Highlights of 30-Years of Bioorganic Chemistry (BOC) Research At Uppsala University (1979-2009)

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BOC was founded at UU in October 1979
Full list of publications from BOC can be found at www.boc.uu.se
The Approach to Bioorganic Chemistry in Uppsala - An Interplay of Synthetic, Structural and Biological Chemistry in Understanding Biological Function

Objectives: Development of post-transcriptional silencing technologies and bringing them to a stage where they can generally be applied for treatment of human diseases via the modulation of gene expression - Nucleic acid based therapeutics.
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Reference list
Acknowledgements

I am very grateful to all of my coworkers for excellent work in giving shape to our ideas, and sharpening them. I also thank my collaborators for giving shape to our common projects. They are summarized here. The names of all coworkers and collaborators appear in the full list of publications (www.boc.uu.se).

Jyoti Chattopadhyaya

LIST OF COWORKERS AND COLLABORATORS

Part 1.0
Development of Protecting Group Chemistry Aimed to Facilitate the Assembly of oligo-DNA and -RNA

Reactive centers that need protection during chain assembly:
(a) Exocyclic amino groups
(b) Lactam-imide function
(c) Terminal hydroxyls
(d) 2'-hydroxyls
(e) Phosphates:

Triester

Phosphoramidite

H-phosphonate

\( R' = \text{CH}_3, \ R'' = \text{H} \)
\( R' = \text{H}, \ R'' = \text{OH} \)

PG\(_1\) = permanent
PG = temporary or none
1.1 Protecting groups for the nucleobases

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Removal condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc</td>
<td>TEA/dry pyridine</td>
</tr>
<tr>
<td></td>
<td>( t_{1/2} = 330 - 60 ) min</td>
</tr>
<tr>
<td>NPS</td>
<td>triethylammonium thiochresolate/dry pyridine</td>
</tr>
<tr>
<td></td>
<td>( t_{99.5} = 10 - 60 ) min</td>
</tr>
<tr>
<td>XPsec</td>
<td>TEA/morpholine/dry pyridine</td>
</tr>
<tr>
<td></td>
<td>( t_{1/2} = 5520 - 1 ) min</td>
</tr>
<tr>
<td>Aroyl</td>
<td>aq. NH(_3)/dioxane (1:1)</td>
</tr>
<tr>
<td></td>
<td>( t_{1/2} = 9 - 30 ) min</td>
</tr>
</tbody>
</table>

Ref.: 1

Ref.: 2-3

Ref.: 4

Ref.: 5

Chattopadhyaya, J. et al., Ref.: 1-5
1.2 Protection of the lactam function of guanine and uracil residues

Reagents: i. 4-nitrobenzaldoxime, N,N,N',N'-tetramethylguanidine in dioxane-water; ii. a nucleophile

Chattopadhyaya, J. et al, Ref.: 6-8
1.3 Protection of the 5'-hydroxyl groups of 2'-deoxynucleosides

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Removal condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flouren-9-methoxycarbonyl (Fmoc)</strong>&lt;br&gt;TEA/dry pyridine&lt;br&gt;T_{99.5} = 90 min</td>
<td><strong>2-(Trimethylsilyl)ethoxycarbonyl (Tmsec)</strong>&lt;br&gt;nitromethane/ZnCl₂&lt;br&gt;t_{99.5} = 10 min</td>
</tr>
<tr>
<td><strong>2-(4-X-phenylsulfonyl)ethoxycarbonyl (XPsec)</strong>&lt;br&gt;TEA/dry pyridine&lt;br&gt;T_{99.5} = 1200 - 60 min</td>
<td><strong>9-Phenylthioxanthyl (SPx)</strong>&lt;br&gt;p-toluenesulfonic acid&lt;br&gt;2% ethanol in chloroform&lt;br&gt;T_{99.5} = 90 sec</td>
</tr>
</tbody>
</table>

Chattopadhyaya, J. et al, Ref.: 9-14
1.4 Protection of the sugar hydroxyl groups of ribonucleosides

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Removal condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-(4-X-phenyl)xanthen-9-yl (XPx)</td>
<td>p-toluenesulfonic acid in CH$_2$Cl$_2$/CH$<em>3$OH (7:3), 0 °C, $t</em>{99.5} = \sim 2$-$3$ min</td>
</tr>
<tr>
<td>3-Methoxy-1,5-dicarbomethoxy-pentan-3-yl (MDMP)</td>
<td>p-toluenesulfonic acid in CH$_2$Cl$_2$/CH$<em>3$OH (7:3), 0 °C, $t</em>{99.5} = \sim 12$ min</td>
</tr>
<tr>
<td>7-Chloro-9-anisoylthioxanthen-9-yl (CAT)</td>
<td>80% aq. acetic acid, $T_{99.5} &lt; 60$ min</td>
</tr>
</tbody>
</table>

Chattopadhyaya, J. et al, Ref.: 15-16, 19
### 1.5 Groups for temporary protection of the 3'-phosphotriester block in phosphotriester chemistry

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Removal cond.</th>
<th>Protecting group</th>
<th>Removal cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flouren-9-methyl (Fm)</td>
<td>Ref.: 17 TEA/dry pyridine $t_{99.5} &lt; 120$ min</td>
<td>4-(Nitrophenyl)thioethyl (Pte)</td>
<td>After oxidation: TEA/dry pyridine $t_{99.5} \sim 10$ min</td>
</tr>
<tr>
<td>2-(Phenylsulfonyl)ethyl (Pse)</td>
<td>Ref.: 18 TEA/dry pyridine $t_{99.5} &lt; 180$ min</td>
<td>5-Benzisoxazolylmethyl (BIM)</td>
<td>Ref.: 20-21 TEA/dry pyridine $t_{99.5} &lt; 60$ min</td>
</tr>
<tr>
<td>2-Oxymethyleneanthraquinone (Maq)</td>
<td>Ref.: 19 $\text{Na}_2\text{S}_2\text{O}<em>4$/pyridine/MeCN/TEAB (1:1:1) $t</em>{99.5} &lt; 2$ min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.6 Protecting groups for internucleotidic phosphate in phosphoramidite chemistry or special assemblage processes (branch point)

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Removal cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(Phenylsulfonyl)ethyl (Pse)</td>
<td>TEA/dry pyridine t&lt;99.5 &lt; 180 min</td>
</tr>
<tr>
<td></td>
<td>Ref.: 23, 54</td>
</tr>
<tr>
<td>Flouren-9-methyl (Fm)</td>
<td>TEA/dry pyridine t&lt;99.5 &lt; 120 min</td>
</tr>
<tr>
<td></td>
<td>Ref.: 54</td>
</tr>
</tbody>
</table>

Chattopadhyaya, J. et al, Ref.: 23, 54
Part 2.0  $^{15}\text{N}-\text{NMR}$ as a tool to optimize the protective group chemistry of nucleobases for the synthesis of pure oligo-DNA and -RNA

Most of the Exocyclic amino/imino PGs are actually anchored at a Nitrogen, and $\delta^{15}\text{N}$ (at natural isotopic abundance) can tell us exactly how the electron-density is altered as a result of an $N$-substituent. We have found that $\delta^{15}\text{N}$ shift also is indicative of placing other PGs in the complex heterocyclic system.
2.1 The effect of $N^1$ versus $O^6$ protection of the lactam function on the suppression of the nucleophilicity of $N^7$ of the guanine residue

<table>
<thead>
<tr>
<th>$N^1$ protection</th>
<th>$O^6$ protection</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="N1 Protection" /></td>
<td><img src="image" alt="O6 Protection" /></td>
</tr>
<tr>
<td>$\Delta \delta^{(15N)} N^7$ upon protonation with 1 eq TFA</td>
<td>$N^7$ methylation</td>
</tr>
<tr>
<td>( R = t\text{-butylbenzamido}, R' = H )</td>
<td>$\sim25 \text{ ppm}$</td>
</tr>
<tr>
<td>( R = t\text{-butylbenzamido}, R' = 2\text{-}(p\text{-nitrophenyl})\text{ethoxy} )</td>
<td>$\sim5.0 \text{ ppm}$</td>
</tr>
<tr>
<td>( R = t\text{-butylbenzamido}, R' = 3\text{-pyridyloxy} )</td>
<td>$\sim0.5 \text{ ppm}$</td>
</tr>
<tr>
<td>( R = t\text{-butylbenzamido}, R' = 2\text{-nitrophenoxy} )</td>
<td>$\sim1.0 \text{ ppm}$</td>
</tr>
</tbody>
</table>

The relative shift of $\Delta \delta^{(15N)} N^7$ upon protonation, above, shows that:
(a) $O^6$ PG suppresses the $N^7$ nucleophilicity/basicity (1-5 ppm shift)
(b) $O^6$-aryl PG suppresses the $N^7$ nucleophilicity more than $O^6$-alkyl (0.5-1 ppm shift)

Remaud, G. and Chattopadhyaya, J. et al, Ref.: 24-25
2.2 $^{15}$N-NMR as a means to distinguish between $N^3/O^4$ or $N^1/O^6$ protections in pyrimidine or purine nucleosides, respectively

(A) Pyrimidines: $\delta^{(15N)}N^3$ indicates the site of attachment ($N^3$ versus $O^4$)

<table>
<thead>
<tr>
<th>$\delta^{(15N)}N^3$</th>
<th>$\delta^{(15N)}N^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R = H$</td>
<td>-225.6 ppm</td>
</tr>
<tr>
<td>$R = \text{benzoyl}$</td>
<td>-193.9 ppm</td>
</tr>
<tr>
<td>$R = 2-(p\text{-nitrophenyl})\text{ sulfonyl}ethyl$</td>
<td>-221.6 ppm</td>
</tr>
<tr>
<td>$R = 2-(p\text{-nitrophenyl})\text{ ethyl}$</td>
<td>-219.9 ppm</td>
</tr>
<tr>
<td>$R = 2-(\text{phenyl})\text{ sulfonyl}ethoxycarbonyl$</td>
<td>-197.4 ppm</td>
</tr>
<tr>
<td>$R = 2-(\text{p-toluene})\text{ sulfonyl}ethoxycarbonyl$</td>
<td>-197.4 ppm</td>
</tr>
<tr>
<td>$R = \text{amino}$</td>
<td>-177.2 ppm</td>
</tr>
<tr>
<td>$R = 4\text{-nitrophenoxo}$</td>
<td>-152.0 ppm</td>
</tr>
<tr>
<td>$R = \text{phenoxy}$</td>
<td>-151.5 ppm</td>
</tr>
<tr>
<td>$R = \text{methoxy}$</td>
<td>-155.9 ppm</td>
</tr>
<tr>
<td>$R = \text{acetamido}$</td>
<td>-152.4 ppm</td>
</tr>
<tr>
<td>$R = \text{benzamido}$</td>
<td>-139.5 ppm</td>
</tr>
</tbody>
</table>

