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主　論　文　の　要　旨

論文題目　Enhancement of Antisense Oligonucleotide Functionality through Conjugation with Reactive Functional Groups

（反応性官能基の導入によるアンチセンス核酸の機能向上）

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論　文　内　容　の　要　旨

In today’s advanced medical landscape, oligonucleotide therapeutics, particularly antisense DNA, are emerging as promising treatments for challenging diseases. Despite their precision and efficiency, antisense therapies encounter obstacles in delivery and gene silencing activity. While lipid nanoparticle systems and GalNAc conjugation address delivery, and chemical modifications improve oligonucleotide’s biological activity, these methods are not entirely effective and present unique challenges. The research hereby introduces a novel approach by conjugating antisense oligonucleotides (ASOs) with biocompatible, reactive functional groups. This approach, adaptable for various ASO designs, highlights the power of chemistry in altering function through molecular structure. It significantly improves ASOs’ functionalities from aspects including cellular uptake, biological stability, and gene silencing efficacy, thus enhancing their therapeutic potential.

In Chapter 1, he studied the development of new antisense oligonucleotide bearing branched intracellular delivery groups. Building on the concept of Membrane Permeable Oligonucleotide (MPON) to address delivery challenges, he explored the use of functional groups to create novel intracellular-deliverable antisense oligonucleotides. Hereby, he specifically focused on a cyclic disulfide, lipoic acid, to enhance the reactivity, in contrast to the first-generation MPON utilizing a linear disulfide moiety. Using a post-synthetic conjugation method, he successfully introduced disulfide units using a modular tri-branched scaffold. This new method not only resolves issues encountered in synthesis and separation from previous studies, but it also offers more flexible design options, thereby expanding the possibilities of antisense applications. He synthesized targeted oligonucleotide conjugates more easily, which could be purified and separated while minimizing potential oxidative decomposition. In evaluations, these structures demonstrated increased cellular uptake and improved knock-down activity. Moreover, the modified antisense oligonucleotides showed enhanced stability without triggering

cytotoxicity and elicited a lower immune response. These advancements position this new method as a promising candidate for *in vivo* and therapeutic applications in antisense technology.

In Chapter 2, he examined various structural derivatives of intracellularly deliverable ASOs, building on the foundations laid in Chapter 1. Specifically, he focused on molecular design involving the linear arrangement of membrane-permeable units in lipophilic acid-based nucleic acids. This included investigating the correlation between the backbone structure incorporating membrane-permeable units and their activity, evaluating boronic acid as a novel membrane-permeable unit alternative to disulfide, and assessing new delivery forms based on the combination of disulfide-modified oligonucleotides and LNPs. In each investigation, he identified structures with potential for future applications. Notably, the combination of LNPs and lipophilic acid-based MPONs exceeded the efficacy of traditional LNP methods, suggesting the possibility of a novel intracellular release mechanism facilitated by disulfide.

In Chapter 3, he engaged in developing topological mRNA capture antisense oligonucleotides. This section of the research shifts the focus to developing topological mRNA capture antisense ASOs. By incorporating reactive functional groups, it is possible to create complex structures between ASOs and mRNA. This goes beyond simple hybridization, addressing challenges related to ASO activity. He designed branched reactive oligonucleotide fragments to initiate topological complex formation (TCF) reactions, aiming at improving gene suppression levels. These fragments, through rigorous testing, have proven effective in forming more stable and efficient complexes with mRNA. Notably, the bifurcated reactive oligonucleotides, which feature double ligation, showed significantly enhanced gene silencing effects compared to conventional antisense oligonucleotides, due to the stability of the pseudorotaxane complex they form. This highlights the TCF strategy's potential to boost gene silencing, offering a novel approach beyond traditional chemical modifications.

In this doctoral thesis, the applicant aimed at addressing the challenges inherent in traditional antisense methods by developing an approach that introduces appropriate reactive functional groups to ASOs. This led to the successful identification of effective molecular designs and the establishment of their synthesis methods. In terms of membrane permeability, a method for the intracellular delivery of oligonucleotides based on cyclic disulfide was established. In terms of activity, the applicant developed a novel gene silencing method that topologically captures target mRNA using fragmented DNA with reactive functional groups, demonstrating its superiority over existing methods. The results of this research significantly contribute to the further development of oligonucleotide therapeutics from the perspectives of both molecular design and synthesis.