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Microelectrode Arrays, Dihydroxylation, and the Development of an Orthogonal Safety-Catch Linker

Nai-Hua Yeh, Ruby Krueger, and Kevin D. Moeller*

Cite This: *Org. Lett.* 2021, 23, 5440–5444 Read Online

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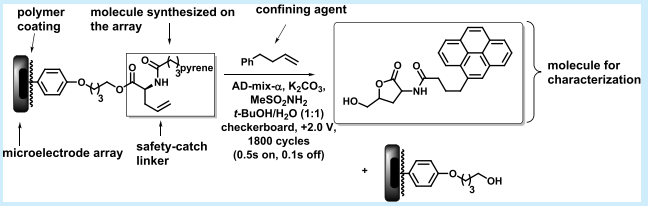
ABSTRACT: Construction of larger molecular libraries on an addressable microelectrode array requires a method for recovering and characterizing molecules from the surface of any electrode in the array. This method must be orthogonal to the synthetic strategies needed to build the array. We report here a method for achieving this goal that employs the site-selective dihydroxylation reaction of a simple olefin.



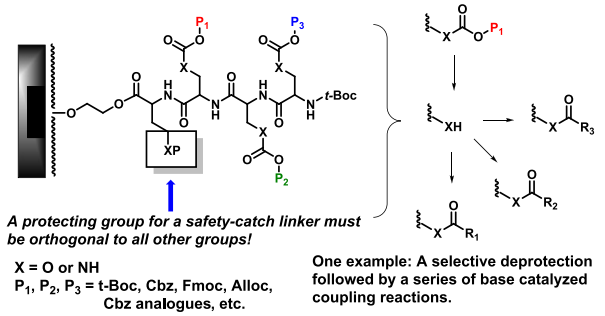
Because they can serve as tools for evaluating biological interactions in real time,[1,2] we have been developing the synthetic chemistry needed to place the members of a molecular library by any electrode or combination of electrodes in a microelectrode array.[3] For each method developed, the transformation uses the selected electrode or electrodes to generate the required catalyst or reagent. That catalyst or reagent is then confined to the surface of the selected electrode with the use of a solution-phase "confining agent" that destroys it before it can migrate to any other site on the array. The strategy allows for the site-selective functionalization of any electrode in the array, even at electrode densities up to 12,544/cm^2.[4]

While the chemistry works well, it is currently limited in terms of the size of the library that can be generated. We can place molecules by any electrode in an array, but to assemble a larger addressable library requires building the molecular library elsewhere and then transferring the individual members of the library to the electrodes on the array one at a time. Obviously, a better method would be to synthesize the molecular library directly on the arrays, a challenge that amounts to the total synthesis of a complex, two-dimensional addressable array.

Of course, the development of synthetic strategies for building molecules directly on the arrays also requires a method for characterizing those molecules once they are made. To do so, the molecules on the surface of any electrode in a high-density microelectrode array must be selectively removed. To ensure the fidelity of the process, the same electrode used for the synthesis and monitoring of the molecules in question must also be used for their recovery. In addition, the method must be orthogonal to the synthetic chemistry used to make the molecules and stable to the analytical methods employed.

To date, characterization methods of this nature have taken advantage of a Kenner-type safety-catch linker strategy.[5] In this approach, a protected alcohol or amine (XP in Scheme 1) is unmasked after the synthesis of a molecule. A subsequent cyclization reaction to form a lactone cleaves the molecule from the surface. For the arrays, the safety-catch linker used has taken advantage of a *t*-Boc protected alcohol (XP = O*t*-Boc) that could be cleaved by generating acid at a selected electrode.[6]

Scheme 1. Requirements for a Safety-Catch Linker to Be Used for Building Molecular Libraries



While the use of the *t*-Boc group worked fine for assessing the success of individual reactions run on an array, it is a poor choice for efforts to build a molecular library directly on an array. Consider the proposal shown in Scheme 1. In this approach, a core scaffold would contain a series of protected amines and/or alcohols that could each be independently deprotected and utilized as a site for diversification. The diversification step would use the alcohol or amine as a nucleophile in a coupling step that is frequently catalyzed with base. To do the coupling step site-selectively on an array, the

