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Building Addressable Libraries: The Use of a Mass Spectrometry Cleavable Linker for Monitoring Reactions on a Microelectrode Array

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Chip-based molecular arrays are important tools for probing interactions between libraries of potential ligands and biological receptors.[1,2] Arrays where the members of the library can be individually addressed are particularly intriguing. For example, building a molecular library on an addressable array of microelectrodes (Figure 1a)[3,4] would allow for the "real-time" monitoring of members in the library using the electrodes.[5a] But how does one build a molecular library so that its individual members all reside proximal to a unique electrode? In order to answer this question, we have been developing the synthetic methodology needed for site-selectively synthesizing molecules proximal to individual microelectrodes in an addressable array.[5,6] Recently, we reported the utility of Pd(II)-initiated reactions for these transformations. As in all microelectrode array reactions performed to date, the array chip was first coated with a polyhydroxylated polymer and then an initial substrate bound to the polymer next to each of the microelectrodes in the array. For the Pd(II) reactions, selected electrodes were then used as anodes to convert a Pd(0) substrate into the desired Pd(II) reagent. Ethyl vinyl ether was added to the solution above the array as a confining agent in order to consume any Pd(II) reagent that diffused away from the selected electrodes. Wacker oxidations (Figure 1b),[5b] alcohol oxidations,[5c] and Pd(II)-catalyzed coumarin syntheses[5a] were all accomplished in this manner. In each case, the success of the confinement strategy was determined using fluorescence techniques.

Yet while the fluorescence techniques used were outstanding for determining that no reaction occurred at unused electrodes, they provided little information as to the extent of reaction that occurred above electrodes that were selected. With this in mind, we turned our attention toward the use of time-of-flight secondary ion mass spectrometry (TOF SIMS) techniques for analyzing the microelectrode array-reactions.[7] In our experiments, a Bi^+ beam is used to ionize molecules on the surface. The ions are then collected and analyzed by mass spectrometry to gather information as to the nature of the molecules on the surface. The lateral resolution of TOF SIMS is approximately 200 nm. Since each microelectrode in the array illustrated in Figure 1 has a diameter of 95 micrometers, TOF SIMS can in principle can determine the structure of the molecules above an individual microelectrode in the array. We report here that with an appropriate linker, reactions conducted using the microelectrodes of an addressable array can be directly monitored with a TOF SIMS experiment.

Initial attempts to use TOF SIMS to directly observe molecules on an array were not successful. The polyhydroxylated polymer used for the experiments was agarose, and all that could be consistently observed in the mass spectrum was fragmentation products from the agarose. Previous application of the TOF SIMS technique to the analysis of molecular arrays solved problems of this nature by cleaving the molecules from the solid support using a preliminary chemical reaction.[7b−d] While this approach worked well, it was complicated by the need to keep the molecules cleaved from the surface of the array close to their initial location. Migration of the molecules meant a loss in the spatial resolution of the array. An alternative strategy would be to use a linker for attaching molecules to the polymer coating the array that would fragment faster than the polymer under the TOF SIMS reaction conditions. Our initial plan called for building a linker that would rapidly undergo a McLafferty fragmentation during the TOF SIMS experiment (Scheme 1).[8]

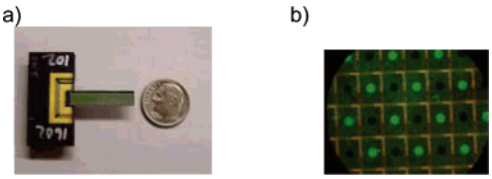
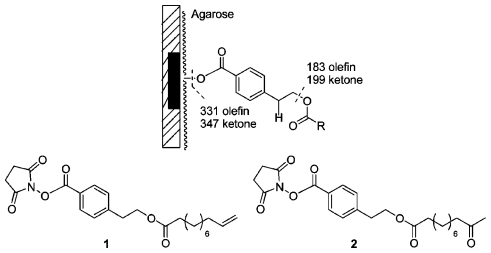


Figure 1. (a) A "1K-chip" having 1024 addressable electrodes in a 1 cm^2 area; (b) a chip utilizing the initially developed Wacker oxidation conditions at every other electrode. The ketone product was imaged by conversion into a 2,4-DNP derivative followed by incubation with a fluorescently labeled anti-2,4-DNP antibody.[5b]

Scheme 1



To this end, 4-(2′-hydroxyethyl)benzoic acid was converted into its *N*-hydroxysuccinimide ester, and then the alcohol coupled to both the olefin substrate (**1**) and the ketone product (**2**) of a Wacker oxidation. The *N*-hydroxysuccinimide ester was then used to place the linker and substrate on the chip using the strategy employed in the earlier studies.[3,4] The use of the aromatic linker immediately proved effective. For an array functionalized with substrate **1**, the TOF SIMS experiment showed a distinct parent peak for the olefin at $m/z = 183$. In addition, the presence of the phenyl ring caused the entire linker + substrate molecule to be lost from the agarose, giving rise to a parent peak at $m/z = 331$. This second peak proved particularly useful since the 183 peak fell in a spot consistent with one of the fragments from agarose. In a similar fashion, the TOF SIMS experiment arising from the placement of **2** on the array gave rise to peaks at $m/z = 199$ and $m/z = 347$. Once again, the $m/z = 347$ peak proved most useful because of its location relative to agarose fragmentation peaks.

