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# Nonenzymatic Synthesis of RNA and DNA Oligomers on Hexitol Nucleic Acid Templates: The Importance of the A Structure

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**Abstract:** Hexitol nucleic acid (HNA) is an analogue of DNA containing the standard nucleoside bases, but with a phosphorylated 1,5-anhydrohexitol backbone. HNA oligomers form duplexes having the nucleic acid A structure with complementary DNA or RNA oligomers. The HNA decacytidylate oligomer is an efficient template for the oligomerization of the 5′-phosphoroimidazolides of guanosine or deoxyguanosine. Comparison of the oligomerization efficiencies on HNA, RNA, and DNA decacytidylate templates under various conditions suggests strongly that only nucleic acid double helices with the A structure support efficient template-directed synthesis when 5′-phosphoroimidazolides of nucleosides are used as substrates.

## Introduction

Nonenzymatic synthesis of RNA oligomers from activated guanosine 5′-phosphates on RNA, DNA, and PNA templates has been studied in detail.[1−5] Hexitol nucleic acids (HNAs) are novel DNA analogues containing the standard nucleoside bases, but with a phosphorylated 1,5-anhydrohexitol backbone (Figure 1a). The six-membered hexitol ring mimics the furanose ring, frozen in its 2′-exo, 3′-endo conformation.[6] Unlike previously described DNA or RNA analogues that have backbones based on a pyranose sugar ring,[7,8] HNA oligomers form duplexes with complementary DNA or RNA oligomers. These duplexes have structures that closely resemble the structure of A-form DNA.[9,10] It seemed possible, therefore, that studies of oligonucleotide synthesis on HNA templates would provide information about the conformation that is required for efficient nonenzymatic oligomerization on DNA and RNA templates. In this work, we compare the oligomerization of guanosine 5′-phosphoro-2-methylimidazole (2-MeImpG, Figure 1b)[11] and the $Pb^{2+}$-

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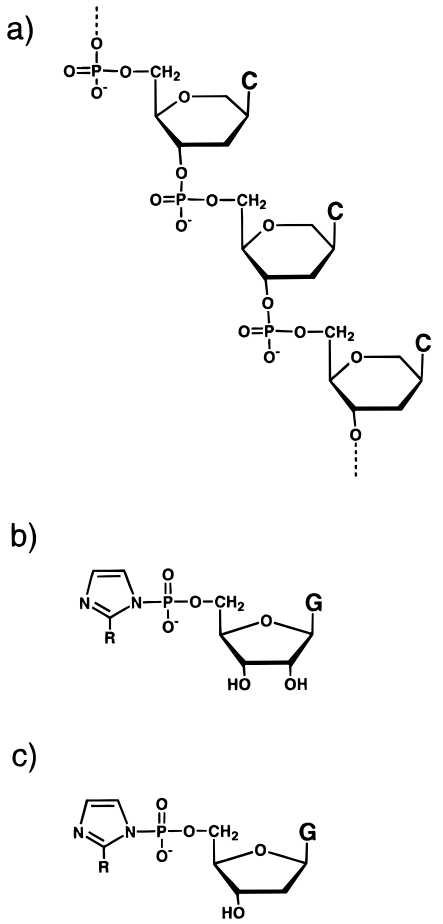


**Figure 1.** Structures of **(**a**)** hexitol nucleic acid (HNA) template, **(**b**)** activated guanosine 5′-phosphates: R═H − ImpG and R═$CH_3$ − 2-MeImpG, and **(**c**)** activated deoxyguanosine 5′-phosphates: R═H − ImpdG and R═$CH_3$ − 2-MeImpdG.

catalyzed oligomerization of guanosine 5′-phosphoroimidazole (ImpG, Figure 1b)[1] on decacytidylate templates of HNA ($hC_{10}$), RNA ($C_{10}$), and DNA ($dC_{10}$). We have also explored the ability of deoxyguanosine 5′-phosphoro-2-methylimidazole (2-MeImpdG, Figure 1c) to form oligodeoxyribonucleotides on the same templates.