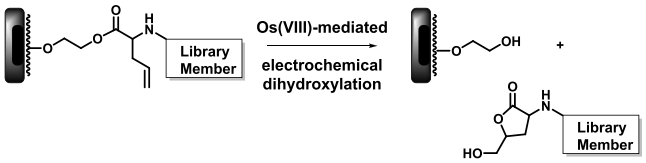
Received: May 28, 2021
Published: June 29, 2021

https://doi.org/10.1021/acs.orglett.1c01675
Org. Lett. 2021, 23, 5440–5444

array needs to be submerged in an acidic solution (the confinement strategy for the generation of a base)[3a] and then the selected electrodes used as a cathode to generate the base-catalyst needed. Under such conditions, a safety-catch linker with an acid cleavable *t*-Boc group would not be stable.

As an alternative, it is tempting to suggest a reductively or oxidatively cleavable protecting group for the alcohol or amine in the safety-catch linker. However, the use of such a group in the linker precludes its use for the diversity oriented synthesis portion of the strategy, a scenario that is again less than ideal given the short supply of such protecting groups and the need to avoid the use of acid- and base-cleavable protecting groups for the coupling steps in the sequence. What is needed is a safety-catch linker that avoids the use of an alcohol or amine protecting group altogether but can still be cleaved with the use of any electrode or set of electrodes in the array. We report here that the dihydroxylation of a simple monosubstituted olefin provides an ideal solution to this challenge (Scheme 2).[7]

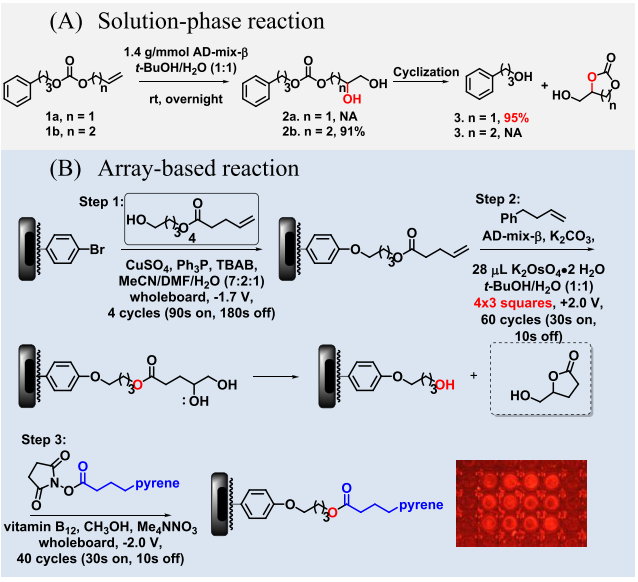
Scheme 2. Use of a Dihydroxylation Strategy



The olefin is stable, the oxidation can be conducted selectively at any electrode in an array,[8] and the subsequent cyclization and cleavage of the molecule from the electrode surface occurs spontaneously.

Development of this approach began with a solution-phase test of the overall concept (Scheme 3A). For the initial "proof

Scheme 3. Initial Studies



of principle" experiment, an Alloc protecting group was used, since it could be synthesized with available reagents (Scheme 3A). Following its synthesis, substrate **1a** was treated with AD-mix-β using the standard tBuOH/water reaction conditions to generate the cis-hydroxylated product. The product could not be isolated but instead immediately underwent the cleavage reaction desired for the safety-catch strategy to afford a 95% isolated yield of the alcohol product **3**.