$\delta^{(15N)}N^3$ is in the range of -140 - -180 ppm suggests $N^3$ protection

$\delta^{(15N)}N^3$ is above -190 ppm suggests $O^4$ protection

Remaud, G. and Chattopadhyaya, J. et al, Ref.: 26-29
(B) Purines: $\delta^{(15N)}N^1$ indicates the site of attachment ($N^1$ versus $O^6$)

$N^1$-protection

$O^6$-protection

$\delta^{(15N)}N^1$ is above $-200$ ppm signifies $N^1$ protection
$\delta^{(15N)}N^1$ is around $\sim -160$ ppm signifies $O^6$ protection

Remaud, G. and Chattopadhyaya, J. et al, Ref.: 26-29
2.3 Investigating the depurination of 2'-deoxyadenosines

\[
\begin{align*}
\Delta\delta^{(15N)}N^1 & \quad \Delta\delta^{(15N)}N^7 \\
\text{Compound} & \quad \text{upon protonation} & \quad \text{upon protonation} \\
& \quad \text{with 1 eq TFA} & \quad \text{with 1 eq TFA} \\
a: R = \text{amino} & \quad 63.4 \text{ ppm} & \quad 0.0 \text{ ppm} \\
b: R = o-\text{nitrophenylsulfenylamino} & \quad 6.9 \text{ ppm} & \quad 2.2 \text{ ppm} \\
c: R = \text{dibenzamido} & \quad 0.0 \text{ ppm} & \quad 0.3 \text{ ppm} \\
d: R = \text{phenoxy} & \quad 0.0 \text{ ppm} & \quad 0.5 \text{ ppm} \\
e: R = \text{benzamido} & \quad 7.5 \text{ ppm} & \quad 18.9 \text{ ppm} \\
f: R = m-\text{chlorobenzamido} & \quad 2.0 \text{ ppm} & \quad 8.8 \text{ ppm} \\
g: R = 9-\text{fluorenlymethoxycarbamoyl} & \quad 8.9 \text{ ppm} & \quad 6.9 \text{ ppm} \\
h: R = 2,2,2-\text{trichloro}-t-\text{butoxycarbamoyl} & \quad 8.3 \text{ ppm} & \quad 6.4 \text{ ppm} \\
\end{align*}
\]

\(^{15}\text{N}\) chemical shift changes indicate change of protonation site from \(N^1\) to \(N^7\) for monoacyl derivatives

Reamaud, G. and Chattopadhyaya, J. et al, Ref.: 30-31
2.4 Efficient synthesis of the hypermodified Y-nucleosides

Regioselective methylation of 4-desmethylwyosine triacetate

\[
\text{AcO} - \text{O} - \text{O} - \text{AcO} \quad \xrightarrow{\text{CH}_2\text{I}_2, \text{ZnEt}_2 \text{ in diethylether with glyme, 20 °C}} \quad \text{AcO} - \text{O} - \text{O} - \text{Ac}
\]

76%

Summary:

1. Y-base is extremely acid-labile and it occurs at the 3'-end of the anticodon loop of tRNA\(^{\text{phe}}\).

2. The synthesis of Y-base has been a challenge for over 30 yrs. The standard methylation of desmethylwyosine mainly gives \(N^5\)-methyl wyosine plus <0.1% wyosine.

3. Our approach with new methylating agent (\(\text{CH}_2\text{I}_2 + \text{Zn(Et)}_2\)) gives exclusively wyosine over 76% yield.

Bazin, H. and Chattopadhyaya, J. et al, Ref.: 32
2.5 Reactivity of wyosine (A) and its $N^5$ isomer (D)

A) Red imidazole is electron rich, blue imidazole is electron deficient
B) Red imidazole is more electron rich in (A) than in (D)

Glemarec, C. and Chattopadhyaya, J. et al, Ref.: 36
2.6 $^{15}\text{N}-\text{NMR}$ as a means to understand the reactivity of wyosine

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta\delta^{(15\text{N})}\text{N}^1$ upon protonation with 1 eq TFA</th>
<th>$\Delta\delta^{(15\text{N})}\text{N}^5$ upon protonation with 1 eq TFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.4 ppm</td>
<td>39.9 ppm</td>
</tr>
<tr>
<td>B</td>
<td>7.5 ppm</td>
<td>-0.4 ppm</td>
</tr>
<tr>
<td>C</td>
<td>-1.2 ppm</td>
<td>45.7 ppm</td>
</tr>
<tr>
<td>D</td>
<td>36.6 ppm</td>
<td>-2.8 ppm</td>
</tr>
</tbody>
</table>

The change of protonation site corroborates that wyosine triacetate (A) has electron rich imidazole containing N$^5$ whereas its N$^5$-methyl analogue (D) has electron rich imidazole containing N$^1$ nitrogen. 

*Glemarec, C. and Chattopadhyaya, J. et al, Ref.: 33-36*
Part 3.0 New synthetic methods developed to modify the 2'-or/and 3'-centers in nucleosides for the synthesis of potential anti HIV agents

Second half of 80's: demanded the preparation of 2',3'-modified nucleoside analogs that can act as anti-HIV agents as reverse-transcriptase inhibitors:

\[ \text{R} = \text{N}_3, \text{F} \]

The vast majority of existing methodologies for achieving 2' and/or 3' modifications in nucleosides involved typical S_N2 type reactions.

We decided to explore the functionalization of 2',3'-double bond of the sugar moiety of Nucleosides.
3.1 Electrophilic addition reaction to achieve 2'-or/and 3'-modification

Welch, C. J. and Chattopadhyaya, J. et al, Ref.: 37
3.2 Nucleophilic addition reactions to achieve 2'- or/and 3'-modifications

The 2',3'-double bond was, however, found rather inactive in electrophilic addition reactions!

\[
\text{Functionalization of 2',3'-double bond}
\]

Chattopadhyaya, J. et al, Ref.: 38-41
3.2.1 Michael addition as means to functionalize Sugar double bond

Wu, J.-C. and Chattopadhyaya, J. et al, Ref.: 38
A

\[ \text{OH} \quad \text{U} \]
\[ R' = H, R'' = \text{NO}_2 (61\%) \]  
\[ R' = \text{Me} (73\%) \]  
\[ R' = \text{CH}_2\text{Ph} (50\%) \]

\[ \text{PhSeO}_2 \quad \text{OR}' \]
\[ R' = \text{Me} (68\%) \]  
\[ R' = \text{Et} (69\%) \]

\[ \text{OH} \quad \text{U} \]
\[ \text{HN} \quad \text{NH} \]
\[ 23\% \]

\[ \text{HN} \quad \text{NH} \]
\[ \text{SCH}_2\text{CH}_2\text{OH} \]
\[ 14\% \]

\[ \text{OH} \quad \text{U} \]
\[ \text{HN} \quad \text{NH} \]
\[ 40\% \]

\[ \text{OH} \quad \text{U} \]
\[ \text{HN} \quad \text{NH} \]
\[ \text{SCH}_2\text{CH}_2\text{OH} \]

\[ \text{B} \]

\[ \text{OMMTr} \quad \text{HN} \quad \text{CO}_2\text{Me} \]
\[ \text{NO}_2 \]
\[ 70\% \]

\[ \text{OMMTr} \quad \text{HN} \quad \text{CO}_2\text{Me} \]
\[ \text{NO}_2 \]
\[ \text{R} = \text{CH}_2\text{OH} (78\%) \]
\[ \text{R} = \text{CH}_2\text{CH}_2\text{COCH}_3 (29\%) \]

\[ \text{OH} \quad \text{U} \]
\[ \text{HN} \quad \text{NH} \]
\[ \text{X} = \text{OH} (73\%) \]
\[ \text{X} = \text{CH}_2\text{COCH}_3 (66\%) \]

\[ \text{Wu, C.-J. and Chattopadhyaya, J. et al, Ref.: 40, 43} \]

\[ \text{Hossain, N. and Chattopadhyaya, J. et al, Ref.: 44} \]
3.2.2 Cycloaddition reactions:

\[
\text{OMMTrO} \quad \text{NO}_2 \quad \text{OMMTrO} \quad \text{O} \quad \text{OEt} \quad \text{OAcO} \quad \text{NO}_2 \quad \text{OAc} \\
\text{T} \quad \text{O} \quad \text{H} \quad \text{OAcO} \quad \text{NO}_2 \quad \text{OAc} \\
\text{U} \quad \text{PhSeO}_2 \quad \text{OH} \quad \text{O} \quad \text{U} \quad \text{PhSeO}_2 \quad \text{OH} \\
\]
Hossain, N. and Chattopadhyaya, J. et al, Ref. : 46
3.3 Stereospecific Free-radical reactions as means to form C-C bond

Chattopadhyaya, J. et al, Ref.: 47-50
Part 4.0  Are All Phosphates in RNA Physico-Chemically Equivalent?
Phosphate has three ionizable $H^+$

First midpoint
$[H_3PO_4] = [H_2PO_4^-]$  
$pK_a = 2.2$

Second midpoint
$[H_2PO_4^-] = [HPO_4^{2-}]$  
$pK_a = 7.2$

Third midpoint
$[HPO_4^{2-}] = [PO_4^{3-}]$  
$pK_a = 12.7$

$pH$ vs. Equivalents of $OH^-$

Chemical structures:

$\text{H}_3\text{PO}_4 \overset{pK_a = 2.2}{\leftrightarrow} \text{H}_2\text{PO}_4^- \overset{pK_a = 7.2}{\leftrightarrow} \text{HPO}_4^{2-} \overset{pK_a = 12.7}{\leftrightarrow} \text{PO}_4^{3-}$
Transesterification Reaction: the key to the processing of Genetic Information

- $k_{eq} \sim 1$;
- $k_f \sim 10^{-6} \text{ min}^{-1}$

Pancreatic ribonuclease
- $k_f \sim 1400 \text{ min}^{-1}$

RNase T₁, T₂, U₂

tRNA Splicing endonuclease

U6 snRNA processing
Catalysis by Group I and Group II Introns

Mechanisms for Catalytic Activity of RNase A, Hammerhead Ribozyme, and Hairpin RNA

Pre-mRNA Processing – Splicing (Group II)

Lariat-RNA formed as an intermediate dictates the fidelity of subsequent transesterification to ligate the Exon 1 (E1) and Exon 2 (E2) with the elimination of the Intervening sequence (IVS)
4.1 Synthetic and structural studies of lariat-RNA modelling both the splicing intermediate and the structure of the self-cleavage site of some catalytic RNAs (Ribozyme)

**Aim**: To reveal why there is a genuine need for the formation of the lariat-RNA as an intermediate for processing of every pre-mRNA to a functional mRNA in the penultimate step of Group II and Nuclear pre-mRNA processing reaction (Splicing) in Eukaryotes?

