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Site-Selective, Cleavable Linkers: Quality Control and the Characterization of Small Molecules on Microelectrode Arrays

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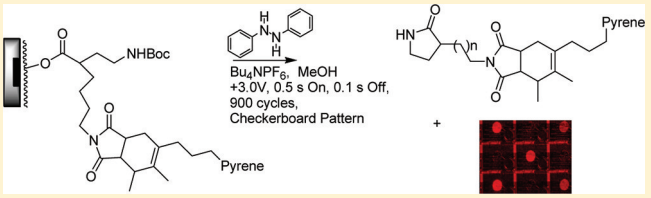
Supporting Information

ABSTRACT: A "safety-catch" linker strategy has been used to release a portion of the products of a Diels–Alder reaction conducted on a microelectrode array for characterization of stereochemistry. The attachment and cleavage of organic compounds from the surface of selected electrodes in the array can be accomplished by site-selective generation of base or acid at the electrode. It was found that the surface of the array had a minor influence on the stereochemistry of the Diels–Alder reaction, leading to slightly more of the exo-product relative to a similar solution-phase reaction.



INTRODUCTION

Microelectrode arrays[1−3] have great potential as platforms for building addressable molecular libraries that can be monitored in "real time".[4] To accomplish this goal, molecules are built or placed proximal to unique, individually addressable microelectrodes in the array. The electrodes are then used to monitor binding events between the molecules in the library and biological targets. The process begins by coating the microelectrode array with a porous reaction layer that provides the functionality needed for anchoring molecules to the surface of the electrodes.[5] The electrodes are then used to conduct site-selective reactions that first attach molecules to the reaction layer at specific locations in the array and then modify the molecules located on the array as needed.[6−11] Once this effort is completed, analytical efforts can start. However, how does one know that reactions run on an array are successful, and how does running a reaction on the surface of an array alter the product formed?

Initial attempts to address these questions focused on the use of mass-spectrometry-cleavable linkers.[12] The linkers were used in connection with TOF-SIMS experiments to determine the mass and fragmentation pattern of molecules attached to the polymer surface coating the array. Because TOF-SIMS has a resolution of approximately 50 μm and the diameter of a microelectrode on an array having 12 544 microelectrodes/cm^2 is 43 μm, the experiments could examine molecules bound next to any given electrode in the array. However, TOF-SIMS analysis would sacrifice the array, making it impossible to characterize molecules in the molecular library prior to biological studies or to reuse a library once the molecules had been characterized. Also, mass spectrometry does not allow for characterization of the stereochemistry of a molecule synthesized on an array. This is a major limitation because our goal is to functionalize arrays with molecules of designed shape and then employ them in efforts to map the three-dimensional binding preferences of biological receptors.

In principle, both issues can be addressed with the use of a linker that can be chemically cleaved using the electrodes in the array. Such a scenario would allow for the recovery of the molecules bound next to any one of the electrodes. Our initial attempt employed an Fmoc-type base-cleavable linker, shown in Scheme 1, placed on the array by a Heck reaction.[13] For the image shown on the left in Scheme 1, the Fmoc linker was labeled with a pyrene group and then coupled to every position in the array. A checkerboard pattern of electrodes was then used to reduce vitamin B_{12} to its radical anion and generate a base at every other electrode in the array.[4b,8] The chemistry was successful, and the checkerboard pattern can be seen in the image on the right in Scheme 1, although a number of complications did arise. Chief among them was the sensitivity of the linker. It is cleaved under very mildly basic conditions. This makes the linker very difficult to synthesize and the modified surface of the array unstable once the linker is placed by the microelectrodes.

A more stable "safety-catch" linker strategy was judged to be preferable.[14] In this strategy, the linker contains either an alcohol or amine functional group that is protected as a masked nucleophile. Deprotection of the nucleophile triggers a cyclization reaction that results in cleavage of the molecule from the solid support or surface. Adaptation of this strategy to a microelectrode array was straightforward (Scheme 2). Linkers containing nucleophiles masked with an acid-cleavable protecting group would be used to attach molecules by the microelectrodes in an array. To recover a molecule from any location on the array would then involve using the electrode at

