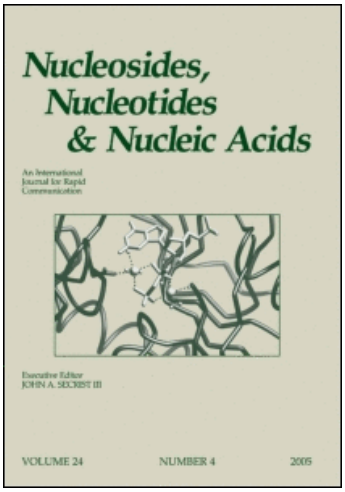

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PRO-OLIGONUCLEOTIDE SYNTHESIS USING ALLYL AND ALLYLOXYCARBONYL PROTECTIONS: DIRECT MALDI-TOF MS ANALYSIS ON SOLID SUPPORT

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ABSTRACT

The solid-support synthesis of pro-oligonucleotide heteropolymer chimeras had been performed with allyloxycarbonyl group (AOC) for the protection of nucleobases and of allyl and S-acetyl-2-thioethyl (MeSATE) for phosphate protections to respectively generate phosphodiester and MeSATE phosphotriester linkages.

To increase nuclease stability and cellular uptake of antisense, we have developed prodrugs of oligonucleotides (pro-oligonucleotides) bearing internucleosidic linkages masked with the enzyme labile protecting group S-acetyl-2-thioethyl (MeSATE) (1). Homo-dT pro-oligos are taken up more efficiently by cells (2) and are selectively hydrolyzed by cell carboxyesterases releasing inside the cell, the free oligonucleotide (3).

Because MeSATE phosphotriester linkages are base-sensitive, the solid-supported synthesis of homo-dT pro-oligos was performed on a photolabile solid support (4,5). The synthesis of pro-oligo heteropolymers requires nucleobase protecting

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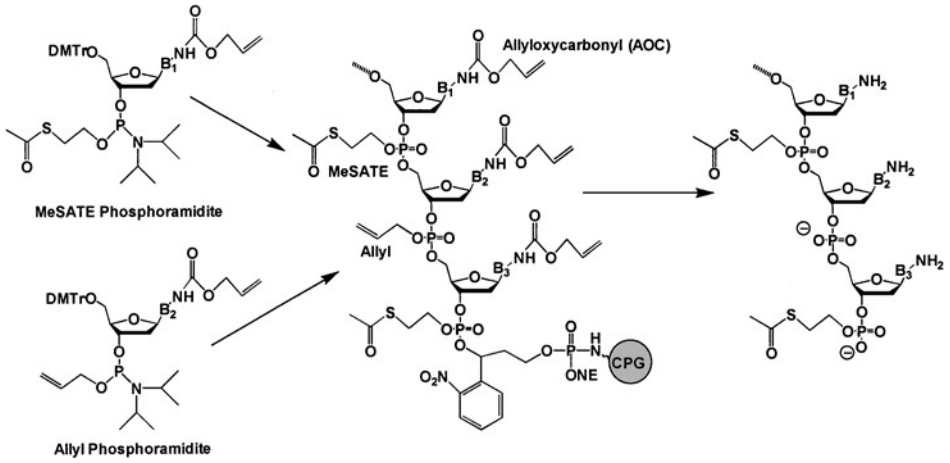

Scheme 1.

groups removable under non-basic conditions. Several protections as sulfenyl (6) and photolabile groups (7) were evaluated but sulfenyls are not efficient (6) and removal of UV-photolabile protections is somewhat sluggish (7) and would give side-reactions.

In this work, we would like to describe the use of allyloxycarbonyl group (AOC) (8) for the protection of exocyclic amines of nucleobases and of allyl (8) and MeSATE phosphate protections for the solid-support synthesis of pro-oligo heteropolymer chimeras bearing phosphodiester as well as MeSATE phosphotriester linkages.

Several conditions of AOC removal were evaluated on short pro-oligos linked to the photolabile solid support (4). Analyses of the oligonucleotides were directly performed by MALDI-TOF MS as laser UV irradiation induced breakage of the photolabile linker releasing the pro-oligo. The stability of pro-oligonucleotides was evaluated upon the palladium (II) complex conditions described by Hayakawa et al. (8), i.e. $Pd_2(dba)_3$-$CHCl_3$/PPh_3 at 55°C with a mixture of formic acid and n-butyl amine as allyl scavengers. Unfortunately, those conditions induced an important loss of MeSATE protections. The nucleophilicity of the amine could be responsible of this instability.

Use of formic acid (150 molar equivalents) without n-butylamine (9) gave better results as AOC were removed without significant MeSATE release. Dimedone (9,10) (150 molar equivalents) as allyl scavenger was as efficient as formic acid and was chosen to avoid the possible depurination of oligo heteropolymers during an acidic treatment.

During synthesis cycle, standard Ac_2O capping induced formation of acetylated byproducts. This reagent was replaced by an alternative one using diallyl N,N-diisopropyl phosphoramidite in presence of tetrazole (11). Moreover, after allyl removal, failure sequences have a 5′-phosphate moiety. Purification of the

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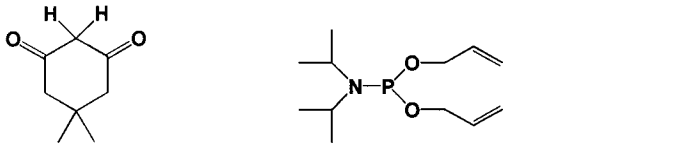

Dimedone **Diallyl N,N-diisopropyl Phosphoramidite**

Scheme 2.

desired hydrophobic pro-oligo from more hydrophilic truncated sequences was easier by reverse-phase HPLC.

The synthesis of mixed SATE-phosphotriester and phosphodiester dT homopolymers was described (12). For that purpose, phosphoramidite and H-phosphonate chemistries were respectively used to generate MeSATE phosphotriester and phosphodiester linkages. Such method had constraints due to changes of reagents (activator, oxidant, capping) during changes in chemistry.

In this work, we have used same phosphoramidite chemistry to create phosphodiester/MeSATE phosphotriester pro-oligos. Nucleoside 3′-Allyl phosphoramidites were used to yield phosphodiester linkages and Nucleoside MeSATE phosphoramidites to generate phosphotriester linkages. The synthesis of a dodecanucleotide d(ACACCCAATTCT) alternatively containing phosphodiester internucleosidic linkages (6) and MeSATE phosphotriester linkages (5) and one 3′-MeSATE phosphodiester moiety demonstrated the efficiency of this method.

During this work, MALDI-MS was used to analyze pro-oligonucleotides still anchored to a solid support through a photolabile linker. Allyloxycarbonyl (AOC) were successfully employed to protect the exocyclic amines of nucleobases during the synthesis of MeSATE pro-oligonucleotides. Their removal under Pd-catalyzed reaction with dimedone as allyl scavenger did not degrade MeSATE phosphotriester linkages.

A new capping reagent, i. e. diallyl N,N-diisopropyl phosphoramidite in presence of tetrazole avoided acetylation and allowed easy purification of the pro-oligos.

A phosphodiester/MeSATE phosphotriester pro-oligo 12 mer was obtained starting from nucleoside Allyl- and MeSATE-phosphoramidites.

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