A similar reaction with the related six-membered ring precursor **1b** was conducted. In this case, the oxidation led to the dihydroxylated product **2b** in a 91% isolated yield, but generation of the diol did not spontaneously lead to the cleavage step. The difference between the two substrates reflected the rate of the cyclization reaction with the five-membered ring cyclization being much faster. The five-membered ring strategy was selected for further development, since cleavage from an array in a single step was highly desirable. It was for this same reason that an ester linkage was chosen for attachment of the substrate to the array rather than an amide linkage. While amide linkages are certainly compatible with Kenner-type safety-catch linker strategies, we wanted to use a leaving group for the required addition–elimination strategy that would depart faster under neutral conditions.

To test the deprotection strategy on an array, substrate **4** was synthesized and placed by every electrode in an array (Scheme 3B). For this experiment, the Alloc group used in substrate **1** was replaced with a 4-pentenoic acid derivative in order to avoid the use of a protecting group that could be employed to mask a site of diversification in future synthetic efforts. The placement of the substrate by the electrodes in the array was achieved by coating the array with a diblock copolymer containing a 4-bromo-polystyrene block and a cinnamate functionalized methacrylate block and then photo-cross-linking the cinnamate groups in order to add stability to the surface.[9] Each electrode in the array was then used as a cathode to drive a Cu(I)-catalyzed cross-coupling reaction between the aryl bromide on the polymer surface and substrate **4**.[10] In this experiment, the potential listed is the potential drop across the cell and therefore controls the current used or the rate at which the catalyst is generated. Typically, the electrode is cycled on and off to help with confinement of the catalyst to the electrode used for its generation. Since this was a first time trial with the substrate being placed by every electrode in the array, the conditions used for previous placement reactions were selected.[3a]

The dihydroxylation reaction was performed on the functionalized array by using blocks of 12 microelectrodes each (a 4 × 3 pattern) as anodes in order to recycle the Fe(III) co-oxidant needed to generate catalytic amounts of the active Os(VIII) reagent.[8] 4-Phenyl-1-butene was added to the solution above the array as a confining agent so that any Os(VIII) that escaped from the surface of the selected electrodes would be consumed before it could reach a remote site on the array. This experiment was accomplished by treating the array with a 1:1 tBuOH/H_2O solution containing 0.07 g of AD-mix-β, 28 μL of 68 mM $K_2OsO_4 \cdot 2H_2O$, K_2CO_3 as base, and 1.0 equiv of the 4-phenyl-1-butene confining agent. This mixture was stirred overnight in order to make sure that all of the Os(VIII) reagent was consumed prior to the start of the array reaction. For the reaction shown in Scheme 3B (a reaction run on a 12K array),[4] the selected electrodes were set at a potential of +2.0 V relative to the counter electrode. This was done for a period of 30 s followed by a period of 10 s where the electrodes were turned off. This was repeated 60 times. The cycling of the electrodes was done in order to balance the rate of Os(VIII) generation with the rate that it was consumed by the surface substrate, and in so doing

https://doi.org/10.1021/acs.orglett.1c01675
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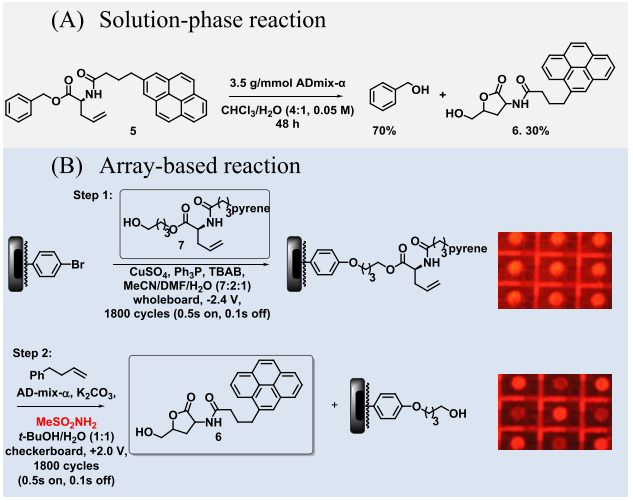
optimize confinement of the reaction to the selected electrodes.