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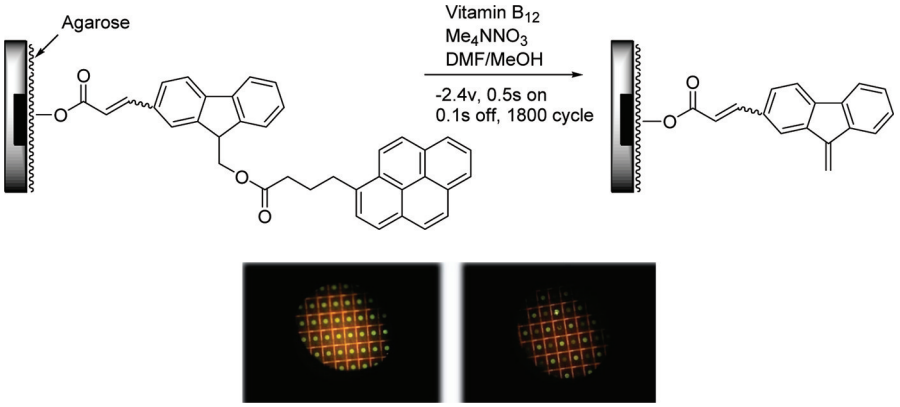
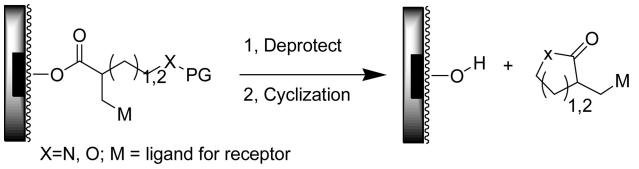
Scheme 1



Scheme 2

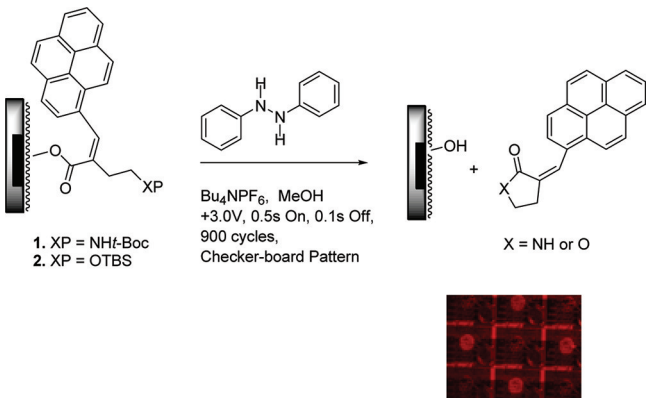


that location to generate acid,[9] deprotect the nucleophile in the linker, and cleave the molecule of interest from the surface.

RESULTS AND DISCUSSION

Proof-of-principle for this plan was gained by examining the two test cases illustrated in Scheme 3.[15] In both cases, the base-catalyzed esterification reaction developed previously[4b] was used to place the substrate by every microelectrode in the array. Because the linker was attached to a pyrene group, a bright fluorescent spot appeared by each electrode. The protecting group was then removed from selected electrodes by using the electrodes as anodes to oxidize diphenylhydrazine, leading to the generation of acid and diazobenzene.[9] The acid was confined to the selected electrodes in the array by using excess hydrazine. With substrate **1**, the acid led to cleavage of the *t*-Boc group followed by formation of a lactam byproduct. In the case of **2**, cleavage of the TBS-protecting group led to the formation of a lactone. In both cases, the solution above the array was analyzed by HPLC and the product lactam or lactone identified by coinjection with independently synthesized material. The image shown in Scheme 3 shows an array that

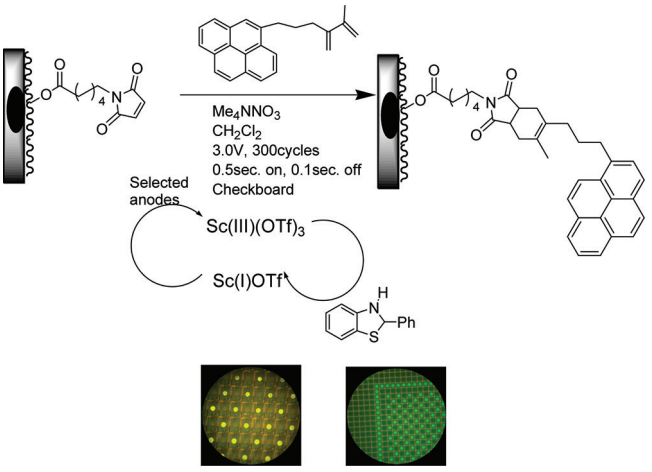
Scheme 3



was functionalized by each microelectrode with **1** and then utilized to cleave the protecting group from every other electrode, leaving a checkerboard pattern of fluorescence on the array. The use of linker **2** led to a similar image.

