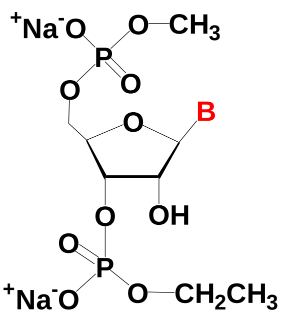
SUMMERY OF THE THESIS WORK ‡

The phosphodiester backbone makes DNA or RNA to behave as polyelectrolyte, the pentose sugar gives the flexibility, and the aglycones help in the self-assembly or the ligand-binding process. The hydrogen bonding, stacking, stereoelectronics and hydration are few of important non-covalent forces dictating the self-assembly of nucleic acids. The aim of my doctoral studies has been to explore the chemical nature of these non-covalent forces on the model nucleic acid system using the tools of NMR spectroscopy and computational chemistry.

The pH-dependent thermodynamics of mononucleosides-3',5'-bisphophate (Figure 1, taking **1a – c** as a mimic of trinucleoside diphosphate, and **1d** as their abasic counterpart for comparison), clearly shows (**Papers I** and **II**) that by changing the electronic character of



(1a): B = 9-guaninyl
(1b): B = 9-adeninyl
(1c): B = 1-cytosinyl
(1d): B = H (abasic)

Figure 1

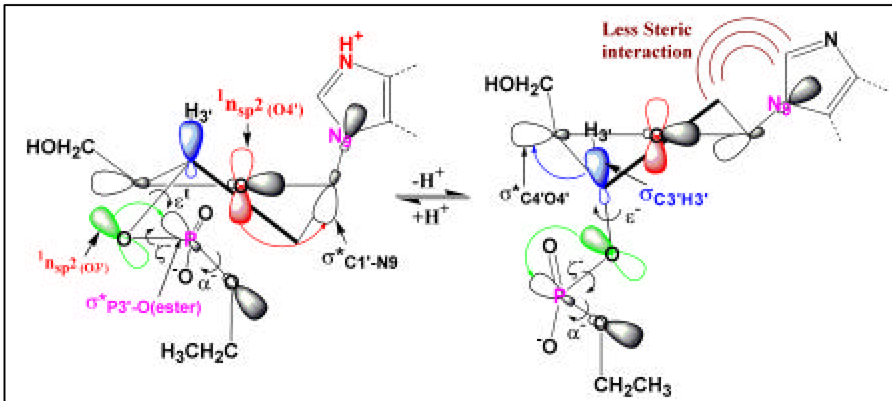
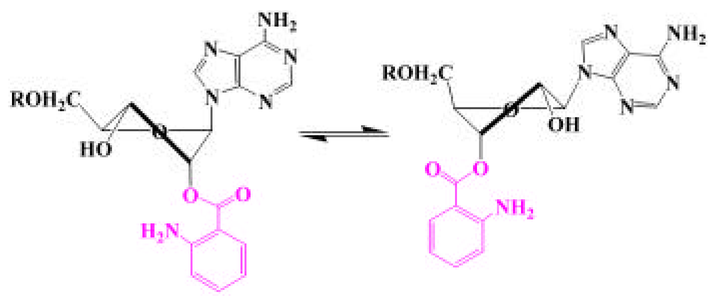


Figure 2. The schematic representation of the RNA molecular wire. Transmission of the free energy of the protonation . deprotonation equilibrium of the 9-guaninyl drives the constituent sugar-phosphate conformations through three consecutive stereoelectronic tunings: anomeric effect ($n_{O4'} \rightarrow \sigma^{*}_{C1'-N1/9}$ orbital mixing), *gauche* effect ($\sigma_{C3'-H3'} \rightarrow \sigma^{*}_{C4'-O4'}$ orbital mixing) and O3'-P3'-O anomeric effect [$^{1}n_{sp}2_{\ (O3')} \rightarrow \sigma^{*}_{P3'-O(ester)}$ orbital mixing]. All orbitals are shown by straight arrows. Smaller curved arrows show the preferred torsional orientation (see Paper **I** for details), whereas the larger curved arrows indicate the mixing of donor and acceptor orbitals.