After the dihydroxylation reaction and cleavage of the linker, all of the electrodes in the array were used to reduce vitamin B_{12} and generate a base for catalyzing an esterification reaction between any alcohol on the surface of the array and a pyrene-labeled activated ester.[11] The array was then examined using fluorescence microscopy. From the image provided in Scheme 3B, it was clear that the esterification reaction only occurred on the electrodes used for the dihydroxylation reaction. This indicated that the dihydroxylation was selective, but it did not prove that the cleavage reaction had occurred, since the esterification reaction used to fluorescently label the product would also occur with the dihydroxylated intermediate.

The issue was addressed by taking advantage of the chemistry the linker was designed for. To this end, the lactone product from the cleavage reactions was independently synthesized (Scheme 4A)[12] and fully characterized. In this case, the instability of the lactone made it difficult to isolate in high yield relative to the corresponding alcohol product. Nevertheless, the solution-phase reaction did provide enough of the lactone product **6** for characterization. Due to the differences between solution-phase reactions and array-based reactions, further optimization of the solution-phase reaction was not pursued.

Scheme 4. Schematic Showing the Array-Based Reaction Does Form the Same Product as the Solution-Phase Study



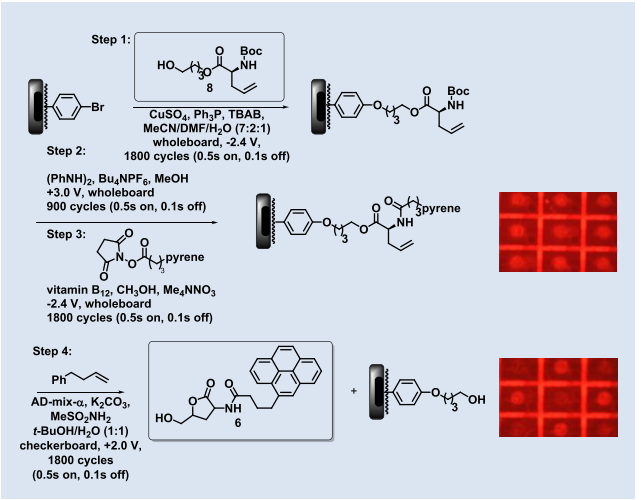
The safety-catch linker substrate **7** was then synthesized and added to the surface of every electrode in an array having 1024 microelectrodes/cm^2, as shown in Scheme 4B. In this case, a longer reaction time (more cycles) and a higher voltage (higher current and faster catalyst generation) were used relative to the experiment shown in Scheme 3B in order to maximize the amount of substrate placed on the array. Note that the image provided in Scheme 3B was not as intense as one might like and showed incomplete coverage of the electrodes. Since our goal for this experiment was to recover material from the electrode surface for characterization, we maximized the amount of material placed by each electrode.

Next, the dihydroxylation was performed using a checkerboard pattern of electrodes. This was done by submerging the array along with a remote Pt-wire counter electrode in a 1:1 solution of tBuOH and water containing AD-mix β, methanesulfonamide, potassium carbonate, and 4-phenyl-1-butene as a confining agent. The selected electrodes in the array were set to a potential of +2.0 V relative to the counter electrode for a period of 0.5 s and then turned off again for 0.1 s. This pattern was repeated for 1800 cycles.

The success of the reaction can be seen by comparing the fluorescence images of the array taken before and after the dihydroxylation. In this case, the substrate for the deprotection reaction contained a pyrene group that was cleaved from the surface of the array, leading to decreased fluorescence at the selected electrodes. A small amount of fluorescence did remain, indicating the potential for an incomplete reaction. This is not a problem as long as enough of the cleavage occurs for the product to be characterized, which turned out to be the case. The material cleaved from the array was collected, examined by HRMS, and shown to be lactone **6**. The cleavage reaction on the array clearly worked as designed and afforded the same product as the solution-phase reaction.

