Building Addressable Libraries: The Use of Electrochemistry for Generating Reactive Pd(II) Reagents at Preselected Sites on a Chip

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Chip-based molecular libraries are an important tool for analyzing the gene products produced by a cell.[1,2] This technology enables the assembly of large libraries of potential ligands within a diminutive area and, hence, allows for the development of systematic, global strategies for evaluating complex mixtures of proteins. Along these lines, approaches to molecular libraries were developed at CombiMatrix that take advantage of chip-based microfabrication technologies stemming from the semiconductor industry.[3,4] The strategy developed utilizes active-semiconductor chips that incorporate arrays of individually addressable micro-electrodes to synthesize the molecules in a library such that each unique set of molecules is proximate to a unique electrode.[5] The electrodes on the chip can then be used to monitor the molecules in the library during subsequent binding studies. To accomplish this, chips containing arrays of microelectrodes were coated with a porous membrane[5,6] and the electrodes were utilized to both attach monomers to the membrane using a linker molecule and to develop larger molecules of interest from monomer units. The synthetic transformations were accomplished by generating acids and bases at the electrodes. The electrochemically generated acids and bases were confined to a region surrounding the electrodes with a solution that neutralizes the acid or base. For example, a basic solution would be placed over the chip where the electrodes were being used for the anodic generation of an acid. In the immediate region sur-rounding an active electrode, rapid generation of acid overwhelms the base, enabling acid-initiated reactions to occur in regions proximate to the electrode surface, in particular reactions with substrates bound to the polymer covering the chip. This effect falls off as the distance from the electrode became larger. Varying both the concentration of the base in solution and the potential at the electrode controls the solution volume that is exposed to the acid. The electrodes on the chip were controlled using proprietary software and hardware connected to a PC, so that changing the pattern of reactions on the chip involved a simple software manipulation. This approach was used to site-selectively deprotect DMT groups on the chips, a development that led to the synthesis of DNA libraries.[5a] Analogous experiments used the electrodes as cathodes to generate bases at preselected sites on the chip, a development that enabled FMOC deprotections on the chips and the resultant construction of peptides.[5b]

In addition to synthesizing acids and bases, electrochemistry can be used to make a host of other reagents. For example, one can readily imagine utilizing an electrochemical platform to synthesize transition metal reagents at preselected sites on the chips. The development of such methodology would allow for the site-selective synthesis of small molecules on the chips using the same reagents that have proven so successful for other solution- and solid-phase syntheses. We report here the site-selective generation and use of a Pd(II) reagent on a chip.

As a starting point for developing chip-based transition-metal chemistry, we selected the well-known electrochemical Wacker oxidation.[7] In the most successful of these reactions, a Pd(II) reagent is recycled at an anode with the use of a triarylamine mediator. In principle, such a process should be directly transferable to a chip-based environment (Scheme 1).

In this scenario, the electrode on the chip would be used as an anode to oxidize the triarylamine and generate a radical cation. The amine radical cation in turn oxidizes Pd(0) to Pd(II), an event that would lead to the Wacker oxidation of a substrate bound to the chip. Any excess Pd(II) reagent generated in this fashion can be prevented from migrating to a neighboring electrode by the addition of a reactive substrate to the solution. For example, if ethyl vinyl ether were added to the reaction solution, any excess Pd(II) escaping the region surrounding the electrode would oxidize the ethyl vinyl ether to ethyl acetate, leading to the formation of Pd(0) reagent and would not initiate a Wacker oxidation at a neighboring electrode.

To test the effectiveness of this approach, a 1 cm^2 chip having an array of 1024 individually addressable platinum electrodes[3] was coated with a porous hydroxylated polymer membrane and then treated with the *N*-hydroxysuccinic ester of 10-undecenoic acid as outlined in Scheme 2. The substrate was concentrated on the chip in the region close to the electrodes by catalyzing the reactions with an electrogenerated base.[8] The base was formed by using the electrodes on the chip to reduce vitamin B_{12}.[5b] To accomplish this, the chip was submerged (along with a Pt-rod counter electrode) into a tetrabutylammonium nitrate in DMF/MeOH electrolyte solution containing the vitamin B_{12}. Selected electrodes were poised at a potential difference of -2.4 V versus the Pt counter electrode for 0.5 s and off for 0.1 s for 300 cycles. These conditions were selected in analogy to earlier coupling reactions using the same chips to ensure selectivity (longer times generate larger quantities of reagent and more chance for migration to neighboring electrodes) and complete coverage of the electrode (extra cycles). Following the coupling reaction, any free hydroxyls remaining on the surface of the chip were capped by exposing the chip to acetic anhydride using the same electrogenerated base conditions. The Wacker oxidation outlined above in Scheme 1 was then performed at selected electrodes by reversing the electrode polarity and utilizing them as anodes. Electrodes not selected for the Wacker oxidation were simply turned off. For this experiment, the chip and counter electrode were submerged in 2.5 mL of 0.5 M Et_4NOTs in 7:1 acetonitrile/water electrolyte solution containing 32 μg of $Pd(OAc)_2$, 1.39 mg of tris-2-bromophenylamine, and 83 μL of ethyl vinyl ether. The oxidation reaction was performed by pulsing the selected electrodes for 0.5 s at $+2.4$ V and 0.5 s at 0 V for either 300 or 600 cycles. The selected electrodes were chosen to form a checkerboard pattern on the chip.

