J|A|C|S
COMMUNICATIONS

Building Addressable Libraries: Spatially Isolated, Chip-Based Reductive Amination Reactions

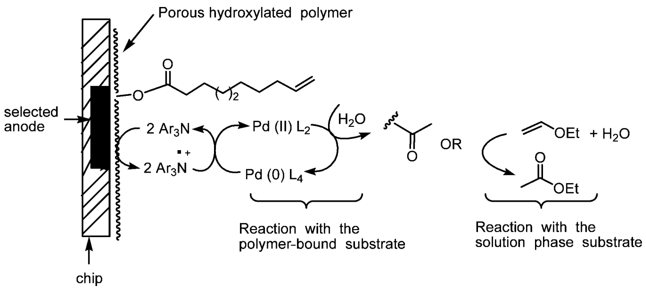
Eden Tesfu,[†] Karl Maurer,[‡] and Kevin D. Moeller*,[†]

Department of Chemistry, Washington University, St. Louis, Missouri 63130, and CombiMatrix Corporation, 6500 Harbor Heights Parkway, Suite 301, Mukilteo, Washington 98275

Received October 20, 2005; E-mail: moeller@wuchem.wustl.edu

We have been working to develop the synthetic chemistry needed for building molecules at specific locations on active semiconductor chips that incorporate arrays of individually addressable microelectrodes.[1,2] The goal of this work is to build molecular libraries so that each unique set of molecules in the library is located next to either a unique electrode or set of electrodes. The electrodes can then be used to monitor the molecules' behavior.[3] The overall synthetic strategy taken is to coat the chip with a permeable polymer and then use the electrodes to first attach a substrate to the polymer and then carry out subsequent transformations on the substrate. Both steps are accomplished by using the electrodes to generate the chemical reagents needed to mediate the desired transformation. The chemistry to be performed is confined to a region surrounding the electrodes by adding a substrate to the solution above the chip that consumes the reagent generated. Recent efforts have shown that this strategy can be used to effect both Pd(II)-mediated Wacker oxidations[4] and Pd(0)-catalyzed Heck reactions[5] at preselected sites on the chips. Having established the potential for conducting transition metal chemistry on the chips, we began to wonder about the generality of the processes being developed. For example, consider the chemistry used for spatially isolating a Wacker oxidation on the chips (Scheme 1). For these transformations, the required Pd(II) reagent was generated by oxidizing a Pd(0) substrate at the selected electrodes and confined with the use of ethyl vinyl ether in the reaction medium. Any Pd(II) reagent generated that was not consumed by an olefin substrate attached to the polymer proximal to the selected electrodes was scavenged by a solution phase Wacker oxidation involving the ethyl vinyl ether. The reaction conditions were designed to ensure that solution phase oxidation of the ethyl vinyl ether was relatively fast compared to that of polymer-bound substrate in the Wacker oxidation. The question remained, could such a strategy be applied to other Pd(II)-mediated processes? To begin addressing this question, the utility of Pd(II) for effecting spatially isolated alcohol oxidations on the chips was examined. This reaction was selected because Pd(II) is known to serve as an effective reagent for the oxidation of alcohols[6] and because the success of such a process would provide a basis for developing a variety of spatially isolated, multistep synthetic strategies on the chips. Of particular interest was a strategy for conducting reductive aminations on the chips. In addition to providing a new synthetic method for building target structures on the chips,[7] the availability of a site-selective reductive amination would afford a new method for attaching both substrates and intact biomolecules next to the electrodes on the chips.[8] We report here that ethyl vinyl ether does serve as a effective confining agent for performing site-selective alcohol oxidations and reductive amination strategies on a chip.

Scheme 1



To test the utility of ethyl vinyl ether as a confining agent for site-selective alcohol oxidations, an experiment examining the utility of the method for placing fluorescent amines on the chip's surface was selected. In this experiment, selected electrodes would be used to generate a Pd(II) reagent that would oxidize the alcohols on the surrounding polyhydroxylated polymer. The newly generated carbonyls would then be utilized to effect a reductive amination reaction on the chips using either sulforhodamine 101 hydrazide (a red fluorophore) or 8-aminopyrene-1,3,6-trisulfate (a green fluorophore). The success of ethyl vinyl ether as a confining agent for the reaction would then be examined with the use of a fluorescence microscope.