In order to verify that the cleavable linker was compatible with array-based coupling reactions, the experiment shown in Scheme 5 was conducted. In this case, the *t*-Boc-protected allyl-glycine substrate **8** was placed by every electrode in a 1K array and then the *t*-Boc group removed by using the electrodes as anodes to oxidize diphenylhydrazine and generate an acid.[6] The electrodes were then used as cathodes to induce a base-catalyzed coupling reaction between the deprotected amine and the NHS-ester of 4-pyrene butanoic acid to form the pyrene-labeled allylglycine.[11]

Scheme 5. Testing Linker Compatibility with Peptide Synthesis



With the substrate for the dihydroxylation reaction assembled, the electrodes were once again used as anodes to conduct the dihydroxylation reaction and cleave the molecule from the surface of the array. As in the previous experiment, the transformation led to both a decrease in fluorescence on the arrays and the isolation and characterization by HRMS of lactone **6** from the solution above the array. The safety-catch linker strategy was clearly compatible with the acid- and base-catalyzed deprotection and coupling steps used for the synthesis of peptides on a microelectrode array.

The synthetic sequence shown in Scheme 5 was intriguing in that it illustrated the versatility of the arrays for synthesis. For the chemistry, the electrodes were used alternatively as

https://doi.org/10.1021/acs.orglett.1c01675
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cathodes (the Cu(I) coupling reaction and the amide coupling) and anodes (the *t*-Boc deprotection and dihydroxylation steps) with no change required to the electrolysis setup. Each reaction was a simple constant current electrolysis where the polarity of the electrode was set either negative or positive and then the working potential of the electrode allowed to adjust to whatever catalyst was being used.[13] In addition, it should be noted that each of the reactions conducted is not actually an "electrochemical reaction". The electrochemistry is used to locate a standard chemical reagent or catalyst at a site on the array. The chemistry takes place on the surface of the polymer coating away from the electrode. Therefore, the selectivity of the reactions should be the same as that for any other solution-phase or solid-phase synthesis. The cis-hydroxylation reaction should show the same selectivity between olefin substrates it normally does.

In conclusion, we have demonstrated that a simple olefin can form the basis of a synthetically orthogonal safety-catch linker. For cleavage of the linker on an addressable array, the electrodes in the array were used to generate an Os(VIII) species that in turn triggered a cis-hydroxylation of the olefin. One of the resulting alcohols then underwent a spontaneous cyclization to generate a lactone and remove the molecule from the surface of the electrode. The success of the reaction was indicated by both fluorescence microscopy and HRMS characterization of the lactone produced. In this fashion, a molecule at any electrode in the array can be cleaved from the array for characterization. This development provides the foundation for constructing molecular libraries directly on the surface of a microelectrode array and then verifying the structure of the synthesized molecules. Since the safety-catch linker is simply allylglycine, allylglycine is used frequently as a building block in peptide synthesis, and no naturally occurring amino acid contains an olefin, the strategy appears particularly well suited for the synthesis, analysis, and characterization of peptides on a microelectrode array.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c01675.

Complete experimental procedures for the site-selective reactions are included (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Kevin D. Moeller − *Washington University in Saint Louis, Saint Louis, Missouri 63130, United States;* orcid.org/0000-0002-3893-5923; Email: moeller@wustl.edu

Authors

Nai-Hua Yeh − *Washington University in Saint Louis, Saint Louis, Missouri 63130, United States*

Ruby Krueger − *Washington University in Saint Louis, Saint Louis, Missouri 63130, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.1c01675

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the National Institutes of Health (1R01 GM122747) for their generous support of our work.

