Electrochemistry Communications 10 (2008) 973–976

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

The use of a detectable, mass-spectrometry-cleavable linker for quality control on an addressable microelectrode array

Ceng Chen [a], Peng Lu [a], Amy Walker [a], Karl Maurer [b], Kevin D. Moeller [a,*]

[a] Department of Chemistry, Washington University, St. Louis, MO 63130, United States
[b] CombiMatrix Corporation, 6500 Harbor Heights Parkway, Suite 303, Mukilteo, Washington 98275, United States

ARTICLE INFO

Article history:
Received 12 March 2008
Received in revised form 17 April 2008
Accepted 21 April 2008
Available online 26 April 2008

Keywords:
Microelectrode array
Fluorescence
Cleavable-linkers

ABSTRACT

The synthesis, site-selective placement, and TOF-SIMS cleavage properties of a new, fluorescent linker for attaching molecules to a microelectrode array are reported. The linker was developed to provide a handle for quality control assessment of the microelectrode arrays being used to probe the binding of molecular libraries with biological receptors.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Microelectrode arrays have the potential to serve as outstanding tools for analyzing the binding of molecular libraries to biological receptors [1–3]. However, to take advantage of this potential we must have the ability to synthesize the individual members of a molecular library by unique, individually addressable microelectrodes in the array. For this reason, we have been developing the tools for both the site-selective synthesis [3–7] and analysis [8] of molecules on microelectrode arrays. While these efforts have been proceeding nicely, several items concerning the use of the microelectrode arrays for quantitative studies have become worrisome.

First, protocols for determining if the microelectrodes in an array are working simply check to see if the electrodes are "active". But what does "active" mean? Are all the microelectrodes in an array equally active? This is a major issue since site-selective reactions on a microelectrode array are accomplished by using the microelectrodes themselves to place substrates onto the surface of the array. Second, molecules are attached to the microelectrode array by first coating the array with a porous, functionalized polymer and then binding the molecules in the library to the polymer [3–8]. How do we know that the polymer coating is of a uniform character for each microelectrode in the array? Both of these issues are critical because they influence the amount of material located by the microelectrodes. How does one use a microelectrode array to measure the relative binding of molecules in a library to a targeted biological receptor if the quantity of material placed on each microelectrode in the array is potentially different and unknown? Finally, we have found that when there is a short circuit on a microelectrode array chip, whole regions of the array become active. The microelectrodes in the affected region cannot be turned off, and the site-selective construction of a molecular library on the array becomes impossible. Simply conducting tests to see if the microelectrodes in the array are active does not identify this problem.

One method for addressing these issues would be to connect the molecular library to the polymer that coats the microelectrode array with a fluorescent linker. The linker could then be used to quantify the amount of material placed above each microelectrode. If the linker was placed onto the array in patterns (for example, two complementary checkerboard patterns to cover the whole microelectrode array), then after the first step the use of a fluorescent linker would allow us to determine if a short circuit existed anywhere on the array.

Any fluorescent linker used for this purpose would also need to be compatible with the use of TOF-SIMS techniques for characterizing the surface-bound molecules in the library being studied [8]. This requires that the linker undergo fragmentation under mass spectrometry conditions faster than does the polymer that coats the surface of the array. Hence, what is needed is a fluorescent, mass-spectrometry-cleavable linker.

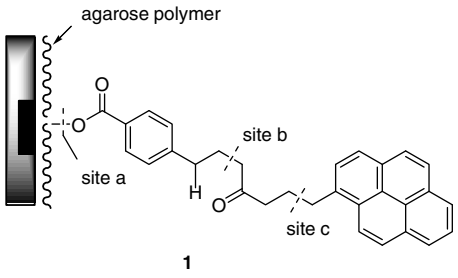
2. Results and discussion

A suggestion for such a linker arose during initial studies to probe the compatibility of a styrene-based, mass-spectrometry-cleavable

* Corresponding author. Tel.: +1 314 935 4270; fax: +1 314 935 4481.
E-mail address: moeller@wustl.edu (K.D. Moeller).