The experiment was conducted, as outlined in Scheme 2, using a chip containing 1024 platinum electrodes in a 1 cm^2 area. The initial oxidation was performed using conditions nearly identical to those used in connection with the previous Wacker oxidation. To this end, the chip was coated with a porous hydroxylated polymer and then submerged into 2.5 mL of a 0.5 M tetraethylammonium tosylate in acetonitrile/water (7:1) electrolyte solution containing 32 μg of palladium acetate, 1.39 mg of tris-4-bromophenylamine, and 500 μL of ethyl vinyl ether.[9] The counter electrode (cathode) for the reaction was a platinum wire that was also inserted into the reaction solution. Oxidation of the Pd(0) substrate (formed by premixing the Pd(OAc)$_2$ and ethyl vinyl ether) was performed by cycling selected electrodes in a checkerboard pattern between a potential of +2.4 V for 0.5 s and 0 V for 0.5 s. The reaction was continued for 1000 cycles. The chip was then removed from the solution, washed with water and acetonitrile, and then inserted into a methanol solution containing the Texas red hydrazine (red) and sodium cyanoborohydride for a period of 12 h.

After completion of this first step, the chip was imaged using a fluorescence microscope. The image obtained is shown in Figure 2. Clearly, the ethyl vinyl ether worked well to confine the alcohol

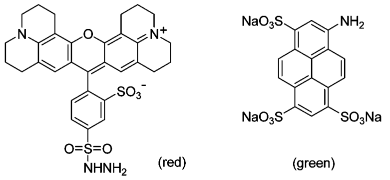
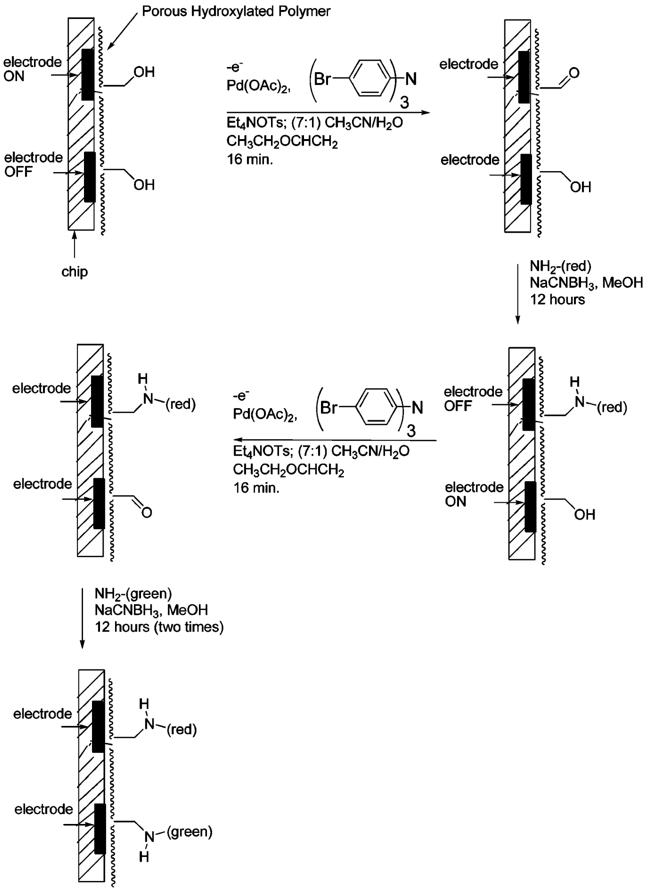


Figure 1. Fluorophore-labeled amines for use in chip-based reductive amination reactions.

[†] Washington University.
[‡] CombiMatrix Corporation.

10.1021/ja057155x CCC: $33.50

Scheme 2



oxidation to the desired electrodes. As can be seen in the image, no apparent "leakage" of the Pd(II) away from the selected electrodes occurred. This was especially striking since, unlike previous reactions that utilized deposited substrates proximal to the electrodes, the alcohol oxidation utilized the polymer coating the entire chip as a substrate. Hence, oxidation between the electrodes was possible. However, the confining agent appeared to be efficient enough to stop even this level of migration.

Having established the potential for the reductive amination using a very reactive hydrazine substrate, attention was turned toward a reaction using the less reactive aryl amine substrate (green).[8c] The procedure was repeated exactly as outlined above. However, this time, the opposite set of alternating electrodes was selected. Initially, only a faint appearance of the green fluorescence was observed. However, when repeated a second time, the chip clearly showed the presence of the second amine through the observed green fluorescence (Figure 3). Once again, confinement of oxidation and the subsequent reductive amination reaction appeared perfect. None of the green fluorescent amine appeared at either unwanted electrodes or the regions between the electrodes.