Key step: regiospecific phosphorylation of the cis-diol of the branch-point nucleoside

![Chemical Diagram](attachment:chemical_diagram.png)
4.1.1 Earlier Syntheses of Branched-RNA

Earlier Syntheses of Branched-RNA

Earlier Syntheses of Branched-RNA

4.1.2 Strategies for the Synthesis of Branched-RNA

Vial, J.-M. and Chattopadhyaya, J. et al, Ref.: 51

Zhou, X.-X. and Chattopadhyaya, J. et al, Ref.: 54
Strategies for the Synthesis of Branched-RNA

Balgobin, N.; Földesi, A and Chattopadhyaya, J. et al, Ref.: 55

Zhou, X.-X. and Chattopadhyaya, J. et al, Ref.: 56
4.1.3 Largest Branched-RNA Synthesized Chemically So Far - in Solution!
Sund, C. and Chattopadhyaya, J. et al, Ref.: 57
4.1.4 SYNTHETIZED BRANCHED-RNAs, Modelling the Lariat of the Group II & Nuclear mRNA introns

Zhou, X.-X. and Chattopadhyaya, J. et al, Ref.: 51-57
4.1.5 First Synthesis of a Lariat-RNA

Sund, C. and Chattopadhyaya, J. et al, Ref.: 58
$^1$H – $^{31}$P Chemical Shift Correlation of Lariat RNA Tetramer

Sund, C. and Chattopadhyaya, J. et al, Ref.: 58
4.1.6 Synthesis of the Lariat Hepta-RNA

Sund, C. and Chattopadhyaya, J. et al, Ref.: 59
Sund, C. and Chattopadhyaya, J. et al, Ref.: 59
Figure 4. (A, B) $^{1}H$-NMR spectrum of human hepatoma (HepG2) (7.17 m d) recorded at 264 K. (B) Expansion of aromatic region ($0.59 - 4.2$ ppm) showing full assignments. (C) Expansion of aromatic region ($0.59 - 3.42$ ppm) showing full assignments. (D) TD $^{13}C$NMR spectrum of human hepatoma (HepG2) showing full assignments.
Figure 5 Clean-TOCSY spectra of the lanat heptamer (19) recorded at 284 K with a sweep width of 4000 Hz. 4K data points were used in F2 and 256 experiments of 64 scans in F1. Quadrature detection in F1 was achieved with TIPPI. A sinc² window was applied in both dimensions before zero-filling and Fourier transformation to give a 2K × 1K matrix. All seven sugar residues are easily assigned in the spectra (reference, 8 (H₂O) = 4.7 ppm). See Table 1.
4.2 Synthetic Lariats - Self-cleaving RNA

Agback, P. and Chattopadhyaya, J. et al, Ref. 60-62
*whereas Nature performs the chemospecific transesterification of a single phosphodiester bond out of a hundreds/thousand or more by specific folding and by interactions with cofactors, We achieved cleavage of One phosphodiester bond specifically out of six others.

See: www.boc.uu.se

Agback, P. and Chattopadhyaya, J. et al, Ref.: 60-62
Agback, P. and Chattopadhyaya, J. et al, Ref.: 61

锤头 DNAzyme:

\[ k_{\text{obs}} = 0.01 \text{ to } 0.83 \text{ min}^{-1} \]

\[ 2',3'-\text{Cyclic Phosphate} \]

\[ \text{Self-Cleavage site} \]

\[ \text{WT-Hammerhead: } k_{\text{obs}} = 0.43 \text{ m}^{-1} \]

\[ \text{DNAzyme: } k_{\text{obs}} = \sim 0.83 \text{ m}^{-1} \]

\[ 0.25 \times 10^{-4} \text{ m}^{-1} \text{ at } 22 \degree \text{C} \]
$^1$H/$^{31}$P Correlation of the self-cleavage of Tetra-loop Lariat RNA

Agback, P. and Chattopadhyaya, J. et al, Ref.: 61
4.3 NMR-constrained Simulated annealing and Molecular Dynamics of the Self-cleavage site

- **Tri-Loop Lariat**
  - No Cleavage

- **Tetra-Loop Lariat – Form A**
  - No Cleavage

- **Cleaving √**
  - **Tetra-Loop Lariat – Form B**
  - Near Cleaving Conformation

- **Cleaving √**
  - **Penta-Loop Lariat**
  - Near Cleaving Conformation

*Agback, P. and Chattopadhyaya, J. et al, Ref.: 61*
RNA Self-cleavage Site Geometry

NMR structure  Near-transition state of the RNA self-cleavage  Ab intio optimization

HF/3-21G basis set  Gaussian 92

Agback, P. and Chattopadhyaya, J. et al, Ref.: 58-61
4.4 A Recent Strategy for the Synthesis of Branched-RNA

Part 5.0 Structural studies of oligo-DNA and RNA by NMR
Site-specific isotope labelling as a means to facilitate NMR studies of large, biologically significant DNA/RNA molecules

The bottleneck of NMR studies: How is it possible to tackle the problem of (i) increasing spectral complexity and (ii) the deleterious relaxation effects of large biopolymers of specific biological function.

5.1 Deuterium labelling:

The "NMR-window" concept

Arrow signifies shift of "NMR-window" across the whole length of molecule. The design of "NMR-windows" with a slight overlap enables one to walk on the molecule by a common nOe or a chemical shift of two adjacent "NMR-windows".

Deuterated part (²H)
Nondeuterated part (¹H) - the window

Földesi, A. and Chattopadhyaya, J. et al, Ref.: 63
5.1 Various nucleoside blocks site specifically deuterated for sequence specific incorporation into oligonucleotides

RNA (R=OH): 2',3',4'(50 atom%),5',5"-d_5
DNA (R=D): 2',2",3',4'(~50 atom%),5',5"-d_6

RNA: (21mer and 31mer) - significant spectral simplification increasing the number of structural constraints obtained from the visible "1H-NMR window".

DNA: (20mer duplex) - drastically decreased spectral overlap (except for the aromatic-H1' and H1'-H4' regions) decreased relaxation rates of the residual protons within the deuterated nucleosides in RED.

5'-d(CGCGGGCU)2-3'

Glemarec, C. and Chattopadhyaya, J. et al, Ref.: 64-66
NOESY spectra of aromatic to H1' regions for the natural 20mer DNA duplex (A) and its deuterated counterpart (B) in $^2$H$_2$O at 21 °C. The boxed areas (green) show that a significant number of resonances arising from the G residues overlap in both duplexes. Arrows (red) show sharp, intense cross-peaks arising from the partially deuterated residues. This change of line-shape indicates an increase of the T2 relaxation which is a consequence of the decreased number of relaxation pathways due to substitution of deuteriums for protons and the absence of coupling to vicinal H2'/2". The sequential connectivity from the 5'-dC to the 3'-dG is shown by the solid lines.

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 66
Aromatic to H1' region of the NOESY spectrum for the selectively deuterated 20mer DNA duplex (A) and the ROESY spectrum of the same region (B). The protons in the natural abundance and in the deuterated nucleotides have very different T2 relaxation time, any experiment which filters away protons with shorter relaxation time (ROESY, MINSY, HAL, HAL-MINSY) can give spectral simplification. This is shown in panel (B), where in the ROESY experiment, cross-peaks arise from the residual H1' of the deuterated residues only. The NOESY pectrum in (A) clearly gives information which complements the ROESY counterpart in (B) resolving two dG cross-peaks in the boxed area.

Agback, P. and Chattopadhyaya, J. et al, Ref.: 67
Aromatic to H$_2$/H$_3$/H$_4$/H$_5$/H$_5''$ regions of NOESY spectra for the natural 21mer RNA (a) and its deuterated counterpart (b) in $^2$H$_2$O at 24 °C. The boxed areas show that a significant number of resonances overlapping in case of the natural 21mer RNA are well resolved in case of the deuterated analogue. Cross-peaks belonging to the same residue are connected and appropriate assignments are labelled.
Contour plots of aromatic to H2'/H3'/H4'/H5'/H5" regions of the NOESY spectra for the natural 31mer RNA (a) and its deuterated counterpart (b) in 2H2O at 26 ºC. The boxed area in (a) is crowded making assignment and accurate NOE volume determination impossible. The cross-peaks are better resolved in the same boxed area for the deuterated counterpart in (b) and they are much sharper allowing easier and more accurate NOE volume determination for molecular modelling.

The NMR structure of the 31mer RNA domain of the catalytic RNase P RNA

RNA: (55mer, block A) – Identification of the helical regions was possible

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 72
Expanded DQF-COSY spectrum of deuterated 55-mer RNA, H1'-H2' area
Expanded DQF-COSY spectrum of deuterated 55-mer RNA, aromatic to H5 region: only residues in the "Window" region are clearly visible.
RNA: (55mer, block A) - the low and varying level of deuterium incorporation at C4' (~85 - ~50 atom%) results in overcrowding of important regions of NOESY spectra.

