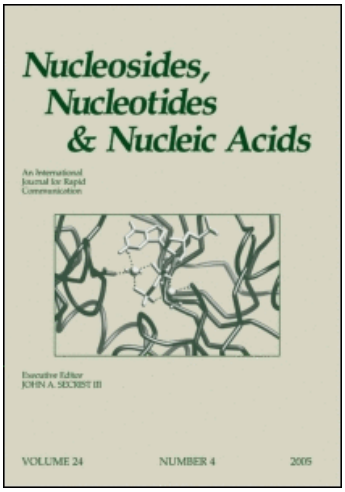

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ABSTRACT

MALDI-TOF mass spectrometry was used to analyze oligonucleotides still anchored to long-chain alkylamine controlled-pore glass (LCAA-CPG) through a photolabile linker. This technique is useful to follow supported chemical reactions in real time and monitor by-products formation.

As it has been previously described for peptides bound to resins through photolabile linkers (1,2), synthetic oligonucleotides still anchored to LCAA-CPG through an *o*-nitrophenyl-1,3-propanediol (3) moiety can be directly analyzed by MALDI-TOF mass spectrometry (Fig. 1) without cleavage of the overall material from the solid support. Upon laser irradiation, the oligonucleotides are simultaneously cleaved from the solid support and ionized.

This method releases a full-length oligonucleotide unlike MALDI-TOF MS of oligonucleotide bound to LCAA-CPG through standard succinyl linker which induces fragmentation of the oligonucleotide resulting in a series of ions (4). This process appeared to be useful for following reactions on the solid-supported oligonucleotide. We would like to report here, three examples dealing with the deprotection of internucleoside linkages of oligonucleotide analogs.

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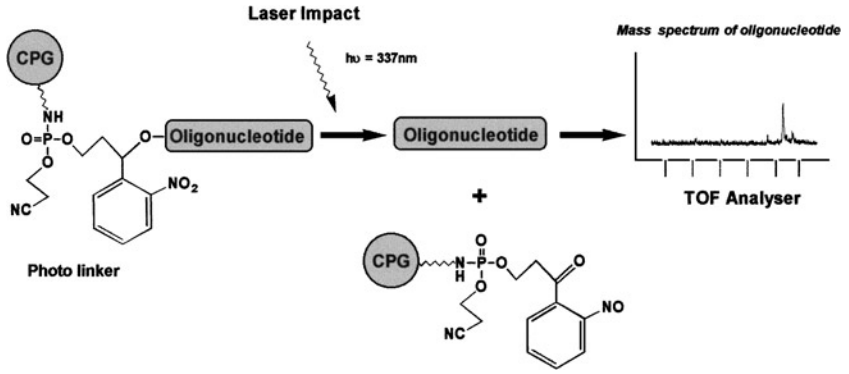
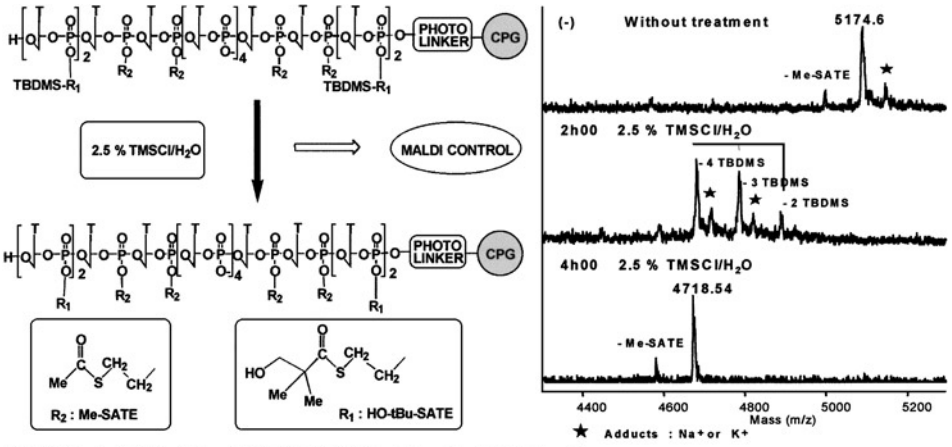


Figure 1.

