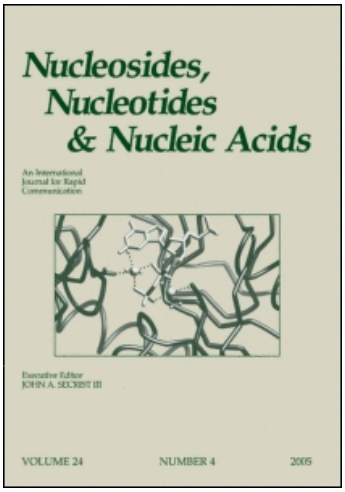

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RNA RECOGNITION BY FLUOR-AROMATIC SUBSTITUTED

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▫ *RNA exhibits a higher structural diversity than DNA and is an important molecule in the biology of life. It shows a number of secondary structures such as duplexes, hairpin loops, bulges, internal loops, etc. However, in natural RNA, bases are limited to the four predominant structures U, C, A, and G and so the number of compounds that can be used for investigation of parameters of base stacking, base pairing, and hydrogen bond is limited. We synthesized different fluoromodifications of RNA building blocks: 1′-deoxy-1′-phenyl-β-D-ribofuranose (B), 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose (4 FB), 1′-deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose (2,4 DFB), 1′-deoxy-1′-(2,4,5-trifluorophenyl)-β-D-ribofuranose (2,4,5 TFB), 1′-deoxy-1′-(2,4,6-trifluorophenyl)-β-D-ribofuranose, 1′-deoxy-1′-(pentafluorophenyl)-β-D-ribofuranose (PFB), 1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (BI), 1′-deoxy-1′-(4-fluoro-1H-benzimidazol-1-yl)-β-D-ribofuranose (4 FBI), 1′-deoxy-1′-(6-fluoro-1H-benzimidazol-1-yl)-β-D-ribofuranose (6 FBI), 1′-deoxy-1′-(4,6-difluoro-1H-benzimidazol-1-yl)-β-D-ribofuranose (4,6 DFBI), 1′-deoxy-1′-(4-trifluoromethyl-1H-benzimidazol-1-yl)-β-D-ribofuranose (4 TFM), 1′-deoxy-1′-(5-trifluoromethyl-1H-benzimidazol-1-yl)-β-D-ribofuranose (5 TFM), and 1′-deoxy-1′-(6-trifluoromethyl-1H-benzimidazol-1-yl)-β-D-ribofuranose (6 TFM). These amidites were incorporated and tested in a defined A, U-rich RNA sequence (12-mer, 5′-CUU UUC XUU CUU-3′ paired with 3′-GAA AAG YAA GAA-5′). Only one position was modified, marked as X and Y, respectively. UV melting profiles of those oligonucleotides were measured.*

Keywords Nucleosides, Base Stacking, Base Pairing, Hydrogen Bonding, RNA Stability

INTRODUCTION

Hydrogen bonds, base stacking, and solvatation are the three predominant forces that are responsible for the stability of secondary structure of nucleic acids. As those interactions are very important, and the number of compounds you can investigate is limited to four predominant structures (U [T], C, G, and A), we decided to synthesize some novel nucleic acid analogues where the nucleobases are replaced by fluorobenzenes and fluorobenzimidazoles. It is important and

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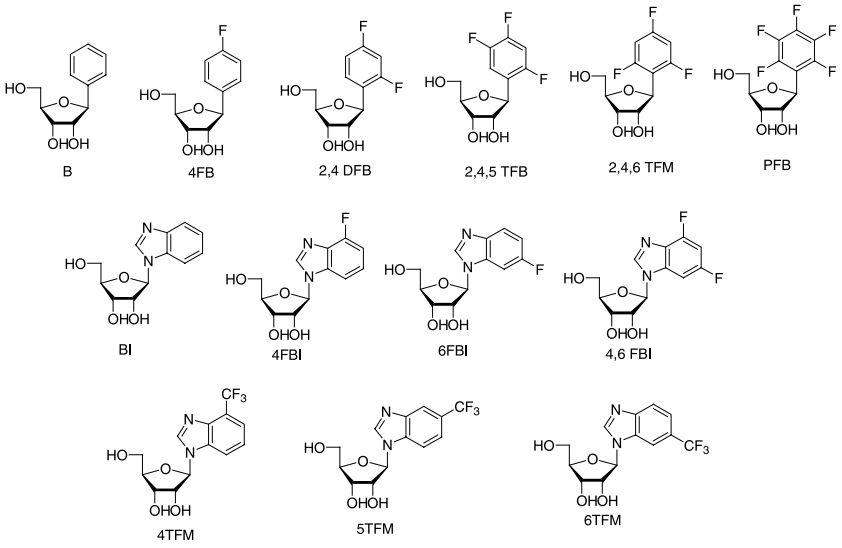

