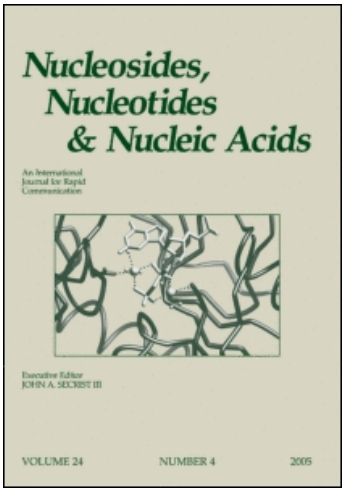

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Synthesis of Modified RNA-Oligonucleotides for Structural Investigations

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ABSTRACT

RNA exhibits a higher structural diversity than DNA and is an important molecule in 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′-(2,4,6-trifluorophenyl)-ß-D-ribofuranose (F), 1′-deoxy-1′-(2,4,5-trifluorophenyl)-ß-D-ribofuranose (M) and 1′-deoxy-1′-(5-trifluoromethyl-1H-benzimidazol-1-yl)-ß-D-ribofuranose (D). Those 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') (Schweitzer, B.A.; Kool, E.T. Aromatic nonpolar nucleosides as hydrophobic isosters of pyrimidine and purine nucleosides. J. Org. Chem. **1994**, *59*, 7238 pp.). Only one position was modified, marked as X and Y respectively. UV melting profiles of those oligonucleotides were measured.

Key Words: RNA; Fluorobenzenes; Fluorobenzimidazoles; Ribonolactone; Duplex melting curve.

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INTRODUCTION

Hydrogen bonds, base stacking and solvatation are three predominant forces which 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 interesting to investigate fluorine due to it's "special" properties such as high electronegativity, relatively small size, low polarizability, hydrogen-bonding ability etc.[1]

CHEMICAL SYNTHESES

The synthesis of 1′-deoxy-1′-(2,4,6-trifluorophenyl)-ß-D-ribofuranose (F) and 1′-deoxy-1′-(2,4,5-trifluorophenyl)-ß-D-ribofuranose (M) starts with C glycosilation (Fig. 1). Lithiation of 1a and 1b was performed with t-BuLi and n-BuLi in either Et2O or THF at −78°C and was followed by addition of 2,3,5-tri-O-benzyl-D-ribono-γ-lactone[2] and gave intermediate lactols (3a and 3b) which were directly dehidroxilated with triethylsilane and $BF_3{\cdot}Et_2O$ to afford stereoselectively 4a and 4b in 52 and 65% yield.[3] The deprotection of benzilated nucleosides (4a and 4b) was performed with $Pd(OH)_2/C$ and cyclohexene to afford 5a and 5b in 98 and 94% yield, respectively (Fig. 2).

The synthesis of 1′-deoxy-1′-(5-trifluoromethyl-1H-benzimidazol-1-yl)-ß-D-ribofuranose followed procedure of Vorbrueggen[4] (57% of 9). Deprotection was done with 0,2 M CH_3ONa in CH_3OH within 3 h to afford 10 in 91% yield (Fig. 3).

Synthesis of corresponding amidites was done by usual procedures.

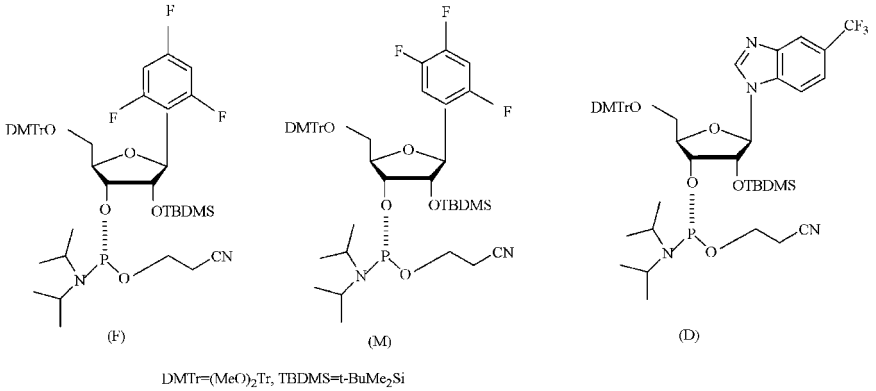

Figure 1. Synthesized modified phosphoramidite and the one-letter abreviations of the nucleoside "bases".