aglycone through protonation of nucleobase, the shift of North (N, C2'-*exo*, C3'-*endo*) ? South (S, C3'-*exo*, C2'-*endo*) pseudorotational equilibrium of the constituent sugar moiety (*in absence of intramolecular base-base stacking interaction*) is modulated by the interplay of stereoelectronic anomeric and *gauche* effects. This is further transmitted to tune the sugar-phosphate backbone conformation (ε^{t} ? ε^{-} equilibrium) in tandem *as evident from pH-dependent cooperative shift of the (N,ε^{t}) ? (S,ε^{-}) equilibrium in* *nucleotides* (Figure 2). However, this aglycone promoted conformational transmission across the nucleotidyl wire depends upon the tunibility of aglycone (9-guaninyl > 9-adeninyl > 1-cytosinyl), thereby showing the cooperative interplay of constituent pentofuranose, nucleobase and phosphodiester moieties in controlling the intrinsic dynamics, hence the function, of nucleic acids.

The 3'-anthraniloyl adenosine (**2c**) and its 5'-phosphate (**2d**) [a mimic of 3'-teminal CC$\underline{A}_{OH}$ of the aminoacyl-tRNA^{Phe}] binds to EF-Tu*GTP (*Elongation Factor Tu complexes with Guanosine Triphospahte*) in preference over 2'-anthraniloyl adenosine (**2a**) and its 5'-phosphate (**2b**) (Figure 3), thereby showing (**Paper III**) that the 2'-*endo* sugar conformation is a more suitable mimic of the transition state geometry than the 3'-*endo* conformation in discriminating between correctly and incorrectly charged aminoacyl-tRNA by EF-Tu. This stereoelectronics dependent recognition switch (-2.9 kJ mol^{-1}) for EF-Tu binding is an important step, besides other recognition elements, during protein synthesis. The preferential stabilization of 2'-*endo* sugar conformation of 3'-anthraniloyl derivatives is further corroborated by the poorer transacylation of the anthraniloyl moiety from 3'- to 2'-position [$\Delta G°$ < 0 for stabilization of 3'-isomers **2c** and **2d** over 2'-isomers **2a** and **2b** respectively: $\Delta G°_{\mathbf{2a}\ ?\ \mathbf{2c}}$ = -1.2 kJ mol^{-1} and $\Delta G°_{\mathbf{2b}\ ?\ \mathbf{2d}}$ = -1.7 kJ mol^{-1}], which is cooperatively dictated by a balance of the tunable *gauche* effect of 2'- or 3'-anthraniloyl substitution. Thus, 3'-*O*-anthraniloyl adenosine derivatives have the potential to be useful as the EF-Tu recognition mimicry.



(2a): R = H
(2b): R = OPO$_3$H$^-$

(2c): R = H
(2d): R = OPO$_3$H$^-$

Figure 3

The 2'-OH distinguishes RNA from DNA both functionally as well as structurally. The NMR studies (**Paper IV**) on the 2'-OH mediated hydrogen bonding and hydration pattern in RNA at the nucleotide level showed (Figure 4): (i) The specific chemical nature of the vicinal 3'-substituent, geometrical factors like sugar pucker dependent spatial orientation of 2'-OH (proximity towards O3') as well as the H-bond angle (non-linearity of ∠O-H···O induced by attached sugar pucker) contribute to the overall strength of the weak non-linear intramolecular H-bonding (O-H2'···O3') in adenosine (**3**) and adenosine 3'-ethylphospahte (**4**). (ii) The presence of hydrophilic 3'-phosphate group in **4** compared to the 3'-OH in **3** causes a much higher water activity in the vicinity of 2'-OH (*i.e.* shorter exchange life time of 2'-hydroxyl proton with bound water, τ) in the former ($\tau_{2'\text{-}OH}$ = 489 ms) compared to the latter ($\tau_{2'\text{-}OH}$ = 6897 ms). Thus, the exposure of vicinal phosphate to the bulk water determines the overall availability of the bound water around its vicinal 2'-OH, thereby dictating the relative propensity of other inter- or intramolecular interactions.

