ORGANIC LETTERS

2009
Vol. 11, No. 6
1273−1276

Building Addressable Libraries: Site-Selective Suzuki Reactions on Microelectrode Arrays

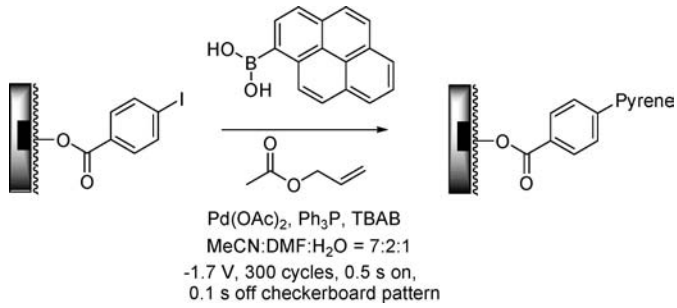
Libo Hu,[†] Karl Maurer,[‡] and Kevin D. Moeller*,[†]

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

moeller@wuchem.wustl.edu

Received January 12, 2009

ABSTRACT



A site-selective Suzuki reaction has been developed for use on microelectrode arrays. The reaction conditions employed are similar to those used to achieve site-selective Heck reactions. The reaction can be run with either an aryliodide attached to the surface of the array and an arylboronic acid in solution or with an arylboronic acid attached to the surface of the array and an arylbromide in solution. Both allyl acetate and air are effective confining agents for the reaction. The reactions are compatible with arrays containing either 1024 microelectrodes cm^{-2} or 12 544 microelectrodes cm^{-2}.

Addressable microelectrode arrays[1,2] are intriguing tools for probing interactions between small molecules and biological receptors[3,4] because they enable monitoring of the interactions in "real-time".[5] For this reason, we have been developing synthetic methods for site-selectively building molecules proximal to the electrodes in a microelectrode array.[6,7] The long-term goal of the work is to use the arrays as a platform for small molecular libraries containing engineered, semirigid peptidomimetics that are useful for probing the conformational requirements of protein receptors.

Triaryl peptidomimetics that mimic helical regions of proteins[8,9] are of interest in this context. Helices on protein surfaces often play key roles in the binding of proteins to

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

(1) For a description of the microelectrode array chips used here, see: Dill, K.; Montgomery, D. D.; Wang, W.; Tsai, J. C. *Anal. Chim. Acta* **2001**, *444*, 69.

(2) For alternative approaches using electrochemical microarrays, 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. *Electroanalysis* **2000**, *12*, 660.

(3) For the use of small molecule arrays for probing biological receptors, see: (a) Lipshutz, R. J.; Fodor, S. P. A.; Gingeras, T. R.; Lockhart, D. J. *Nat. Genet.* **1999**, *21*, 20. (b) Pirrung, M. C. *Chem. Rev.* **1997**, *97*, 473. For more recent examples, see: (c) Webb, M. S.; Miller, A. L.; Johnson, B. H.; Fofanov, Y.; Li, T.; Wood, T. G.; Thompson, E. B. *J. Steroid Biochem. Mol. Biol.* **2003**, *85*, 183. (d) Shih, S.-R.; Wang, Y.-W.; Chen, G.-W.; Chang, L.-Y.; Lin, T.-Y.; Tseng, M.-C.; Chiang, C.; Tsao, K.-C.; Huang, C. G.; Shio, M.-R.; Tai, J.-H.; Wang, S.-H.; Kuo, T.-L.; Liu, W.-T. *J. Virol. Methods* **2003**, *111*, 55.

(4) For reviews, see: (a) Duffner, J. L.; Clemons, P. A.; Koehler, A. N. *Curr. Opin. Chem. Biol.* **2007**, *11*, 74. (b) Taipa, M. A. *Comb. Chem. High Throughput Screening* **2008**, *11*, 325.

(5) (a) Tesfu, E.; Roth, K.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2006**, *8*, 709. (b) Stuart, M.; Maurer, K.; Moeller, K. D. *Bioconjugate Chem.* **2008**, *19*, 1514.

