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Building Addressable Libraries: The Use of "Safety-Catch" Linkers on Microelectrode Arrays

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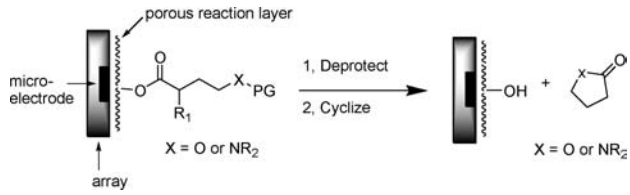
Abstract: A "safety-catch" linker strategy has been used to site-selectively cleave and characterize molecules from a microelectrode array. The linkers are attached to the array by means of an ester and contain either a protected amine or protected alcohol nucleophile that can be released using acid generated at the microelectrodes.

Microelectrode arrays have great potential as a platform for building "addressable" molecular libraries.[1−3] This potential is derived from the ability to run cyclic voltammetry experiments at each individual microelectrode in the array. Hence, if molecular libraries are built or placed on the array such that each individual member of the library is by a unique, addressable microelectrode, then the electrodes can be used to monitor the behavior of the molecules. To this end, site-selective reactions using a variety of mediators to place or build molecules by individual microelectrodes have been explored,[4−10] a stable porous reaction layer for attaching molecules to the surface of the arrays has been developed,[11] and proof-of-principle experiments demonstrating the capability of the arrays for monitoring small-molecule−receptor binding in real time have been completed.[12] However, one of the main barriers to utilizing the data from any small-molecule library is quality-control. Is a molecule that gives rise to a signal in a library really the molecule that one thinks it is? To really understand the data obtained from a small molecule library, one must have the ability to fully characterize the molecules in the library. For a small-molecule library on a microelectrode array, this means characterizing the molecules that are located next to each of the microelectrodes used in an analysis. To date, we have shown that the use of a mass-spectrometry cleavable linker allows TOF-SIMS experiments to be used for this purpose.[13] Yet while this work has been effective, it is limited in that it "sacrifices" the array and does not provide us with a handle to examine the chemo-, regio-, or stereoselectivity of reactions run on the arrays. Since we hope to use the arrays to probe the three-dimensional binding preferences of biological receptors, the inability to determine the stereochemistry of molecules on the arrays is particularly bothersome. Hence, an alternative approach that both allows us to examine the molecules on the array, while retaining the array for further biological studies, and allows us to more fully characterize molecules on the array is of utmost importance.

One approach to this problem would be to develop linkers that allow us to site-selectively recover molecules located on the surface of any microelectrode in an array. To accomplish this requires a linker between a molecule and the surface of the electrode that can be cleaved using the microelectrode itself. Such a linker would require two main features: (1) it needs to be stable to the chemistry used to build molecules libraries but readily cleavable under mild conditions when needed, and (2) since each microelectrode in the array has only 20−50 fmole of material on the polymer coating its surface, it is best if the linker contains a label to aid in detection of the product by HPLC. The structures of the molecules can be characterized by independent synthesis. Because reactions run on microelectrode arrays can be run at larger scales in solution using identical conditions, characterization can be done for a larger-scale reaction and then HPLC used to make sure the products on the array are the same as the products from the solution-phase synthesis.

Scheme 1. "Safety-Catch" Linker Strategy



With these things in mind, it appeared that a "safety-catch" linker strategy for attaching molecules to the surface of a microelectrode array might be ideal.[14] In this strategy, molecules are attached to the surface through either an ester or an amide linkage. The linker contains a protected alcohol or amine that when released can cyclize onto the ester or amide to form a lactone or lactam, thereby releasing the molecule from the surface. We report herein, the success of this strategy for site-selectively removing and characterizing molecules from the surface of a microelectrode array.

For an initial trial, the linker was attached to the surface of the array using the ester chemistry employed on numerous occasions in the past (Scheme 1).[5−10,12] The plan is to functionalize this ester with a substituent (R_1) on the α-carbon of the ester that will provide a handle for attaching fluorescence active groups, small molecules of interest for binding studies, or both. A protected amine or alcohol would be located in the γ-position to provide the masked nucleophile for removing the linker and associated molecule from the array. The use of a protected amine in this position would be particularly attractive because it would provide a second site (R_2) for locating a biological substrate or fluorescence label.

