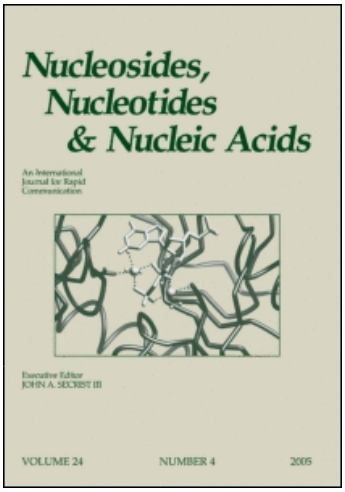

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NEW STRUCTURAL MOTIFS FOR HAMMERHEAD RIBOZYMES. CATALYTIC ACTIVITY OF ABASIC NUCLEOTIDE SUBSTITUTED RIBOZYMES

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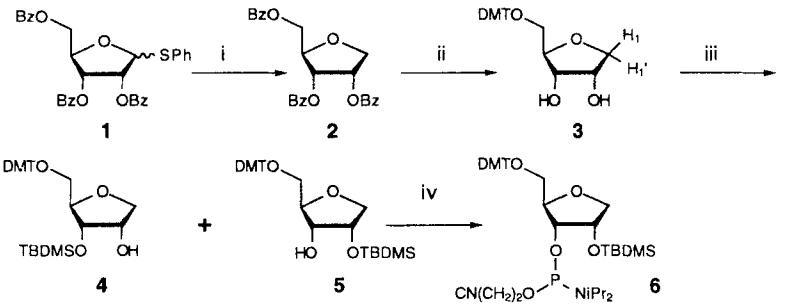
Abstract: The synthesis of 1-deoxy-D-ribofuranose-3-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (**6**) from D-ribose and its incorporation into a hammerhead ribozyme is described.

Hammerhead ribozymes[1] are among the smallest catalytic RNAs with sequence-specific endoribonuclease activity. Their highly specific cleavage activity suggests their use as therapeutic agents for the inhibition of gene expression.[2] As a part of our studies on the structure-activity relationships and molecular mechanism of action of hammerhead ribozymes, we describe the synthesis of an abasic monomer, the incorporation of ribo- and deoxyribo-abasic sites into a hammerhead ribozyme model sequence ***Rz 1*** and the catalytic properties of the modified ribozymes (***Rzs 2-6*** abasic sites shown as **H**).

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite **6** is shown in Figure 1. The synthesis of the related phosphoramidite of 1,2-dideoxy-D-ribofuranose has been described.[3,4] Phenylthioglycosides, successfully employed in the Keck reaction,[5] appeared to be a convenient starting material for the synthesis of 1-deoxy-D-ribofuranose. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on Bu_3SnH concentration[6,7]). Therefore, we subjected phenylthioglycoside **1** (prepared from commercially available 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-D-ribofuranose according to Ferrier[8]) to radical reduction with Bu_3SnH (6.1 eq) in the presence of Bz_2O_2 (2.1 eq) resulting in the isolation of tribenzoate **2** in 63% yield.[9] Subsequent debenzoylation and dimethoxytritylation led to synthon **3** in 70% yield. Introduction of the TBDMS group under standard conditions resulted in the formation of a 4:1 ratio of 2- and 3-isomers **5** and **4**. The two regioisomers were separated



Reagents and Conditions: *i)* Bu_3SnH, Bz_2O_2/toluene, *ii)* 2M NaOH/Pyr/MeOH, DMT-Cl/Pyr, *iii)* TBDMS-Cl, $AgNO_3$, Pyr/THF, *iv)* 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite, DIPEA/CH_2Cl_2.

FIGURE 1

Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-N,N-diisopropylphosphoramidite)-1-Deoxy-D-Ribofuranose (6)

