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# A base-stable dithiomethyl linker for solid-phase synthesis of oligonucleotides

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**Abstract**—A novel linkage, useful for the synthesis of oligonucleotides is described. The linking function is compatible with all conditions used for oligonucleotide synthesis, orthogonal to all other protecting groups, but regenerates 3′-OH rapidly upon mild reduction under aqueous conditions. This method is employed in the removal of depurinated fragments during the synthesis of oligonucleotides.


Oligonucleotides conjugated to other oligonucleotides, proteins, labels, haptens or separation tags by means of a linker that can be cleaved with liberation of the native oligonucleotide, have potential to find application in all fields of molecular biology. Solid-phase based methods for chemical synthesis are the most attractive routes for the synthesis of such conjugates. This requires, however, an access to a linkage that can withstand all reaction conditions for oligonucleotide synthesis and deprotection, being at the same time cleavable under mild and preferably aqueous conditions. Preferentially, the cleavage of the conjugate should liberate 3′-OH of the oligonucleotide, since it is often needed as a starting point for template-based DNA extension. These highly demanding conditions are not easy to realize. It is thus not surprising that amongst all existing linkages only photolabile[1] and silyl-based linkers[2] tend to approach these demands. However, photolabile functions often demand a long deprotection time, and are inadequate for application in sites which are nonaccessible by light. The cleavage of silyl-based functions proceeds under nonaqueous conditions, thus it is cumbersome and incompatible with most biological systems.

Recently, we reported the use of *tert*-butyldithiomethyl as a 2′-OH protecting group for solid-phase RNA synthesis.[3] Herein, a strategy for the conjugation of oligonucleotides via the 3′-*O*-dithiomethyl linkage is described and applied for the removal of depurinated oligonucleotides during the synthesis and purification of synthetic DNA.

Introduction of the dithiomethyl group onto a nucleoside was achieved via 5′-*O*-MMTr-3′-*O*-(methylthiomethyl)thymidine **1** obtained according to the published procedure[4] from 5′-*O*-MMTr-thymidine. This synthon, upon activation, reacted with 6-mercapto-1-hexanol, 3-mercapto-3-methyl-1-butanol[5] or 6-mercapto-6-methyl-1-heptanol (prepared according to the modified procedure)[6] to produce several nucleoside derivatives (**2**, **4**, **5**, and **6**) (Scheme 1). These nucleosides were converted to respective phosphoramidites (**3**, **7**, **8**, and **9**) bearing the dithiomethyl group. The stability of both nucleosides and nucleotide amidites was examined. It was found that tertiary alkyl substituents had pronounced stabilizing effects on the disulfide bond toward aqueous ammonia and iodine compared to the less stable primary analogues. Additionally, the stability of the disulfide bond in the obtained phosphoramidites was dependent on the presence of a bulky, tertiary carbon atom, flanking the dithio function. Thus, nucleoside **2** was not completely stable in 0.02 M iodine oxidation solution and phosphoramidite **3** had limited stability when dissolved in acetonitrile.

It was found that phosphoramidite **7** was prone to degradation. After isolation, derivative **7** underwent decomposition within few hours at room temperature in dry acetonitrile. HPLC studies showed the formation of intermediate **10**, which was further converted to **11**