## Results

**Oligomerization of 2-MeImpG on $hC_{10}$, $C_{10}$, and $dC_{10}$ Templates.** In the absence of a template, 0.1 M aqueous solutions of 2-MeImpG yield as products only dimers and smaller amounts of trimers. We found that the presence of $hC_{10}$, $C_{10}$, or $dC_{10}$ templates leads to efficient oligomerization of 2-MeImpG (Figure 2a−c). The results obtained on $C_{10}$ and $dC_{10}$ templates (Figure 2b,c) are analogous to those previously reported for poly(C) and $(dC)_{5-8}$ templates.[12] The major peaks on the HPLC profiles (Figure 2b,c) correspond to all 3′-5′-linked oligo$(G)_n$s ranging in length from the dimer to 10-mer. The major products formed on an $hC_{10}$ template (Figure 2a) were shown to be identical by cochromatography with oligo$(G)_n$s synthesized in a reaction on a $C_{10}$ template.

**Oligomerization of ImpG on $hC_{10}$, $C_{10}$, and $dC_{10}$ Templates in the Presence of $Pb^{2+}$ Ions.** We found that an $hC_{10}$ or a $C_{10}$ template in the presence of $Pb^{2+}$ catalyses oligomerization of ImpG (Figure 3a,b). This is consistent with earlier observations that $Pb^{2+}$ ions catalyze oligomerization of ImpG on a poly(C) template[1] to give all 2′−5′-linked oligo$(G)_n$. The major products of ImpG oligomerization on an $hC_{10}$ template were established to be 2′−5′-linked oligo$(G)_n$s by cochromatography with oligo$(G)_n$s synthesized in the $Pb^{2+}$-catalyzed oligomerization of ImpG on poly(C). The oligomerization of ImpG on a $dC_{10}$ template in the presence of $Pb^{2+}$ is much less efficient and regiospecific (Figure 3c). ImpdG does not oligomerize significantly on $dC_{10}$, $C_{10}$, or $hC_{10}$ templates (data not shown).

**Oligomerization of 2-MeImpdG on $hC_{10}$, $C_{10}$, and $dC_{10}$ Templates.** In the absence of a template, 2-MeImpdG yields only dimers and smaller amounts of trimers. The presence of a $C_{10}$ or an $hC_{10}$ template leads to oligomerization of 2-MeImpdG (Figure 4a,b), but the presence of $dC_{10}$ template does not catalyze the oligomerization (Figure 4c). The peaks in the HPLC profiles (Figure 4a,b) represent oligo$(dG)_n$s capped with a pyrophosphate-linked pdG group at the 5′-terminus. Hydrolysis by $Zr^{4+}$ which specifically cleaves pyrophosphate linkages[13] converts the products to oligodeoxyribonucleotides up to $(dG)_5$.

Although 2-MeImpdG does not oligomerize on a $dC_{10}$ template under our standard conditions, it does oligomerize in the presence of 0.2 M $[Co(NH_3)_6]^{3+}$ ions under conditions in which a DNA duplex is known to be present in the A form.[14] The same result was observed when 0.2 M $[Co(NH_3)_6]^{3+}$ ions were replaced by 0.2 M $Mg^{2+}$ ions. A combination of 0.2 M $[Co(NH_3)_6]^{3+}$ and 0.2 M $Mg^{2+}$ ions leads to the most efficient oligomerization. Under these conditions the oligomerization on a $dC_{10}$ template is as efficient as on a $C_{10}$ template (data not shown).

## Discussion

The oligomerization of 2-MeImpG yields similar patterns of products on $hC_{10}$, $C_{10}$, and $dC_{10}$ templates. The reaction is slightly more efficient on the $hC_{10}$ template, and after longer times, the $hC_{10}$ template gives rise to significantly larger amounts of the longest products $G_{10}$. The $Pb^{2+}$-catalyzed reaction of ImpG is somewhat more efficient on the $hC_{10}$ template than on the $C_{10}$ template and, as in the previous case, yields substantially more $G_{10}$. The $Pb^{2+}$-catalyzed oligomerization of ImpG is much less efficient and much less regiospecific on a $dC_{10}$ template than on an $hC_{10}$ or a $C_{10}$ template.

