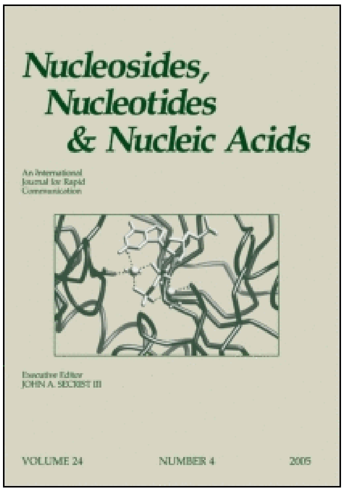

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SYNTHESIS AND PURIFICATION OF FLUORINATED BENZIMIDAZOLE AND BENZENE NUCLEOSIDE-5′-TRIPHOSPHATES

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▫ *The expression "universal base" is very often used to express hybridization properties and recognition patterns of nucleosides. Their behaviour in biological applications, however, is of great interest regarding, e.g., their incorporation by polymerases. The 4,6-difluorobenzimidazole and the 2,4-difluorobenzene nucleoside analogues have proven to be universal bases that do not discriminate between the four natural nucleobases in RNA duplexes. Therefore, we synthesized the corresponding triphosphates to evaluate their behavior in polymerase catalyzed reactions and to investigate their ability to serve as substrates for the T7 RNA polymerase.*

INTRODUCTION

Universal bases are nucleoside analogues that do not differentiate between the four natural nucleosides in base pairs.[1,2] In RNA duplexes universal bases mimic the natural nucleosides without forming specific interactions. Most commonly, isosteric structures with hydrophobic nucleobases are used to determine the influence of hydrogen bonds in oligonucleotide duplexes. It has been shown that the fluorinated nucleoside analogues **1** and **2** (Figure 1) are universal bases.[3,4] Upon incorporation in a RNA 12-mer duplex, the modifications do not discriminate between the four natural nucleobases and the resulting T_m values are nearly identical for all four duplexes ($\Delta T_m = 0.6–1°C$). However, the overall stability of the duplex decreased due to a lower solvation and the absence of strong hydrogen bonds.

These findings raised the question if the artificial nucleosides **1** and **2** behave like universal bases, too, when used in biological systems, i.e., polymerase catalyzed reactions. Therefore, we intended to synthesize the corresponding triphosphates to

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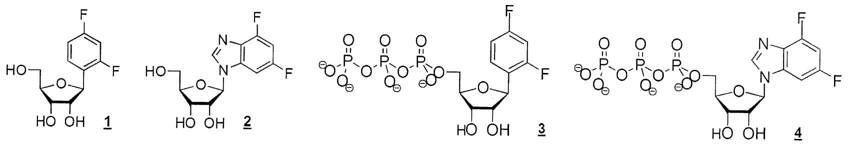

FIGURE 1 The universal, fluorinated nucleobases **1** and **2** and the corresponding triphosphates **3** and **4**.

evaluate their behavior in polymerase catalyzed reactions and to investigate their ability to serve as substrates for the T7 RNA polymerase.

TRIPHOSPHATE SYNTHESIS

The two fluorinated nucleosides shown in Figure 1 were synthesized via a C-glycosilation reaction (**1**) and the Vorbrüggen procedure (**2**), respectively.[3–5] The synthesis of the corresponding nucleoside-5′-triphosphates **3** and **4** was performed with Yoshikawa's procedure.[6] This one-pot reaction is a very effective method for the synthesis of nucleoside-5′-triphosphates (Figure 2). Due to the high regioselectivity it is not necessary to protect the 2′- and 3′-hydroxy groups. This selectivity can be assigned to the use of trimethylphosphate which forms a highly reactive, intermediate complex with the phosphoryl oxychloride. This complex leads to the activated monophosphate that can be directly converted into a cyclic nucleoside 5′-triphosphate upon reaction with inorganic pyrophosphate. The linearization reaction is then performed by the addition of a 1-M triethylammonium bicarbonate solution to the reaction mixture.

TRIPHOSPHATE PURIFICATION AND ANALYSIS

As the crude product contains different polar and nonpolar compounds several steps for the isolation of the triphosphate are necessary: 1) ion exchange FPLC,

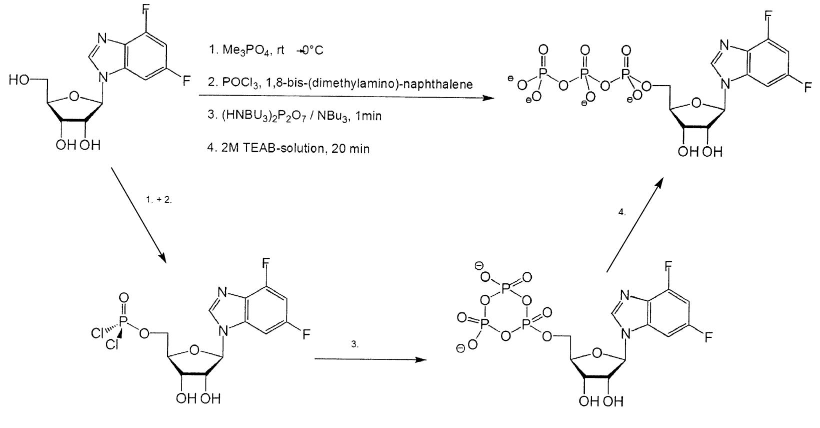

FIGURE 2 Synthesis of the triphosphate **4** with Yoshikawa's procedure.

