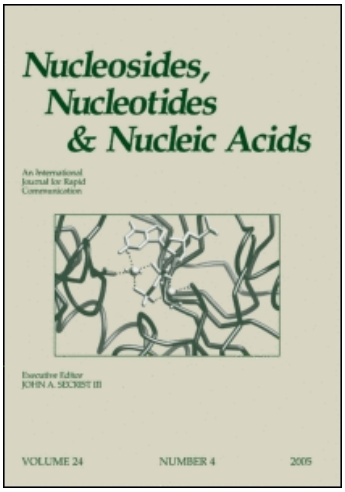

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Modified Nucleoside Triphosphates: Synthesis and Applications for RNA In Vitro Selection

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Modified Nucleoside Triphosphates: Synthesis and Applications for RNA In Vitro Selection

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Key Words: RNA in vitro selection; Nucleoside triphosphate synthesis; Melting point synthesis.

The in vitro selection process is used to enrich aptamers from combinatorial nucleic acid libraries against many classes of molecular targets.[1] In the last years deoxy- and ribozymes have been selected to catalyze various reactions (e.g., ligation of RNA,[2] Diels-Alder-reaction[3]).

Although complex the functional diversity of nucleic acid libraries is limited, consisting of four different nucleotide monomers only, whereas peptide libraries are made up of 20 different amino acids, adding basic, acidic and lipophilic diversity to the complexity of the library.

The suitability of various modified dNTP's, e.g., 5-modified dUTP[4–7] and 8-modified dATP[8] using different linkers and functional groups, was shown in the DNA SELEX process. However, only few investigations show the suitability of modified ribonucleotides for the RNA SELEX procedure.[9–11] Here, we have studied two commercially available nucleobase-modified NTP's **5**, **6** with 5-aminoallyl-2′-fluoro-dUTP **4** for their suitability in the SELEX process.

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CONCEPT

Which requirements must be met for the effective utilisation of modified nucleotides in the SELEX process?

- RNA polymerases must accept and incorporate the modified nucleoside triphosphates as building blocks for a growing transcript in high fidelity.
- The modified transcription products must be recognized and copied with high fidelity by reverse transcriptase.
- NTP's must be easily accessible by synthesis.

SYNTHESIS

After acetylation and detritylation of nucleoside **1** the nucleoside triphosphate **3** (Fig. 1) is obtained in 49% yield. Transformation of **3** to 5-AA-2′-F-dUTP [**4**] was achieved in 29% yield by alkylation of the intermediate Hg-derivative with allylamine. The UV of **4** clearly shows a shift of λ_{max} to 289 nm indicating the presence of an exocyclic double bond (Fig. 1).

RESULTS

All three analogues proved to be compatible with all enzymatic steps of the RNA in vitro selection process which proceeds in high fidelity (Table 2). All modified

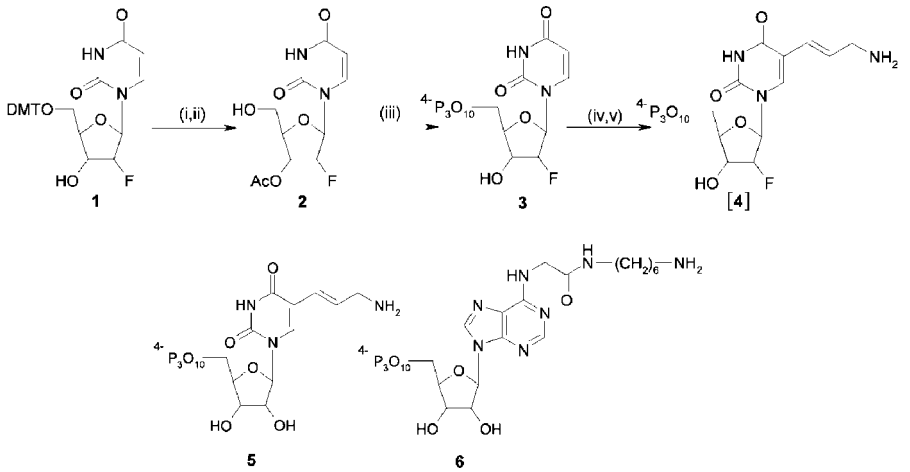

Figure 1. Synthesis of 5-AA-2′-F-dUTP 4 and commercial available modified NTP's used. (i) acetic anhydride, DMAP, Pyr; (ii) acetic acid; (iii) 2-chloro-4H-1,2,3-dioxaphosphorin-4-one, dioxane, pyrophosphate, tributyl-amine, DMF; iv) mercuric acetate, 0.1 M sodium acetate buffer (pH 6.0); (v) allylamine, K_2PdCl_4, 0.1 M sodium acetate buffer (pH 5).

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Table 1. T_m (°C) of different 17-mer RNA duplexes containing the modified nucleotides.

RNA duplex	Modification	T_m (°C)	ΔT_m (°C)
5′-GGA CUG ACU GAC UGA CC-3′ 3′-CCU GAC UGA CUG ACU GG-5′	RNA	75.0	–
5′-GGA C**U**G AC**U** GAC **U**GA CC-3′ 3′-CC**U** GAC **U**GA C**U**G AC**U** GG-5′	2′-F	80.3	+5.3
5′-GGA C**U**G AC**U** GAC **U**GA CC-3′ 3′-CC**U** GAC **U**GA C**U**G AC**U** GG-5′	**4**	> 85	–
5′-GGA C**U**G AC**U** GAC **U**GA CC-3′ 3′-CC**U** GAC **U**GA C**U**G AC**U** GG-5′	**5**	> 85	–
5′-GG**A** CUG **A**CU G**A**C UG**A** CC-3′ 3′-CCU G**A**C UG**A** CUG **A**CU GG-5′	**6**	57.7	−17.3

Modified bases U and A are underlined and boldface.

Table 2. Summary of substrate properties of the nucleotide analogues.

Modified RNA	RNA transcription	Reverse transcription	DNA sequencing
4	+	+	+
5	++	+	+
6	+++	+	+

+ indicates that desired product was obtained.

nucleotides are accepted and incorporated by *Taq* mutant RNA polymerase (Table 2). In the case of the RNA duplex containing the double substituted uridine **4** the increase of the T_m (>85°C) seems to be an additive combination of the 2′-fluoro and 5-aminoallyl substitutions. The 2′-fluoro-modification confers a RNA-like 3′-endo sugar conformation to oligonucleotides, thus forming an A-form duplex structure which improves binding affinity to RNA.[12] Additionally, the modification of the 5-position in **4** and **5** leads also to the increased stability of the duplex which can be explained by stacking of the nucleobases[13] (Table 1). The thermal melting points of the RNA containing the modified nucleotide **6** was significantly decreased by 17.3°C. The steric hinderence of the larger N6-linker modification as bulky functional group can cause the lower stability of the duplex.

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