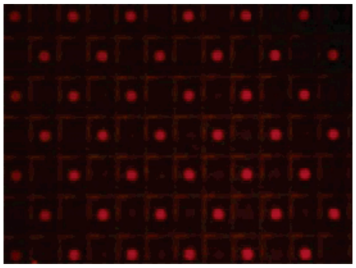


Figure 2. Fluorescent image of a chip having a checkerboard pattern of amine (red) on the surface.[10]

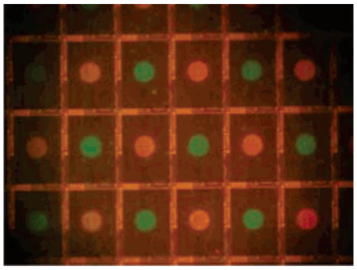


Figure 3. Fluorescent image of a chip having an alternating pattern of amines (red and green) on the surface.[10]

In conclusion, we have found that the confinement strategy developed for the Wacker oxidation is general and can be directly applied to the site-selective oxidation of alcohols. The confinement in these reactions is outstanding and not only prevents oxidations at neighboring electrodes but also prevents oxidation of the polymer between the electrodes. The availability of spatially isolated alcohol oxidations on the chips has enabled the development of a site-selective strategy for effecting reductive aminations, a process that provides a new method for placing molecules on the chip's surface proximal to the addressable electrodes.

Acknowledgment. We thank the National Science Foundation (CHE-0314057) and CombiMatrix for their generous support of this work. We also gratefully acknowledge the Washington University High Resolution NMR facility, partially supported by NIH Grants RR02004, RR05018, and RR07155, and the Washington University Mass Spectrometry Resource Center, partially supported by NIHRR00954, for their assistance.

Supporting Information Available: A sample procedures and images for the control experiments conducted are included. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) For the lead patent on DNA related work, see: Montgomery, D. D. PCT Int. Appl. 1998, 91 pages (CODEN: PIXXD2 WO 9801221 A1 19980115). (b) For peptide-based libraries, see: Rossi, F. M.; Montgomery, D. D. PCT Int. Appl. 2000, 52 pages (CODEN: PIXXD2 WO 0053625 A2 20000914). (c) For recent work, see: Oleinikov, A. V.; Gray, M. D.; Zhao, J.; Montgomery, D. D.; Ghindilis, A. L.; Dill, K. *J. Proteome Res.* **2003**, *2*, 313 as well as ref 3.
(2) For alternative approaches, see: (a) Sullivan, M. G.; Utomo, H.; Fagan, P. J.; Ward, M. D. *Anal. Chem.* **1999**, *71*, 4369. (b) Zhang, S.; Zhao, H.; John, R. *Anal. Chim. Acta* **2000**, *421*, 175. (c) Hintsche, R.; Albers, J.; Bernt, H.; Eder, A. *Electroanal.* **2000**, *12*, 660.
(3) For a description of the chips, see: Dill, K.; Montgomery, D. D.; Wang, W.; Tsai, J. C. *Anal. Chim. Acta* **2001**, *444*, 69.
(4) Tesfu, E.; Maurer, K.; Ragsdale, S. R.; Moeller, K. D. *J. Am. Chem. Soc.* **2004**, *126*, 6212.
(5) Tian, J.; Maurer, K.; Tesfu, E.; Moeller, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 1392.
(6) For leading references, see: Schultz, M. J.; Alder, R. S.; Zierkiewicz, W.; Privalov, T.; Sigman, M. S. *J. Am. Chem. Soc.* **2005**, *127*, 8499 and references therein.
(7) For a review of the reductive amination reaction, see: Baxter, E. W.; Reitz, A. B. *Org. React.* **2002**, *59*, 1.
(8) For selected examples using reductive amination reactions to place amines onto solid phase surfaces, see: (a) Hwang, J. Y.; Choi, H.-S.; Gong, Y.-D. *Tetrahedron Lett.* **2005**, *46*, 3107. (b) Bettinetti, L.; Loeber, S.; Huebner, H.; Gmeiner, P. *J. Comb. Chem.* **2005**, *7*, 309. (c) Shannon, S. K.; Peacock, M. J.; Kates, St. A.; Barany, G. *J. Comb. Chem.* **2003**, *5*, 860.
(9) The optimal confinement was obtained for a reaction using twice the amount of ethyl vinyl ether used for the Wacker oxidation.
(10) The fluorescent images were viewed using an inverted fluorescent microscope (Olympus Ix70) with a mercury lamp source, a XF 105-2 filter set (OMEGA Optical, Inc.), and a 4X (Figure 1) or 10X (Figure 2) objective lens (UM PlanFL, Olympus). The images were captured by an Olympus C5060 wide zoom digital camera (5.1 megapixel effective resolution).

JA057155X