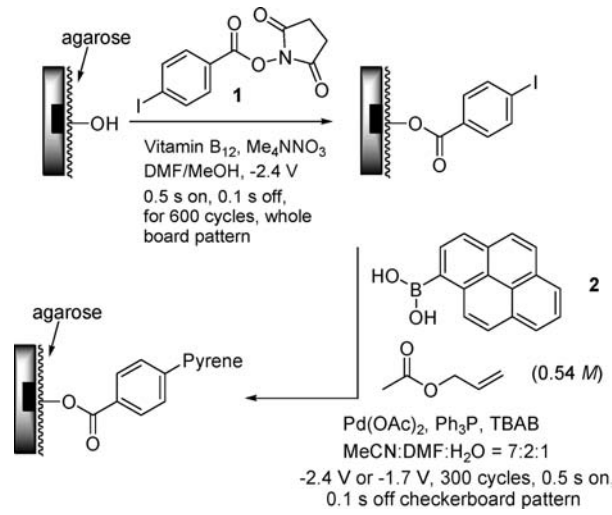
10.1021/ol900056u CCC: $40.75
Published on Web 02/24/2009

other proteins, DNA, and RNA. Hence, they represent attractive targets for developing new methods for treating a variety of diseases. Triaryl ring systems can mimic sections of these helices by orientating peptide side-chains in either the *i*, *i*+*3*, and *i*+*7* or *i*, *i*+*4*, and *i*+*7* positions. They have the potential to be particularly useful in this regard because they represent typical drug-like scaffolds. With this in mind, we became interested in developing the synthetic methodology needed to build microelectrode array-supported libraries of triaryl peptidomimetics.

The key to constructing triaryl ring skeletons is the Suzuki reaction.[8,10] The Suzuki reaction allows for a "building-block" approach to the molecules using preconstructed, individual aryl rings that are then coupled to each other. But can such an approach be employed site-selectively on a microelectrode array? Microelectrode array reactions involve a delicate balance between the electrochemical generation of a catalyst, the rate of the desired reaction, and the destruction of the catalyst generated in the solution above the array by a "confining" agent. In this balance, the confining agent must be reactive enough to prevent migration of the catalyst to nonselected sites in the array. This is a challenging task since the arrays can contain up to 12 544 microelectrodes cm^{-2}. On the other hand, the confining agent must not be too reactive or it will prevent the desired reaction from occurring at the selected electrodes. Hence, the critical step in the development of any new reaction on a microelectrode array is identifying an effective confining agent. For the Suzuki reaction, the need for a confining agent immediately raised questions about the generality of the strategy used to confine the earlier Pd(0)-catalyzed reactions.[7] Does changing the reaction catalyzed by a reagent on a microelectrode array require the identification of a new confining agent, or can the parameters of the electrolysis be adjusted to account for differences in the reactions so that the same confining agents can be employed? The answer to this question is essential for planning new reactions and synthetic strategies on the arrays. We report here that site-selective Suzuki reactions can be accomplished using the confining strategy developed earlier for the Heck reaction if the rate of Pd(0) generation at the microelectrodes is reduced and the concentration of the confining agent increased to account for a faster reaction.

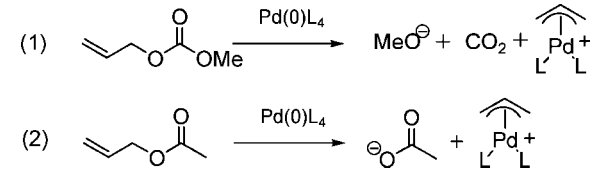
The study of site-selective Suzuki reactions began with the placement of a 4-iodobenzoate substrate (**1**) onto the surface of an array having 1024 microelectrodes cm^{-2} (Scheme 1). The substrate was placed proximal to each of