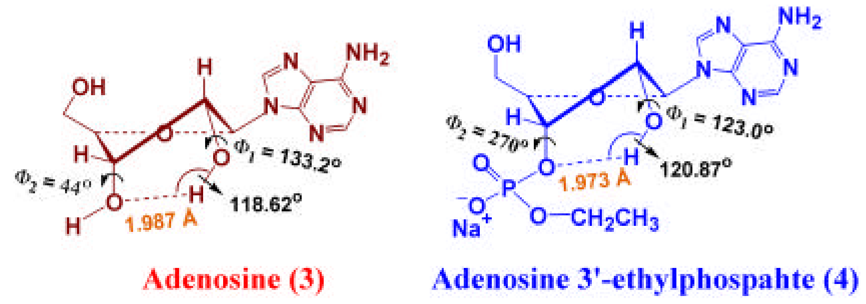


Figure 4. The 2'-OH mediated intramolecular H-bonding in adenosine (**3**) and adenosine 3'-ethylphosphate (**4**) [both predominantly with S-type sugar conformation] as calculated from ^{1}H NMR in DMSO-d_6 (with 2 mol% water) and NMR-constrained *ab initio* calculations. The torsion angles [(Φ_1 (H2'-C2'-O2'-H) and Φ_2 (H3'-C3'-O3'-H for **3** and C4'-C3'-O3'-P for **4**), the curved arrows show the rotation across the bond] at global minima of the NMR-constrained *ab initio* geometry optimization for $^3J_{H2',OH}$ = 4.1 Hz and $^3J_{H3',OH}$ = 6.1 Hz for **3** as well as $^3J_{H2',OH}$ = 4.5 Hz for **4**. The H-bond distance ($d_{2'\text{-}OH\cdots O3'}$): 1.987 Å in **3** and 1.973 Å in **4** as well as hydrogen bond angle (/O-H···O): 118.62° in **3** and 120.87° in **4**.

The pH-dependent ^{1}H NMR study with nicotinamide derivatives (**5** and **6**, Figure 5) shows (**Paper V**) the experimental strength of the intramolecular cation (pyridinium)-π (phenyl) interaction (-2.1 kJ mol^{-1}) in **5**. It has been unequivocally demonstarted that this electrostatic interaction between pyridine and the neighboring aromatic phenyl moiety perturbs the pK_a of the pyridine-nitrogen (ΔpK_a ~0.4, *becoming more basic*). The methyl protons of the linker 2,2-

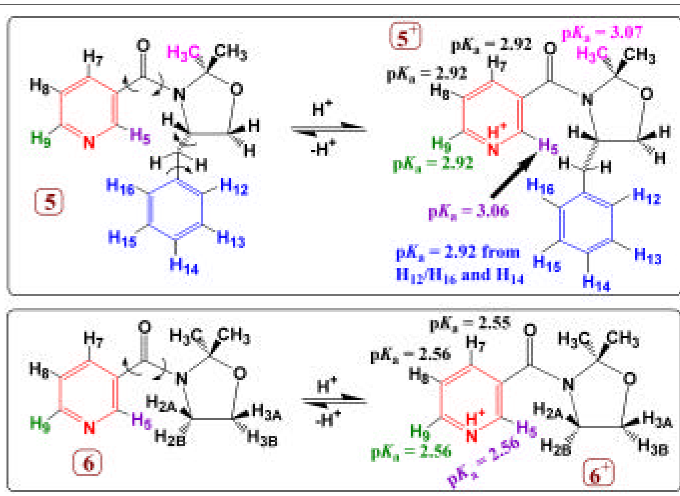


Figure 5. Nicotinamide derivatives (**5** and **6**) used in NMR and *ab intito* studies. The small arrow indicates possible rotational torsions. The pK_a values of pydinium moiety as measured from all assigned marker protons by pH–dependent ^{1}H NMR titration.

