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Abstract
Zhou, C. 2010. Conformationally Constrained Nucleic Acids as Potential RNA Targeting Therapeutics. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 717. 67 pp. Uppsala.
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Synthetic, physicochemical and biochemical studies of oligoncucleotides containing conformationally constrained nucleos(t)ide modifications have been performed to evaluate their abilities as potential RNA targeting therapeutics. Conformationally constrained nucleosides such as carba-LNA derivatives (C6′ and/or C7′ are modified with OH and/or Me), carba-ENA, BHNA and nucleotide D2-CNA have been synthesized through a common radical cyclization step. NMR and *ab initio* calculations studies showed that these nucleos(t)ides are indeed tightly constrained in specific conformations. Antisense oligonucleotides (AONs) modified with carba-LNA derivatives exhibited higher (+2 to +5˚C/modification) affinities but AONs containing BHNA or D2-CNA modifications showed much decreased affinities (-4 to -7˚C/modification) toward complementary RNA compared with native counterpart. D2-CNA was found that it could not be degraded by nucleases, but its immense chemical instability limits its further therapeutic application. BHNA or carba-LNA derivatives modified AONs were found to be nucleolytically more stable than unmodified one. All these modified AONs have been shown to initiate efficient RNase H-mediated RNA degradation. We also found both the target affinities and nucleolytical stabilities of AONs modified with carba-LNA derivatives were significantly modulated by both the nature and orientation of the functional groups at C6′. 6′*S*-OH-carba-LNA or 6′*R*-OH-6′-Me-carba-LNA modified AONs showed even better antisense properties than LNA modified counterpart; hence both of them are excellent candidates as antisense therapeutic agents.

2-(4-Tolylsulfonyl)ethoxymethyl (TEM) has been developed as a new 2′-OH protecting group for solid-supported RNA synthesis. RNA synthesis based on TEM strategy was compatible with the standard synthesis cycles and reagents. Only 120s was needed for the coupling step, giving high coupling yields (97-99%). After post-synthetical treatment, the obtained crude products were in high purities and can be used for biological study without further purification. Using TEM strategy, siRNA with carba-LNA, carba-ENA and aza-ENA modifications have been synthesized. Carba-LNA modifed siRNAs exhibited very good target RNA silencing activities in HeLa cells, as efficient as native one. But all of these modified siRNAs were found more toxic than native counterpart.

Keywords: antisense oligonucleotide, conformationally constrained nucleic acid, Locked Nucleic Acid, RNA synthesis, RNA targeting, siRNA

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