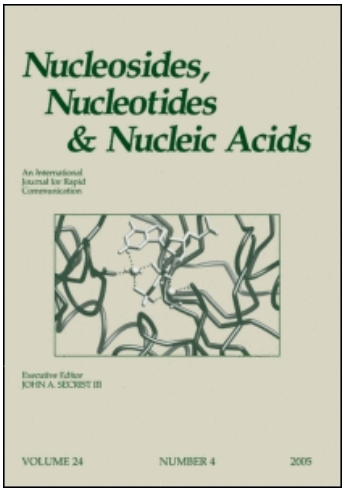

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Novel Method for the Covalent Immobilization of Oligonucleotides via Diels-Alder Bioconjugation

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ABSTRACT

The synthesis of cyclohexadiene and maleimide derivatives and their use for the functionalization of oligonucleotides and the coating of glass surfaces is reported. A method for the covalent attachment of diene or maleimide modified oligonucleotides to the coated glass surfaces via aqueous Diels-Alder reactions is presented.

Key Words: Surface immobilization; Bioconjugation; Aqueous Diels-Alder reaction.

Applications for surface immobilized nucleic acids are widespread and include nucleic acid sequencing, gene expression profiling, genotyping (e.g. single nucleotide polymorphism analysis), capture probes and affinity chromatography. The most accepted technologies in this field are based on chemically modified glass surfaces including microscope slides, the oxidized surface of silicon wafers, silica gel or controlled pore glass that allow the covalent attachment of synthetic oligonucleotides,

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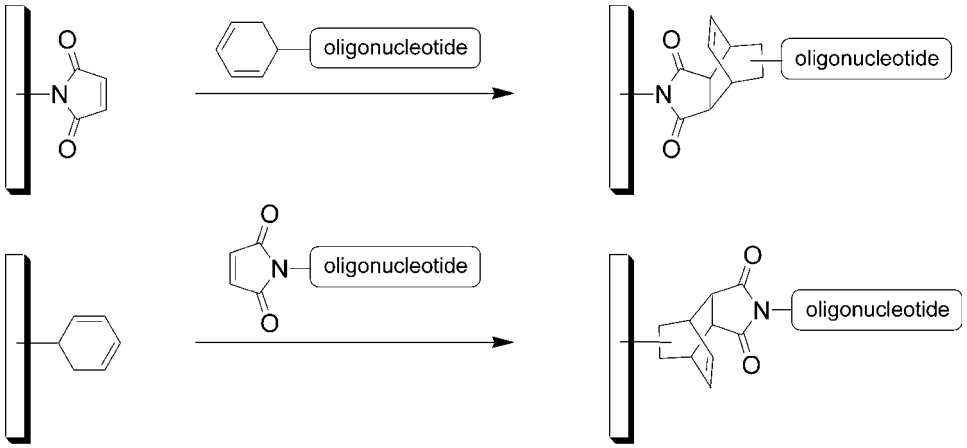

Scheme 1.

PCR products or other desired nucleic acid sequences in a controlled manner. A variety of conjugation chemistries have been applied in the attachment process and some of these approaches are employed in commercial applications including the fabrication of nucleic acid microarrays.

We have demonstrated the utility of aqueous Diels-Alder reactions for the bioconjugation of diene-modified oligonucleotide probes[1] and for the covalent attachment of diene and maleimide functionalized oligonucleotides to a variety of glass

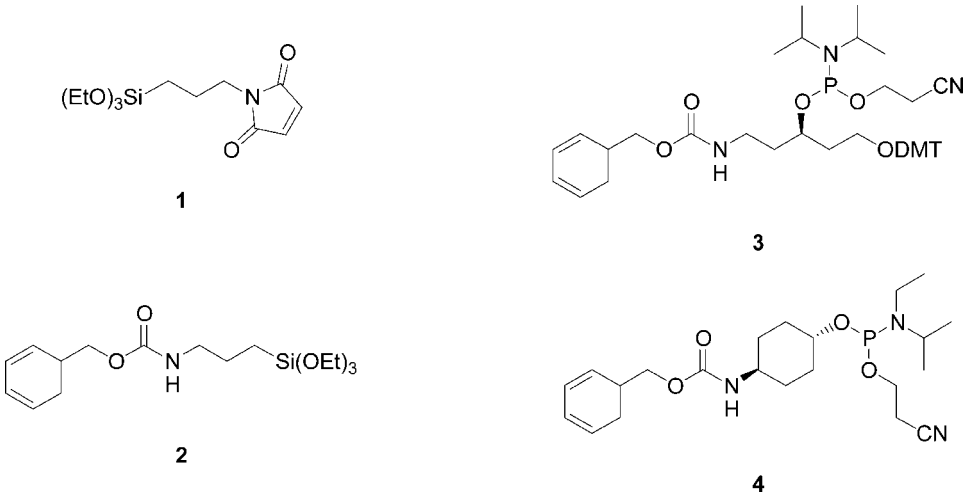

Figure 1. Reagents for the derivatization of glass surfaces (**1**, **2**) and the functionalization of oligonucleotides (**3**, **4**) through Diels-Alder reactions.

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surfaces.[2] This method is compatible with the presence of other chemical functionalities like amino groups and can be applied under particularly mild conditions, such as aqueous buffers without co-solvents, neutral pH and moderate temperature (Sch. 1).

In this communication we present the synthesis of cyclohexadiene and maleimide derivatives, their application in the functionalization of oligonucleotides and glass surfaces, and the demonstration of covalent immobilization of oligonucleotides to glass surfaces via Diels-Alder reactions (Fig. 1).

The maleimide-silane **1** and the cyclohexadiene-silane **2** were synthesized from aminopropyltriethoxysilane with maleic anhydride or with 5-hydroxymethylcyclohexadiene and carbonyldiimidazole. The surface functionalization of glass slides was conducted with a 1% solution of each silane in toluene for 16 hours at room temperature or for 5 hours at 55°C. AFM experiments indicated that maleimide coated glass slides have a comparable surface roughness then commercial SuperAmine and SuperAldehyde standard slides.

The diene-amidite **3** was prepared as described before[1] and the diene-amidite **4** was synthesized in two steps through the conjugation of 5-hydroxymethylcyclohexadiene to *trans*-4-aminocyclohexanol with carbonyldiimidazole followed by the phosphitylation of the secondary hydroxyl group. The functionalization of oligo nucleotides with dienes or dienophiles was achieved by the terminal coupling of the diene-amidites **3** or **4** to support bound oligonucleotide (2×10 min coupling time) or by the post-synthetic reaction of an 5′-aminomodified oligonucleotide with a maleimide-NHS ester. The conjugation to a glass surface was conducted with a solution of the 5′-modified oligonucleotide in 100 mM Na_2HPO_4 buffer (pH 6.5) on the coated surface at 37°C for 1 h. The immobilization of oligonucleotides was demonstrated by the hybridization of the surface bound oligonucleotide with a 5′-FAM labeled complementary sequence followed by washing of the slides and measuring of the surface bound fluorescence with a Typhoon fluorescence scanner.

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