dimethyloxazolidine moiety in **5** (but not in absence of neighboring phenyl group as in **6**) is involved with CH (methyl)-π (phenyl) interaction (-0.8 kJ mol^{-1}), showing the electronically coupled nature of the pyridine-phenyl-methyl system. This edge-to-face electrostatic interaction between pyridinium and neighboring phenyl moiety is further supported by the T_1 relaxation studies and *ab initio* calculations.

The pH-dependent NMR studies of single stranded (ss) di-ribonucleotides (**7a – f**, Figure 6) demonstrates that pK_a of the ionized nucleobase can also be measured from the aromatic marker protons of the neighboring nucleobase in a electronically-coupled π system

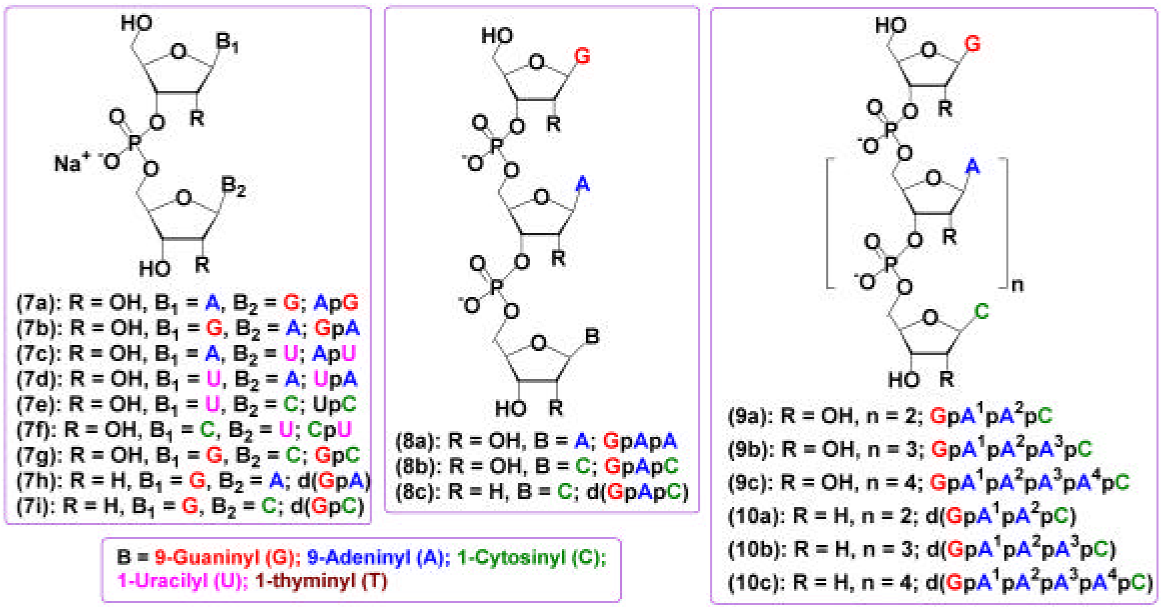


Figure 6

via intramolecular offset stacking [**Paper VI**]. Similar studies with single stranded tri-ribonucleotides (**8a** and **8b,** Figure 6) show [**Paper VII**] that this electrostatic nearest-neighbor interaction propagates over ~6.8Å in a step-wise manner [from first to third nucleobase via the second, *i.e.* **G** ⇨ **A** ⇨ **A** (or **C**)]. The further pH-dependent NMR studies of oligo-ssRNAs (**9a – c**, Figure 6) and oligo-ssDNAs (**10a – c**, Figure 6) with single ionization of 5'-(9-guaninyl) over a particular pH range titration show (**Papers VIII** and **IX**) that the propagation