With the safety-catch linker strategy in place, efforts to answer specific questions about array-based reactions could begin. Recently, we demonstrated that Diels–Alder reactions can be conducted on microelectrode arrays by generating a Sc(III) catalyst at selected electrodes (Scheme 4).[11] The Lewis

Scheme 4. (Left) Reaction on an Array Having 1024 microelectrodes/cm^2; (Right) Reaction on an Array Having 12 544 microelectrodes/cm^2



acid catalyst was confined to the selected electrodes with the use of a 2-arylbenzothiazole in solution, which reduces any Sc(III) catalyst that escapes from the region surrounding the electrode where it was generated. In this work, the presence of the product on the array was tentatively identified by fluorescence. Typically, Lewis-acid-catalyzed Diels–Alder reactions give predominately endo products, but the surface of the array may alter this result.

In order to address this issue, the fluorescent group used for the analysis of **1** and **2** needed to be replaced with either a diene or a dienophile. This was accomplished as shown in Scheme 5. On the basis of the model systems highlighted in Scheme 3, a protected amine was selected as the masked nucleophile for the linker. Use of the masked amine leads to cleaner removal of the linker from the array.

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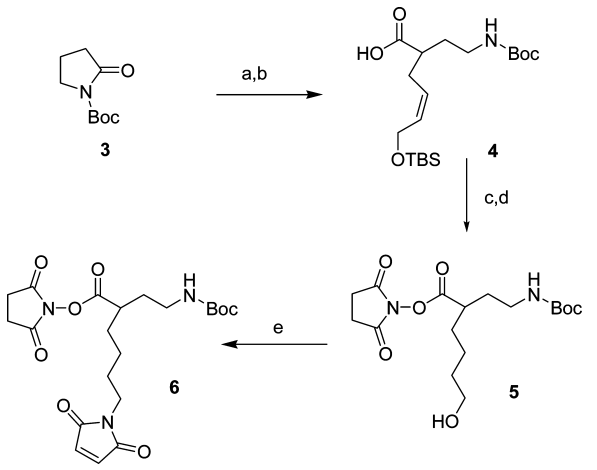
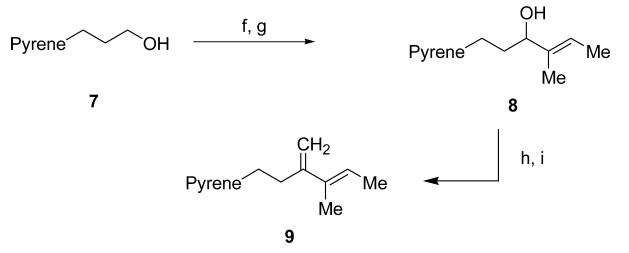
Scheme 5 [a]



[a]Reagents and Conditions: (a) LDA, $BrCH_2CH{=}CHCH_2OTBS$, −78 °C, 84%. (b) LiOH, THF/H_2O (5:1), RT, 89%. (c) *N*-hydroxysuccinimide, DCC, DMF, RT, 81%. (d) H_2/ Pd−C, MeOH, RT, 76%. (e) DEAD, PPh_3, neopentyl alcohol, maleimide, RT, 79%.

The diene (**9**) needed for the Diels−Alder reaction was designed so that it contained both the substituents needed for generating exo and endo products in the Diels−Alder reaction and a fluorescent group for making sure that reactions run on the array proceeded as previously observed (Scheme 6).

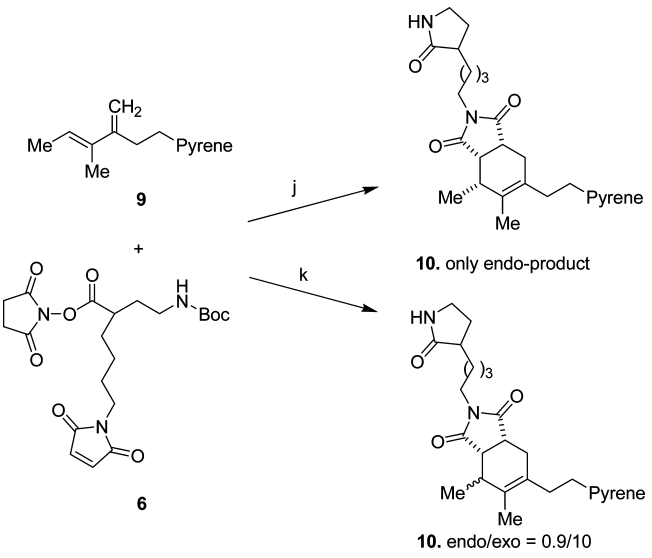
Scheme 6 [a]



[a]Reagents and Conditions: (f) PCC, CH_2Cl_2, RT. (g) (*Z*)-but-2-en-2-yllithium, THF, −78 °C. (h) Dess−Martin periodiane, CH_2Cl_2, RT. (i) $Ph_3P{=}CH_2$, THF, RT, 46% from **7**.

