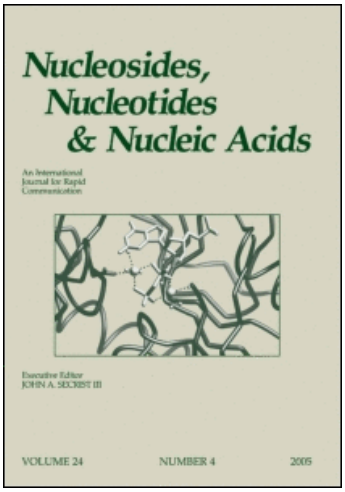

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NMI Linkage Modification Increases Potency and Stability of H-*RAS* Antisense Oligonucleotides

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MMI LINKAGE MODIFICATION INCREASES POTENCY AND STABILITY OF H-*RAS* ANTISENSE OLIGONUCLEOTIDES

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Phosphorothioate antisense oligodeoxyribonucleotides (PS-ASOs) have proven to be useful first generation antisense tools for *in vitro* and *in vivo* uses and now show great promise as human therapeutic agents. However, there are two characteristics of PS-ASOs that make it desirable to continue to attempt to improve their biophysical characteristics through chemical modification. First, PS-ASOs have been reported, at very high concentrations, to have some nonspecific activities, both *in vitro* and *in vivo*, usually attributed to their protein binding properties. Second, while significantly more stable than their phosphodiester analogues, the in vivo stability of phosphorothioate oligonucleotides can still be improved. This instability is primarily due to 3' exonucleases, 5' exonucleases, and to a lesser degree, endonucleases. There is a strong rationale for exploring backbone modifications that can reduce the P=S content and maintain or

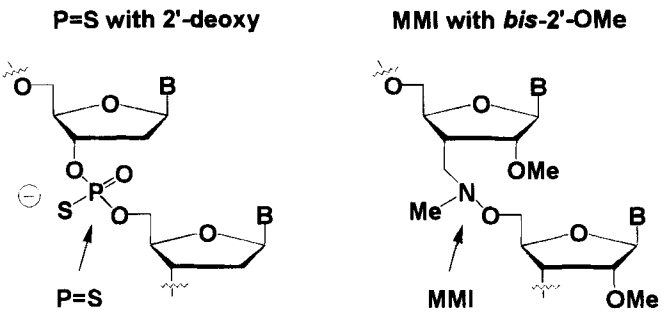

Figure 1

increase nuclease resistance of antisense oligonucleotides. One such modification, methylene(methyl)imino (MMI), allows for complete substitution of the phosphate backbone while maintaining high affinity for the target RNA and enhanced nuclease resistance.[1,2] This modification is incorporated into the oligonucleotide as MMI-dimers.

ISIS 2503 is a 20 mer phosphorothioate antisense oligonucleotide targeting the initiation of translation region of human Ha-*ras*.[3] ISIS 2503 has been demonstrated to discriminate between the H-, K-, and N- isoforms of *ras in vitro* and to have potent antitumor activity *in vivo*. ISIS 2503 is nearing completion of Phase I clinical trials as an antitumour agent.

As part of an ongoing strategy to improve the activity or duration of effect of antisense oligonucleotides, MMI modifications were incorporated into ISIS 2503 followed by *in vitro* biological assays to monitor activity. In this approach, MMI-dimers were incorporated at both the 5' and 3' ends of the oligonucleotide as either a single dimer (1+1), two dimers (2+2), or three dimers (3+3). Dimers were spaced with either phosphorothioate or phosphodiester linkages. Non-MMI-containing portions of the oligonucleotides were phosphorothioate DNA providing opportunity for RNase H activity (Gapmer Technology).

Oligonucleotides containing MMI in the (1+1) configuration were found to be at least 5 times more potent than the phosphorothioate DNA parent oligonucleotide. Increasing the MMI content (2+2) or (3+3) reduced activity relative to the (1+1) configuration but were still more active than the parent molecule. The relative order of activity may be rationalized based on the efficiency of transfection with these novel compounds. Substitution of a phosphodiester linkage for a phosphorothioate linkage at the inter-MMI-dimer position resulted in a slight reduction in activity. Duration effect studies suggest the half-life of MMI is increased at least 2 fold over phosphorothioate DNA. Together these data suggest that MMI is a viable backbone modification offering significant advantages over first generation phosphorothioate DNA.

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