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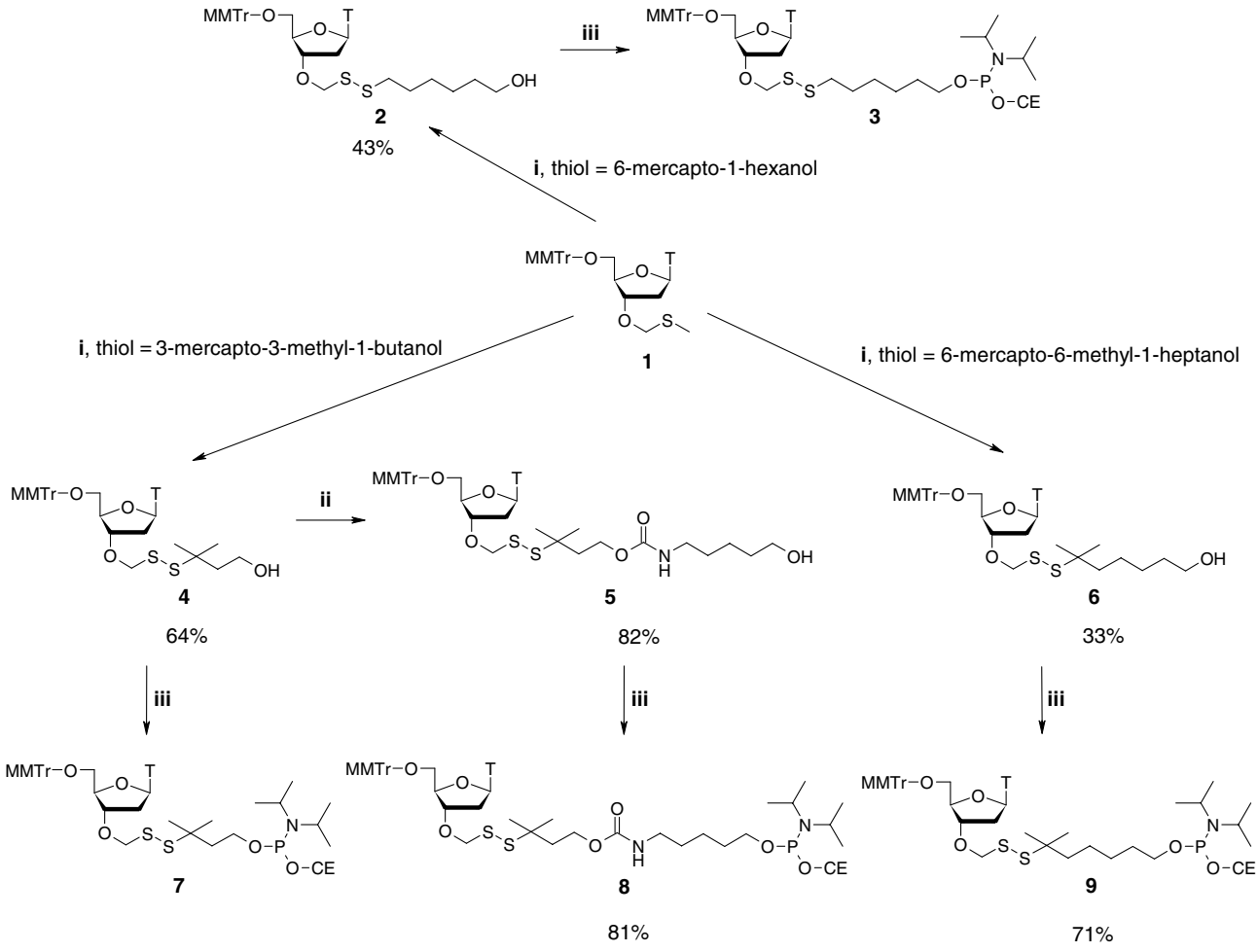


**Scheme 1.** Synthesis of phosphoramidites used in the present study: (i) a. $SO_2Cl_2$ (1.1 equiv), TEA (1.2 equiv) in 1,2-dichloroethane; b. *p*-$MeC_6H_4SK$ (3.0 equiv) in DMF; c. thiol (5.0 equiv), DIEA (5.0 equiv); (ii) a. CDI (2.0 equiv) in Py; b. 5-aminopentanol (5.0 equiv); (iii) $(i\text{Pr})_2$NP(Cl)$OCH_2CH_2CN$ (1.5 equiv), TEA (4.5 equiv) in $CH_2Cl_2$.

after the addition of pH 8.0 aqueous buffer. A possible explanation of such rapid conversion of **7** could be made by assuming neighboring attack of the trivalent phosphorus atom on the disulfide bond, favored by a six-membered ring intramolecular conformation (Scheme 2). This hypothesis was supported by the observation that phosphoramidites **8** and **9** were much more stable in acetonitrile at room temperature.

The destabilizing effect of the hydroxyl group located in close proximity to the disulfide bond was also observed, similar to the described effect of the neighboring amino group on the stability of the disulfide bond.[7,8] Thus, compound **4** degraded completely after 14 h incubation in 32% aq $NH_3$–EtOH (1:1) at 55 °C. In contrast, derivative **5** obtained from **4**, and nucleoside **6** did not show any sign of disulfide bond cleavage under identical conditions. The fast decomposition of **4** cannot be explained only by the electronegativity of the hydroxyl group destabilizing the dithio bond, since the presence of the carbamido group in compound **5** did not result in a similar rate of decomposition. The stability of oligonucleotides bearing dithiomethyl linkers toward ammonia was verified by the synthesis of oligonucleotide **12** using the standard conditions for DNA synthesis and deprotection.