Scheme 1



the electrodes in the array using the electrogenerated base catalyzed procedure previously developed for coupling activated esters to the agarose polymer coating the array.[6,7] The Suzuki reaction was then initiated using nearly the same conditions utilized to accomplish site-selective Heck reactions.[7a] Accordingly, the entire surface of the array was treated with an acetonitrile/dimethylformamide/water solution containing pyrene-1-boronic acid **2**, palladium acetate, triphenylphosphine, and tetrabutylammonium bromide. The Pd(II) reagent was then reduced to the required Pd(0)-catalyst at selected locations on the array by using the microelectrodes as cathodes. The potential at the selected electrodes was set at −2.4 V relative to a remote Pt-wire anode for 0.5 s and then the electrodes turned off for 0.1 s. Nonselected electrodes were left off for the entire reaction. The only change in the reaction relative to the site-selective Heck reaction was the use of allyl acetate as the confining agent instead of the allyl methyl carbonate employed previously. The confining agent is added to the solution above the array in order to oxidize any Pd(0) that begins to migrate away from the microelectrodes selected as cathodes. This prevents unwanted reactions from occurring proximal to electrodes that were not selected. Both allyl acetate and allyl methyl

Scheme 2



(6) For Pd(II) reactions, see: (a) Tesfu, E.; Maurer, K.; Ragsdale, S. R.; Moeller, K. D. *J. Am. Chem. Soc.* **2004**, *126*, 6212–6213. (b) Tesfu, E.; Maurer, K.; McShae, A.; Moeller, K. D. *J. Am. Chem. Soc.* **2006**, *128*, 70. For examples using electrogenerated base, see: Maurer, K.; McShea, A.; Strathmann, M.; Dill, K. *J. Comb. Chem.* **2005**, *7*, 637. For the site-selective generation of acid, see: Kesselring, D.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2008**, *10*, 2501. For the use site-selective use of ceric ammonium nitrate, see: Kesselring, D.; Maurer, K.; Moeller, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 11290.

(7) For a Pd(0) reactions see: (a) Tian, J.; Maurer, K.; Tesfu, E.; Moeller, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 1392. (b) Tang, F.; Chen, C.; Moeller, K. D. *Synthesis* **2007**, 3411. (c) Chen, C.; Lu, P.; Walker, A.; Maurer, K.; Moeller, K. D. *Electrochem. Commun.* **2008**, *10*, 973. (d) Tian, J.; Maurer, K.; Moeller, K. D. *Tetrahedron Lett.* **2008**, *49*, 5664.

(8) For the use of triaryl systems as helix mimics, see: Yin, H.; Lee, G.; Seedy, K. A.; Kutzki, O.; Park, H. S.; Orner, B. P.; Ernst, J. T.; Wang, H.-G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 10192.

(9) For the phenyl dipyridyl systems, see: Che, Y.; Brooks, B. R.; Marshall, G. R. *Biopolymers* **2007**, *86* (4), 288.

(10) For **2**: Unpublished results with Bourne, G. and Marshall, G. R.

carbonate work as confining agents by converting the Pd(0) catalyst into a π-allyl-Pd(II) species (Scheme 2). The switch to allyl acetate was made to avoid the generation of methoxide during this process. In the Heck reaction, it was thought that the methoxide generated cleaved the ester connecting the product to the agarose polymer.[7a] This decreased the amount of product on the surface of the array with time. The switch to allyl acetate was made to eliminate this possibility.

The use of these conditions with a checkerboard pattern of microelectrodes employed as cathodes led to a moderate degree of success (Figure 1a). When the array used to

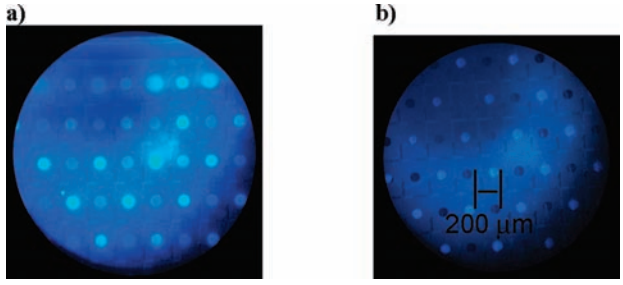


Figure 1. Fluorescence image of the site-selective Suzuki reaction (a) checkerboard pattern run using −2.4 V (b) checkerboard pattern run using −1.7 V.

conduct the Suzuki reaction was imaged using a fluorescence microscope, a checkerboard pattern for the pyrene product could be seen. However, significant reaction also occurred at electrodes that were not selected. The checkerboard pattern was only visible because the pyrene-derived fluorescence associated with the selected electrodes was brighter than that associated with the nonselected electrodes. Clearly, confinement on the array was being lost and migration of the Pd(0) generated at the selected electrodes was not being completely prevented. The loss of confinement for the Suzuki reaction relative to the Heck reaction run earlier suggested that either the Suzuki reaction is faster than the Heck reaction, the allyl acetate is less effective as a confining agent than the allyl methylcarbonate employed in the Heck reaction, or both.