New synthesis:

>97 atom% 2',3',4',5',5''-d$_5$

Földesi, A. and Chattopadhyaya, J. et al, Ref.: 73-75
Achieved $^1$H-NMR spectral simplification (A) compared to type A block (C) and to the U at natural abundance (B)
DNA: (12 & 20mer duplexes, blocks B+C) - considerable simplification of the spectral overlap providing both the J-coupling and the nOe information allowing the extraction of quantitative interproton distance information, the residual proton at C2' shows a ca 2-fold increase of T2 compared to the natural non-deuterated residue.

\[
5'-d(\text{C6G7C8G9A10A11T12T13C14G15C16G})_2-3' \\
5'-d(\text{C2G3C4G5C6G7C8G9A10A11T12T13C14G15C16G17C18G19C20G})_2-3'
\]

DNA: (12-mer duplex, blocks D+E) - 3JH1'H2', 3JH4'H5', 3JH4'H5'', 3JPH4', 3JPH5' and 3JPH5'' values, H1'-H5'/5'', H1'-H4', H1'-H2'', H2''-H4', H2''-H5'/H5'', H4'-H5'/H5'', H1'i-H5'/5''i+1, H1'i-H4'i+1, Ar-H5'/5'' nOe volumes with less affect from spin diffusion are extractable

\[
5'-d(\text{C2G3C4G5A6A7T8T9C10G11C12G})_2-3'
\]

DNA: 12-mer duplexes - measurement of T1 relaxation time of the C-2' carbon at natural abundance, giving information about the dynamics of the 2'-methylene fragment.

\[
5'-d(\text{C2G3C4G5A6A7T8T9C10G11C12G})_2-3'
\]

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 68-71
Extracting $J$-couplings from the $^1$H-NMR-window-II

5'-d($^5$C$^6$G$^7$C$^8$G$^9$A$^{10}$A$^{11}$T$^{12}$T$^{13}$C$^{14}$G$^{15}$C$^{16}$G)$_2$-3'

Sections of the DQF-COSY spectra of the labelled 12mer duplex and its nondeuterated analogue: (A) selectively labelled $7^C$ and nonlabelled $13^C$ H1'-H2' cross-peaks; (B) Same region for the nondeuterated oligomer; (C) and (D) show the "down-off diagonal" same regions. The cross sections (taken at the arrows) show the improved signal intensity for the labelled residue due to the restricted $J$-network.

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 68
Extracting the $^3J_{4',5'}$, $^3J_{4',5''}$, $^3J_{4',p}$ couplings from the $^1$H-NMR-window-III

$5'$-d($^{1}$C$^{2}$G$^{3}$C$^{4}$G$^{5}$A$^{6}$A$^{7}$T$^{8}$T$^{9}$C$^{10}$G$^{11}$C$^{12}$G)$_2$-3'

Expansions of the H4'-H5'/5" regions of the DQF-COSY spectra of the labelled 12mer duplex: (a) cross section at the arrow through the cross-peaks gives $^3J_{4',5''}$, (b) cross section at the arrow through the cross-peaks gives $^3J_{4',5''}$.

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 70
5.2 $^{13}$C-labelling: relaxation window

**RNA:**

$^{13}$C$_6$-D-Glucose → $^{13}$C$_5$ OTBDMS

B = U  
B = A$_{Bz}$  
B = C$_{Bz}$  
B = G$_{Bu}$

29mer HIV-1 TAR RNA

full T1 and T2 relaxation measurements for the labelled nucleotides

*Milecki, J. and Chattopadhyaya, J. et al, Ref. 76*
HSQC-CT spectrum of the site specifically $^{13}$C$_5$-sugar labelled 29mer TAR RNA. The chemical shift ranges for different types of sugar protons (top) and carbons (right) are given. Four sets of $^1$H-$^{13}$C cross-peaks corresponding to the carbon labelled residues are clearly observed.

*Milecki, J. and Chattopadhyaya, J. et al, Ref.: 76*
5.3 DNA: additional $^2$H-labelling at C2' (R/S: ~15/85) and C5' (R/S: ~1:1)

5'-d(1C2G3A4T5T6A7A8T9C10G)$_2$-3'
5'-d(1C2C3A4T5T6A7A8T9G10G)$_2$-3'

Földesi, A. and Chattopadyhaya, J. et al, Ref.: 77

Made it possible to investigate the relaxation properties of the labelled oligo-DNAs

Maltseva, T. V. and Chattopadyhaya, J. et al, Ref.: 78-79
Comparison of expanded regions [(A) and (C)] of the \[^{1}H, {2}H, {13}C\] correlation spectrum of the double labelled DNA duplex and the standard HSQC spectrum [(B) and (D)] of the natural abundance counterpart. The triple correlation experiment successfully filters away those C2' and C5' carbons which are not covalently attached to deuterium.

*Maltseva, T. V. and Chattopadyhaya, J. et al, Ref.: 78*
Part 6.0 Physicochemical properties of oligonucleotides using NMR
6.1 Are All 2′-OH in RNA Equivalent?
Screening of the $^{31}$P nucleus in the Nuclear Magnetic Field

**Applied magnetic field**
- Spinning $^{31}$P nucleus with a larmour frequency

The magnitude of $^{31}$P chemical shift depends on the electronic environment of the nuclei.

**Electronic configuration in the outermost electronic orbital of $^{31}$P in ground state**
- $3s^2$
- $3p^3$
- $3d$

**Electrostatic repulsion**
- Extra Charge around $^{31}$P moves to the vacant $d\pi$ orbital
- Electronic environment around $^{31}$P nucleus becomes depleted of negative charge hence $\delta^{31}$P is deshielded

With pH G$^-$ interacts with negatively charged phosphates.
6.1.1 Titration of Hepta-RNA from pH 7.0 – 12.6

\[ \Delta \delta = 0.036 \]

\[ \Delta \delta = 0.119 \]

\[ \Delta \delta = 0.168 \]

\[ \Delta \delta = 0.102 \]

\[ \Delta \delta = 0.092 \]

G\textsuperscript{−}/2′-O\textsuperscript{−} | PO\textsubscript{2}^- | G\textsuperscript{Me}/2′-O\textsuperscript{−} | PO\textsubscript{2}^-
Do all 2'-OH in an RNA has the same pKₐ? Titration from pH 11.6 – 12.5

2'-OHs in 8c are more acidic as a result of electrostatic repulsion between G⁻ and O⁻.
6.2 Does an Aromatic Residue Feel the Presence of the Neighbor?

Acharya, P. and Chattopadhyaya, J. et al, Ref.: 81
**TOOL:** \( pK_a \) of Heterocyclic Compounds Speaks for Their Aromatic Characters.

\[
\begin{align*}
\text{pK}_a &= 5.2 & \text{pK}_a &= 1.3 & \text{pK}_a &= 3.54 & \text{pK}_a &= 2.83 & \text{pK}_a &= 5.71 \\
pK_a &= 2.24 & \text{pK}_a &= 2.5 & pK_a &= 9.17 & pK_a &= 1.2 & pK_a &= 8.9
\end{align*}
\]

\[pH = pK_a + \log \frac{a(\text{conj. base})}{m[\text{weak acid}]}.\]

\[pK_a = pH - \log \frac{a(\text{HCOO}^-)}{m[\text{HCOOH}]}\]

\[pK_a = -\log K_a, \text{ Which is dependent on the Aromatic Character}\]
$^1$H-NMR titration
Giving the $pK_a$
Error 0.02-0.04

Pyridine is More Basic

(A)

pKa modulation of pyridyl group as a result of neighboring Phenyl group:
Compare (A) and (B)

(Acharya, P. and Chattopadhyaya, J. et al, Ref.: 81)
Ab initio calculation (HF/6-31G**) based molecular model building

$\text{d}(\text{H}_5 - \text{Ph}) = 4.36 \, \text{Å}$

$\text{d}(\text{H}_5 - \text{Ph}) = 3.65 \, \text{Å}$

Edge-to-face aromatic Interaction between pyridinium and phenyl moieties

Acharya, P. and Chattopadhyaya, J. et al, Ref.: 81
Geometries of the Aromatic Interaction

Aromatic Interactions in Non-biological Model System

T-shaped (-2.8 kJ mol\(^{-1}\))

Interaction in benzene dimer

**1H NMR of dipolar coupling in liquid benzene**

-1.4 kJ mol\(^{-1}\)


**Edge-to-face Interaction**

Aromatic and polar group interaction

pK\(_a\) = 6.86 when aromatic moiety is trans w.r.t

-COOH: pKa = 6.36


**Aromatic and polar group interaction**

CH/\(\pi\) Interaction

Nishio et al. *JCS Perkin Trans. 2* 2000, 1243

**CH/\(\pi\) Interaction**

Hydrocarbon/heterocycle

Aromatic Interaction

**Parallel-stacked Polar/\(\pi\) interaction interaction**

Crystal structure of the CT (1:1) complex between aniline and \(p\)-dinitrobenzene

*The first structural report showing aromatic rings in stacked arrangement*


**Offset stacking**

R = Adenine, Phenyl Naphthyl, Methyl

Electrostatic Potential Energy ($E$):

$$E = \frac{Q_1 \times Q_2}{4\pi \varepsilon_0 r}$$

Where,

$Q_1 = \text{Pyridinyl}$ and $Q_2 = \text{Phenyl}$

$\varepsilon_0 = \text{Permitivity factor}$

($\text{Microenvironment}$ around Pyridinyl and Phenyl)

$r = \text{Distance between the Pyridinyl and Phenyl.}$
Stronger H-Bonding in DMSO
Weaker H-Bonding in Water

6.3 Can Electrostatic Interactions Dictate the Chemical Reactivity & Preorganization of RNA?

⇒ The Nearest-neighbor Effect.
⇒ How would You see this?
⇒ The $pK_a$ Modulation?

Acharya, P. and Chattopadhyaya, J. et al, Ref.: 81-84
6.3.1 Chemical Shift Titration of r(C1A2C3G4C5A6C7)

**Signature of a Coupled System**
In the given pH range, only the G base is getting ionised, but this process is observable all alongside the RNA chain!

Marker protons of the different nucleobases give a slightly varying value for $pK_a$ of G (Perturbed $pK_a$)

The $pK_a$ of G can be calculated from the pH dependent $^{31}$P chemical shift variations of the phosphate moieties
6.3.2 Sequence-dependent pK\textsubscript{a} Modulation of G in r(C\textsuperscript{5}'A\textsuperscript{5}'Q\textsuperscript{5}GQ\textsuperscript{3}A\textsuperscript{3}'C\textsuperscript{3}') is Considerable !!

-A\textsuperscript{5}GA\textsuperscript{3}- (10.58/H8G; 10.58/H8A\textsuperscript{5}; 10.35/H2A\textsuperscript{5}; 10.42/H8A\textsuperscript{3}; 10.40/H2A\textsuperscript{5'}; 10.46/H8A\textsuperscript{3'}; 10.41/H6C\textsuperscript{5'}).

-C\textsuperscript{5}GC\textsuperscript{3}- (10.09/H8G; 9.79/H5/6C\textsuperscript{5}; 11.01/H5/6C\textsuperscript{3}; 10.46/H8A\textsuperscript{3'}; 10.73/H8A\textsuperscript{5'}; 10.62/H2A\textsuperscript{5'}; 10.40/H5C\textsuperscript{3'}; 10.67/H6C\textsuperscript{5'}).

