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Microelectrode Arrays and Ceric Ammonium Nitrate: A Simple Strategy for Developing New Site-Selective Synthetic Methods

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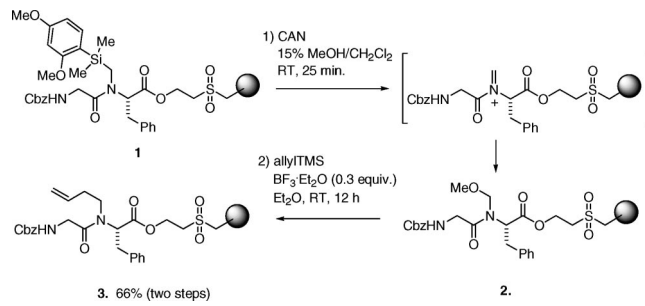
The construction of a molecular library on a microelectrode array[1,2] opens up the possibility for monitoring the binding of molecules in the library with biological receptors in "real time".[3] Accomplishing this task means that each unique member of the library must be built next to a unique, individually addressable microelectrode in the array, even when the array has a density of 1024 or 12 554 microelectrodes cm^{-2}. For this reason, we have been developing tools for site-selectively synthesizing[4−7] and characterizing[8] molecules on microelectrode arrays. From a synthetic standpoint, each new site-selective reaction requires the development of both an electrochemical means for generating the reagent used to effect the transformation and a solution phase substrate for destroying the reagent generated before it migrates away from the electrode used in its synthesis. To date, much of this work has focused on the use of palladium reactions in a site-selective fashion.[4,5] This was done because palladium can be easily cycled between its nucleophilic Pd(0) and electrophilic Pd(II) oxidation states, a fact that had already led to the development of effective preparative reactions that recycle palladium at an electrode.[9] In addition, the availability of a variety of Pd(0) and Pd(II) reagents and a broad knowledge of how to chemically oxidize and reduce them (most stoichiometric reactions involving Pd either oxidize or reduce the corresponding reagent) made adapting Pd chemistry to the microelectrode arrays straightforward. However, not all synthetic reactions needed for building molecular libraries have such readily identifiable reduction−oxidation cycles and available reagents. For example, ceric ammonium nitrate is a very useful oxidant for a wide range of chemical reactions.[10] We have found that the cleavage of a silylelectroauxiliary substituted amino acid using ceric ammonium nitrate provides an ideal method for inserting *N*-acyliminium ions into peptides (Scheme 1).[11] Hence, the use of ceric ammonium nitrate in a site-selective fashion might provide a pathway for building addressable libraries of functionalized peptidomimetics. But how would one utilize ceric ammonium nitrate in a site-selective fashion? What is the reduced cerium reagent that one can oxidize at a microelectrode to site-selectively form a Ce(IV) oxidant, and what is the chemical reducing agent that can be used in solution to confine the ceric ammonium nitrate generated to the region surrounding a preselected microelectrode?[12] We report here a very simple strategy for developing new site-selective reactions and its application to the site-selective use of Ce(IV).

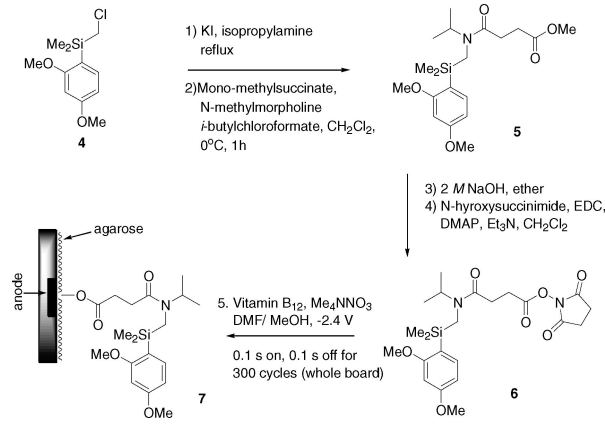
The oxidative cleavage of a silylelectroauxiliary substituted amino acid was an ideal reaction for probing site-selective ceric ammonium nitrate reactions because a method for determining the site-selective formation of an *N*-acyliminium ion on a microelectrode array was already available.[7] In addition, the substrate attached to the agarose polymer coating the microelectrode array is insulated from the electrode surface by the polymer. Hence, no background oxidation of the substrate in the absence of the Ce(IV) mediator is possible.[13] With this in mind, a silylelectroauxiliary-substituted amide was constructed as a test substrate and then placed by each of the microelectrodes in a 1 cm^2 array having 1024 electrodes (a 1K array, Scheme 2). Placement of the substrate on the microelectrode array took advantage of an electrochemically generated base-catalyzed esterification reaction.[3−7] In this reaction, the microelectrodes were used as cathodes to generate the radical anion from vitamin B_{12} and in turn catalyze an esterification reaction between the agarose polymer coating the microelectrode array and the activated ester in substrate **6**. Use of the electrogenerated base ensured that the substrate was placed proximal to the electrodes (**7**).