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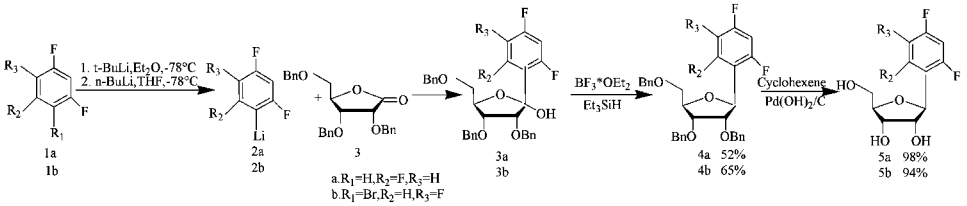

Figure 2. Synthesis of 1′-deoxy-1′-(2,4,6-trifluorophenyl)-ß-D-ribofuranose and 1′-deoxy-1′-(2,4,5-trifluorophenyl)-ß-D-ribofuranose.

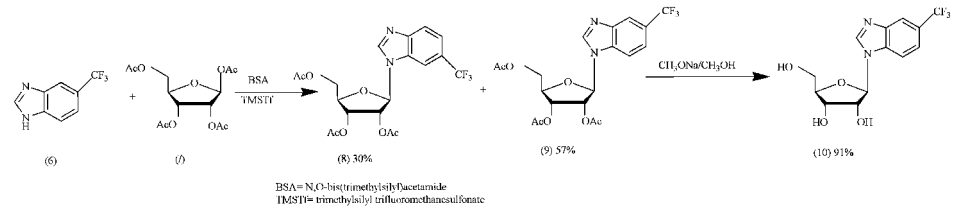

Figure 3. Synthesis of 1′-deoxy-1′-(5-trifluoromethyl-1H-benzimidazol-1-yl)-ß-D-ribofuranose.

RESULTS AND DISCUSSION

The modified nucleosides were tested in a definied RNA sequence. In the 12mer 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.[5] 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 –same results at 274 nm). First we measured only RNA duplexes containing natural bases. The Wobble base pair U·G shows the highest Tm (38,6°C, Table 1). The U·C and U·U mismatches show nearly the same stability (Tm= 30,4°C and Tm=30,1°C, Table 1).

In a second series we measured oligonucleotides with fluorobenzene modifications paired with natural bases (Fig. 4). In all cases Tm values are lower than those for natural bases. Possible explanation for this could be failure of hydrogen bonding

Table 1. Synthesized Double Modified Duplex RNA (5′-CUU UUCXUU CUU paired with 3′-GAA AAG YAA GAA-5′) and their thermodynamical properties (Errors: Tm = ±0,2°C; ΔG° = ±2%).

	Y=A		Y=C		Y=G		Y=U		Y=D	
X	Tm (°C)	ΔG° (kcal/mol)	Tm (°C)	ΔG° (kcal/mol)	Tm (°C)	ΔG° (kcal/mol)	Tm (°C)	ΔG° (kcal/mol)	Tm (°C)	ΔG° (kcal/mol)
F	23,3	7,8	20,6	7,1	22,8	7,7	22,9	13	25	8,3
M	25,6	8,4	26,7	8,8	28,7	9,3	27,5	8,9	26,1	8,5

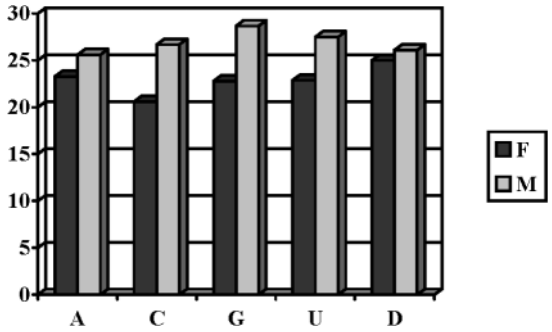

Figure 4. Pairing of nucleosides F and M with the natural bases and modification D in the center of base pair RNA duplex measured by thermal melting point temperature.

between modified and natural bases and that the modified bases are less solvated by water molecules than the natural ones. Very small differences in Tm values by pairing for example M against purine or pyrimidine bases indicate that there are no hydrogen bonds. It can be noticed that M is pairing much better than F against natural bases (Tm values higher up to 6,1°C (Fig. 4)). Explanation for this is not yet clear. F is pairing better with modified imidazole D than against the natural bases but with modification M it is opposite (still M is pairing better against D than F).

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