

List of Original Publications

This thesis is comprised of the following original publications, which will be referred in the text by their Roman numerals.

- I. Honcharenko, D.; Barman, J.; Varghese, O. P.; Chattopadhyaya, J. Comparison of the RNase H cleavage kinetics and blood serum stability of the north conformationally constrained and 2'-alkoxy modified oligonucleotides
Biochemistry **2007**, *46*, 5635 – 5646
- II. Varghese, O. P.; Barman, J.; Pathmasiri, W.; Plashkevych, O.; Honcharenko, D.; Chattopadhyaya, J. Conformationally constrained 2'-N,4'-C-ethylene-bridged thymidine (aza-ENA-T): synthesis, structure, physical, and biochemical studies of aza-ENA-T modified oligonucleotides
J. Am. Chem. Soc. **2006**, *128*, 15173 – 15187
- III. Srivastava, P.; Barman, J.; Pathmasiri, W.; Plashkevych, O.; Wenska, M.; Chattopadhyaya, J. The five- and six-membered conformationally-locked 2',4'-carbocyclic ribo-thymidine: synthesis, structure and biochemical studies
J. Am. Chem. Soc. **2007**, *129*, 8362 – 8379
- IV. Acharya, S.; Barman, J.; Cheruku, P.; Chatterjee, S.; Acharya, P.; Isaksson, J.; Chattopadhyaya, J. Significant pKa perturbation of nucleobases is an intrinsic property of the sequence context in DNA and RNA
J. Am. Chem. Soc. **2004**, *126*, 8674 – 8681
- V. Barman, J.; Acharya, S.; Zhou, C.; Chatterjee, S.; Engstroem, A.; Chattopadhyaya, J. Non-identical electronic characters of the internucleotidic phosphates in RNA modulate the chemical reactivity of the phosphodiester bonds
Org. Biomol. Chem. **2006**, *4*, 928 – 941

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3.2 The ^{31}P chemical shifts of the internucleotidic phosphorous nuclei

The internucleotidic phosphodiester (p*K*_a = 1.5) in ssDNA and ssRNA are fully ionized in the pH range (6.6 – 12.5) used in the present study (**Paper V**). Yet the ^{31}P resonances for each of the internucleotidic phosphates are shifted downfield due to the formation of G^- and show sigmoidal behavior giving an inflection point typical of a titration curve. The p*K*_a of G^- obtained from the pH dependent ^{31}P chemical shifts [Table 2] of various ^{31}P resonances in the trimeric ssDNAs and ssRNAs, and in the heptameric ssDNAs is a result of the through-space repulsive electrostatic interaction. The central G is gradually transformed to G^- upon titration which results in repulsive interaction with already negatively charged phosphates. In the case of heptameric ssRNA sequences ^{31}P chemical shifts are the results of electrostatic interactions between the phosphate and the 9-guaninyl anion as well as of the interaction between the phosphate anion and the 2'-oxyanion.

It is well known that the chemical shift is dictated by the screening of a nucleus, which in turn is directly correlated to the diamagnetic shielding by the neighboring electrons. This would normally mean that the phosphate ionization would be expected to shield the phosphorus to a higher field as for protons. However, it is well known that for various types of phosphates,^{132,133,134} phosphonates,¹³⁵ and aminophosphonates¹³⁵ the resonances are shifted downfield in alkaline pH compared to those under neutral conditions. The downfield shift of the ^{31}P resonances reflects weaker screening of the ^{31}P nucleus owing to delocalization of charge into its d*π* orbitals.¹³⁶

This is also true for some of the internucleotidic phosphorus nuclei, in our short model sequences. The ^{31}P NMR shifts of those phosphorus atoms are shifted downfield with an increase in pH, because of the excess negative charge accumulation (charge repulsion occurs between the electron cloud in the outermost orbitals of phosphorus and the central 9-guanylate ion/2'-oxyanion) around the phosphorus nucleus leads to the delocalization of the excess negative charge into its own d*π* orbitals through p*π*-d*π* orbital overlap.

3.2.1 Non-identical electronic environment around internucleotidic phosphodiester in ssRNAs

3.2.1.1 The p*K*_a of central guanine residue obtained from ^{31}P NMR

It was possible to obtain the p*K*_{a(31P)}[‡] values from almost all the internucleotidic phosphorus nuclei in case of the trimeric sequences. However, in the

[‡] The p*K*_a values obtained from ^1H NMR is mentioned as p*K*_{a(1H)} and that from ^{31}P NMR is mentioned as p*K*_{a(31P)} in this thesis. They were denoted as p*K*_{a1} in paper IV and p*K*_{a2} in paper V respectively.