FIGURE 1 Synthesized modified phosphoramidites and their abreviations of the nucleoside "bases."

interesting to investigate fluorine due to its special properties such as high electronegativity, relatively small size, low polarizability and hydrogen-bond accepting.[1]

CHEMICAL SYNTHESES

The synthesis of fluorophenyl-β-D-ribofuranose modifications (13–18) start with C glycosilation. Lithiation of fluorobenzenes (5 and 6) or bromofluorobenzenes (1–4) was performed with t-BuLi (5 and 6) and n-BuLi (1–4) in either Et_2O(3,4,6) or THF(1,2,5) at −78°C and was followed by addition of 2,3,5-tri-O-benzyl-D-ribono-γ-lactone and gave intermediate lactols that were directly dehidroxilated with triethylsilane and $BF_3 \cdot Et_2O$ to afford (7–12) stereoselectively in good yields (Figure 2).[2,3] The deprotection of benzilated nucleosides (7–12) was performed with $Pd(OH)_2$/C and cyclohexene to afford (13–18) in high yields.[4,5]

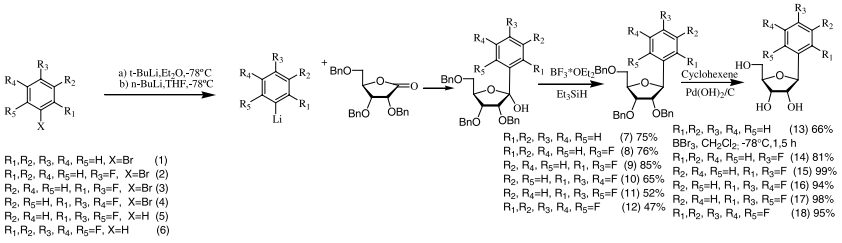

FIGURE 2 Synthesis ofl fluorophenyl-β-D-ribofuranose modifications.

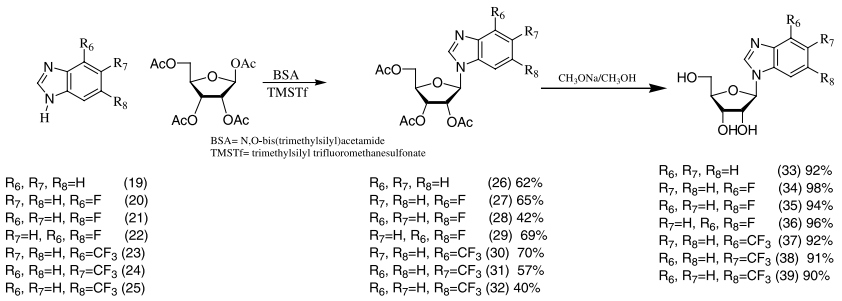

FIGURE 3 Synthesis of benzimidazole nucleosides.

The synthesis of benzimidazole modifications followed procedure of Vorbrueggen (Figure 3).[6] Deprotection was done with 0.2 M CH_3ONa in CH_3OH within 3 h to afford modified nucleosides in high yields.[2,7]

Synthesis of corresponding amidites was done by usual procedures.[3]

RESULTS AND DISCUSSION

The modified nucleosides were tested in a defined RNA sequence. In the 12-mer oligoribonucleotides (5′-CUU UUC XUU CUU-3′ paired with 3′-GAA AAG YAA GAA-5′) only one position was modified, marked as X and Y, respectively.[8] All measurements were done in phosphate (pH = 7) buffer containing 140 mM NaCl, 10 mM Na_2HPO_4 and 10 mM NaH_2PO_4 (at wavelength of 260, the same results at 274 nm). First we measured only RNA duplexes containing natural bases. The Wobble base pair U · G shows the highest T_m (38.6°C, Figure 4). The U · C