At this point, known ratios of the substrate and product were placed onto the polymer coating the microelectrode array and TOF SIMS experiments conducted in order to generate a calibration curve

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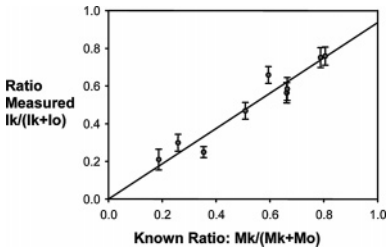

Figure 2. Calibration curve for the Wacker oxidation (Ik = intensity of ketone, Io = intensity of olefin, Mk = molar concentration of ketone, Mo = molar concentration of olefin).

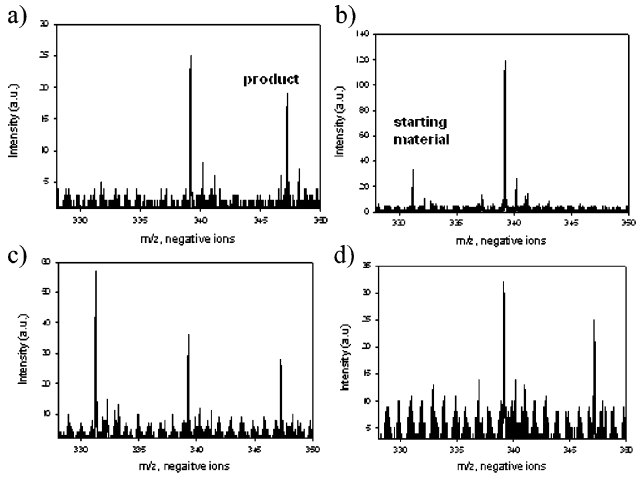

Figure 3. High-resolution negative ion TOF SIMS mass spectra of Wacker oxidation with (a) 0.32 mg $Pd(OAc)_2$ and no confining agent;[8] (b) 0.32 mg $Pd(OAc)_2$ and 50 μL confining agent; (c) 1 mg $Pd(OAc)_2$ and 0.5 μL confining agent; (d) 3.2 mg $Pd(OAc)_2$ and 0.5 μL confining agent.

for determining the product/starting material ratio over an electrode (Figure 2). The data obtained showed that TOF SIMS experiments could be used to provide a good, qualitative assessment of the percent conversion for a reaction that takes place over a microelectrode in an addressable array.

With a calibration curve in place, we were ready to begin exploring the quality of a microelectrode array-based Wacker oxidation. The reaction was run in a fashion identical to that used in the previously described Pd(II) mediated reactions.[5b] For the initial experiment, 0.32 mg of $Pd(OAc)_2$ (in 1.5 mL of a 0.5 M tetraethylammonium *p*-toluenesulfonate in 7:1 acetonitrile to water electrolyte solution)[8] was used along with no confining agent. Following the reaction, the microelectrode array was washed, dried, and then a TOF SIMS experiment was used to show that the starting material (*m*/*z* 331) had been totally consumed and the expected product (*m*/*z* 347) formed (Figure 3a, the peak at *m*/*z* = 339 is from the agarose polymer).

A second experiment (Figure 3b) was conducted in the same manner[5b] except in this case 50 μL of the ethyl vinyl ether confining agent was added. The TOF SIMS experiment showed no signal (*m*/*z* 347) for product formation. This was very surprising since the conditions used were identical to those employed for the earlier Wacker oxidations. Apparently, the previous reactions only proceeded to a slight extent. The fluorescence images taken showed a positive reaction on the electrodes used for Pd(II) generation owing to the very high sensitivity of fluorescent imaging for the product generated. The extremely high level of confinement observed[3b] was a result of the confining agent not only interfering with migration of the Pd(II) reagent to neighboring electrodes, but also with the reaction on the selected electrodes. Optimization of the process led to the most ideal fluorescent image possible, but not the most efficient reaction possible!

Fortunately, the development of a mass spectrometry cleavable linker and the use of the TOF SIMS approach allows for a change in this strategy. The reactions can be optimized by making changes in the amount of palladium used in the reactions, the amount and reactivity of the confining agent employed, the length of time the positive potential is applied in each cycle, and the number of cycles used. For example, an increase in the amount of $Pd(OAc)_2$ to 1 mg and a decrease in the ethyl vinyl ether to 0.5 μL led to an increase in the percent conversion for the microarray reaction to approximately 50% (Figure 3c). Increasing the amount of $Pd(OAc)_2$ even further to 3.2 mg led to complete conversion of the olefin (loss of *m*/*z* 331) to the ketone product (*m*/*z* 347) (Figure 3d). While product formation was complete using these conditions, confinement of the reaction across the chip was not maintained and further optimization is needed.

At this point, it is clear that a combination of fluorescence and TOF SIMS techniques is needed to truly optimize reactions that take place on a microelectrode array. In this way, both the confinement of reagents to selected electrodes on the addressable array as well as the percent conversion of the reaction that takes place on the electrodes selected can be monitored. Key to this approach is the use of a mass spectrometry cleavable linker for attaching the reaction substrate to the polymer coating the surface of the microelectrode array. This strategy should be amenable to the use of a variety of cleavable linkers and the analysis of molecules on a variety of different surfaces. Work to explore the scope of mass spectrometry cleavable linkers that can be used on a microelectrode array is underway.

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Supporting Information Available: The procedure for synthesizing the linker, procedures for conducting the microelectrode array reactions, and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) For a synthesis of the linker and experimental details for the electrolyses reactions please see the Supporting Information.

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