With **7** in hand, attention was turned to the ceric ammonium nitrate oxidation (Scheme 3). Our plan for developing a site-selective Ce(IV) oxidation called for the simplest possible approach. The reduced cerium reagent needed for the reaction was generated by incubating a catalytic amount of the commercially available ceric ammonium nitrate with an excess of a solution phase substrate that was identical to the substrate placed on the surface of the microelectrode array for the site-selective reaction. The resulting solution was then placed above the array, and selected microelectrodes were used as anodes to regenerate a Ce(IV) oxidant where it was needed. The hope was that the excess solution

Scheme 1



Scheme 2



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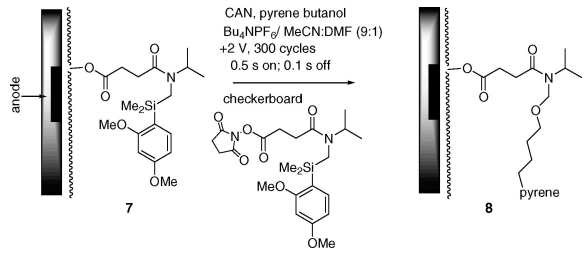
Scheme 3



phase substrate would serve as the confining agent in solution needed for preventing the migration of any Ce(IV) reagent generated to remote locations on the array. The result would be an approach for developing a site-selective microelectrode-array oxidation that capitalized on the same reagents and substrates used for the solution phase reaction. One would simply take the solution-phase reaction, reduce the amount of oxidant used to a catalytic amount, add an electrolyte, and then use the solution on the microelectrode array to effect the site-selective reaction.

In practice, 1 equiv of ceric ammonium nitrate and 10 equiv of activated ester **6** were added to dichloromethane and stirred overnight. To this mixture were added tetrabutylammonium hexafluorophosphate as an electrolyte for the electrolysis and pyrene butanol in order to trap any *N*-acyliminium ion generated. The surface functionalized microelectrode array **7** was then inserted into the solution and a checkerboard pattern of microelectrodes used as anodes at a potential of +2.0 V relative to a remote platinum wire cathode. The reaction was run for 300 cycles where the selected microelectrodes were turned on for 0.5 s and off for 0.1 s. It was found that turning the current on for longer periods of time led to a deterioration in the amount of current that could be passed. Following the reaction, the array was removed from the solution, washed to remove any unbound pyrene butanol, and then imaged using a fluorescence microscope (Figure 1a).

A checkerboard pattern of fluorescence on the microelectrode array was obtained indicating site selective formation of the *N*-acyliminium ion intermediate from the silyl-substituted amide. No loss of confinement was observed. Clearly, the simple strategy taken for generating a reduced cerium substrate for the electrolysis and confining Ce(IV) to the selected microelectrodes was very effective.

The site-selective ceric ammonium nitrate oxidation could also be accomplished using an array having 12 544 microelectrodes cm^{-2} (a 12K array). This was important because the 12K arrays are used in electrochemical signaling experiments.[3] Using the same conditions employed for the 1K array, the image in Figure 1b was generated using a 12K array by employing a checkerboard pattern of electrodes inside a box as anodes for the oxidation. Once again, a high level of confinement of the reaction to the selected electrodes was observed. The very simple strategy used for developing the site-selective Ce(IV) oxidation on the 1K array was also effective for developing site-selective Ce(IV) oxidations on the more dense 12K microelectrode array.

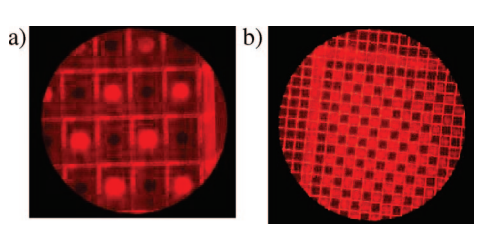


Figure 1. Site-selective Ce(IV) oxidation reactions. (a) Site-selective pattern on a 1K microelectrode array. (b) Site-selective pattern on a 12K array.

In conclusion, we have found that Ce(IV) oxidations can be performed in a site-selective fashion on both 1K- and 12K-microelectrode arrays. Additionally, it was found that the site-selective reactions could be performed using the same reagents employed for a solution-phase reaction. This allowed for a very simple approach to developing site-selective reactions. To this end, a solution phase oxidation was modified by removing the stoichiometric amount of ceric ammonium nitrate normally used and replacing it with a catalytic amount of the oxidant. The mixture was stirred overnight to allow for complete consumption of the oxidant. An electrolyte was added followed by insertion into the solution of a microelectrode array functionalized with a substrate for the oxidation. Selected microelectrodes in the array were then turned on as anodes to regenerate the oxidant exclusively at sites where it was desired. The excess substrate in the reaction served as the confining agent. Both the use of ceric ammonium nitrate on the microelectrodes and the overall strategy used in its development should prove extremely useful for the construction of addressable molecular libraries in the future. Efforts along these lines are currently underway.

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Supporting Information Available: Spectral data for all new compounds are provided along with sample procedures for conducting the microelectrode array reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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