1. Base	2.Base	T_m (°C)	ΔH° (kcal/mol)	TΔS° (kcal/mol) T = 298 K	ΔG°(kcal/mol) T = 298 K
U	A	37.8	87.8	75.9	11.9
	C	30.4	84.5	74.8	9.8
	G	38.6	83	71.1	11.9
	U	30.1	89.5	79.8	9.7
2,4,6 TFB	4,6 DFBI	29.1	80.1	70.7	9.4
	4 FBI	29.6	84.9	75.5	9.4
	6 FBI	28.2	85.2	75.9	9.3
2,4,5 TFB	4,6 DFBI	33.5	76.7	66.3	10.4
	4 FBI	32.9	73.1	63	10.1
	6 FBI	34.7	96.7	85.3	11.4
2,4 DFB	4,6 DFBI	34.6	94.4	83.2	11.2
	4 FBI	33.5	88.1	77.4	10.7
	6 FBI	33.1	84.9	74.4	10.5

FIGURE 4 Some of the synthesized double modified duplex RNA (5′-CUU UUC XUU CUU paired with 3′-GAA AAG YAA GAA-5′) and their thermodynamic properties (Errors: $T_m = \pm 0.2$°C; $\Delta G° = \pm 2\%$).

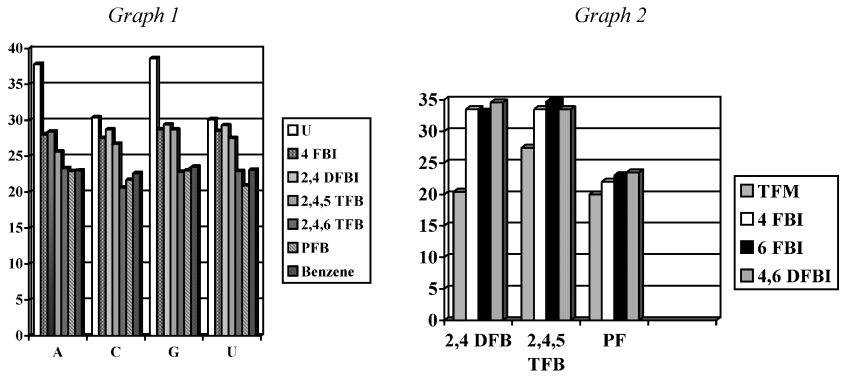

FIGURE 5 Graph 1. Pairing of some of the nucleosides with the natural bases; Graph 2: Pairing of some modified nucleosides.

and U · U mismatches show nearly the same stability (T_m = 30.4°C and T_m = 30.1°C, Figure 4).

In a second series we measured oligonucleotides with modifications paired with natural bases and some results are shown in Figure 4 and Figure 5. Comparing the thermodynamic data obtained from UV measurements brought us to the following conclusions:

1. All modifications show much greater stability paired in-between themselves than paired with natural bases (U, C, G, and A).
2. 2,4,6 TFB modification shows much lower stability than 2,4 DFB and 2,4,5 TFB modifications, although 2,4 DFB has also higher stability compared to 2,4, TFB (0.4–1.5°C).
3. 4,6 DFBI is so far the best fluorobenzimidazole modification we synthesized; lately, synthesised 6FBI modification can compete with it, while all TFM nucleosides show greater decrease of T_m values probably due to the size of trifluoromethyl group, which is rather more similar to isopropyl than methyl group.
4. All imidazole nucleosides inhibited the primase activity with remarkably similar potency. Changing the shape of the base from one that closely resembled normal bases (4,6 DFBI) to ones whose shape substantially varied from that of normal bases (5 and 6 TFM for example) did not greatly affect inhibition.[7]

Comparing the results achieved in our group on the subject of fluoro modifications we decided to employ NMR and crystal structure studies of both nucleosides and oligonucleotides to proof binding and behavior of modifications in duplexes.[9]

For the first investigation we decided to use 2,4 DFB as fluorobenzene modification and 4,6 DFBI as fluorobenzimidazole modification (one opposite the other) as they have show the best thermodinamical results from the compounds tested up to

now. We based our NMR studies on the work of Varani&coworkers and decided to use the following 20-mer sequence: 5′-GGG AXA ACU UCG GUU YUC CC-3′.

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