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Building Addressable Libraries: Site-Selective Formation of an *N*-Acyliminium Ion Intermediate

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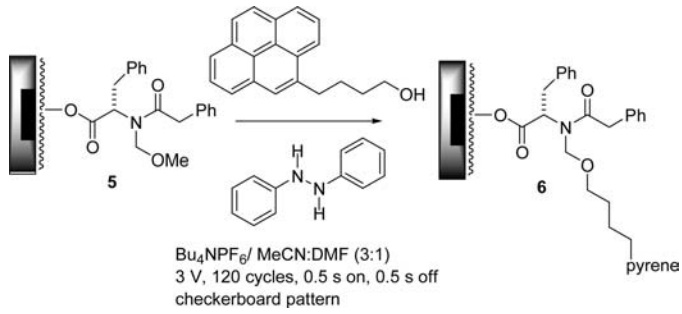
ABSTRACT



A strategy for site-selectively generating reactive *N*-acyliminium ion intermediates on a microelectrode array has been developed. The route capitalizes on the use of an electroauxiliary for building a methoxylated amino acid substrate, and then the electrochemical generation and solution phase confinement of acid in order to form the *N*-acyliminium ion. Keys to this strategy were the stability of an *N*-α-methoxyalkyl amide to basic reaction conditions and the generality of the electrogenerated acid conditions for conducting microelectrode array reactions in a site-selective fashion.

Chip-based molecular arrays are important tools for probing interactions between libraries of potential ligands and biological receptors.[1,2] Of particular interest are addressable microelectrode arrays that have the potential to monitor binding events between a molecular library and a target receptor in "real-time".[3] In order to take advantage of microelectrode arrays for this purpose, we must first have the synthetic tools needed for building the unique members of a molecular library by unique, individually addressable microelectrodes in the array.[4,5] For this reason, our group has been developing methods for conducting site-selective syntheses on microelectrode arrays.[5,6] In this effort, the microelectrode array is coated with a porous polymer and

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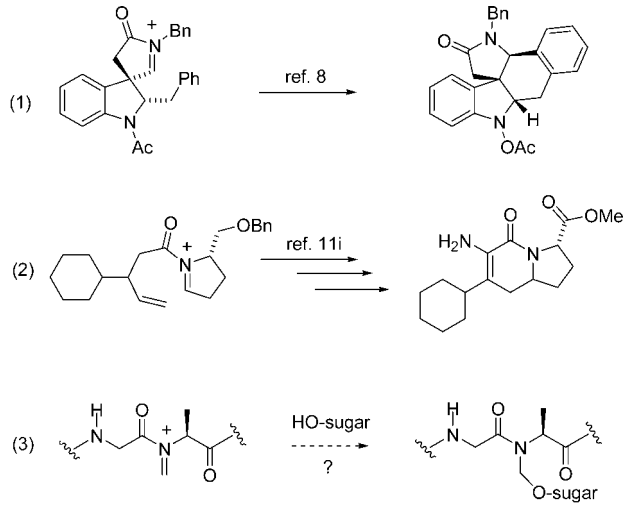
then substrates for building the library are covalently attached to the polymer proximal to the electrodes. Site-selective chemical reactions are then conducted to further develop the substrates. These site-selective reactions are essentially competition experiments between the electrochemical generation of a chemical reagent or catalyst and the solution phase destruction of the reagent or catalyst before it can migrate away from the electrodes employed for its formation. Hence, each new microelectrode array reaction requires two new developments; an electrochemical method for generating a reagent or catalyst and a solution phase "confining-agent" that destroys the reagent or catalyst generated.

Because reactive *N*-acyliminium ion intermediates have proven very useful for building alkaloids (Scheme 1, eq 1)[7]

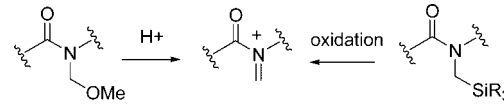
Scheme 1



and constrained peptidomimetics (Scheme 1, eq 2),[8–10] and because they are potentially useful for building peptide-based bioconjugates (Scheme 1, eq 3), a method for site-selectively generating *N*-acyliminium ions on a microelectrode array would represent an important addition to this effort. We report herein a synthetic strategy for accomplishing this task.

In principle, site-selective *N*-acyliminium ion generation can be achieved by using the electrode to generate either an acid for catalyzing the departure of a leaving group from a suitable precursor or an oxidant for converting an amide with an silyl-electroauxiliary into the *N*-acyliminium ion (Scheme 2).[11,12] For the first, a confining agent would be needed to

Scheme 2



scavenge acid generated at selected electrodes in the microelectrode array. For the second, a confining agent would be needed to scavenge an oxidant generated at the electrodes. While both approaches are attractive, the recent demonstration that *t*-Boc groups can be deprotected in a site-selective fashion using acid on a microelectrode array[13] led us to start our investigation by examining the acid-catalyzed approach. Our twin goals were to both develop an effective route for site-selectively producing *N*-acyliminium ions on a microelectrode array and demonstrate the generality of the electrochemical generation of acid/ confining-agent strategy developed for the *t*-Boc deprotection reaction. Can reaction conditions worked out for the site-selective generation of a chemical reagent on a microelectrode array be extended to new applications?