of the interplay of nearest-neighbor electrostatic interactions (Figure 7) across the hexameric ssDNA chain is considerably less favourable (*NMR detectable effectively up to the fourth nucleobase*) compared to that of the isosequential ssRNA (*NMR detectable up to the sixth nucleobase residue*). It therefore shows that the stacking is more easily perturbed in ssRNAs, upon the generation of 9-guanylate ion, compared to those in isosequential ssDNAs. Moreover, the pseudoaromatic character of the nucleobases in ssDNA is much less tunable compared to those in ssRNA. Each nucleobase across the oligomeric

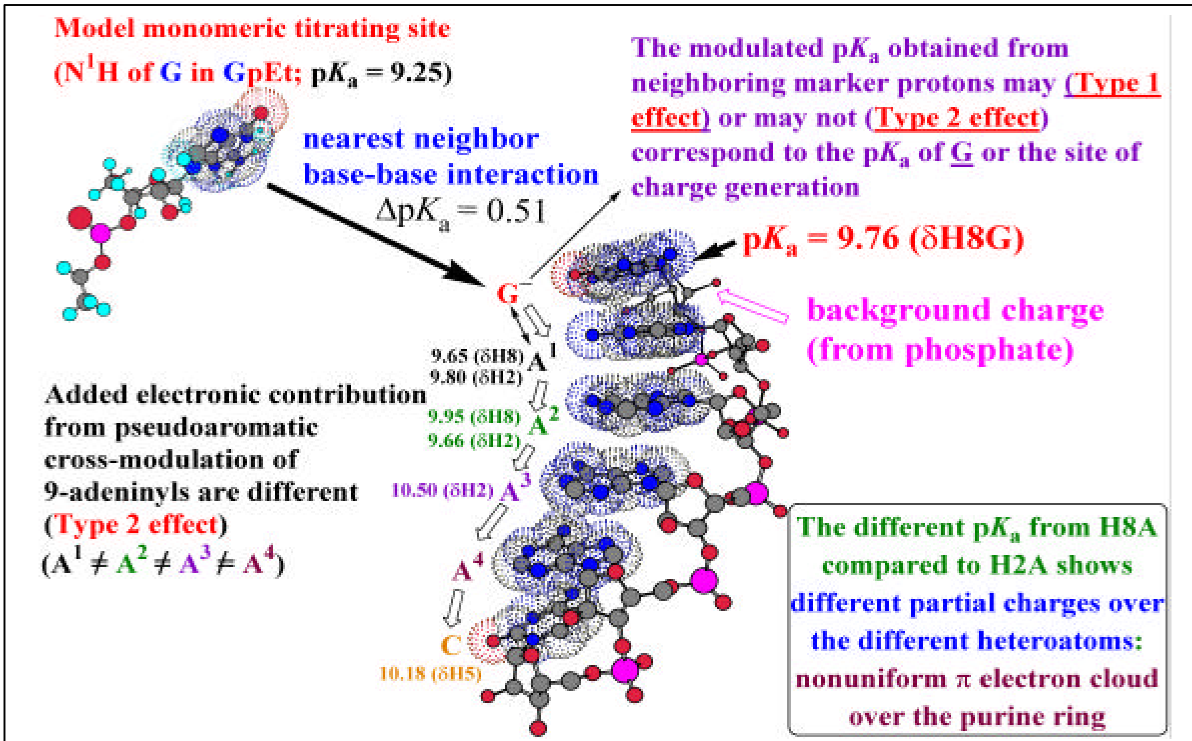


Figure 7. A schematic representation of the nearest-neighbor electrostatic interaction in hexameric ssRNA (**9c**), showing the pK_a perturbation of 9-guaninyl by the local microenvironment as well as demonstrating the nearest neighbor electrostatic interactions across the single stand.

single strand is engaged through a cascade of variable nearest neighbor electrostatic interactions, thereby cross-modulating each others pseudoaromatic character. This results into the creation of a unique set of aglycones in an oligo or polynucleotide, whose physico-chemical properties are completely dependent upon the propensity and geometry of the nearest neighbor stacking interactions.