The slow and nonregiospecific addition of the last G residue on oligo(C) or oligo(dC) templates has been attributed to growing instability of the template−substrate double helix as the 5′-terminus of the template is approached.[12] Our present results suggest that the $hC_{10}$ template is preadapted to adopt the backbone conformation of A-DNA, permitting efficient chain elongation of the oligomeric products all the way to the 5′-terminus of the template.

We do not understand the mechanism of $Pb^{2+}$ catalysis in detail. It seems likely that the $Pb^{2+}$ ion directly activates the 2′-OH group, since $Pb^{2+}$ ion is known to activate hydroxyl groups in other contexts. It is not clear why $Pb^{2+}$ ion activation is ineffective on a DNA template.

The oligomerization of activated derivatives of deoxyribonucleotides is much less efficient than the oligomerization of the corresponding ribonucleotides.[15−17] This has usually been attributed to the much easier ionization of a *cis*-glycol than of

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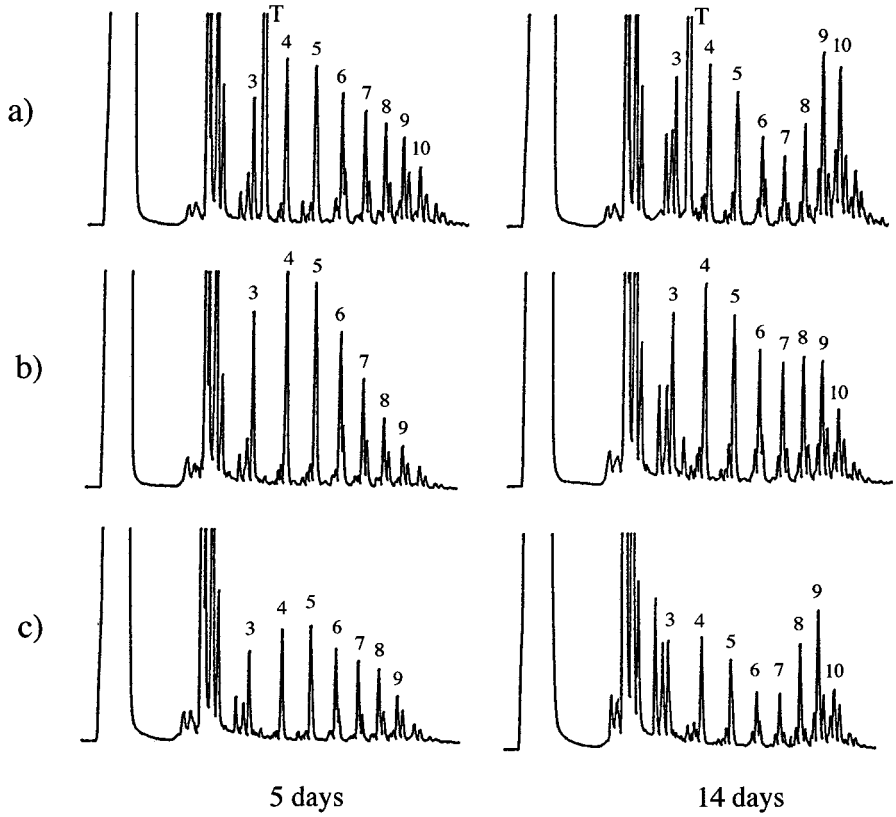


**Figure 2.** Oligomerization of 2-MeImpG on different templates after 5 days and 14 days: **(a)** an $hC_{10}$ template; **(b)** a $C_{10}$ template; **(c)** a $dC_{10}$ template. The numbers above the peaks indicate the length of the all 3′−5′-linked oligo$(G)_n$ products, and T indicates the template. The $C_{10}$ template was cleaved with RNase A before analysis.[22] The profiles in this figure were obtained using a different RPC5 column from that used in other experiments. The template $dC_{10}$ peak appears in Figures 3 and 4 but is absent here because it came after the end of our recording.