■ REFERENCES

(1) For selected examples, see: (a) Chandra, S.; Siraj, S.; Wong, D. K. Y. Recent Advances in Biosensing for Neurotransmitters and Disease Biomarkers using Microelectrodes. *ChemElectroChem* **2017**, *4* (4), 822−833. (b) Lee, E.-j.; Chan, E. W. L.; Luo, W.; Yousaf, M. N. Ligand slope, density and affinity direct cell polarity and migration on molecular gradient surfaces. *RSC Adv.* **2014**, *4* (60), 31581−31588. (c) Li, J.; Sun, C.-L.; An, P.; Liu, X.; Dong, R.; Sun, J.; Zhang, X.; Xie, Y.; Qin, C.; Zheng, W.; Zhang, H.-L.; Jiang, X. Construction of Dopamine-Releasing Gold Surfaces Mimicking Presynaptic Membrane by On-Chip Electrochemistry. *J. Am. Chem. Soc.* **2019**, *141* (22), 8816−8824. (d) Soscia, D. A.; Lam, D.; Tooker, A. C.; Enright, H. A.; Triplett, M.; Karande, P.; Peters, S. K. G.; Sales, A. P.; Wheeler, E. K.; Fischer, N. O. A flexible 3-dimensional microelectrode array for in vitro brain models. *Lab Chip* **2020**, *20* (5), 901−911.

(2) For examples using the arrays employed here, see: (a) Stuart, M.; Maurer, K.; Moeller, K. D. Moving Known Libraries to an Addressable Array: A Site-Selective Hetero-Michael Reaction. *Bioconjugate Chem.* **2008**, *19*, 1514. (b) Fellet, M. S.; Bartels, J. L.; Bi, B.; Moeller, K. D. Site-Selective Chemistry and the Attachment of Peptides to the Surface of a Microelectrode Array. *J. Am. Chem. Soc.* **2012**, *134* (40), 16891−16898. (c) Graaf, M. D.; Marquez, B. V.; Yeh, N.-H.; Lapi, S. E.; Moeller, K. D. New Methods for the Site-Selective Placement of Peptides on a Microelectrode Array: Probing VEGF−v107 Binding as Proof of Concept. *ACS Chem. Biol.* **2016**, *11* (10), 2829−2837.

(3) For selected reviews, see: (a) Graaf, M. D.; Moeller, K. D. Introduction to Microelectrode Arrays, the Site-Selective Functionalization of Electrode Surfaces, and the Real-Time Detection of Binding Events. *Langmuir* **2015**, *31* (28), 7697−7706. (b) Moeller, K. D. Using Physical Organic Chemistry To Shape the Course of Electrochemical Reactions. *Chem. Rev.* **2018**, *118* (9), 4817−4833. (c) Yeh, N.-H.; Zhu, Y.; Moeller, K. D. Electroorganic Synthesis and the Construction of Addressable Molecular Surfaces. *ChemElectroChem* **2019**, *6* (16), 4134−4143.

(4) For a description of the microelectrode arrays used here and a detailed discussion of how the array reactions are run on these devices, see: (a) Dill, K.; Montgomery, D. D.; Wang, W.; Tsai, J. C. Antigen detection using microelectrode array microchips. *Anal. Chim. Acta* **2001**, *444*, 69−78. (b) Bartels, J.; Lu, P.; Maurer, K.; Walker, A. V.; Moeller, K. D. Site-Selectively Functionalizing Microelectrode Arrays: The Use of Cu(I)-Catalysts. *Langmuir* **2011**, *27*, 11199−11205. [Supporting Information - 12K slide: diameter = 44 μm; distance between the Pt-electrodes (square cells) = 33 μm]

(5) Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. The safety catch principle in solid phase peptide synthesis. *J. Chem. Soc. D* **1971**, 636.

(6) (a) Bi, B.; Maurer, K.; Moeller, K. D. Building Addressable Libraries: The Use of "Safety-Catch" Linkers on Microelectrode Arrays. *J. Am. Chem. Soc.* **2010**, *132*, 17405. (b) Bi, B.; Huang, R. Y.-C.; Maurer, K.; Chen, C.; Moeller, K. D. Site-Selective, Cleavable Linkers: Quality Control and the Characterization of Small Molecules on Microelectrode Arrays. *J. Org. Chem.* **2011**, *76* (21), 9053−9059.