Once this experiment was completed, the ketones that were generated were then converted to their 2,4-DNP derivatives by
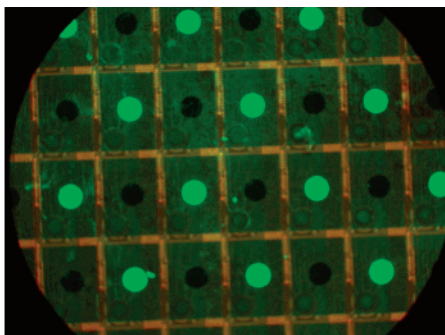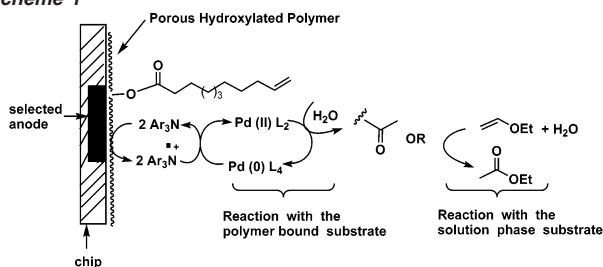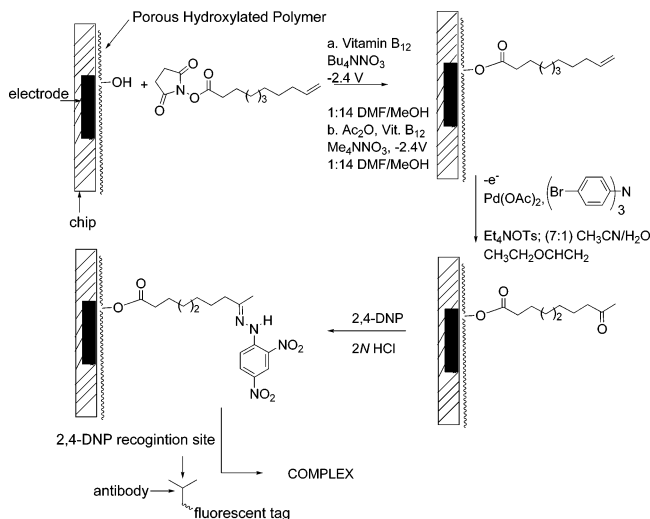

Figure 1. Selective Pd(II) oxidation on a chip.

Scheme 1

Scheme 2

treating the chip with a 0.5% DNP in 2 N HCl solution, and the chip was incubated with a 5% BSA in PBS buffer solution containing commercially available rabbit anti-2,4-dinitrophenol antibody that is conjugated to the fluorescent probe Alexa Fluor 488 (1/16 antibody to buffer).[9] Next, the surface of the chip was washed with PBS buffer to remove excess antibody, and the chip was imaged with an epifluorescence microscope using a blue filter (PBS buffer was needed on the surface of the chip to ensure a successful image). The image shown in Figure 1 suggests that the experiment worked perfectly. It appears the reaction led only to the formation of ketones at the selected electrodes as demonstrated by the checkerboard pattern of fluorescence (indicated by the bright spots) on the chip.

In Figure 1, the dark spots are electrodes that were not utilized for the oxidation (the Pt electrodes block the background fluorescent originating from the chip itself).

Subsequent control experiments yielded two important observations about this initial experiment. First, when Pd(II) was generated at selected electrodes on a chip without the olefin substrate, a faint checkerboard pattern was still observed. It appears that the acetic anhydride capping step was not completely effective and Pd(II)

generated at the electrode led to oxidation of the unprotected alcohols in the polymer membrane.[10] An additional experiment compared "side-by-side" electrodes that had associated olefin substrate and electrodes that were devoid of any olefin substrate. In this experiment, the intensity of the fluorescent spots was significantly greater for the electrodes having the olefin substrate present. This indicates that the intensity of the fluorescent spots in the initial experiment (Figure 1) was due primarily to the initially planned Wacker oxidation.

In a second control experiment, the ethyl vinyl ether was removed from the solution over the chip. In this case, the experiment led to fluorescence at many electrodes that were not utilized for the oxidation as well as at a variety of random sites on the surface of the chip. Clearly, the use of ethyl vinyl ether was required for confining the Pd(II) to the preselected sites on the chip.

In conclusion, we have found that the mediated electrochemical conditions developed for recycling a Pd(II) reagent in a preparative-scale oxidation can be used without modification for generating Pd(II) reagents on the surface of a chip. The Pd(II) reagent generated in this fashion can be confined to preselected locations on a chip by adding a reactive substrate to the solution over the chip. In principle, one can imagine using analogous strategies to conduct a wide variety of transition-metal-based chemistry in a chip-based environment. Efforts to explore the scope of this chemistry and its overall utility for building chip-based molecular libraries are currently underway.

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

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