The dissection of relative strength of basepairing vis-à-vis stacking in a duplex shows (**Paper X**) that stability of DNA-DNA duplex weakens over the corresponding RNA-RNA duplexes with the increasing content of A-T base pairs, while the strength of stacking of A-T rich DNA-DNA duplex increases in comparison with A-U rich sequence in RNA-RNA duplexes.

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‡ Full text is available at http://www.boc.uu.se/boc14www/res_proj/List_of_thesis.html

THE PUBLICATION LIST

The thesis is based on the following original publications

I. **Acharya, P.**; Trifonova, A.; Thibaudeau, C.; Földesi, A. and Chattopadhyaya, J. The Transmission of the Electronic Character of Guanin-9-yl Drives the Sugar-phosphate Backbone Torsions in Guanosine 3',5'-bisphosphate.
Angew Chem. Int. Ed. **1999**, *38*, 3645-3650.

II. Velikian, I.; **Acharya, P.**; Trifonova, A.; Földesi A. and Chattopadhyaya, J. The RNA Molecular Wire: The pH-Dependent Change in Electronic Character of Adenine-9-yl is Transmitted to Drive the Sugar-Phosphate Backbone Torsions in Adenosine 3', 5'-bisphosphate.
J. Phys. Org. Chem. **2000**, *13*, 300-305.

III. **Acharya, P.**; Nawrot, B.; Sprinzl, M.; Thibaudeau C. and Chattopadhyaya, J. The Strength of the 3'-*gauche* effect Dictates the Structure of 3'-anthraniloyladenosine and its 5'-phosphate, Two Analogues of the 3'-end of Aminoacyl tRNA.
J. Chem. Soc. Perkin 2, **1999**, 1531-1536.

IV. **Acharya, P.** and Chattopadhyaya, J. The Hydrogen Bonding and Hydration of 2'-OH in Adenosine and Adenosine 3'-ethylphosphate.
J. Org. Chem. **2002**, *67*, 1852-1865.

V. **Acharya, P.**; Plashkevych, O.; Morita, C.; Yamada, S. and Chattopadhyaya. J. A Repertoire of Pyridinium-Phenyl-Methyl Cross-Talk through a Cascade of Intramolecular Electrostatic Interactions.
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VI. Acharya, S.; **Acharya, P.**; Földesi, A. and Chattopadhyaya, J. Cross-Modulation of Physicochemical Character of Aglycones in Dinucleoside (3'? 5') monophosphates by the Nearest Neighbor Interaction in the Stacked State.
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VII. **Acharya, P.**; Acharya, S.; Földesi, A. and Chattopadhyaya, J. Tandem Electrostatic Effect From the First to the Third Aglycon in the Trimeric RNA Owing to the Nearest-neighbor Stacking.
J. Am. Chem. Soc. **2003**, *125*, 2094-2100.

VIII. **Acharya, P.**; Acharya, S.; Amirkhanov, N. V.; Cheruku, P.; Földesi, A. and Chattopadhyaya, J. The Cross-modulation of the pK_a of Nucleobases in a Single Stranded Hexameric-RNA Due to Tandem Electrostatic Nearest-neighbor Interactions.
J. Am. Chem. Soc. **2003**, *125*, 9948-9961.