To stop the migration of Pd(0) to microelectrodes not selected for the Suzuki reaction, the rate of Pd(0) generation at the selected electrodes was decreased. This was accomplished by reducing the potential at the electrodes from −2.4 V to −1.7 V relative to the remote Pt counter electrode. Once again, the selected electrodes were cycled on for 0.5 s and off for 0.1 s. The change resulted in far superior confinement (Figure 1b). In this case, no fluorescence was observed at electrodes not selected as cathodes for the generation of Pd(0).

To demonstrate that the fluorescence observed in Figure 2 was a result of the Suzuki reaction and not a reaction between the pyrenyl boronic acid and the agarose polymer coating the array, a control experiment was performed. In this study, the initial base catalyzed esterification reaction was performed using a boxes of eight electrodes. The Suzuki reaction was performed using all of the electrodes in the array as cathodes. After

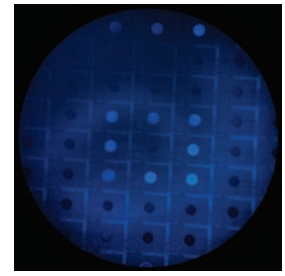


Figure 2. Fluorescence image of an experiment that ran the VB_{12} esterification reaction in box pattern and the Suzuki reaction on all of the electrodes in the array. The spots at the top of the image are part of a second box pattern.

completion of the reaction, the microelectrode array was examined with a fluorescence microscope giving rise to the image shown in Figure 2. Only the box of electrodes employed in the initial esterification reaction showed the presence of pyrene. There was no background reaction between the pyrene boronic acid and the agarose polymer.

With the reactions working well on arrays having 1024 microelectrodes cm^{-2} (a 1 K-array), attention was turned to the use of more dense arrays having 12 544 microelectrodes cm^{-2} (a 12 K-array). This work was done because it is the more dense arrays that are used in signaling studies.[5] Initially, the experiment on the 12 K-array was attempted using reaction conditions that were identical to those used on the 1 K-array. In this case, the initial substrate placement reaction was performed at each microelectrode in the array. The Suzuki reaction was then initiated using a checkerboard inside of a box pattern of microelectrodes. While the checkerboard pattern could be seen in places on the array, confinement was lost leading to reactions at electrodes that were not selected (Figure 3a). The pattern was difficult to

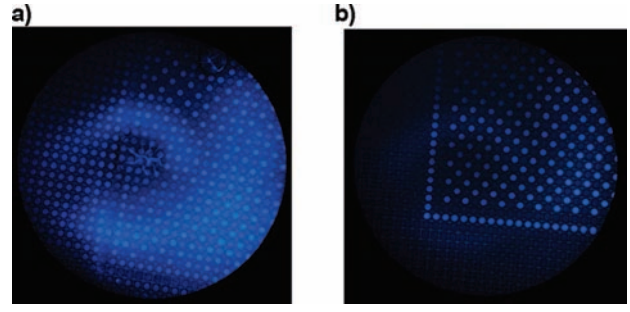


Figure 3. Fluorescence image of a site-selective Suzuki reaction on a 12 K-array: (a) checkerboard in a box pattern run with the conditions developed for the 1 K-array; (b) same experiment run with double the confining reagent.

observe. For example, compare the array illustrated in Figure 3a with the array in Figure 3b that clearly shows the checkerboard in a box pattern. The image shown in Figure 3b was obtained by doubling the concentration of allyl