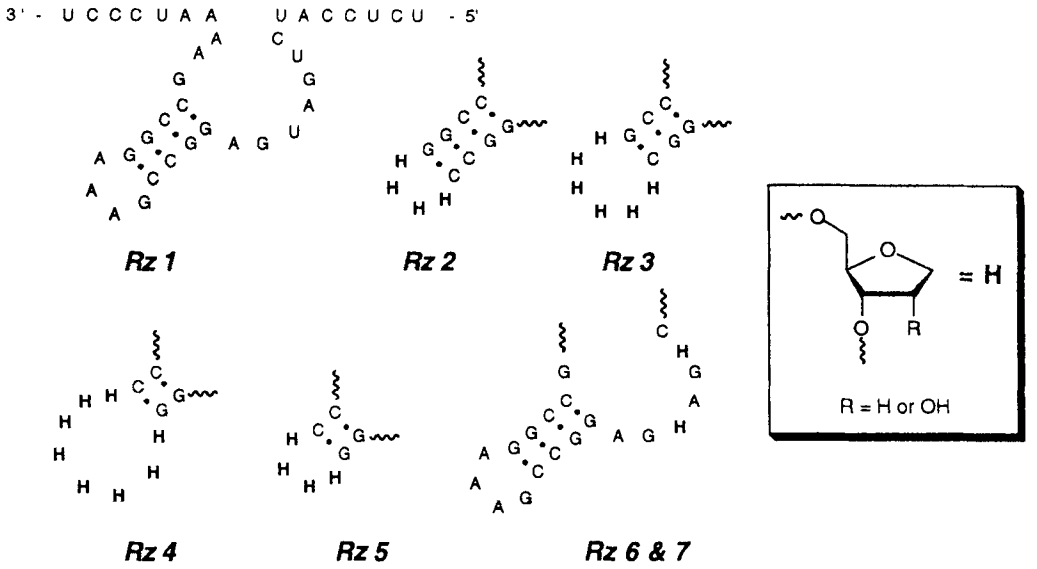


FIGURE 2

Hammerhead Ribozymes Containing Abasic Nucleotides H

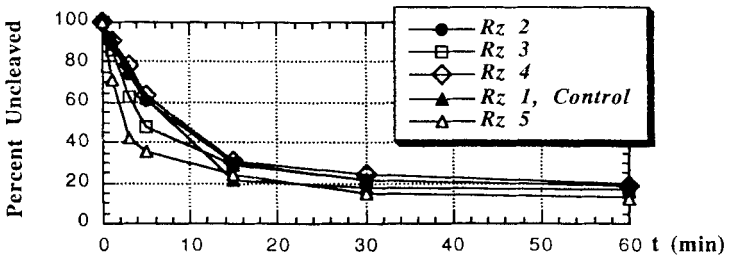


FIGURE 3

Substrate Cleavage by Ribo-Abasic Nucleotide Containing Ribozymes

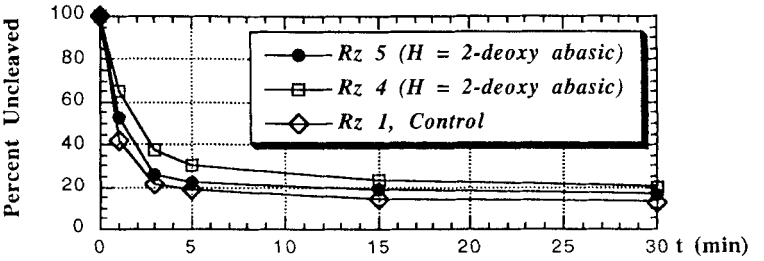


FIGURE 4

Substrate Cleavage by Deoxy-Abasic Nucleotide Containing Ribozymes

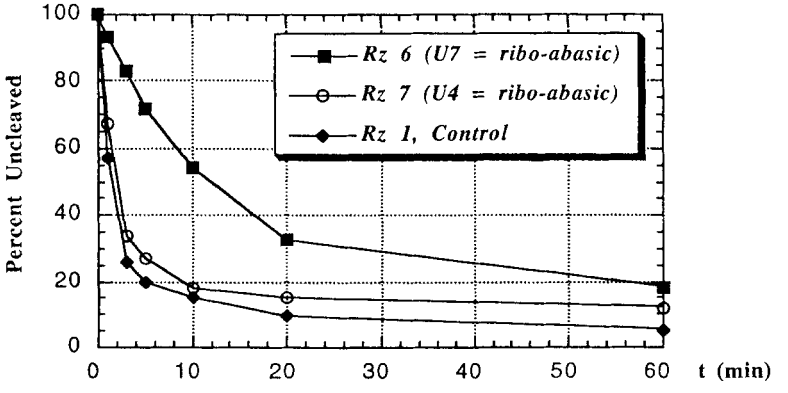


FIGURE 5

Substrate Cleavage by Ribozymes Containing Abasic Sites in the Catalytic Core

by silica gel chromatography and 2-*O*-*t*-butyldimethylsilyl derivative **5** was phosphitylated to provide phosphoramidite **6** in 82% yield. Abasic residues were incorporated into the hammerhead ribozyme shown in Figure 2 utilizing the standard RNA synthesis protocol[10] with coupling efficiencies of 98.5%.

Figure 3 shows a time course of cleavage of a 17-mer substrate by ***Rzs 2-5*** containing 4 (***Rz 2***), 6 (***Rz 3***) and 8 (***Rz 4***) ribo-abasic residues in Stem II and a shortened stem II with 4 ribo-abasic residues (***Rz 5***) (Figure 2). These modifications had little effect on catalytic activity indicating that the majority of the Stem II-Loop II region serves only a general structural role in maintaining or allowing a certain conformation in the single-stranded catalytic core.[11,12] There are no specific required base-base or base-metal interactions in this stem-loop. The similar cleavage activity of ***Rzs 4*** and ***5*** with identical deoxy-abasic substitutions (Figure 4) supports the hypothesis that the 2'-OH groups of nucleosides in positions 10.3 to 11.3 of the Stem-loop II are also not involved in interactions important for catalysis.

To probe the importance of specific bases we sequentially replaced nucleotides in the catalytic core with ribo-abasic residues and assayed the cleavage activity of all 11 ribozymes. Nine of these demonstrated a complete loss of cleavage activity, in agreement with catalytic core mutagenesis data.[13] However, ribozymes containing U7 (***Rz 6***) or U4 (***Rz 7***) ribo-abasic residues showed high cleavage activity (Figure 5). This observation indicates that it may be possible to modify uracil bases in these positions to increase the activity and stability of hammerhead ribozymes.

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