The study began with the synthesis of a suitable test substrate for use on the microelectrode array (Scheme 3).

To this end, phenylalanine methyl ester was alkylated with trimethylsilylmethyl chloride and the resulting product acyl-

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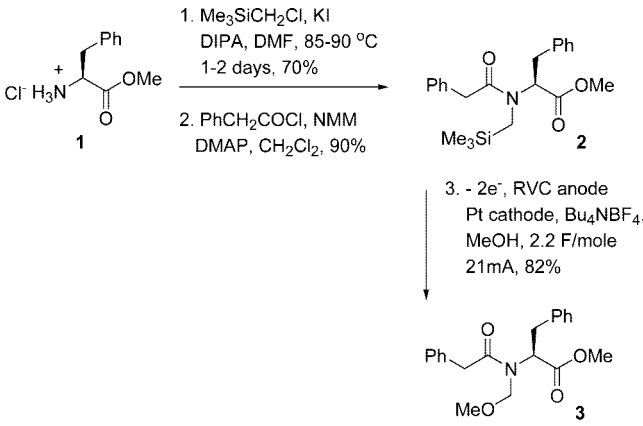
(11) For the generation of *N*-acyliminium ions using a silyl group as an electroauxiliary, see: (a) Yoshida, J.; Isoe, S. *Tetrahedron Lett.* **1987**, *28*, 6621. (b) Suga, S.; Watanabe, M.; Yoshida, J. *J. Am. Chem. Soc.* **2002**, *124*, 14825. For electroauxiliary as a general concept, see: (c) Yoshida, J.; Nishiwaki, K. *J. Chem. Soc., Dalton Trans.* **1998**, 2589.

(12) For applications to the synthesis of peptide derivatives, see: Sun, H.; Martin, C.; Kesselring, D.; Keller, R.; Moeller, K. D. *J. Am. Chem. Soc.* **2006**, *128*, 13761.

(13) For the use of acid on the chips, see: (a) Rossi, F. M. ; Montgomery, D. D. ,PCT Int. Appl. (2000), 52 pp. CODEN: PIXXD2 WO 0053625 A2 20000914. (b) Maurer, K.; McShea, A.; Strathmann, M.; Dill, K. *J. Comb. Chem.* **2005**, *7*, 637.

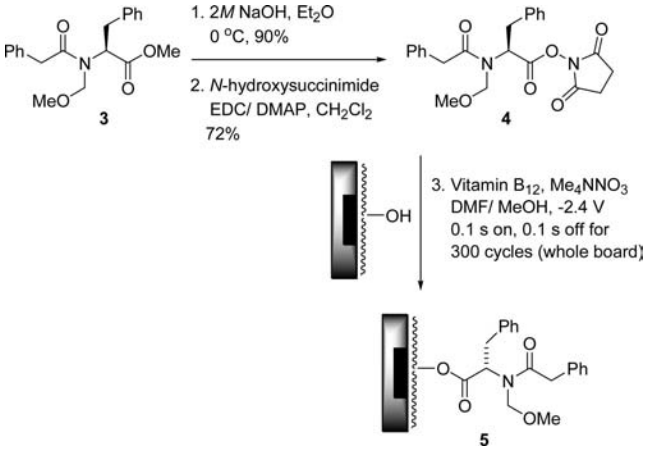
Scheme 3



ated to form **2**. The silyl group in **2** was then oxidatively exchanged for a methoxy group using a constant current electrolysis in an undivided cell.[12] The use of the silyl electroauxiliary-based approach to **3** was essential for making the methoxylated amino acid building block in that it avoided any elimination problems associated with incorporating the leaving group into the amino acid substrate prior to forming the amide while aiding the anodic oxidation reaction.[12]

The methoxylated amide **3** was converted into an activated ester and placed onto a microelectrode array as outlined in Scheme 4. Key to this sequence was the compatibility of

Scheme 4

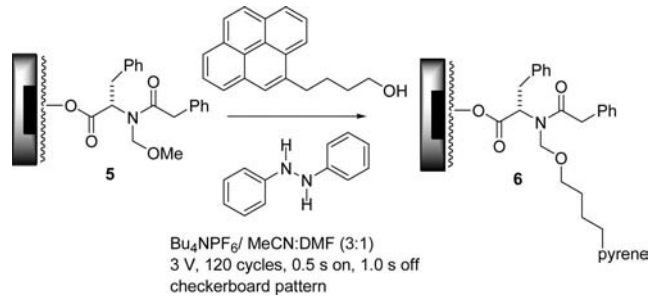


the α-methoxy alkylamide with saponification of the methyl ester to deprotect the C-terminus of the amino acid derivative, conversion of the resulting acid into an activated ester, and placement of the substrate onto the agarose polymer coating the surface of the microelectrode array using the electrogenerated base conditions (reduction of vitamin B_{12} in methanol/DMF). In the microelectrode array reaction, the electrodes in the array were cycled on at a potential of −2.4 V for 0.1 s and off for 0.1 s. Three hundred such cycles were used.[5,6]