-A\textsuperscript{5}GC\textsuperscript{3}- (10.25/H8G; 10.80/H8A\textsuperscript{5}; 10.31/H6C\textsuperscript{3}; 10.34/H8A\textsuperscript{3}; 10.46/H8A\textsuperscript{5'}; 10.31/H5C\textsuperscript{3}; 10.87/H6C\textsuperscript{3'}; 10.53/H6C\textsuperscript{5'}).

-C\textsuperscript{5}GA\textsuperscript{3}- (10.4/H8G; 10.60/H8A\textsuperscript{5}; 10.57/H2A\textsuperscript{5}; 10.39/H8A\textsuperscript{3}; 10.57/H2A\textsuperscript{3'}; 10.59/H8A\textsuperscript{5}; 10.54/H2A\textsuperscript{5'}; 10.50/H6C\textsuperscript{5'}).

Acharya, P. and Chattopadhyaya et al, Ref.: 83-85
6.3.3 Sequence-dependent pK$_a$ Modulation of G in d(C$^5'$A$^5'$Q$^5$GQ$^3$A$^3'$C$^3'$) is Negligible!!

-A$^5$GA$^3$- (11.06/H8G; 11.13/H2A$^5$; 10.86/H8A$^3$; 11.21/H2A$^3$; 11.19/H2A$^5'$; 11.15/H8A$^3'$; 11.13/H5C$^3$; 11.01/H6C$^3$').

-C$^5$GC$^3$- (10.40/H8G; 10.40/H5C$^5$; 10.36/H6C$^5$; 10.33/H5C$^3$; 10.68/H6C$^3$; 10.38/H2A$^5$; 10.44/H8A$^3$; 10.40/H5C$^5$; 10.56/H6C$^5$; 10.39/H5C$^3$; 10.43/H6C$^3$').

-A$^5$GC$^3$- (10.76/H8G; 10.71/H8A$^5$; 11.85/H2A$^5$; 10.80/H5C$^3$; 10.88/H2A$^5$; 10.64/H8A$^3$; 10.82/H2A$^3$; 10.68/H5C$^3$; 10.74/H6C$^3$').

-C$^5$GA$^3$- (10.76/H8G; 10.72/H5C$^5$; 10.68/H6C$^5$; 10.73/H8A$^3$; 10.80/H5/6C$^5$; 10.73/H5C$^3$').

*Acharya, S. and Chattopadhyaya et al*, Ref.: 85
6.3.4 Propagation of the electrostatic interaction ($pK_{a2}$\textsuperscript{1}) at the 3'- and the 5'-ends as a result of $G^-$ formation

\begin{align*}
5'\text{-}r(C\!A\!A\!G^-\!A\!A\!C)-3' & \ (\Delta pK_{a2} \ 0.1) \\
5'\text{-}r(C\!A\!A\!G^-\!C\!A\!C)-3' & \ (\Delta pK_{a2} \ 0.56) \\
5'\text{-}r(C\!A\!C\!G^-\!A\!A\!C)-3' & \ (\Delta pK_{a2} \ 0.46) \\
5'\text{-}r(C\!A\!C\!G^-\!C\!A\!C)-3' & \ (\Delta pK_{a2} \ 1.25)
\end{align*}

1. \textit{This means that the $pK_{a2}$ perturbation is maximum when the $G$ is sandwiched between two Pyrimidines, and}

2. \textit{It is minimum when the $G$ is between two Purines.}

\textsuperscript{1) $pK_{a2}$ is the $pK_a$ obtained from neighbors

\textit{Acharya, S. and Chattopadhyaya et al, Ref.: 85}
The $pK_a$ of a Nucleobase can be measured both from its own protons and also from the protons of the neighboring nucleobases as a result of:

- **Electrostatic** cross-modulation of two-coupled $\pi$ systems.
- The Nearest Neighbor effect

_Acharya, P. and Chattopadhyaya et al, Ref.: 81-84_
Extended Genetic Code?

Stacking ⇔ Destacking in any two adjacent nucleotides triggers the ON-OFF switch to tune the pseudoaromatic character of a particular nucleobase, $N$.

$N$ varies as the $Q^1$ and $Q^2$ varies, then $N = 2^4 = 16$.

If again, $P^1$ and $P^2$ varies with $Q^1$ and $Q^2$ then, $N = 4^4 = 256$ variations.

Nearest-neighbor interaction would also be dictated by the intrinsic dynamics of folding and unfolding within the molecule owing to the sequence context or interaction with an external ligand.

Variable Pseudoaromatic Character ($pK_a$)
6.4 Single-Stranded Adenine-Rich DNA and RNA Retain Structural Characteristics of Their Respective Double-Stranded Conformations and Show Directional Differences in Stacking Pattern

ss-5′-GAAAAAC

Isaksson, J. and Chattopadhyaya, J. et al, Ref.: 86
Structures of the G-A basestep in a canonical B-DNA compared to that of the canonical A-RNA.

In the B-DNA step: the H6/H8n-H1'(n-1) distance (3.0 Å) is shorter and H6/H8n-H3'(n-1) distance (5.1 Å) is longer.

while the A-RNA step shows the opposite characteristics, longer H6/H8n-H1'(n-1) and shorter H6/H8n-H3'(n-1) This is reflected in their respective NOESY crosspeak intensities.
Simulated Annealing Cycles (30ps Step) of Eight ssRNA/DNA with full NMR constraints

Start with A- or B-type DNA or RNA

Heating Step with Full NMR constraints (100K to 400K for 10ps)

Cooling Step with Full NMR constraints from 400K to 100K in 10ps

Heating at 400K with Full NMR constraints for 10ps
5′-GAAAAC-3′

ssDNA

ssRNA
5′-GAAAC-3′

ssDNA

ssRNA
5′-GAAAC-3′

ssDNA  ssRNA
5′-GAAAC-3′

ssDNA

ssRNA
5′-GAAAC-3′

ssDNA

ssRNA
Electrostatic complementarity steers conformation

5'-d(GpA1p)-3'

5'-d(A1pA2p)-3'

5'-GpA1p-3'

5'-[imidazole] over [pyrimidine]

5'-[pyrimidine] over [imidazole]

ssDNA

ssRNA

δ− δ+ δ− δ− δ+ δ−
"......Single-stranded RNA, however, lacks such three-dimensional character. Therefore, only specific interactions through RNA bases can determine specificity. Given the limited number of variants (only four different nucleotides), specificity would require long RNA segments of specific sequence, with relatively weak protein interactions at each single nucleotide within such a sequence in order to avoid nonspecific binding....."
In Contrast, our NMR data show that the **Origin of the Helicity** is in fact in the Single-strand arising from the Electrostatic interaction.

**Double-strand merely strengthens this by H-bonding.**

Chatterjee, S. and Chattopadhyaya, J. et al,  Ref.: 87
`r(C^1A^1A^2GA^3A^4C^2)`  
`r(C^1A^1A^2GC^2A^3C^3)`  
`r(C^1A^1C^2GA^2A^3C^3)`  
`r(C^1A^1C^2GC^3A^2C^4)`

Strong NOE : 32  
Medium NOE : 19  
Weak NOE : 4  
Total : 55

Strong NOE : 36  
Medium NOE : 20  
Weak NOE : 6  
Total : 62

Strong NOE : 41  
Medium NOE : 12  
Weak NOE : 5  
Total : 58

Strong NOE : 45  
Medium NOE : 8  
Weak NOE : 7  
Total : 60
d(C¹A¹²G A³A⁴C²)

Strong NOE : 40
Medium NOE : 25
Weak NOE : 15
Total : 80

d(C¹A¹²G C²A³C³)

Strong NOE : 37
Medium NOE : 23
Weak NOE : 3
Total : 63

d(C¹A²G A³A⁴C³)

Strong NOE : 57
Medium NOE : 17
Weak NOE : 3
Total : 77

d(C¹A²G C³A²C⁴)

Strong NOE : 47
Medium NOE : 43
Weak NOE : 18
Total : 108
NOESY Footprint (Anomeric-Aromatic Contacts) for d(C₁A₁A₂GA₃A₄C₂)

- Strong NOE: 40
- Medium NOE: 25
- Weak NOE: 15
- Total: 80
NOESY Footprint (Anomeric-Aromatic Contacts) for r(C1A1A2GA3A4C2)

- Strong NOE : 32
- Medium NOE : 19
- Weak NOE : 4
- Total : 55
NOESY Footprint (Anomeric-Aromatic Contacts) for d(C1A1C2GCA3A2C4)

- Strong Contact: 47
- Medium NOE: 43
- Weak NOE: 18
- Total: 108
NOESY Footprint (Anomeric-Aromatic Contacts) for r(C¹A¹C²G₃A²C⁴)

- Strong NOE: 45
- Medium NOE: 8
- Weak NOE: 7
- Total: 60

ppm

- H1'A2
- H1'A1
- H1'C3
- H1'C4
- H1'C1
- H2A2
- H2A1
- H5C2
- H5C3
- H5C4
- H6C1
- H6C2
- H6C3
- H6C4
- H8A2
- H8A1
- H8G
- C1
- C2
- C3
- C4
- A1
- A2
- G

- Strong Contact
- Weak Contact
NOESY Footprint (Anomeric-Aromatic Contacts) for d(C¹A¹C²G²A²A³C²)

Strong NOE : 57
Medium NOE : 17
Weak NOE : 3
Total : 77
NOESY Footprint (Anomeric-Aromatic Contacts) for r(C1A1C2G A2A3C2)

- Strong NOE: 41
- Medium NOE: 12
- Weak NOE: 5
- Total: 58
NOESY Footprint (Anomeric-Aromatic Contacts) for d(C¹A²G²C³A³C³)
NOESY Footprint (Anomeric-Aromatic Contacts) for r(C\textsuperscript{1}A\textsuperscript{1}A\textsuperscript{2}G\textsuperscript{2}C\textsuperscript{2}A\textsuperscript{3}C\textsuperscript{3})
Simulated Annealing Cycles (30ps Step) of Eight ssRNA/DNA with full NMR constraints (60 Cycles)

Start with A- or B-type DNA or RNA

Heating Step with Full NMR constraints (100K to 400K for 10ps)