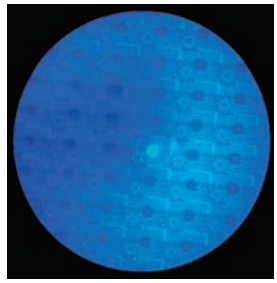


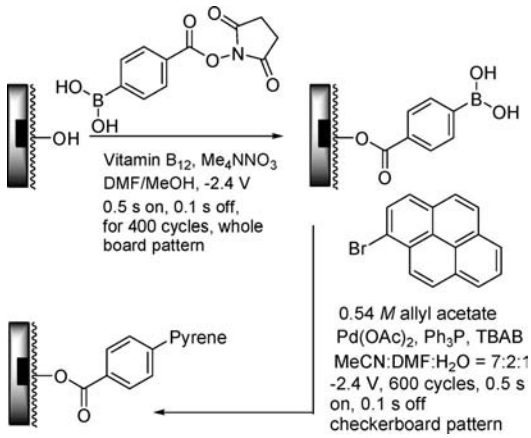
Figure 4. Fluorescence image of an air confined Suzuki reaction run on a 1 K-array using a potential at the working electrode of −1.4 V relative to the counter electrode.

acetate confining agent used for the reaction from 0.54 *M* to 1.08 *M*. Clearly, site-selective Suzuki reactions can be accomplished on a 12 K-microelectrode array.

Next, a Suzuki reaction was run using air as the confining agent. No allyl acetate was added to the reaction. In this case, the current used for the reaction needed to be reduced further in order to maintain confinement to a preselected electrode. This was accomplished by decreasing the potential at the working microelectrode to −1.4 V relative to the counter electrode (Figure 4). In this experiment, a single microelectrode in a 1 K-array was used as a cathode to generate the Pd(0) catalyst. The need to reduce the rate at which the Pd(0) was generated in order to maintain confinement suggests that oxygen is not as an effective scavenger for Pd(0) as is the allyl acetate used in the previous experiments.

Finally, an "inverse-Suzuki" reaction was done on a 1 K-microelectrode array. In this experiment, a boronic acid coupling partner was placed on the array and a bromopyrene used as the aromatic ring in solution (Scheme 3). The challenge

Scheme 3



of running the reaction in this direction is that the generation of Pd(0) at the selected microelectrodes is followed by insertion of the catalyst into a solution phase aryl bromide bond. This results in a reactive aryl-Pd(II) species that is not tied to the surface of the array. The confining agent, which is an oxidant for Pd(0), will have no effect on this reactive intermediate. Instead confinement of the aryl-Pd(II) species depends solely on the relative rates of diffusion away from the selected cathode and capture of the aryl-Pd(II) intermediate by the boronic acid on the surface of the array. If the rate of the Suzuki reaction is significantly faster than diffusion away from the electrode, then the reaction will be confined. If it is not, then the reaction will lose confinement. In practice, the reaction was performed in the same manner as the earlier 1 K-array reaction. A high level of confinement to the selected microelectrodes was obtained (Figure 5) indicating that the Suzuki reaction is fast enough to

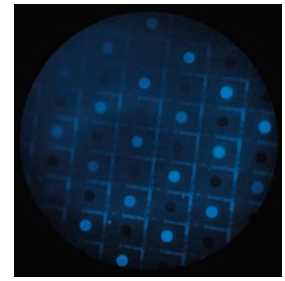


Figure 5. Fluorescence image of the inverse Suzuki reaction.

avoid migration of the aryl-Pd(II) intermediate away from its point of origin. Unlike the earlier Suzuki reaction, the "inverse-Suzuki" worked well even when the Pd(0) reagent needed is generated at a fast rate (electrode potential of −2.4 V vs the counter electrode). This observation appears to be a result of the high concentration of bromopyrene used in the reaction. The bromopyrene also reacts with the Pd(0) generated to prevent its migration to neighboring electrodes.

In conclusion, the reaction conditions developed for achieving site-selective Heck reactions on a microelectrode array have been successfully extended to the Suzuki reaction. Both allyl acetate and oxygen can be used as confining agents for the faster Suzuki reaction if the rate of reagent generation is limited and the concentration on the confining agent raised. The reactions work well with either an aryliodide or an arylboronic acid attached to the surface of the microelectrode array and the other coupling partner in solution. Finally, site-selective Suzuki reactions can be accomplished on either 1 K- or 12 K-arrays.

Acknowledgment. We thank the National Science Foundation (CHE-0613077) for their generous support of our 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: Sample experimental procedures are included for both placing substrates onto the microelectrode arrays and the site-selective Suzuki reaction along with characterization data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL900056U