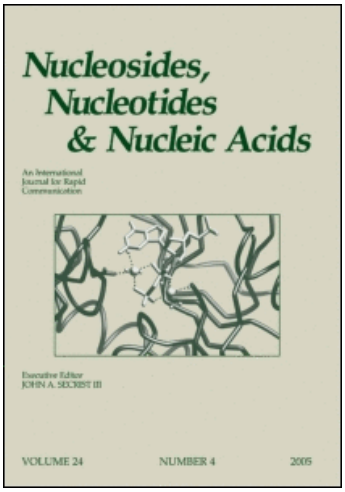

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Elucidation of the Hydrolytical Properties of α-Hydroxybenzylphosphonates as a New Potential Pro-Oligonucleotide Concept

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ELUCIDATION OF THE HYDROLYTICAL PROPERTIES OF α-HYDROXYBENZYLPHOSPHONATES AS A NEW POTENTIAL PRO-OLIGONUCLEOTIDE CONCEPT

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Abstract: The synthesis of Fpmp-protected α-hydroxybenzylphosphonate modified oligonucleotides as potential new pro-oligonucleotides is described. The proposed hydrolytic pathways of the oligonucleotides were studied using two dimers **2** and **4** and the tetramer **6** containing one α-hydroxybenzyl modification as model compounds.

In previous studies[1], we showed that the stability of different 3'-α-hydroxybenzyl-phosphonate-modified oligonucleotides exhibit a significant stability enhancement in degradation studies with 3'-exonucleases in contrast to the natural $(T)_{15}$-oligonucleotide. The T_m-values of the modified oligonucleotides hybridized with DNA and RNA were identical with those of the unmodified oligonucleotide. The new modified oligonucleotides may act as pro-oligonucleotides due to the hydrolytic pathways of α-hydroxybenzyl-phosphonates in aqueous alkaline media; It was shown before[2], that strong electron-withdrawing substituents bearing 5',5'-α-hydroxybenzylphosphonates rearrange to the benzylphosphotriesters which lead after hydrolysis to the unmodified phosphodiesters. However, introduction of an electron-donating substituent lead to direct cleavage with formation of a H-phosphonate diester. To study these properties for oligonucleotides, we synthesized two different 3',5'-α-hydroxybenzylphosphonate dimers **2** and **4** and the tetramer **6** containing one α-hydroxy-2-nitrobenzyl modification within the backbone as model compounds. For the synthesis of α-hydroxybenzylphosphonate-modified oligo-nucleotides, the α-hydroxybenzyl moiety was protected with the acid-labile 1-(2-fluorophenyl)-4-methoxypiperidine-4-yl (Fpmp) group, which was introduced by Reese[3] for the protection of the 2'-hydroxy function in RNA synthesis.

To verify the degradation pathways of α-hydroxybenzylphosphonates first, the unsubstituted α-hydroxybenzylphosphonates **1,2** were prepared to elucidate the direct cleavage pathway and second, α-hydroxy-2-nitrobenzylphosphonates **3,4** were prepared to study the rearrangement pathway. Furthermore, the tetramers **5,6** were synthesized. The syntheses of the dimers **1** and **3** were performed as shown before[1], tetramer **5** was synthesized in solution using a α-hydroxyphosphonate-modified dimer phosphoamidite.

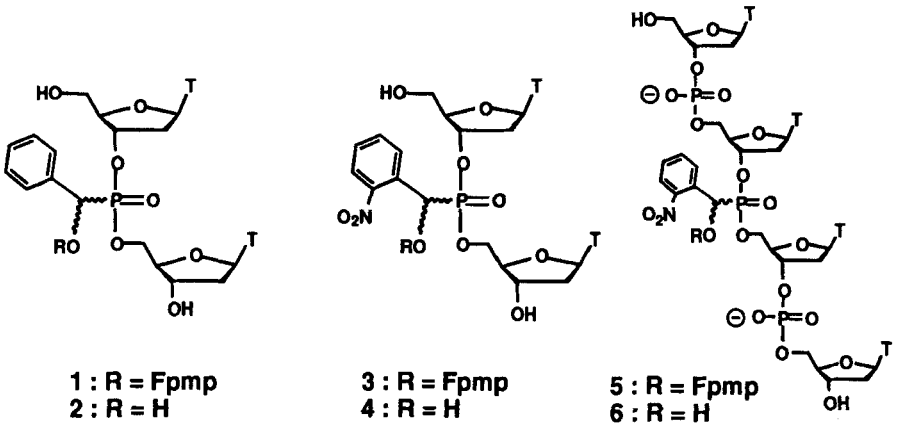

Figure 1: Structures of the dimers **1-4** and the tetramers **5, 6**

To deprotect the hydroxy groups in **1, 3** and **5**, a 0.5 M glycine/NaCl/HCl buffer, pH 3.0 at 37 °C was used. After one to three days, the Fpmp group was cleaved and the α-hydroxybenzylphosphonates **2, 4** and **6** were isolated in moderate yields. The hydrolysis pathways were studied by ^{31}P-NMR in a 0.5 M TRIS/HCl buffer, pH 8.6 at room temperature. The four characteristic ^{31}P-signals of the α-hydroxybenzylphosphonate **2** at 23 ppm disappeared while two new signals at 5 and 6 ppm appeared. These are characteristic for the corresponding H-phosphonate monoesters. The α-hydroxy-2-nitrobenzylphosphonate dimer **4** (four signals at 21 ppm) rearranged to the 2-nitrobenzyl-phosphotriester (two signals at -2 ppm), which was finally hydrolyzed to the phosphodiester (0 ppm). The same hydrolytic characteristics were observed for tetramer **6**.

Fpmp-protected α-hydroxybenzylphosphonate-modified oligonucleotides were synthesized using the standard amidite protocol. After cleavage of the Fpmp group and HPLC purification, the unprotected oligonucleotides could be isolated and characterized by ESI mass spectrometry. Hydrolytic studies are still in progress and were published elsewhere.

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