Cooling Step with Full NMR constraints from 400K to 100K in 10ps

Heating at 400K with Full NMR constraints for 10ps
NOE footprint of rA\textsuperscript{1}A\textsuperscript{2}A\textsuperscript{3}A\textsuperscript{4}A\textsuperscript{5}A\textsuperscript{6}A\textsuperscript{7}

![Diagram showing NOE footprint with specific contacts indicated by arrows: Anti contact and Syn contact.](diagram.png)

- H1'A7
- H1'A1
- H1'A2
- H1'A6
- H1'A3
- H1'A4
- H1'A5
- H2A5
- H2A6
- H2A4
- H2A3
- H2A2
- H8A5, H2A7
- H8A6
- H8A4
- H8A3
- H2A1
- H8A7
- H8A2
- H8A1
Microenvironment of each 9-adeninyl in ss-ribo-A\textsubscript{7} is different

It is found A\textsuperscript{4}, A\textsuperscript{5}, A\textsuperscript{6} are having more North-sugar populated

Signature of A-type RNA for ss-ribo-A\textsubscript{7}
Simulated Annealing Cycles (30ps Step; 60 Cycles : 100K-400K-100K) of ss-r(AAAAAAAA) with full NMR constraints
6.5 How much hydration is necessary for the stabilization of DNA-duplex

\[ 5'-(T-G-T-T-G-G-C) \quad 3'-(A-C-A-A-A-C-C)-PZN \]
Dangling/Matched: \( T_m = 30^\circ C \)

(Duplex 1)

\[ 5'-(T-G-T-T-G-G-C) \quad 3'-(A-C-A-A-A-C-A)-PZN \]
Dangling/Mismatched: \( T_m = 25^\circ C \)

(Duplex 3)

\[ 5'-(T-G-T-T-G-G-C) \quad 3'-(A-C-A-A-A-C-C)-PZN \]

(Duplex 2)

\[ 5'-(T-G-T-T-G-G-C) \quad 3'-(A-C-A-A-A-C-A)-PZN \]

(Duplex 4)

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 88
$^1$H-NMR spectra showing NOEs through the cross-section of the ROESY spectrum

$^5$-p(T-T-T-G-G-C)
Dangling/Matched: $T_m = 30^0C$

$^5$-p(T-G-T-T-G-G-C)
$T_m = 50^0C$

$^5$-p(T-G-T-T-G-G-C)
Dangling/Mismatched: $T_m = 25^0C$

$^5$-p(T-G-T-T-G-G-C)
$T_m = 34^0C$
Imino proton temperature dependence

\[ 5'\text{-p}^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C\text{-}3' \]
\[ 3'\text{- (A C A A A C C)}\text{- PZN-5'} \]

\[ 5'\text{-p}^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C\text{-}3' \]
\[ 3'\text{- (A C A A A C C)p-5'} \]

Maltseva, T. V. and Chattopadhyaya, J. et al, Ref.: 89-91
5'-r(1G2A3U4U5G6A7A)-3'  
3'-Phz-pd( C T A A C T)-5'
Imino proton temperature dependence
Imino proton temperature dependence

5'-p(TGTTTG\text{GC})-3'  5'-p(TGTTTG\text{GC})-3'
3'-(\text{ACAAACC})-\text{Phe}-5'  3'-(\text{ACAAACC})p-5'

275 K  278 K  285 K  289 K  294 K  298 K
6.6 A Uniform Mechanism Correlating Dangling-end Stabilization and Stacking Geometry

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jyoti@boc.uu.se

Isaksson, J. and Chattopadhyaya, J., Ref.: 92
Dangling ends

$-\Delta \Delta G^\circ$ of dangling end stabilization

Isaksson, J. and Chattopadhyaya, J., Ref.: 92
Dangling ends

$-\Delta\Delta G^o$ of dangling end stabilization

<table>
<thead>
<tr>
<th></th>
<th>DNA$^1$</th>
<th>RNA$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′</td>
<td>-0.28 – 0.92</td>
<td>0.1 – 1.7</td>
</tr>
<tr>
<td>5′</td>
<td>-0.48 – 0.96</td>
<td>0.0 – 0.5</td>
</tr>
</tbody>
</table>

Closing basepair

G-C: -1.9 kcalmol$^{-1}$
A-U/T: -0.7 kcalmol$^{-1}$

---


Trends in RNA dangling end data

- **3'-end > 5'-end**
  - 3'-end < 1.8 kcal/mol
  - 5'-end < 0.5 kcal/mol

- **Purine (R) > Pyrimidine (Y)**
  - Purine < 1.8 kcal/mol
  - Pyrimidine < 1.2 kcal/mol

- **YR > RR = YY > RY**
  - Y-R > 1.5 kcal/mol
  - R-R/Y-Y ~ 1.1 kcal/mol
  - R-Y < 0.6 kcal/mol

- **G≡C > A=U**
  - G≡C < 1.8 kcal/mol
  - A=U < 0.7 kcal/mol
ΔΔGº = -0.2 kcal/mol

5'-GC-3'
3' - G-5'

3'-end > 5'-end

ΔΔGº = -1.8 kcal/mol

5'-CA-3'
3' - G-5'

(1L2X [RNA pseudoknot])

(1TRA [tRNA^Phe])
5'-CC-3’
3’-G -5’

ΔΔG° = -0.9 kcal/mol

(1NA2 [Telomerase RNA])

5'-CA-3’
3’-G -5’

ΔΔG° = -1.8 kcal/mol

(1TRA [tRNA^{Phe}])

Purine > Pyrimidine
5'- A C G C G C A -3'
3'- G C G C G G -5'

YR > RR = YY > RY

ΔΔG° = -0.9 kcal/mol

ΔΔG° = -1.1 kcal/mol

5'-CC-3'
3'-G -5'

ΔΔG° = -1.8 kcal/mol

5'-GA-3'
3'-C -5'

ΔΔG° = -1.8 kcal/mol

5'-GU-3'
3'-C -5'

ΔΔG° = -0.6 kcal/mol
- 3'-end > 5'-end
- Purine (R) > Pyrimidine (Y)
- YR > RR = YY > RY
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$5'$-UG-3'

3'-A -5'

ΔΔ$G^\circ$ = -0.6 kcal/mol

(1GKV [RNA/MS2])

$G=C > A=U$

ΔΔ$G^\circ$ = -1.8 kcal/mol

(1TRA [tRNA$^{\text{Phe}}$])

$5'$-CA-3'

3'-G -5'

$C$ $G$

$G$ $C$

$C$ $G$

$C$ $G$

$C$

$G$ $C$

$C$

$G$

$C$

$G$

$A=U$

$G≡C > A=U$

$A$ $G$

$G$ $A$

$G$

$A$

$G$
Figure showing a graph with data points representing the stabilization (ΔΔG) and screening values. The correlation coefficient R=0.873 indicates a strong correlation. Different markers are used to distinguish between 3' RNA, 5' RNA, 3' DNA, and 5' DNA samples.
Data points from 3' RNA (♦), 5' RNA (■), 3' DNA (◇) and 5' DNA (□) dangling-ends fall on the correlation line and gives a correlation factor, $R = 0.873$. Considering only the points from RNA gives a better correlation, $R = 0.934$, showing that dangling-ends on RNA are more ordered than dangling-ends on DNA, $R=0.376$ (C).

Correlation of Geometry of the dangling base in 105 published structures (from X-RAY/NMR) to the thermodynamic stabilization found (UV) for the corresponding dangling-base/closing basepair combination
Similarity of the stacking pattern of the single-stranded bases applies to dangling bases.
Part 7.0 Study on the Intramolecular Stereoelectronic anomeric and gauche effects contributing to the energetics of the self-organisation of DNA and RNA

😊 Why there is conformational control in DNA versus RNA?
😊 How to exploit these Stereoelectronic Principles in synthetic molecules to drive Function?

A Monograph on Stereoelectronic Effects in Nucleosides and Nucleotides and their structural implications

Chattopadhyaya, J.; Acharya, P. and Thibadeau, C.

Free Download (~200 pages) available at my website: www.boc.uu.se
The Pseudorotational Wheel for Pentofuranosyl of $\text{D}$-nucleosides

The diagram illustrates the conformational energy landscapes of pyranose and furanose sugars. It shows the energy barriers and transition states between different conformations, such as chair and boat, with ΔE_\text{pyranose} and ΔE_\text{furanose} representing the energy differences between these states. The energy components ΔG_N/S and ΔG_\text{ax/eq} are also depicted, indicating the energy changes associated with the different conformations.
7.1 Structural properties of 2'-dA & 3'-dA in solution. Impact of gauche and anomeric effects on the furanose conformation

Van't Hoff plot based on data of 2'-dA & 3'-dA

\[ \Delta H^0 = 17 \text{ kJ/mol} \]

\[ \Delta H^0 = 14 \text{ kJ/mol} \]

Favorable axial orientation of 2'OH/3'OH group & nucleobase in North conformer

Unfavorable equatorial orientation of nucleobase and 2'OH/3'OH group in South conformer

The N=S equilibrium is temperature dependant and favors North conformer with lower enthalpy.