The placement of substrate on the microelectrode array was done so that the substrate was located proximal to each of the electrodes in the array. In this case, there was little choice in this matter. In sharp contrast to previous studies using this same electrogenerated-base catalyzed coupling,[14] the activated amino acid substrate used here could not be placed onto the microelectrode array in a site-selective fashion. This suggests that placement of the amino acid substrate on the microelectrode array proceeds through a different mechanism than the earlier activated ester reactions, perhaps involving formation of a ketene intermediate that would react with alcohols in the absence of a base catalyst. Such a reaction would not be confined to electrodes used to generate the catalyst. While this observation leads to obvious concerns about the stereochemistry of the substrate and the need for a more gentle approach to placing amino acid derivatives onto a microelectrode array, it did not interfere with our plan to investigate the potential for site-selectively generating *N*-acyliminium ions on the array.

For site-selective *N*-acyliminium ion formation, the substrate modified microelectrode array was inserted into a solution of diphenylhydrazine and 4-pyrenebutanol in acetonitrile/DMF containing tetrabutylammonium hexafluorophosphate electrolyte (Scheme 5). Selected electrodes in the

Scheme 5



array were then used as anodes to oxidize the diphenylhydrazine and generate an acid in a manner analogous to the previously reported *t*-Boc deprotection.[14] The acid was confined to the region of the microelectrode array surrounding the selected electrodes with the use of excess phenylhydrazine. The excess phenylhydrazine served as a base to neutralize any acid that migrated away from the selected electrodes. In the region of the array proximal to the active electrodes, the generation of acid occurred at a rate fast enough to overwhelm the base and lead to the formation of an *N*-acyliminium ion, an event that was detected by an exchange of the methoxy group on the carbon alpha to the amide nitrogen with 4-pyrene butanol. For this transformation, the selected electrodes were cycled; on at a potential of + 3 V for 0.5 s and off for 1.0 s for 400 cycles. The longer off cycle is necessary to allow for substrate diffusion and maintain high current flow. Longer reaction times, up

(14) Unpublished results with Melissae Stuart.

to 1000 cycles (500s on) did not seem to improve the intensity of the fluorescence from the surface of the array above the microelectrodes utilized. Key to the success of this reaction appears to be adequate swelling the polymer coating the array. The presence of a measurable current flow proved to be a useful monitor of polymer swelling since an unswelled polymer coating effectively quenched the current flow. When dichloromethane was used as a solvent for the reaction and a checkerboard pattern of electrodes selected, the current flow for the reaction was $<10\ \mu$A and no reaction was observed. The use of acetonitrile as solvent improved both swelling of the polymer and the current flow, especially when a small amount of DMF was employed as a cosolvent. In addition, the reactions benefited from soaking the chip in ethanol for a minute before beginning the reaction. When these conditions were employed, a current of $>200\ \mu$A could be consistently obtained when activating a checkerboard pattern of microelectrodes on the array.

Following the reaction, the microelectrode array was washed by dipping the chip into vials of ethanol, water, and then ethanol. The arrayed pattern was viewed using a fluorescence microscope (Figure 1a). The pattern observed clearly shows the checkerboard pattern of electrodes that were used as anodes. A closer view shows the level of confinement that was obtained with this reaction (Figure 1b). The success of this experiment demonstrates that, like the Pd(II) reactions studied earlier,[5] the conditions for acid generation and confinement on the microelectrode array are generally applicable.

In conclusion, a synthetic strategy for generating *N*-acyliminium ion intermediates site-selectively on a microelectrode array has been developed. The reaction uses an electrochemically generated acid to trigger formation of the reactive intermediate and excess phenylhydrazine as a confining agent for preventing the migration of acid away from the selected electrodes. The success of the chemistry not only demonstrates the potential for doing *N*-acyliminium ion based syntheses on the microelectrode arrays, but also shows the generality of the strategy for electrochemically generating and confining acid to preselected sites on a microelectrode array. Efforts to develop oxidative strategies for *N*-acyliminium ion formation and to take advantage of the reactive intermediates generated for making substituted peptides are underway.

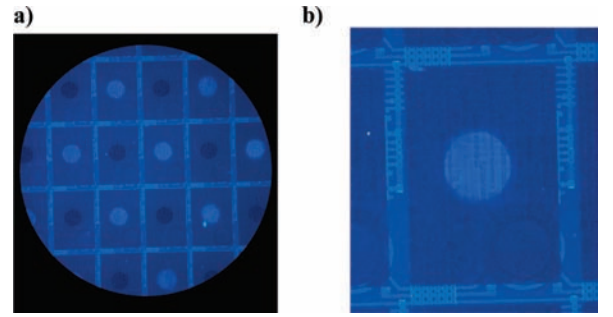


Figure 1. Site-selective reaction. (a) Fluorescence image of the checkerboard pattern. (b) Confinement around a single electrode in the array.

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Supporting Information Available: Experimental procedures and NMR spectra for obtained compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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