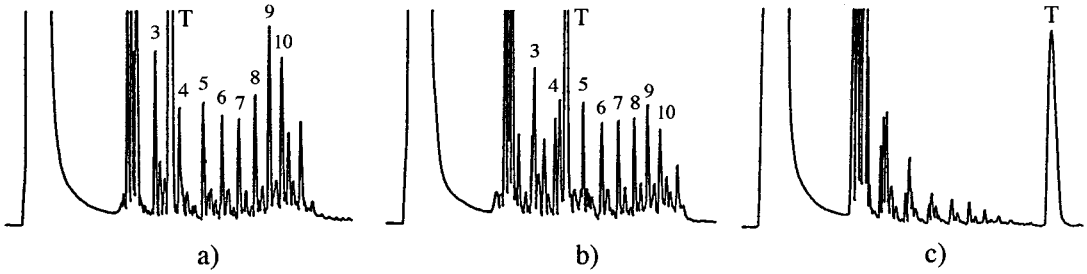


**Figure 3.** Oligomerization of ImpG in the presence of $Pb^{2+}$ ions on different templates after 5 days: **(a)** an $hC_{10}$ template; **(b)** a $C_{10}$ template; **(c)** a $dC_{10}$ template. The numbers above the peaks indicate the length of the all 2′−5′-linked oligo$(G)_n$ products, and T indicates the template.

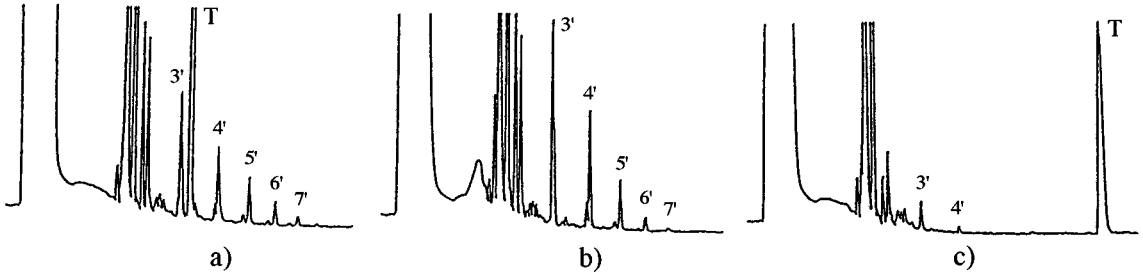


**Figure 4.** Oligomerization of 2-MeImpdG on different templates after 3 days: **(a)** an $hC_{10}$ template; **(b)** a $C_{10}$ template; **(c)** a $dC_{10}$ template. The numbers above the peaks indicate the total number of G residues in the oligo$(dG)_n$ products capped with an extra pdG group at the 5′-terminus, and T indicates the template. The $C_{10}$ template was cleaved with RNase A before analysis.[22]

a monohydric alcohol.[15] Consequently, the oligomerization of 2-MeImpdG does not seem to have been investigated in any detail. We have now detected the formation of oligo(dG)s on $C_{10}$ and $hC_{10}$ templates. No similar oligomers were formed on a $dC_{10}$ template under standard conditions.

It is well-known that right-handed nucleic acid double helixes in dilute aqueous solution adopt two well-defined structures.[18] RNA double helixes and RNA−DNA hybrid helixes usually adopt the A structure with the ribose ring in its 3′-endo

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conformation, while DNA double helixes generally adopt the B structure with the ribose ring in its 2′-endo conformation. Since template-directed synthesis with 2-methylimidazole derivatives of nucleotides is remarkably substrate specific, it is almost certain that it takes place efficiently in only one of these two very different structures. Our result supports the view that the 3′-endo conformation of the ribose ring in the A structure of the double helix is the productive conformation.