Koole, L. H. and Chattopadhyaya, J. et al, Ref.: 93
With Plavec and Thibadeau

8: Aristeromycin (R = R' = OH)
9: 2'-deoxy-aristeromycin (R = OH; R' = H)
10: 3'-deoxy-aristeromycin (R = H; R' = OH)
11: 2',3'-dideoxy-aristeromycin (R = R' = H)

12: R = R' = R" = H
13: R = R" = H; R' = OH
14: R = H; R' = R" = OH
15: R = R" = H; R' = OMe

16: R = Me; R' = OMe; R" = H

17: α-D-ddA (R = R' = H)
18: α-D-ddG
19: α-D-ddC
20: α-D-ddT
21: α-D-ddA (R = R' = H)
22: 3'-OMe-α-D-ddA (R = Me; R' = H)
23: 3',5'-diOMe-α-D-ddA (R = R' = Me)
24: α-D-ddG (R = R' = H)
25: α-D-ddC (R = R' = H)
26: α-D-ddT (R = R' = H)

27: α-L-ddA
28: α-L-ddC
29: α-L-ddT

30: β-D-ddA (R = H)
31: β-D-ddG (R = H)
32: 5'-OMe-β-D-ddG (R = Me)
33: β-D-ddC (R = H)
34: β-D-ddT (R = H)
35: β-D-ddU (R = H)
36: β-D-ddU (R = H)

37: β-D-dA (R = R' = H)
38: 3'-OMe-β-D-dA (R = Me; R' = H)
39: 3',5'-diOMe-β-D-dA (R = R' = Me)
40: β-D-dIm (R = R' = H)
41: β-D-dG (R = R' = H)
42: β-D-dC (R = R' = H)
43: β-D-dT (R = R' = H)
44: β-D-dU (R = R' = H)
45: 5-F-β-D-dU (R = R' = H)

46: β-L-dA
47: β-L-dG
48: β-L-dC
49: β-L-dT

50: β-D-A
51: β-D-G
52: β-D-C
53: β-D-T
54: β-D-U
55: 5-F-β-D-U

56: Formycin B
57: Formycin A
58: 9-deaza-A

60: ψ-U: R = R' = H
61: 1-Me-ψ-U: R = Me; R' = H
62: 1,3-dMe-ψ-U: R = R' = Me

63: β-D-3'-dA

64: β-D-dAMP
65: β-D-dGMP
66: β-D-dCMP
67: β-D-dTMP
68: β-D-dUMP

69: β-D-dAMP Et
70: β-D-dGMP Et
71: β-D-dCMP Et
72: β-D-dTMP Et
73: β-D-dUMP Et

74: β-D-AMP
75: β-D-GMP
76: β-D-CMP
77: β-D-TMP
78: β-D-UMP

79: β-D-AMP Et
80: β-D-GMP Et
81: β-D-CMP Et
82: β-D-TMP Et
83: β-D-UMP Et

84: adenosin-9-yl (A)
85: guanosin-9-yl (G)
86: imidazol-1-yl (Im)
87: hypoxanthin-9-yl (I)
88: cytosin-1-yl (C)
89: thymin-1-yl (T)
90: uracil-1-yl (U)
91: 5-F-uracil-1-yl [5-F-U (45)] & 5-F-U (55)

Chattopadhyaya, J. et al, "Stereoelectronic Effects in Nucleosides & Nucleotides and their Structural Implications", Dept of Bioorganic Chemistry, Box 581, Uppsala University, S-75123 Uppsala, Sweden, Ver 160205 jyoti@boc.uu.se

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Figure 13: Estimates for the anomeric and gauche effects in 12 - 83 from pairwise comparisons

Chattopadhyaya, J. et al, "Stereoelectronic Effects in Nucleosides & Nucleotides and their Structural Implications", Dept of Bioorganic Chemistry, Box 581, Uppsala University, S-75123 Uppsala, Sweden, Ver 160205 jyoti@boc.uu.se
7.2 Stereoelectronic effects in nucleosides and nucleotides

Anomeric effect  Gauche effect

Interplay of Gauche and Anomeric effects dictating the overall sugar geometry

Conformational Cooperativity of Aglycone-sugar-phosphate

Chattopadhyaya, J. et al, "Stereoelectronic Effects in Nucleosides & Nucleotides and their Structural Implications", Dept of Bioorganic Chemistry, Box 581, Uppsala University, S-75123 Uppsala, Sweden, Ver 160205 jyoti@boc.uu.se
7.2.1 Anomeric Effect in Nucleos(t)ides

- **N-type sugar**
  - More Steric interaction
  - $^{1}\text{n}_{\text{sp}^2}$ (p-type)
  - $\sigma^* C'1\cdot N9$

- **S-type sugar**
  - Less Steric interaction
  - $^{1}\text{n}_{\text{sp}^2}$ (p-type)
  - $\sigma^* C'1\cdot N9$

**AE Stabilization**

- Optimal $^{1}\text{n}_{\text{sp}^2}$ (p-type) $\rightarrow \sigma^* C'1\cdot N9$

- $^{2}\text{n}_{\text{sp}^2}$ (s-type)
  - $\beta_2 = 168^\circ$

- $^{1}\text{n}_{\text{sp}^2}$ (p-type)

- $^{2}\text{n}_{\text{sp}^2}$ (s-type)
  - $\beta_2 = 126^\circ$
7.2.2 Gauche Effect in Nucleos(t)ides

**N-type sugar**

![Diagram of N-type sugar showing the gauche effect and the optimal orientation of σ₃'H₃' → σ*₄'O₄'.](image)

**S-type sugar**

![Diagram of S-type sugar showing the gauche effect and the optimal orientation of σ₃'H₃' → σ*₄'O₄'.](image)

No Optimal σ₃'H₃' → σ*₄'O₄'

3'-Gauche Effect Stabilization Optimal σ₃'H₃' → σ*₄'O₄'

HF/6-31G* geometry; X = F
Various  

Gauche Effects in Nucleosides

\[ \beta-D-2'-deoxyadenosine \]

\[ \beta-D-adenosine \]

3'GE \[O3'-C3'-C4'-O4'] \rightarrow S-type conformation

2'GE \[O2'-C2'-C1'-O4'] \rightarrow N-type

\[O2'-C2'-C1'-N9] \rightarrow S-type
7.3 pD-dependent \(^1\)H NMR Titration

\[ pK_a = 3.9 \]

\[ pK_a = 4.0 \]

\[ pK_a = 2.4 \]

\[ pK_a = 2.3 \]

\[ pK_a = 3.7 \]
pD-dependent N $\ncong$ S and $\varepsilon^+ \ncong \varepsilon^-$ equilibria

\[
\Delta G^0 (N/S) \text{ at } 298 \text{ K}
\]

\[
\delta H^8 G
\]

\[
\delta H^8 A/H^2 A
\]

\[
\Delta G^0 (N/S) \text{ at } 298 \text{ K}
\]

\[
\delta H^8 G
\]

\[
\Delta G^0 (\varepsilon^+/\varepsilon^-) \text{ at } 298 \text{ K}
\]

\[
\delta H^8 G
\]
Co-operative shift of \((N, \varepsilon^t) \rightleftharpoons (S, \varepsilon^-)\)

\[ \Delta G^0(N/S) \text{ at 298 K} \]

\[ \delta^{31}P \]

\[ \Delta G^0(\varepsilon^t/\varepsilon^-) \text{ at 298 K} \]

\[ \Delta G^0_{(\varepsilon^t/\varepsilon^-)} \text{ at 298 K} \]

\( R = 0.97 \)

\( R = 0.98 \)
7.4 3'-Gauche Effect Dependent EF-Tu Recognition of Aminoacyl-tRNA

Acharya, P. and Chattopadhyaya, J. et al, Ref.: 94
Aminoacyl-tRNA

aa-tRNA complexed with EF-Tu

Nawrot Reagent

(2a) R = H; 2'-O-antAdo
(2b) R = PO₃H; 2'-O-antAMP
(2c) R = H; 3'-O-antAdo
(2d) R = PO₃H; 3'-O-antAMP

aminoacyl tRNA (aa-tRNA)
For aa-tRNA\textsuperscript{Phe}
R = \[
\begin{array}{c}
\text{phenyl group}
\end{array}
\]
$^1$H-NMR based Thermodynamics

\[ \Delta G^\circ = -2.1 \text{ kJ mol}^{-1} \]

(2a) \( R = H \) (2c) \( R = H \)
(2b) \( R = \cdot \text{PO}_3\text{H} \) (2d) \( R = \cdot \text{PO}_3\text{H} \)

\[ \Delta G^\circ = -2.9 \text{ kJ mol}^{-1} \]

Sugar moieties in 2c and 2d are more S-type due to enhanced 3'-GE

Conformational switch (-2.9 kJ mol$^{-1}$) for EF-Tu recognition

\[ \Delta \Delta G^\circ_{298} \text{ (in kJ mol}^{-1}) = [\Delta G^\circ_{298} \text{ K}]_{2a/2b/2c/2d} - [\Delta G^\circ_{298} \text{ K}]_{4a/7b} \]
Conformational Switch is $-2.9 \text{ kJ mol}^{-1}$ for EF-TU Recognition to Thermodynamically More preferred South-type 3'-Acyl Adenosine Derivative.

More S-type Sugar Stabilization is due to enhanced 3'-GE

Acharya, P. and Chattopadhyaya, J. et al. Ref.: 94
Some representative conformationally constrained polycyclic β-D-nucleosides
8.1 1',2'-oxetane modifications gives North-sugar Constrained Nucleosides

RNase H cleavage pattern of single and multiple oxetane-\(\text{T}\) modified AON/RNA hybrids

Footprints of conformational change in DNA/RNA hybrid

AON (1): \(T_m = 44.5^\circ\text{C}\)

AON (3): \(T_m = 39.5^\circ\text{C}\)

AON (5): \(T_m = 39.3^\circ\text{C}\)

AON (2): \(T_m = 37.7^\circ\text{C}\)

AON (15): \(T_m = \text{n. d.}\)
Footprints of Conformational Change as a result of implantation of a North-constrained Nucleotide in the Antisense/RNA Hybrid duplex

The North-constrained oxetane unit forces the AON to adopt RNA type local conformation preventing the action of RNase H. This conformational change extends to 5 nucleotide unit (see boxed areas corresponding to the "empty" areas in the digestion PAGE analyses).
North-East type conformation ($40^\circ < P < 43^\circ$, $35^\circ < \Psi_m < 37^\circ$)

The oxetane modified AONs met the following antisense criteria:

(i) optimal target binding
(ii) efficient RNase H recruitment (mixmer strategies)
(iii) 3'-exonuclease resistance
(iv) blood serum stability
(v) endonuclease resistance (Oxetane-\text{T} and -\text{C} only)

upon combination of oxetane-modification and 3'-conjugation of dipyridophenazine (DPPZ)

Pradeepkumar, P. I. and Chattopadhyaya, J. et al. Ref.: 95-100
8.2 1',2'-azetidine modification

Honcharenko, D. and Chattopadhyaya, J. et al. Ref.: 102
The azetidine-T or -C modification in the AON:

(i) improved RNA target affinity ($T_m$ increase of 1 to 2 °C per modification compared to isosequential oxetane modified counterpart)

(ii) higher RNase H catalytic activity compared to that of the native AON/RNA duplexes

(iii) higher 3'-exonuclease stability

(iv) higher blood serum stability (degraded in 12 h compared to the unmodified AON which is fully degraded in 4 h)

Honcharenko, D. and Chattopadhyaya, J. et al. Ref.: 102, 105
8.3 4',2'-aza-ENA modification

Varghese, O. P. and Chattopadhyaya, J. et al. Ref.: 103-104
RNase H cleavage pattern of single aza-ENA-T or aze-T modified AON/RNA hybrids

Footprints of conformational change in DNA/RNA hybrid
North-type conformation ($7^\circ < P < 27^\circ$, $44^\circ < \Psi_m < 52^\circ$)