With a potential strategy in place, we set out to determine if the cleavage reaction could be accomplished in a site-selective fashion. Linker **1**[15] (Scheme 2) was selected as an initial substrate. Pyrene was used as the fluorescent group for monitoring the reactions on the array, and a *t*-Boc protected amine was used as the masked nucleophile.[16] For future efforts, such a linker can be converted into one containing a biological substrate by either using a disubstituted pyrene[13b] or the nitrogen as a site for attaching the ligand of interest.

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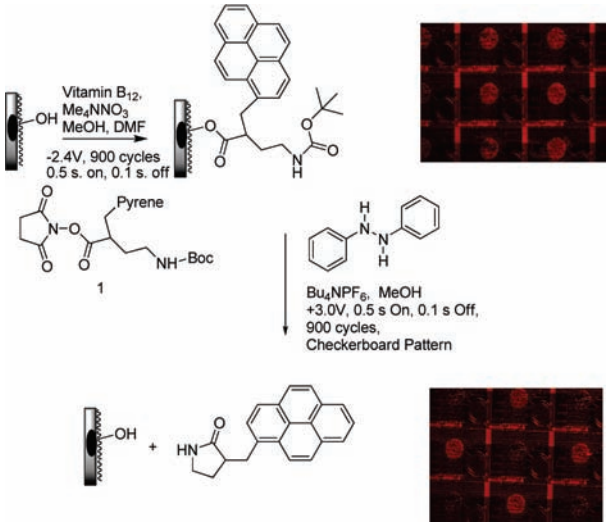
Scheme 2. Initial Trial



The experiment was begun by placing the activated ester[15] proximal to every microelectrode in an agarose-coated 1k-array (1024 microelectrodes/cm^2). This was accomplished using the previously developed electrogenerated base procedure. The cleavage reaction was then performed by site-selectively generating acid on the array.[8,16] To this end, the array was submerged along with a remote Pt-wire into 1.5 mL of a methanol solution containing both 1,2-diphenylhydrazine and tetrabutylhexafluorophosphate electrolyte. Microelectrodes in a checkerboard pattern were then used as anodes to oxidize the diphenylhydrazine to form diazobenzene and 2 equiv of acid by applying a potential of +3.0 V to the microelectrode relative to the remote Pt-wire in solution. The selected microelectrodes were turned on for a period of 0.5 s and off for a period of 0.1 s for a total of 900 cycles. In this case, the success of the reaction was determined by a loss of fluorescence by the selected microelectrodes. As can be seen in the image, a small amount of fluorescence did remain by the selected electrode, but most of the substrate on the surface of the electrode was removed.

Confinement of the acid to the selected microelectrodes was accomplished by using excess 1,2-diphenylhydrazine in the reaction. The excess hydrazine served as a base to neutralize any acid escaping from the region of the array surrounding the selected microelectrodes.

To prove that the molecule being released from the array was the expected lactam derivative, the lactam was independently synthesized by simply mimicking the chemistry on the array in solution. This was done by treating the methyl ester derivative of **1** with trifluoroacetic acid to remove the Boc-group and then triethylamine to trigger the cyclization.[15] HPLC analysis was then used to compare the independently synthesized lactam with the material being removed from the array (Figure 1). Four HPLC chromatograms are shown. In Figure 1a, the retention time for the independently synthesized lactam is shown. Figure 1b shows the HPLC trace obtained for reaction solution prior to the electrochemical cleavage reaction. The two peaks observed arise from the electrolyte used for the reaction (retention time =7.05 min) and the excess 1,2-diphenylhydrazine (retention time = 29.5 min) used as the confining agent for the electrolysis. Figure 1c shows the HPLC trace obtained for the reaction solution following the electrolysis reaction. Figure 1d shows the HPLC trace obtained for coinjection of the solution used for Figure 1c and the independently synthesized lactam. Clearly, the peak with a retention time of 20.6 min in Figure 1c is the lactam indicating that the electrochemical cleavage reaction on the array did give rise to the expected lactam. The peak at 10.7 min in Figure 1c is thought to be a side product derived from hydrolysis of the agarose surface coating the array.

