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

3'-deoxyadenosine (3'-dA)–(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