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The synthesis of 3′- and 5′-iminodiacetic acid derivatives of thymidine and their incorporation into synthetic oligonucleotides

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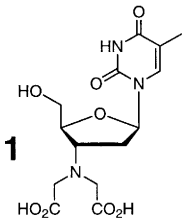
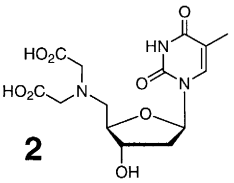
Abstract

The syntheses of thymidines modified with iminodiacetic acid (IDA) at the 3′ (**1**) or 5′ (**2**) positions have been accomplished. These modified nucleotides have been incorporated into synthetic oligonucleotides.





Metal ions play important structural and functional roles in oligonucleotide chemistry and biology due to the myriad complex interactions between various metal ions and the nucleobases or anionic phosphodiester backbone.[1–7] The tools of organic synthesis have played a significant role in the study of such interactions, especially by facilitating the construction and modification of designed ion/oligonucleotide complexes via the introduction of unnatural metal binding sites into predetermined sites within synthetic oligonucleotides.[8–11]

Our group's interest in this area is focused upon developing methods to utilize multiple ligands, predictably organized in space, to bind metal ions in precise locations. This work is inspired by reports from the groups of Ghadiri[12,13] and Hopkins,[14] who demonstrated that multipoint metal ion binding could be used to stabilize secondary structure in small peptides. We have taken a complimentary approach, in that we intend to use the secondary structure of small synthetic oligonucleotides to template the formation of metal-binding sites. We have chosen to introduce the metal-binding residues into synthetic oligonucleotides during their solid-phase synthesis as novel phosphoramidite monomers. Accordingly, we herein report the synthesis of iminodiacetic acid modified thymidines, their conversion

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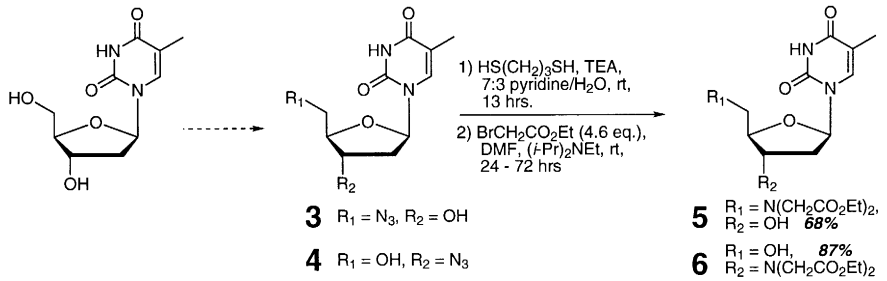
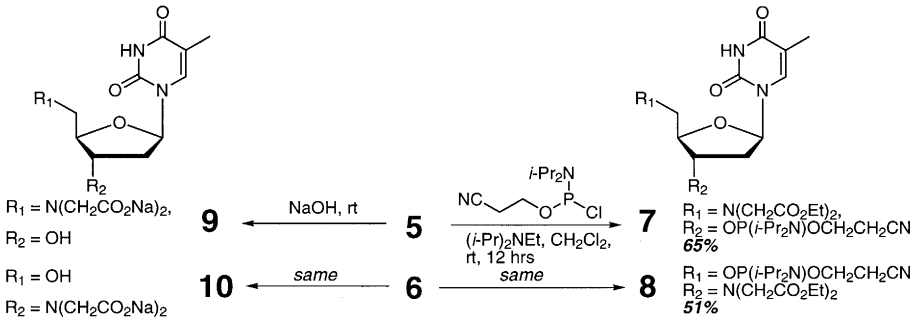
to phosphoramidites suitable for use in solid-phase DNA synthesis, and their incorporation into small synthetic oligonucleotides.

Both syntheses are straightforward, beginning with the known 3′ or 5′-azido thymidines (**3** or **4**, respectively; Scheme 1), which were cleanly reduced to the corresponding amino nucleosides by treatment with 1,3-propanedithiol.[15] Dialkylation of these amines with ethyl bromoacetate provided the penultimate esters **5** and **6**. These esters were prepared for incorporation into synthetic oligonucleotides by reaction of the free hydroxyl groups (5′ or 3′, respectively), with 2-cyanoethyl diisopropylchlorophosphoramidite, thereby converting them to phosphoramidites **7** and **8** (Scheme 2).



Scheme 1. Synthesis of iminodiesters **5** and **6**



Scheme 2. Synthesis of free acids **9** and **10** and phosphoramidites **7** and **8**

These phosphoramidites are efficiently installed as the terminal residue during oligonucleotide synthesis, and the free iminodiacetate ligand is revealed by a careful two-step saponification/oligo deprotection protocol.[9] In order to preserve the natural 3′-5′ linkages, the 5′-IDA amidite **7** was added in the final step of a *reverse-orientation* 5′-3′ oligonucleotide synthesis (Scheme 3). Oligonucleotide compositions were verified using MALDI mass spectrometry, and the oligo purities were evaluated using capillary gel electrophoresis. Diesters **5** and **6** were also directly hydrolyzed to afford the monomeric nucleoside ligands **9** and **10**.

With oligonucleotides fitted with the iminodiaceticacid ligands in hand, we are presently evaluating the ability of these macromoleculecules to preorganize high affinity metal ion binding sites. Results from these studies will be reported elsewhere.

Acknowledgements

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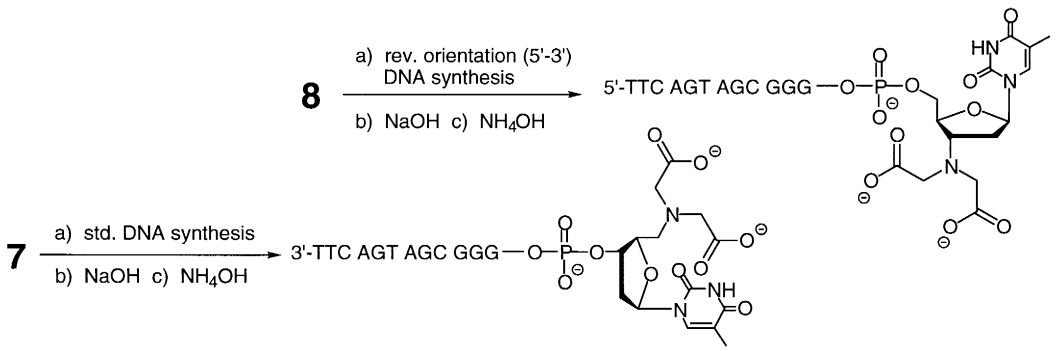

Scheme 3. Synthesis of iminodiacetic acid substituted oligonucleotides

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