The substrates for the Diels−Alder were used to conduct two solution-phase reactions (Scheme 7). The first utilized Sc(III) to catalyze the reaction in order to provide the pure endo adduct. The second was conducted thermally in the absence of the Lewis acid in order to generate a mixture of endo and exo stereoisomers and identify the exo product. The products from the two reactions were analyzed by LC-MS after being converted to lactam derivatives. This was accomplished by acid-catalyzed removal of the *t*-Boc group followed by a base-catalyzed cyclization. This was done so that the products could be directly compared with products cleaved from an array. When the product from the Lewis-acid-catalyzed reaction was analyzed by LC-MS, a single peak was observed having the correct mass for the product (Figure 2). The stereochemistry of this product was established with a NOESY experiment (Figure 1). When the thermal reaction was analyzed by LC-MS, two peaks having the correct mass for the product were observed in a ratio of 0.09:1 (Figure 2). Coinjection with the product from the $Sc(OTf)_3$-catalyzed reaction showed that the major isomer formed from the thermal reaction was the same as

Scheme 7 [a]



[a]Reagents and Conditions: (j) $Sc(OTf)_3$, CH_2Cl_2, RT, TFA, CH_2Cl_2, Et_3N, RT, 71%. (k) Toluene, reflux, TFA, CH_2Cl_2, Et_3N, RT, 68%.

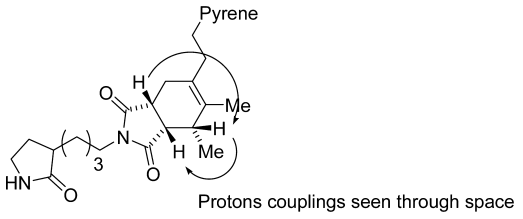


Figure 1. NOESY cross peaks for assignment of the endoproduct.

the sole product formed when the Lewis acid was used. In both cases, two stereogenic centers were formed on the lactam ring because the racemic precursor **3** was used. However, these isomers were not separated.

The corresponding microelectrode array reactions were performed as shown in Scheme 8. The process was started by using a solution of **6** in methanol/DMF solvent to place the dienophile by each microelectrode in an array with a density of 1024 microelectrodes/cm^2. The reaction between the activated ester in **6** and the agarose polymer coating the surface of the array was catalyzed by base generated at the electrodes by the reduction of vitamin B_{12}.[4b] The Diels−Alder reaction was then conducted by treating the array with a Sc(I)−reagent solution made by premixing $Sc(OTf)_3$ and 2-arylbenzothiazole. To this mixture was added diene **9** and tetramethylammonium nitrate (electrolyte). The Diels−Alder reaction was then conducted at all of the microelectrodes in the array by setting each at a potential of +3.5 V versus a remote Pt wire for 0.5 s and then turning each off again for 0.1 s. This was repeated for 600 cycles. Cycling the electrodes in this manner was done in order to control the rate at which Sc(III) was generated. Faster generation of the Sc(III) damaged the agarose surface. The success of the Diels−Alder reaction at each of the electrodes was ascertained with the use of a fluorescence microscope. The image of the array following the Diels−Alder reaction is shown on the left in Scheme 8.

Cleavage of the product from half of the electrodes was performed by submerging the array along with a remote Pt wire in 1.5 mL of a methanol solution containing both

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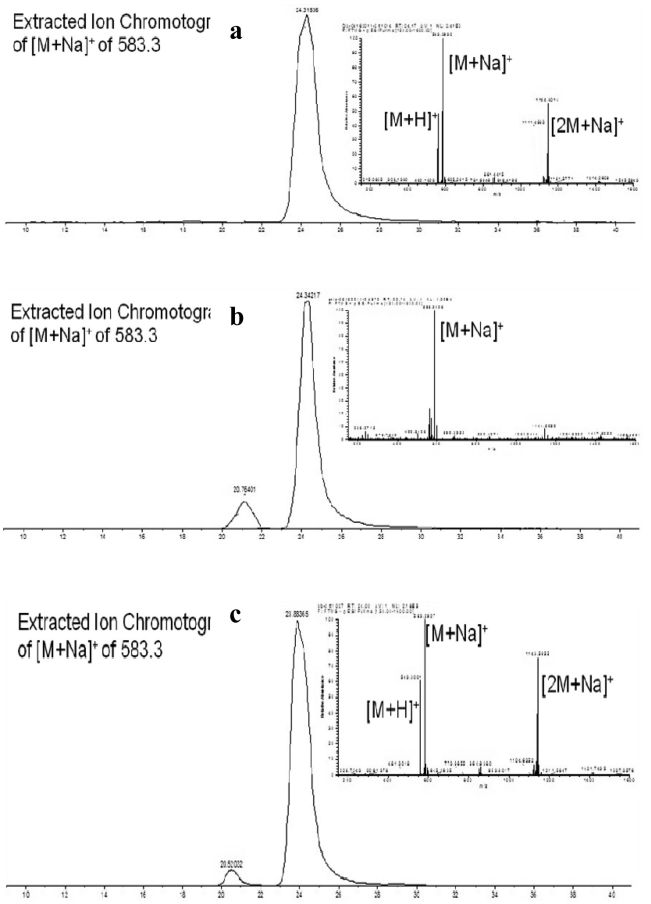