Analysis of Pro-oligonucleotide

Pro-oligonucleotides were designed as a new class of antisense analogs with several internucleosidic phosphates masked by enzymolabile protecting groups (5). Reduction of negative charges and increase of lipophilicity improve the cellular uptake of these oligonucleotide prodrugs. Unfortunately this results in poor solubility of pro-oligonucleotides. To circumvent this problem, a new phosphate protecting group, i.e. HO-tBu-SATE was introduced (6). During oligonucleotide synthesis, the TBDMS group was used to protect the hydroxyl function of every HO-tBu-SATE. The aim of this study, in this first example, was to check that an acidic treatment using 2.5 % trimethylsilylchloride in water (7) was able to remove the silyl groups without affecting the Me-SATE internucleotide linkage (Fig. 2).

The mass spectrum obtained after 2 hrs of reaction displayed a ladder of peaks that differ from another by 114 Da, corresponding to a mixture of the fully



TBDMS : tert-Butyldimethylsilyl, TMSCl : trimethylsilylchloride

Figure 2.

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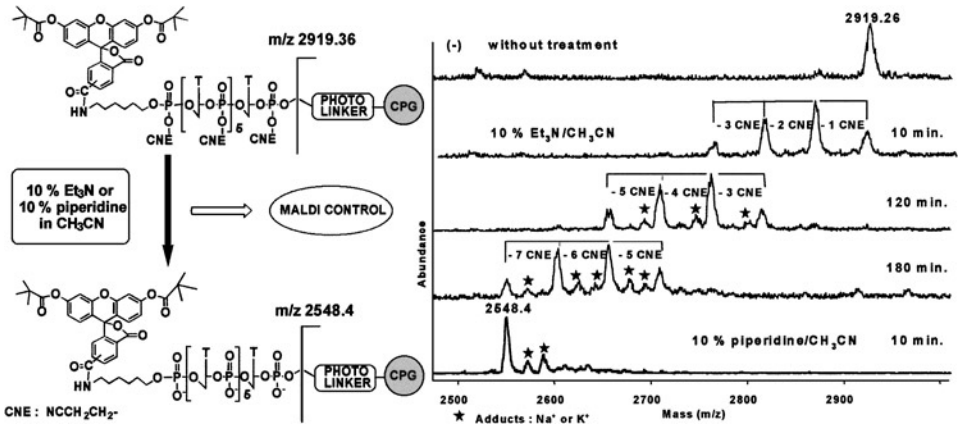

Figure 3.

TBDMS deprotected oligonucleotide and analogs bearing one and two TBDMS residues ($+C_6H_{14}Si$; +114). After 4 hrs of reaction, the spectrum showed a full desilylation. Moreover, no significant loss of Me-SATE was observed as compared to the starting material.

Two other experiments were carried out in Parallel. First, thanks to MALDI-TOF MS analysis we showed the instability of Me-SATE pro-oligonucleotides under allyloxycarbonyl deprotection conditions (8). Secondly, MALDI-TOF MS was used to follow the deprotection of β-eliminable CNE groups and the stability of pivaloyl groups of a fluorogenic oligonucleotide (9) under two different basic conditions, i.e. 10% Et_3N/CH_3CN and 10% piperidine/CH_3CN (Fig. 3).

MALDI sample preparation (4)

The supported oligonucleotides (few beads) were suspended in 10 μl of the matrix solution [45 mg, 2,4,6-trihydroxyacetophenone (THAP); 4 mg, ammonium citrate in 500 μl of acetonitrile/water (1:1, v/v)]. One μl of the beads/matrix mixture was spotted on the stainless steel probe tip and allowed to air dry. The samples were then subjected to MALDI analyses using a MALDI-TOF mass spectra *Voyager DE (Perseptive Biosystems)*, spectrometer equipped with an N_2 laser power (337nm).

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