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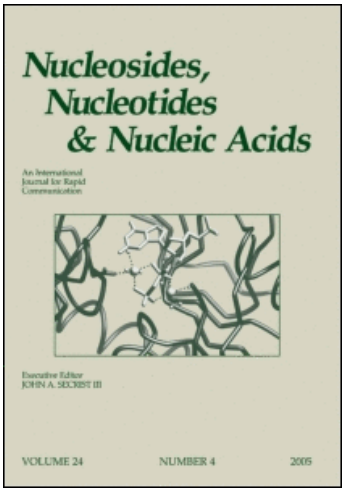

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Site-Specific Introduction of Functional Groups onto Bases in Synthetic Oligonucleotides for Biological Applications

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Site-Specific Introduction of Functional Groups onto Bases in Synthetic Oligonucleotides for Biological Applications

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Abstract: *Postsynthetic functionization of bases in oligonucleotides provides a useful approach to the preparation of DNA and RNA containing modified bases. In this communication is presented our recent work on this subject.*

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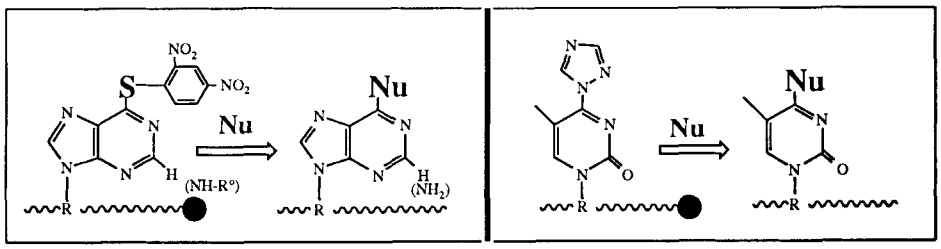
The availability of base-modified oligonucleotides has greatly stimulated research in many aspects such as DNA-DNA, DNA-RNA, and DNA-protein interactions[1]. We[2] and others[3] have developed several methods of postsynthetic functionization of pre-determined bases in DNA. Our results are summarized in the figure below (Nu standing for nucleophiles):



The main advantages of postsynthetic functionization are a) it provides the possibility to prepare oligomers containing chemically reactive or labile bases which may be unstable in monomer preparation, in oligomer assembly, or during deprotection; b) it could utilize effectively rare materials (eg. isotopes) or hazardous agents (eg. carcinogens) by introducing them at the last step.

Very recently we have developed two methods for chemical synthesis of oligodeoxynucleotides containing 6-methylthiopurine (or 6-methylthioguanine)[4]:
i) by conventional monomer incorporation: In this method the phosphoramidite monomer

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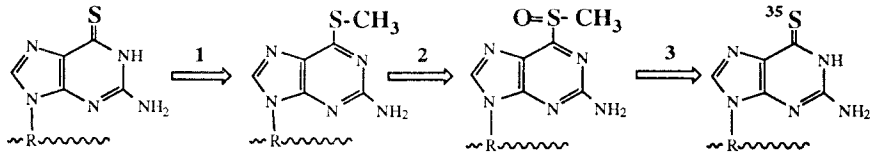
of 6-methylthiopurine was synthesized and incorporated into oligodeoxynucleotides as normal base monomers, but the deprotection was carried out at room temperature;

ii) by direct methylation: In this method oligomers containing 6-thioguanine were first prepared, then the sulphur selectively methylated with methyl iodide. The method is straightforward and simple, as the precursor thioguanine monomer which we previously developed[2a] is now commercially available from Glen Research. In addition this S-alkylation could readily introduce other functional groups. This methodological development has enabled us to show that methylation of thioguanine in DNA by S-adenosylmethionine and recognition of base-pairs involving methylthioguanine are the crucial steps in the mechanism of cytotoxic action of the important cancer drug thioguanine[5].

As after oxidation the methylthio group on purine residues is a good leaving group, we have devised two approaches for the introduction of stable and labile functional groups on purine residues at the oligodeoxynucleotide level:

i) oxidation-conversion *prior to* deprotection. In this approach, the 6-methylthio group on a purine residue in a protected oligomer was activated with an oxidizing agent, then replaced with nucleophiles. This approach is only suitable for the introduction of stable functional groups. As an example, a 20 mer containing 6-methylthiopurine was transformed into the 20 mer containing ^{15}N-adenine and then used for NMR studies[6];

ii) oxidation-conversion *after* deprotection. This approach is particularly suitable for the introduction of labile functional groups. As the functional group is introduced as the last step in the synthesis to the oligomer which has already been deprotected and purified, the resultant base-modified oligomer could be directly used for biological studies. An example: oligodeoxynucleotide containing ^{35}S-6-thioguanine



1: CH_3I ; 2: Monoperoxyphthalic acid (Magnesium salt); 3: ^{35}S-NaSH

At the nucleoside level 6-methylthiopurine 2'-deoxyribonucleosides could be easily converted into 6-sulphinyl and 6-sulphonyl derivatives, both of which are reactive[7]. The 6-sulphinyl derivative is more stable than the 6-sulphonyl derivative. The former compound remains unchanged in neutral aqueous buffer for an extended period, but the methylsulphinyl group can be readily replaced with many nucleophiles such as methanol, ammonia, or N-methylamine.

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We are currently further exploiting the unique properties of 6-methylsulphinyl group on purine residues in nucleosides and in oligonucleotides. When 6-methylsulphinylpurine-2'-deoxyribonucleoside was treated with lysine or arginine in aqueous solution, new compounds were formed and tentatively identified as amino acid-linked 2'-deoxyriboadenosines. If this reaction also takes place when the 6-methylsulphinylpurine in an oligodeoxynucleotide, it might provide the possibility of site-specific crosslinking of oligodeoxynucleotides with DNA binding proteins. We are optimizing the approach for biological studies.

We are also extending the chemistry of postsynthetic functionization to base-modified RNA synthesis. 6-Thioinosine and 6-thioguanosine have been converted into their 6-dinitrophenylthio derivatives respectively. These conversions are efficient and nearly quantitative as their deoxyribo analogues[2a,b]. The resultant 6-dinitrophenyl group on ribonucleosides have also been found good as a leaving group and replaceable by nucleophiles. These useful properties could be expected to be retained at the oligonucleotide level. This could provide a facile method to prepare RNA containing modified bases.

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