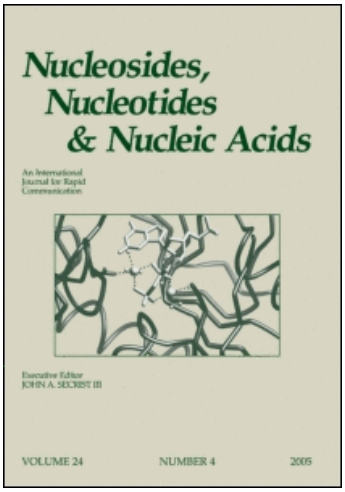


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# STACKING AND STABILITY OF RNA DUPLEXES CONTAINING FLUOROBENZENE AND FLUOROBENZIMIDAZOLE NUCLEOSIDES

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## ABSTRACT

Six different fluorobenzene or fluorobenzimidazole ribonucleosides and one abasic site were incorporated in oligoribonucleotides. Individual contributions of base stacking and solvation of the modified nucleosides could be determined. In fluorobenzene · fluorobenzimidazole-modified base pairs a duplex stabilizing force was found that points to a weak F · · · H hydrogen bond. The lipophilicity of the unprotected nucleosides were investigated by determination of 1-octanol water partition coefficients.

## INTRODUCTION

In natural RNA bases are limited to the four predominant structures U, C, A and G. So the number of compounds which can be used to investigate the parameters of base pairing and base stacking is limited. To address this problem we decided to synthesise some new nucleic acid analogues (Fig. 1).

The nucleic bases are substituted by fluorobenzimidazoles or fluorobenzenes. Compound **2** is an isostere of the natural inosine and compound **8** of uridine. The aromatic ring was designed to be the closest possible steric mimic of the natural bases avoiding the presence of hydrophilic oxygen or nitrogen containing groups (1,2). In compounds **5**, **6** and **7** the natural bases are substituted by mono

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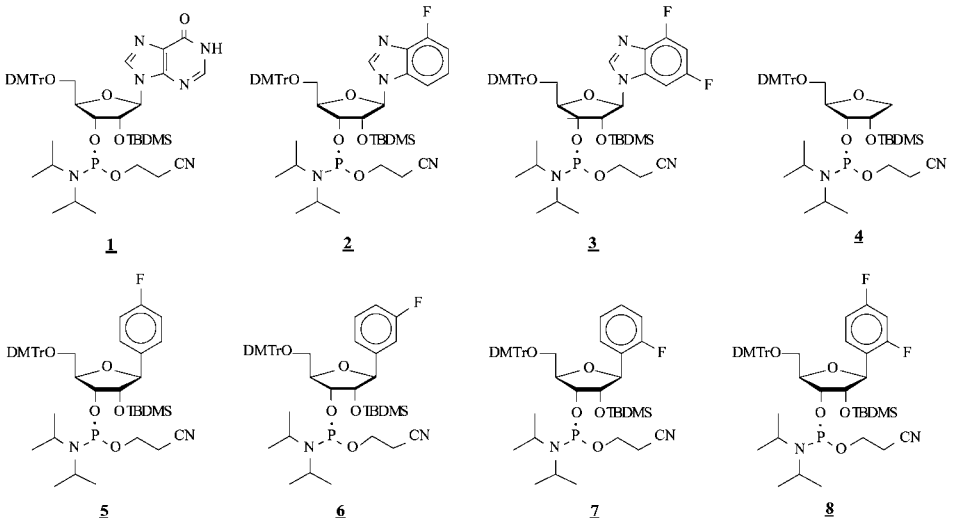


DMTr = $(MeO)_2Tr$, TBDMS = $^tBuMe_2Si$

***Figure 1.*** Synthesized monomer building blocks for the incorporation into oligonucleotides.

fluorobenzenes (3). It is very interesting to investigate the influence of the fluorine atom to hydrogen bonding because in its crystal structure **5** shows a very short C−F · · · H−C distance of 230 pm (4). This is significantly shorter than the sum of the van der Waals radii of fluorine and hydrogen. In order to evaluate the contribution of base stacking effects to the stability of duplex RNA and the influence of the fluorine atom we also synthesised an abasic site **4**.