First, the template $hC_{10}$, which is restricted to the A conformation, is as effective as or more effective than $C_{10}$ and $dC_{10}$ templates for the oligomerization of 2-MeImpG. Second, $hC_{10}$ and $C_{10}$ templates, which form A-type double helixes with $(dG)_n$ oligomers, are effective templates for the oligomerization of 2-MeImpdG, while the $dC_{10}$ template, which forms a B-type double-helix structure is ineffective. Third, a PNA−$C_{10}$ template fails to catalyze the oligomerization of 2-MeImpdG (unpublished data), although it catalyzes the oligomerization of 2-MeImpG.[19] It is known that DNA−PNA double helixes adopt the B structure.[20] Fourth, $[Co(NH_3)_6]^{3+}$ under conditions in which it converts B structure of a DNA duplex to the A structure[14] converts $dC_{10}$ from an ineffective to an effective template.

Our results are not conclusive since we have no detailed knowledge of the conformation at 3′-terminus of the growing oligo(G) or oligo(dG) chains. Furthermore, when we replaced 0.2 M $[Co(NH_3)_6]^{3+}$ by 0.2 M $Mg^{2+}$, we obtained almost the same product distribution and there is no good evidence that 0.2 M $Mg^{2+}$ converts the B structure of DNA into A structure, although it may.[21] Despite these reservations, we strongly suspect that only the A structure of a nucleic acid double helix brings the 3′-OH group of the growing oligomer in line with the imidazole group of the activated 5′-phosphate in such way as to promote oligomerization.

## Experimental Section

Unless otherwise noted, all chemicals were reagent grade, were purchased from commercial sources, and were used without further purification. Guanosine 5′-monophosphate and deoxyguanosine 5′-monophosphate were obtained from Sigma. The oligoribonucleotide $C_{10}$ was purchased from Xeragon (Switzerland). The syntheses of guanosine 5′-phosphoro-2-methylimidazole, guanosine 5′-phosphoroimidazole, deoxyguanosine 5′-phosphoro-2-methylimidazole, and deoxyguanosine 5′-phosphoroimidazole were performed by obvious modifications of a published method[22] with at least 95% yields. The oligodeoxyribonucleotide $dC_{10}$ was synthesized on a 391A DNA synthesizer (Applied Biosystems), deprotected in concentrated ammonia at 55 °C, purified by 20% PAGE, and desalted on a Nensorb column (Nen DuPont). The HNA template ($hC_{10}$) was synthesized as described.[10] HPLC analyses of the reaction mixtures were performed on an RPC5 column as previously described.[22] Reaction products were eluted with a linear gradient of $NaClO_4$ (pH 12, 0−0.06 M in 60 min) and monitored by UV absorption at 254 nm.

Reaction conditions for the oligomerization of 2-MeImpG (or 2-MeImpdG) on various templates were chosen to permit comparison with earlier published work.[23] The reactions were run at 0 °C in 0.2 M 2,6-lutidine−HCl buffer (pH 7.9 at 25 °C) containing 1.2 M NaCl, 0.2 M $MgCl_2$, 0.5 mM of a template, and 0.1 M of an activated mononucleotide.

Reaction conditions for the oligomerization of ImpG (or ImpdG) in the presence of $Pb^{2+}$ ions on various templates were chosen to permit comparison with earlier published work[1]. The reactions were run at 0 °C in 0.4 M 2,6-lutidine−$HNO_3$ buffer (pH 7.5 at 0 °C) containing 0.01 M $Pb(NO_3)_2$, 0.4 M $NaNO_3$, 0.5 M $Mg(NO_3)_2$, 0.5 mM of a template, and 20 mM of an activated mononucleotide.

Oligomerization reactions of 2-MeImpdG on $dC_{10}$ and $C_{10}$ templates in the presence of $[Co(NH_3)_6]^{3+}$ ions[14] were run in 20 mM phosphate buffer (pH 7.0) in the presence of 0.5 mM of a template, 0.1 M of 2-MeImpdG, 0.2 M $[Co(NH_3)_6]Cl_3$, or 0.2 M $MgCl_2$ (or 0.2 M $[Co(NH_3)_6]Cl_3$ and 0.2 M $MgCl_2$).

Hydrolysis of oligo$(dG)_n$s capped with an extra pdG group at the 5′-terminus was performed with 0.04 M $ZrCl_4$ in 0.4 M sodium acetate buffer (pH 4.5) for 1 h at 50 °C as described.[13]

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