This article was downloaded by:

On: 26 January 2011

Access details: Access Details: Free Access

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-

41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597286

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

Hallie A. Latham-Timmons^a; Andreas Wolter^a; J. Shawn Roach^a; Rubina Giare^a; Michael Leuck^{ab}
^a Proligo LLC, Boulder, Colorado, USA ^b Proligo LLC, Boulder, CO, USA

Online publication date: 09 August 2003

To cite this Article Latham-Timmons, Hallie A., Wolter, Andreas , Roach, J. Shawn , Giare, Rubina and Leuck, Michael (2003) 'Novel Method for the Covalent Immobilization of Oligonucleotides via Diels-Alder Bioconjugation', Nucleosides, Nucleotides and Nucleic Acids, 22:5, 1495-1497

To link to this Article: DOI: 10.1081/NCN-120023019 URL: http://dx.doi.org/10.1081/NCN-120023019

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 22, Nos. 5–8, pp. 1495–1497, 2003

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

Hallie A. Latham-Timmons, Andreas Wolter, J. Shawn Roach, Rubina Giare, and Michael Leuck*

Proligo LLC, Boulder, Colorado, USA

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,

1495

DOI: 10.1081/NCN-120023019 Copyright © 2003 by Marcel Dekker, Inc.

> Marcel Dekker, Inc. 270 Madison Avenue, New York, New York 10016

1525-7770 (Print); 1532-2335 (Online)

www.dekker.com

^{*}Correspondence: Michael Leuck, Proligo LLC, 6200 Lookout Road, Boulder, CO 80301, USA; Fax: +1 303 516 0975; E-mail: mleuck@proligo.com.

1496 Latham-Timmons et al.

Reaction conditions: 100 mM Na₂HPO₄, pH 6.5, 37°C, 1 h

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.

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 Na₂HPO₄ 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.

REFERENCES

- Hill, K.W.; Taunton-Rigby, J.; Carter, J.D.; Kropp, E.; Vagle, K.; Pieken, W.; McGee, D.P.C.; Husar, G.M.; Leuck, M.; Anziano, D.J.; Sebesta, D.P. Diels-Alder bioconjugation of diene-modified oligonucleotides. J. Org. Chem. 2001, 66, 5352–5358.
- 2. Pieken, W.; Wolter, A.; Sebesta, D.P.; Leuck, M.; Latham-Timmons, H.A.; Pilon, J.; Husar, G.M. Method for immobilizing oligonucleotides employing the cycloaddition bioconjugation method. PCT Int. Appl. WO 01/84234 A1, November 8, 2001.