs containing triazole dimers were highly active and nuclease resistant. Surprisingly, the *xylo*LNA-modified siRNA was the most active of the series.

Oligonucleotides are emerging as an important class of therapeutics for genetically well-defined diseases. There are two main classes of gene-silencing oligonucleotides – single-stranded "antisense oligonucleotides" (ASOs) and double-stranded small interfering RNAs (siRNAs). For clinical utility, both of these classes of oligonucleotides depend on chemical modifications to increase nuclease resistance.[1]

Oligonucleotides containing triazole in place of the phosphate linkage have been known since as early as the late 1990s.[2] However, interest in these analogues dramatically increased when mild conditions for triazole formation *via* the Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC) were applied to nucleic acids.[3,4] The mild, biorthogonal conditions of the CuAAC have allowed a number of creative applications including in synthetic biology and nanotechnology.[3,5]

Two main triazole linkage designs have been explored as phosphate mimics within oligonucleotides. The first of these, a four-atom linkage (Fig. 1b), was first described by Isobe *et al.*[6]

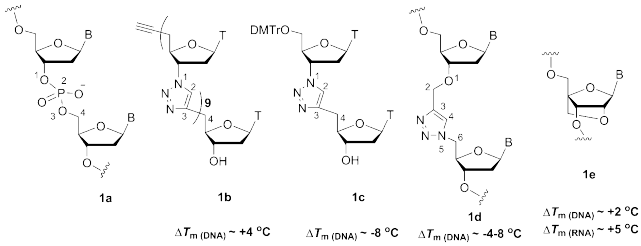

Fig. 1 Structure of DNA **1a**, triazole-linked decamer **1b**, triazole-linked dimer **1c**, biocompatible triazole linkage **1d**, and LNA **1e**; B = nucleobase, T = thymin-1-yl, ΔT_m = change in melting temperature per modification (°C).

A fully triazole-modified 10-mer oligonucleotide based on this linkage forms a highly stable duplex with complementary DNA (ΔT_m = ~+4 °C/modification).[6] However, fully modified electroneutral ASOs impose a number of issues including low water solubility. When the same triazole linkage was tested as a dinucleotide block within a phosphate backbone (Fig. 1c), low binding affinity was observed (ΔT_m = ~−8 °C/modification).[7] The linkage has been applied to the 3′-overhangs of siRNAs, where nuclease stability is essential but binding affinity is unimportant.[8]

The second main triazole linkage design was developed by Brown *et al.*: this six-atom linkage (Fig. 1d) can be correctly read by both DNA and RNA polymerases.[5] Nevertheless, this linkage, like the Isobe linkage, reduces binding affinity (ΔT_m = ~−4–8 °C/modification).[9–11]

In a complex biological environment, an ASO requires high binding affinity to invade structured targets. Combining triazole linkages with high affinity sugar or nucleobase modifications may mitigate the low binding affinity of the triazole linkage itself.[10]

Locked Nucleic Acid (LNA, Fig. 1e) is a bicyclic RNA analogue with very high affinity for complementary targets; it has found numerous applications in nucleic acid therapeutics.[12,13] We set out to study the impact of LNA on the binding affinity of the four-atom Isobe triazole-linkage. Therefore we have synthesized triazole-linked, LNA-modified dinucleotides (Scheme 1) and incorporated these into ASOs and siRNAs. We now present

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