The aza-ENA-T modification in the AON fulfilled three important antisense criteria:

(i) improved RNA target affinity ($T_m$ increase of 2.5 to 4 °C per modification)

(ii) RNase H cleavage rate comparable to that of the native AON/RNA duplexes

(iii) higher blood serum stability (~85% stable over 48 h compared to the unmodified AON which is fully degraded in 4 h)

_Varghese, O. P. and Chattopadhyaya, J. et al._ Ref.: 103, 105
8.4 Carbocyclic analogues – require new C-C bond

8.4.1 C-C bond formation, ionic mechanism

\[
\begin{align*}
\text{Intermolecular} & \quad \text{Intramolecular} \\
\text{Nu} = \text{Oxygen, Sulfur, Nitrogen etc}
\end{align*}
\]

Wu, J.-C. and Chattopadhyaya, J. et al, Ref.: 38-41

Varghese, O. P. and Chattopadhyaya, J. et al, Ref.: 103
8.4.2 C-C bond formation, free-radical mechanism

Intramolecular

\[ \text{Wu, J.-C. and Chattopadhyaya, J. et al, Ref.: 47} \]

Intermolecular

Free-radical reaction: intermolecular C-C bond forming

\[
\begin{align*}
Z^+ & + \text{alkene} \\
\rightarrow & \text{alkene}^+ \\
\rightarrow & \text{alkene}
\end{align*}
\]


Free-radical reactions applied to furanose scaffold in Nucleoside from Chattopadhyaya lab

The 1st strategy: Ring-closure giving C-C bond formation

Chattopadhyaya, J. et al, Ref.: 47, 106
The 2\textsuperscript{nd} strategy: Alkylation at C3$\gamma$

$\text{Xi, Z. and Chattopadhyaya, J. et al, Ref.: 49}$

The 3\textsuperscript{rd} strategy: Alkylation at C2$\gamma$ and C3$\gamma$

$\text{Xi, Z. and Chattopadhyaya, J. et al, Ref.: 48}$
8.4.3 Free-radical reaction: A new efficient strategy to Locked 2',4'-Carbocyclic Ribo-Thymidines

(A) Five-membered ring

(B) Six-membered ring

Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
1,3-Free-radical Cyclization

Forming methyl group (pink) is in pseudoequatorial position and thus favored owing to the absence of 1,3-diaxial interaction with 3'-O-Bn (pink).

Forming methyl group (pink) is in pseudoaxial position and thus disfavored owing to the presence of 1,3-diaxial interaction with 3'-O-Bn (pink).

Forming methyl group (pink) is in equatorial position and thus favored owing to the absence of 1,3-diaxial interaction with 3'-O-Bn (pink).

Forming methyl group (pink) is in axial position thus disfavored owing to the presence of 1,3-diaxial interaction with 3'-O-Bn (pink).

Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
Expansion of the HMBC spectrum. Connectivities evidencing the ring fusion between C2' and C7'. Violet represents the major diastereomer, red is for the minor diastereomer. H2' overlaps with H7'.
Expansion of the TOCSY spectrum. Connectivities evidencing the ring closure between C2' and C8'.

Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
8.4.4 Blood Serum Degradation Patterns of Oligos with Single Modification of LNA, Carbo-LNA, Carbo-ENA and aza-ENA, Compared to the Native

AONs: 2/3/4/5
AONs: 6/7/8/9
AONs: 10/11/12/13
AONs: 14/15/16/17

AONs: 2/6/10/14 = [3'-d(CTTCTTTTTTACTTC)],
AONs: 3/7/11/15 = [3'-d(CTTCTTTTTTACTTC)],
AONs: 4/8/12/16 = [3'-d(CTTCTTTTTTACTTC)],
AONs: 5/9/13/17 = [3'-d(CTTCTTTTTTACTTC)]

Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
Blood serum stability of LNA/Carbo-LNA/Carbo-ENA/aza-ENA modified AONs

[3'-d(CTT\textsubscript{(LNA)}CTTTTTTACTTC)]

[3'-d(CTT\textsubscript{(carbo-LNA)}CTTTTTTACTTC)]

[3'-d(CTT\textsubscript{(carbo-ENA)}CTTTTTTACTTC)]

[3'-d(CTT\textsubscript{(aza-ENA)}CTTTTTTACTTC)]

% AON left vs Time (h)
Hydration versus Nuclease Stability

- Steric nature of the 2'-substituent in the Minor groove
- Accessibility of the Metal ion activated water in the Minor groove
- Nuclease resistance of modified oligonucleotides decreases from:
  Carbo-ENA-T > Carbo-LNA-T > aza-ENA-T > LNA-T > deoxy-T

Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107
8.4.5 Cleavage of target RNA in the modified AONs/RNA Duplex by *E. coli* RNase H

Native = RED

Footprints of conformational change in DNA/RNA hybrid
Cleavage of target RNA in the modified AONs/RNA Duplex by *E. coli* RNase H

Footprints of conformational change in DNA/RNA hybrid
Cleavage of target RNA in the modified AONs/RNA Duplex by *E. coli* RNase H

LNA  |  Carbo-LNA

Footprints of conformational change in DNA/RNA hybrid

Native

5'→r (G A A G A A A A A A U G A A G)  
3'→d (C T T C T T T T T T T A C T T C)

LNA/Carbo-LNA/Carbo-ENA/aza-ENA

Footprints of conformational change in DNA/RNA hybrid

Native

5'→r (G A A G A A A A A A U G A A G)  
3'→d (C T T C T T T T T T T A C T T C)

LNA/Carbo-LNA/Carbo-ENA/aza-ENA

Footprints of conformational change in DNA/RNA hybrid

Native

5'→r (G A A G A A A A A A U G A A G)  
3'→d (C T T C T T T T T T A C T T C)
Rates of the RNase H degradation of modified-AON/RNA heteroduplexes

<table>
<thead>
<tr>
<th>Modification at position from 3'-end</th>
<th>Observed rates $k$ (sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>0.0000</td>
</tr>
<tr>
<td>LNA</td>
<td>0.0005</td>
</tr>
<tr>
<td>Carbocyclic-LNA</td>
<td>0.0010</td>
</tr>
<tr>
<td>Carbocyclic-ENA</td>
<td>0.0015</td>
</tr>
<tr>
<td>aza-ENA</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

- Modification at position 3 from 3'-end
- Modification at position 6 from 3'-end
- Modification at position 8 from 3'-end
- Modification at position 10 from 3'-end

![Graph showing observed rates $k$ (sec$^{-1}$) for different modifications and positions](image-url)
8.4.6 Modified antisense Strand of the siRNA sequence against Green Fluorescent Protein (GFP) RNA:

JC-A1  5'-AC\textbf{X} UGU GGC CGU UUA CG\textbf{X} CGC
JC-A2  5'-ACU \textbf{X}GU GGC CGU UUA CG\textbf{X} CGC
JC-A3  5'-AC\textbf{X} \textbf{X}GU GGC CGU UUA CG\textbf{X} CGC

JC-S1  5'-AC\textbf{Y} UGU GGC CGU UUA CG\textbf{Y} CGC
JC-S2  5'-ACU \textbf{Y}GU GGC CGU UUA CG\textbf{Y} CGC
JC-S3  5'-AC\textbf{Y} \textbf{Y}GU GGC CGU UUA CG\textbf{Y} CGC

JC-F1  5'-AC\textbf{Z} UGU GGC CGU UUA CG\textbf{Z} CGC
JC-F2  5'-ACU \textbf{Z}GU GGC CGU UUA CG\textbf{Z} CGC
JC-F3  5'-AC\textbf{Z} \textbf{Z}GU GGC CGU UUA CG\textbf{Z} CGC

\textbf{X} = \includegraphics{X}
\textbf{Y} = \includegraphics{Y}
\textbf{Z} = \includegraphics{Z}

\textit{Varghese, O. P. and Chattopadhyaya, J. et al, Ref.: 103}

\textit{Srivastava, P. and Chattopadhyaya, J. et al, Ref.: 107}
Modified RNA sequences against GFP RNA:

JC-A1/S1/F1: 5'-r(AC\textcircled{O} UGU GGC CGU UUA CG\textcircled{O} CGC)
JC-A2/S2/F2: 5'-r(ACU \textcircled{O} GU GGC CGU UUA CG\textcircled{O} CGC)
JC-A3/S3/F3: 5'-r(AC\textcircled{O} \textcircled{O} GU GGC CGU UUA CG\textcircled{O} CGC)

The siRNA sequence against c-myb is:
JC-S  5'-r(GCU GAA GAA GCU GGU GGA AZt)
JC-AS  5'-r(UZC CAC CAG CUU CUU CAG CZt)

Inhibition Green Fluorescent Protein (GFP) RNA

In collaboration with Prof J. Keims et al /Aarhus Univ, Denmark (The EU RIGHT project)

JC-F1  5'-AC\textbf{Z} UGU GGC CGU UUA CG\textbf{Z} CGC ; \textbf{Z} = 5-Carbo
JC-S1  5'-AC\textbf{Y} UGU GGC CGU UUA CG\textbf{Y} CGC; \textbf{Y} = 6-Carbo
Concentration dependent siRNA efficiency test

In collaboration with Prof J. Keims et al
/Aarhus Univ, Denmark (The EU RIGHT project)

JC-F1  5'-ACZ UGU GGC CGU UUA CGZ CGC ;  Z = 5-Carbo
JC-S1  5'-ACY UGU GGC CGU UUA CGY CGC;  Y = 6-Carbo
Minimal off-target activity of JC's latest oligos

100 nM siRNA efficiency test

In collaboration with Prof J. Keims et al /Aarhus Univ, Denmark (The EU RIGHT project)

**siRNA Candidates**

JC-F1  5'-AC[Z UGU GGC CGU UUA CG[Z CGC ;  Z = 5-Carbo
JC-S1  5'-ACY UGU GGC CGU UUA CG[Y CGC;  Y = 6-Carbo
8.4.6 A β-hydroxyl group in the olefin alters the stereochemistry of the radical cyclization from 5-\textit{exo} to 6-\textit{endo} eliminating the unwanted C7'-exocyclic methyl group

\begin{equation*}
\text{North-type conformation (} P = 19.6^\circ, \Psi_m = 45.9^\circ \text{)}
\end{equation*}

\textit{Zhou, C. and Chattopadhyaya, J. et al, Ref.: 110}
Thank you
Reference list