1388-2481/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.elecom.2008.04.029

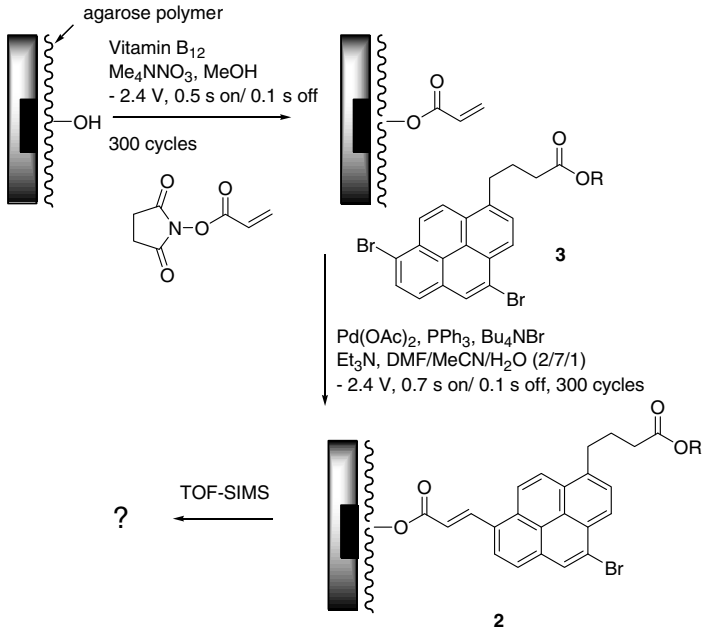
linker with the electrogenerated base-catalyzed esterification reaction typically used to place molecules onto the surface of an array [3–5,8]. The styrene linker was attached to a pyrene moiety so that its stability could be monitored (Scheme 1). When TOF-SIMS experiments were conducted on **1**, negative ions were produced that were consistent with both the expected cleavage sites (a and b) and a new cleavage site (c) that was consistent with a McLafferty fragmentation originating from the pyrene. This observation indicated that a pyrene group might provide a starting point for designing a fluorescent, mass-spectrometry-cleavable linker. With this in mind, linker **2** was designed (Scheme 2). An ester was placed at the end of the linker as a future attachment site for the molecular libraries to be analyzed on the microelectrode arrays. The plan called for attaching the linker onto the array by means of an electrochemically driven Heck reaction [4,9]. For this reaction, the polymer that coats the array would first be functionalized with acrylic acid using earlier activated ester protocols.



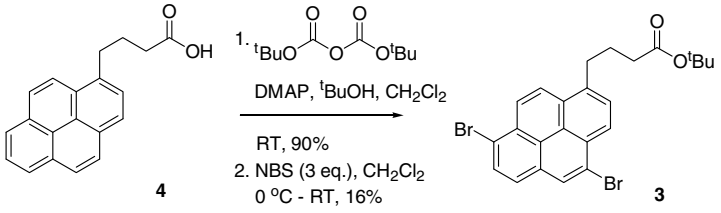
Scheme 1.

The synthesis of substrate **3** is outlined in Scheme 3. The synthesis was straightforward except for the bromination step. In this step, monobromination could not be accomplished. Instead, the reaction led to a complex mixture. When three equivalents of *N*-bromosuccinimide were used, the major product isolable from this mixture was **3**. Although the yield for the reaction was very low, it could be run on a scale large enough to provide ample amounts of the desired linker. A *t*-butyl ester group was used at the end of the linker so that molecules could be added to the linker at a later stage (either prior to or after placement on the surface of a microelectrode array [7]).

With the brominated pyrene in hand, the two-step procedure outlined in Scheme 2 for placing the linker onto an agarose-coated microelectrode array chip was performed. In the first step, the chip was placed into a 0.5 M tetramethylammonium nitrate in methanol electrolyte solution containing vitamin B_{12}. To this mixture was added the *N*-hydroxysuccinimide ester of acrylic acid. All of the microelectrodes in the array were then used as cathodes (−2.4 V relative to a Pt wire counter electrode for 0.5 s followed by 0.1 s off for 300 cycles) in order to reduce the vitamin B_{12}, generate a base, and trigger an esterification reaction between the activated ester in solution and the agarose polymer coating the electrodes. The second step was then used to place the linker onto



Scheme 2.



Scheme 3.

Download English Version:

https://daneshyari.com/en/article/181227

Download Persian Version:

https://daneshyari.com/article/181227

Daneshyari.com