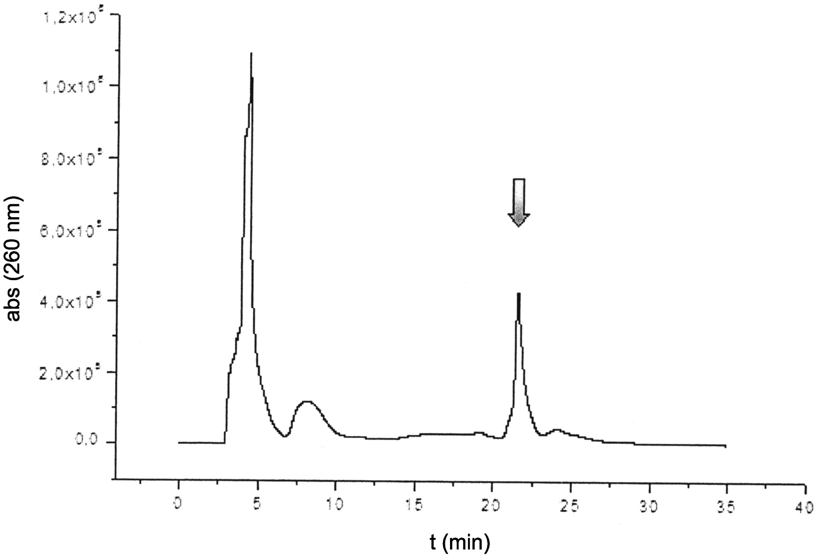

FIGURE 3 Anion exchange HPLC, the arrow marks the triphosphate **3**.

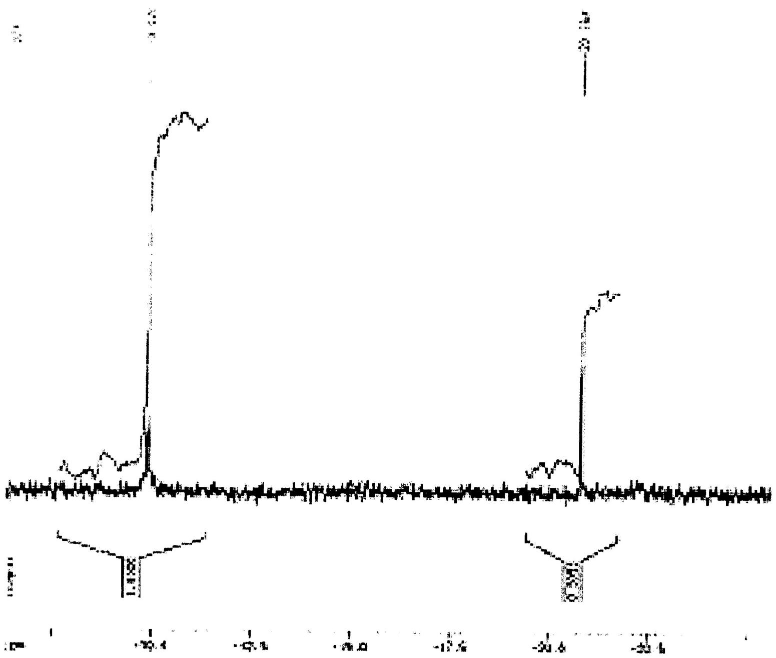

FIGURE 4 ^{31}P-NMR of compound **3**.

2) HPLC, and 3) RP-HPLC. The FPL-chromatography separates the triphosphate from the less polar compounds, i.e., the intermediate product monophosphate. Due to the fact that triphosphates and pyrophosphate carry the same charge they have to be separated in an additional chromatographic procedure, the anion exchange HPLC with 0.05 M potassiumdihydrogen phosphate buffer in formamide (Figure 3).

The elution of the product is carried out with an increasing amount of a 1-M solution of ammonium sulfate. The third step in the purification course is the reversed-phase HPLC with TEAA buffer, which is carried out for removing salts and the formamide. The characterization of the triphosphates was performed with ^{31}P-NMR (Figure 4) and ESI-MS.

OUTLOOK

The successful synthesis of both fluorinated nucleoside-5′-triphosphates **3** and **4** enables us to investigate if these artificial nucleosides behave like universal bases, too, when they are used in biological systems, i.e., in polymerase catalyzed reactions. Therefore, we developed two different enzyme based assay systems to evaluate the template properties of the modified nucleosides as well as their ability to act as a substrate for the T7 RNA polymerase.

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