Chemistry of Carba-LNA modified siRNA for HIV-specific mRNA Targeting

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Fine tuning of the electrostatic properties around the internucleotidic phosphate can be achieved by incorporations of lipophilic *vs* hydrophobic substituents on the Carba-LNAs and –ENAs leading to significant modulation of the antisense and small interferring RNA (siRNA) properties, such as target affinity, nuclease resistance and RNase H or the ago protein elicitation. This study, with synthetic chemistry, enzymology and NMR structure, gives an insight on the importance of chemical characters of the substituent-type in the carbocyclic moiety of carba-LNA and carba-ENA in the minor groove for the design of the RNA targeted therapeutics.

Upon screening of 52 modified antisense oligonucleotides, containing 13 differently functionalized carba-LNA/ENA derivatives, two excellent modifications have been found, which facilitate excellent target RNA affinity, nuclease resistance and RNase H activity, and they are deemed to be excellent candidates as potential antisense and siRNA therapeutic agents against target mRNA.

This study finally shows how the appropriate RNA target selection in the HIV genome and their specific inhibition by the siRNA approach by the choice of appropriate chemistry can also successfully modulate the expression and inhibition of HIV-specific proteins. In summary, We will discuss here the key role of innovative chemistry responsible in steering of the biological function (Chemistry-Biology interplay).

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Some Current Relevant References (For full list, see: www.boc.uu.se)

1. Carba-LNA-5MeC/A/G/T modified Oligos Show Nucleobase-specific Modulation of 3′-Exonuclease Activity, Thermodinamic Stability, RNA-Selectivity and RNAse H Elicitation: Synthesis and BiochemistryUpadhayaya, R.; Deshpande, S.; Li, Q.; Kardile, R.; Sayyed, A.; Kshirsagar, E.; Salunke, R.; Dixit, S.;Zhou, C.; Földesi, A.; Chattopadhyaya, J. *Journal of Organic Chemistry*, **76**, 4408–4431 (2011).
   The carba-LNA modified siRNAs targeting HIV-1 TAR region downregulates HIV-1 replication successfully with Enhanced Potency. S. Dutta, N. Bhaduri, N. Rastogi, S. G. Chandel, J. Vandavasi, R. S. Upadhayaya, and J. Chattopadhyaya. *Med. Chem. Comm.*, **2**, 206-216 (2011) ; *Med. Chem. Commun.*, **2** (11) 1110-1119 (2011)
2. Allele-Selective Inhibition of Mutant Huntingtin Expression with Antisense Oligonucleotides Targeting the Expanded CAG Repeat. K. Gagnon, H. Pendergraff, G. Deleavey, E. Swayze, P. Potier, J. Randolph, E. Roesch, J. Chattopadhyaya, M. Damha, F. C. Benett, C. Montailler, M. Lemaitre and D. Corey. *Biochemistry*, **9**(47), 10166 (2010).
3. Synthesis of 2′,4′-Propylene-Bridged (Carba-ENA) Thymidine and Its Analogues: The Engineering of Electrostatic and Steric Effects at the Bottom of the Minor Groove for Nuclease and Thermodynamic Stabilities and Elicitation of RNase H. Y. Liu, J. Xu, M. Karimiahmadabadi, C. Zhou and J. Chattopadhyaya. *Journal of Organic Chemistry*, **75**, 7112 (2010).
4. Free-radical Ring Closure to Conformationally-locked α-LCarba-LNAs and Synthesis of their Oligos: Nuclease Stability, Target RNA Specificity, and Elicitation of RNaseH. Q. Li, F. Yuan, C. Zhou, O. Plashkevych and J. Chattopadhyaya. *Journal of Organic Chemistry* **75**, 6122-6140 (2010).
5. A screen of chemical modifications identifies positionspecific modification by UNA to most potently reduce siRNA off-target effects. J. B. Bramsen, M. M. Pakula, T. B. Hansen, C. Bus, N.Langkjær, J. Chattopadhyaya, J. W. Engels, P. Herdewijn, J.Wengel and J. Kjems *Nucleic Acids Research*, **38** (17), 5761-5773 (2010).
7. Synthesis of Conformationally Locked Carba-LNAs through Intramolecular Free-Radical Addition to C=N. Electrostatic and Steric Implication of the Carba-LNA Substituents in the Modified Oligos for Nuclease and Thermodynamic Stabilities. Jianfeng Xu, Yi Liu, Christelle Dupouy, and Jyoti Chattopadhyaya, *Journal of Organic Chemistry* ***74***, 6534-6554, 2009.
8. Fine Tuning of Electrostatics Around the Internucleotidic Phosphate through Incorporations of Functionalized 2', 4'-Carbocyclic-LNAs and –ENAs Lead to Significant Modulation of Antisense Properties. C. Zhou, Y. Liu, M. Andaloussi, N. Badgujar, O. Plashkevych, J. Chattopadhyaya, *Journal of Organic Chemistry*. **2009**, *74*, 118–134.