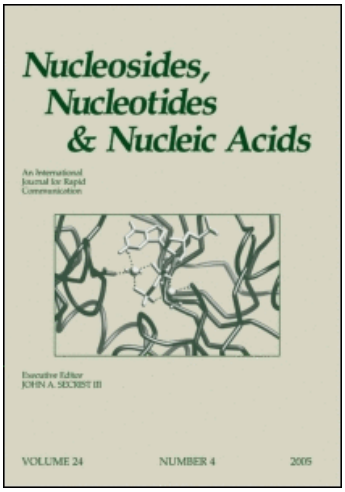


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# Preparation of 6-Thioguanosine Phosphoramidite for Oligoribonucleotide Synthesis

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# Preparation of 6-Thioguanosine Phosphoramidite for Oligoribonucleotide Synthesis

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## ABSTRACT

6-Thioguanosine phosphoramidite was prepared, using 2,4-dinitrophenyl as protection group for thio-function, and its stability towards conditions of RNA synthesis was investigated. The results show that the monomer was stable under the conditions of RNA synthesis and suitable for incorporation of thioguanine into oligoribonucleotides.

*Key Words:* 6-Thioguanosine; RNA Synthesis; Modified RNA; Modified base.

Oligonucleotides containing thio-substituted base are useful for various purposes in different areas, such as molecular biology and cancer study.[1,2] In concert with the goal of developing methods for chemical synthesis of oligonucleotides containing thiobases, we[3–5] and others[6,7] have established methods by which various thiobases are successfully incorporated into DNA and RNA. Here we describe synthesis of 6-thioguanosine phosphoramidite suitable for synthesis of oligoribonucleotides containing thioguanine.

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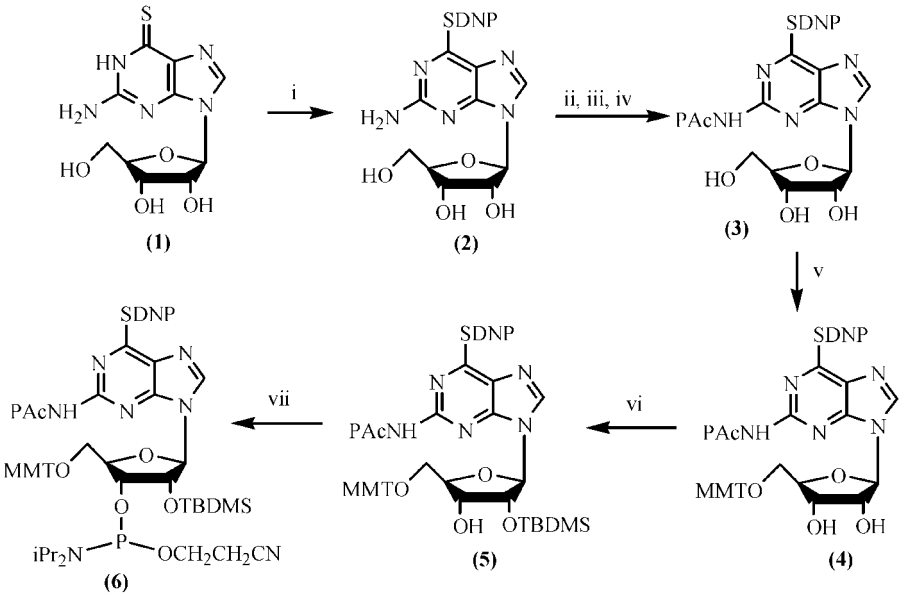


***Scheme 1.*** i, dinitrofluorobenzen/$Et_3N$; ii, $(CH_3)_3SiCl$; iii, phenylacetyl chloride; iv, 30% $NH_3H_2O$; V, MMTCl/pyridine; vi, t-butyldimetylsily chloride/AgNO3/pyridine; vii, i-$Pr_2NP(Cl)OCH_2CH_2CN$/i-$Pr_2NEt$.

The synthetic route for 6-thioguanosine phosphoramidite monomer (**6**) is shown in Scheme 1. Commercially available 6-thioguanosine (**1**) was reacted with 2,4-dinitrofluorobenzene in the presence of triethylamine to give compound **2** with isolated yields of 70–80%. The reaction was highly selective because 6-thio function is much more nucleophilic than 2′-, 3′- and 5′- hydroxyl groups and 2-amino group. Phenoxylacetyl group was chosen to protect 2-amino group of the nucleoside because it can be removed under mild conditions. It was introduced into the 2-amino group using transient protection scheme, in which 3 hydroxyl groups were first protected temporally with silyl groups that were removed by treatment of 30% aqueous ammonia after phenoxyacetyl group had been introduced. N-acylated compound **3** was tritylated with monomethoxyltrityl chloride (MMT-Cl). Although there are three free hydroxyl groups, due to its bulky structure, the MMT group was dominantly reacted with 5′-OH group (1° OH group) in regioselective fashion. Using standard methods, compound **4** was treated with t-butyldimethylsilyl chroride, then phosphitilating reagent to give the desired phosphoramidite monomer (**6**).

The stability of 6-(2,4-dinitrophenyl)thioguanine monomer (**6**) to the reagents used in oligonucleotide synthesis was studied before the monomer was used for RNA synthesis. Compound **3** was dissolved in 3% dichloroacetic acid/dichloroethane (deblocking reagent); and compound **5** in acetic anhydride/lutidine/THF (1 : 1 : 8) (capping reagent A), 4.4% N-methylimidazole in THF (capping reagent B), and 0.1 M iodine in THF/pyridine/water (40 : 9 : 1) (oxidation reagent). By monitoring changes in these solutions, it was shown that compound **5** was stable towards





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the reagents used in oligomer assembly for at least 24 h at room temperature, and compound **3** was stable towards 3% dichloroacetic acid in dichloroethane at room temperature for at least 3 h. It was also found that the 2,4-dinitrophenyl group protecting the 6-thioketo function can be removed completely by 1 M mercaptoethanol in $CH_3CN$ in the presence of triethylamine within 30 min. These results shown that monomer **6** would be stable during the synthesis, the protecting group easily removable after synthesis. We are currently investigating its incorporation into oligomers and use of the thio function as a platform for further post-synthetic modification of the oligonucleotides.

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