## RESULTS AND DISCUSSION

We incorporated the modified ribonucleoside phosphoramidites into 12 mer RNA (5). In the oligonucleotides (5′-CUU UUC XUU CUU paired with 3′-GAA AAG YAA GAA) only one position was modified. We measured the fluorobenzene

***Table 1.*** The Needed Results to Calculate the Individual Contributions of Base Stacking and Solvation in the Case of Nucleoside **8**

| | Base Pair | Tm [°C] | □$G^0$ [kcal/mol] |
|---|---|---|---|
| 1 | **4** · uridine | 18.2 | 6.6 |
| 2 | uridine · uridine | 30.1 | 9.7 |
| 3 | uridine · **8** | 27.9 | 9.1 |
| 4 | **8** · **8** | 32.5 | 10.2 |
| 5 | **8** · **4** | 22.6 | 7.7 |





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***Table 2.*** Individual Contributions for Base Stacking and Solvation

| | Solvation | Stacking |
|---|---|---|
| 2,4-difluorobenzene (**7**) compared with U | −6.6°C (1.7 kcal/mol) | 4.4°C (1.1 kcal/mol) |
| 4-fluorobenzimidazole (**2**) compared with G | −6.3°C (1.6 kcal/mol) | 4.4°C (1.0 kcal/mol) |
| 4,6-difluorobenzimidazole (**3**) compared with G | −6.5°C (1.5 kcal/mol) | 5.4°C (1.2 kcal/mol) |

***Table 3.*** Partition Coefficients

| | Uridine | **1** | **2** | **3** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| Partition coefficient P | 0.022 | 0.019 | 1.781 | 4.235 | 1.497 | 1.369 | 0.809 | 1.683 |



and the fluorobenzimidazole nucleosides paired against natural bases. In these cases all $T_m$ values are lower than those for the natural bases. Possible explanations for these findings are: (1) there are no hydrogen bonds between the modified and the natural bases and (2) the modified bases are less solvated by water molecules. As for the 2,4-difluorobenzene, the uridine analogue, we found a new universal base (6), which paired with all natural bases without energy discrimination. In a second series we measured the modified nucleosides against each other. We paired the 2,4-difluorobenzene (uridine analogue) and the two fluorobenzimidazoles against the fluorobenzene modified nucleosides and the abasic site. The RNA duplexes with the 4,6-difluorobenzimidazole nucleoside are even approximately 1°C more stable than the ones with the 4-fluorobenzimidazole nucleoside. From all these results we calculated the individual contributions of base stacking and solvation. Therefore we compared the $T_m$ values shown in Table 1.

Table 2 shows the contributions of base stacking and solvation of nucleosides **2**, **3** and **8**. We found in the 2,4-difluorobenzene · 4-fluorobenzimidazole and the 2,4-difluorobenzene · 4,6-difluorobenzimidazole base pair a weak force, which stabilizes the RNA duplex about 0.6°C (0.4 kcal/mol) and 0.9°C (0.6 kcal/mol), respectively.

It may be possible that this increase of $T_m$ results from a weak F · · · H hydrogen bond between the modified nucleosides. The existence of such F · · · H hydrogen bonds in this class of molecules has been shown in the crystal structure of **5** with a F · · · H distance of 230 pm (4). This hydrogen bond would be one of the first F · · · H hydrogen bonds of so called "organic fluorine" (7) in aqueous solution.

Beside improving the stability these modifications have an influence on the lipophilicity of the RNA duplex. The partition coefficients between 1-octanol and water reflect the change in lipophilicity (Table 3) (8,9). The modified nucleosides are between 40 to 200 times more lipophilic than the natural bases. This explains the reduced solvation of the bases by water molecules and the loss of stability of RNA duplexes containing fluoro modified nucleosides.

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