IX. Barman, J.; **Acharya, P.**; Isaksson, J.; Acharya, S.; Cheruku, P.; Földesi, A. and Chattopadhyaya J. The Nucleobases in Single-stranded DNA are Better Stacked and Yet Their Pseudoaromatic Characters are More Poorly Cross-modulated Than in the RNA Counterparts Due to Variable Tandem Nearest-neighbour Electrostatic Interactions.
J. Am. Chem. Soc. **2003** (*submitted*) ♣

X. **Acharya, P.**; Cheruku, P.; Chatterjee, S.; Karthick Babu, S.; Acharya, S. and Chattopadhyaya J. The Measurement of Nucleobase pK_a of the Model Mononucleotides shows why RNA-RNA duplex is more stable than DNA-DNA duplex.
J. Am. Chem. Soc. **2003** (*submitted*) ♣

♣*By the time of submission of this application, Paper X has been accepted and Paper IX is under review.*

The following original publications were not included in the thesis

XI. Luzhkov, V. B.; Österberg, F.; **Acharya, P.**; Chattopadhyaya, J. and Åqvist, J. Computational and NMR study of Quaternary Ammonium Ion Conformations in Solutions.
Phys. Chem. Chem. Phys. **2002**, *4*, 4640-4647.

XII. **Acharya, P.**; Thibaudeau, C. and Chattopadhyaya, J. An Energetic Correlation of *Ab initio* and NMR Studies of the 3'-*gauche* effect in 3'-substituted Thymidines.
Nucleosides, Nucleotides & Nucleic Acids, **2001**, *20*, 1229-1234.

XIII. Zamaratski, E.; Trifonova, A.; **Acharya, P.**; Isaksson, J.; Maltseva, T. and J. Chattopadhyaya, Do the 16mer, 5'-GUGGUCUGAUGAGGCC-3' and the 25mer, 5'-CGCCGAACUCGUAAGAGUCACCAC-3', Form a Hammerhead Ribozyme Structure In Physiological Condition? An NMR and UV thermodynamic study.
Nucleosides, Nucleotides & Nucleic Acids, **2001**, *20*,1219-1224.

XIV. **Acharya, P.**; Velikian, I.; Acharya, S. and Chattopadhyaya, J. Molecular modeling of 2'-OH Mediated Hydrogen Bonding in Ribonucleos(t)ides by NMR Constrained AM1 and MMX Calculations.
Nucleosides, Nucleotides & Nucleic Acids, **2001**, *20*, 1211-1218.

XV. <u>Review article</u>: **Acharya, P.**; Issakson, J.; Pradeepkumar P.I. and Chattopadhyaya, J. Experimental Evidences Unequivocally Prove the Role of Stereoelectronics as One of the Major Forces Responsible for the Self-assembly of DNA and RNA.
Collect. Czech. Chem. Commun.,Collection Symposium Series (Chemistry of Nucleic Acid Components), **2002**, *5*, 99-120.

ACADEMIC AND PROFESSIONAL AWARDS

1. 1989-'91: **National Merit Scholarship**, awarded by Department of Education, Govt. of West Bengal, India, during the study years of Higher Secondary School Examination (on the basis of Secondary School Examination results).

2. 1993-'96: **National Merit Scholarship**, awarded by Department of Education, Ministry of Human Resource Development, Govt. of India, during the 2^{nd} and 3^{rd} year of studies of Bachelor Degree (on the basis of 1^{st} year of Bachelor degree result) as well as during the Masters degree studies (1994-'96, on the basis of B. Sc. results).

3. 1997: Awarded **Junior Research Fellowship** sponsored by Department of Science & Technology, Govt. of India for pursuing pre-doctoral research at the Department of Organic Chemistry, Indian Association for the Cultivation of Science, India.

4. 1999: Awarded **Doktorandtjänst (PhD Fellowship)** by Uppsala University to pursue doctoral studies at Bioorganic Chemistry department.

5. 2003: Awarded **IUPAC Travel Grant for Young Scientist** to participate and present poster in the 39^{th} IUPAC Congress held in Ottawa, Canada, during August 10-16, 2003.