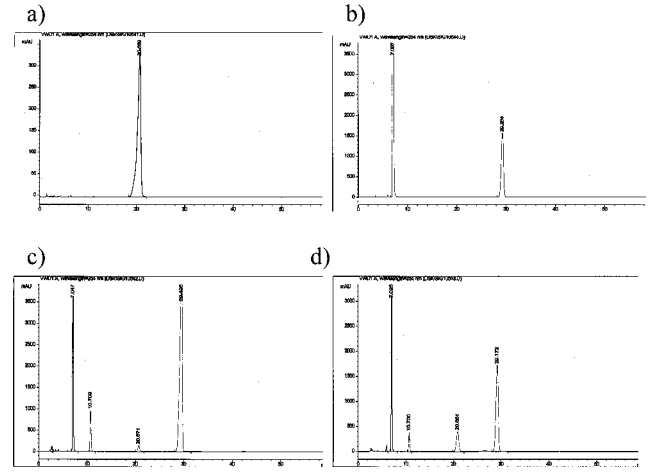
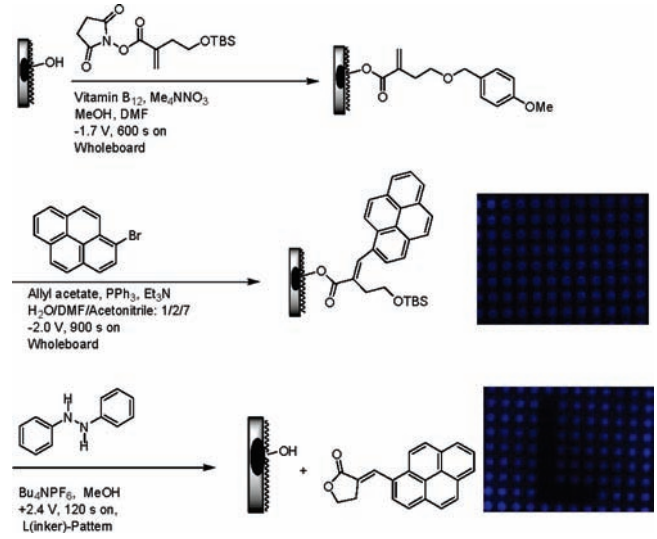


Figure 1. HPLC test results. The chromatograms were obtained using a Discovery HS C18, 5 μm; 25 cm $\times$4.6 mm column with a flow rate of 1 mL/min; 70% methanol:30% water was used as eluant with a 10.0 μL injection. A UV detector was used at 254 nm. (a) Independently synthesized lactam; (b) reaction solution containing 1,2-diphenylhydrazine and tetrabutylammonium hexafluorophosphate prior to electrolysis; (c) reaction solution following the electrolysis; (d) co-injection of independently synthesized lactam and the electrolysis product solution.

Scheme 3. Lactone-Based System



With the success of the nitrogen-nucleophile based system, attention was turned toward examining the use of an oxygen nucleophile based system and establishing the generality of the method, both in terms of the nucleophile and the type of microelectrode array used. In this case, we wanted to put a precursor for the linker onto the array and then demonstrate that the linker was compatible with conducting reactions by the electrodes prior to releasing the molecule from the surface. For this reason, the pyrene group was added to the linker following its placement on the array. The plan started by placing 4-*tert*- butyldimethylsiloxy-2-methylidene butanoic acid (**2**) by each of the microelectrodes in an agarose coated 12k (12,544 microelectrodes/cm^2) array (Scheme 3). This was accomplished by again capitalizing on a base-catalyzed coupling reaction between an activated ester and the agarose surface coating the array. As in the earlier experiments, the potential was

held constant relative to the counter electrode. Each enoate on the array was then used as a substrate for a Heck reaction with bromopyrene. This placed a fluorescent group by each of the microelectrodes in the array. The strategy was intriguing because the same strategy could be used for adding a variety of different fluorescent groups and/or biological probes to the linker. Using this chemistry, the nature of the linker on the array can be varied without building each linker independently and then transferring them one at a time to the array. Instead, they can be built directly on the array.

Cleavage of the linker from the array was accomplished using reaction conditions that were identical to those employed in the lactam case. In this case, an L-pattern (L for linker) of electrodes was used to cleave the linker from the surface. The success of the reaction was monitored using a fluorescence microscope and can clearly be seen in the image provided.

As in the lactam case, the reaction was checked to make sure it led to the desired product by independently synthesizing the lactone and then using the lactone to identify the product in the crude reaction mixture by HPLC.[15]

In conclusion, a "safety-catch" linker strategy has been used to site-selectively cleave molecules from preselected, individual microelectrodes in a microelectrode array. Both amine and alcohol nucleophile strategies work well. The chemistry is compatible with arrays having either 1024 or 12,544 microelectrodes/cm^2. The use of the "safety-catch" linkers will allow for characterization of the molecules built by the electrodes in the array, and in this way opens door for doing quality-control analysis of the molecules in addressable molecular libraries.

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Supporting Information Available: Sample experimental procedure for the site-selective reactions along with HPLC information and characterization data for all new compounds This material is available free of charge via the Internet at http://pubs.acs.org.

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