(7) For selected electrochemical dihydroxylation examples, see: (a) Amundsen, A. R.; Balko, E. N. Preparation of chiral diols by the osmium-catalysed, indirect anodic oxidation of olefins. *J. Appl. Electrochem.* **1992**, *22* (9), 810−816. (b) Torii, S.; Liu, P.; Tanaka, H. Electrochemical Os-Catalyzed Asymmetric Dihydroxylation of Olefins with Sharpless' Ligand. *Chem. Lett.* **1995**, *24*, 319−320. (c) Torii, S.; Liu, P.; Bhuvaneswari, N.; Amatore, C.; Jutand, A. Chemical and Electrochemical Asymmetric Dihydroxylation of Olefins in I2−K2CO3−K2OsO2(OH)4 and I2−K3PO4/K2HPO4−K2OsO2(OH)4 Systems with Sharpless' Ligand. *J. Org. Chem.* **1996**, *61* (9), 3055−3060. (d) Nguyen, B. H.; Perkins, R. J.;

Smith, J. A.; Moeller, K. D. Photovoltaic-driven organic electrosynthesis and efforts toward more sustainable oxidation reactions. *Beilstein J. Org. Chem.* **2015**, *11*, 280−287.
(8) Nguyen, B. H.; Kesselring, D.; Tesfu, E.; Moeller, K. D. Microelectrode Arrays: A General Strategy for Using Oxidation Reactions To Site Selectively Modify Electrode Surfaces. *Langmuir* **2014**, *30* (8), 2280−2286.
(9) For the diblock copolymer used on arrays, see: (a) Hu, L.; Bartels, J. L.; Bartels, J. W.; Maurer, K.; Moeller, K. D. A New Porous Reaction Layer for Developing Addressable Molecular Libraries. *J. Am. Chem. Soc.* **2009**, *131* (46), 16638−16639. (b) Hu, L. B.; Graaf, M. D.; Moeller, K. D. The Use of UV-Cross-Linkable Di-Block Copolymers as Functional Reaction Surfaces for Microelectrode Arrays. *J. Electrochem. Soc.* **2013**, *160* (7), G3020−G3029. (c) Yeh, N.-H.; Medcalf, M.; Moeller, K. D. Organic Electrochemistry and a Role Reversal: Using Synthesis to Optimize Electrochemical Methods. *J. Am. Chem. Soc.* **2018**, *140* (24), 7395−7398.
(10) Bartels, J.; Lu, P.; Maurer, K.; Walker, A. V.; Moeller, K. D. Site-Selectively Functionalizing Microelectrode Arrays: The Use of Cu(I)-Catalysts. *Langmuir* **2011**, *27* (17), 11199−11205.
(11) For a general procedure, please see refs 2a and 3.
(12) (a) Girard, A.; Greck, C.; Genet, J. P. Rapid syntheses of 3-amino-5-hydroxymethyl-γ-lactones from L-allylglycine. *Tetrahedron Lett.* **1998**, *39* (24), 4259−4260. (b) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. Practical asymmetric syntheses of.alpha.-amino acids through carbon-carbon bond constructions on electrophilic glycine templates. *J. Am. Chem. Soc.* **1988**, *110* (5), 1547−1557.
(13) For a description of basic electrochemical concepts for synthetic chemists, see: (a) Moeller, K. D. Synthetic Applications of Anodic Electrochemistry. *Tetrahedron* **2000**, *56*, 9527−9554. (b) Hilt, G. Basic Strategies and Types of Applications in Organic Electrochemistry. *ChemElectroChem* **2020**, *7*, 395−405. (c) Kingston, C.; Palkowitz, M. D.; Takahira, Y.; Vantourout, J. C.; Peters, B. K.; Kawamata, T.; Baran, P. S. A Survival Guide for the "Electro-curious. *Acc. Chem. Res.* **2020**, *53*, 72−83. For a detailed discussion and reviews, see: Hammerich, O.; Speiser, B. *Organic Electrochemistry*, 5th ed.; CRC Press: Boca Raton, FL, 2016.

https://doi.org/10.1021/acs.orglett.1c01675
Org. Lett. 2021, 23, 5440−5444