Figure 2. LC-MS data. (a) Diels–Alder product from the solution-phase reaction with the $Sc(OTf)_3$ catalyst. (b) Diels–Alder product from the solution-phase thermal reaction. (c) Diels–Alder product removed from the microelectrode array, as illustrated in Scheme 8.

1,2-diphenylhydrazine and tetrabutylammonium hexafluorophosphate (electrolyte). As in the earlier reactions,[9] excess hydrazine was used as a confining agent for the acid generated at the electrodes. A checkerboard pattern of electrodes was set at +3.0 V relative to the Pt wire in solution for a period of 0.5 s and then off again for 0.1 s. The reaction was conducted for 900 such cycles. The solution above the array was then collected and the array imaged using a fluorescence microscope, as shown on the right in Scheme 8.

LC-MS analysis of the solution revealed both endo and exo Diels–Alder products to have been formed on the array, with the endo isomer again predominating. As can be seen in Figure 2, the ratio of exo to endo product was a little smaller (approximate ratio: 0.04/1) than that obtained for the thermal solution-phase reaction. However, the presence of the array did apparently alter the stereochemical outcome of the Sc(III)-catalyzed reaction, perhaps because of increased steric demand in the surface-associated reaction.

CONCLUSIONS

One of the key challenges for any small-molecule library is quality control. How do you know that the molecules in the library are what you think they are? This question is particularly important for small-molecule libraries that contain conformational probes. In such cases, both the composition and stereochemistry of the molecules need to be characterized. With the development of site-selectively cleavable safety-catch linkers, we now have the ability to carry out such characterization studies for small-molecule libraries that are synthesized on microelectrode arrays. This ability has been demonstrated with the characterization of a Diels–Alder reaction product synthesized on an array.

One intriguing aspect of the work is that the overall strategy should be general. There is nothing "magical" about a reaction (like the Diels–Alder reaction run above) performed on an array. It employs a solution-phase chemical reagent. For a site-selective reaction, that reagent is generated at an electrode, but the reaction does not have to be run in this manner. The strategy used can be employed to examine the outcome of any reaction run on the surface. Hence, the development of the cleavable linker described above will allow us to use the arrays as an analytical platform to understand how various polymer surfaces influence any number of chemical reactions. In addition, we are now in a position to determine how long a linker between a surface and a reaction substrate needs to be in order to minimize the effect of the surface on subsequent reactions that involve the substrate.

EXPERIMENTAL SECTION

(*Z*)-Tert-butyl 3-(4-(tert-butyldimethylsilyloxy)but-2-enyl)-2-oxopyrrolidine-1-carboxylate. Diisopropyl amine (0.6 mL, 4 mmol) was dissolved in 20 mL of anhydrous THF. The solution was cooled to −78 °C, and 2.5 mL (4 mmol) of a 1.6 M *n*-BuLi in hexane solution was added in a dropwise fashion. The mixture was stirred for 30 min before adding 741 mg (4 mmol) of 1-(tert-butoxycarbonyl)-2-pyrrolidinone. The reaction was stirred at −78 °C for another 30 min before slowly adding a solution of 1.06 g (4 mmol) of (*Z*)-(4-bromobut-2-enyloxy)(tert-butyl)dimethylsilane in 10 mL of cold anhydrous THF. The mixture was stirred at −78 °C for 7 h and then quenched with saturated ammonium chloride. The resulting solution was diluted with ethyl acetate and washed with water. The organic phase was concentrated in vacuo and then chromatographed through silica gel using 15% ethyl acetate in hexane as the eluent to afford 1.24 g (84%) of the desired product. ^{1}H NMR ($CDCl_3$/300 MHz) δ5.65–5.56 (m, 1H), 5.42–5.33 (m, 1H), 4.18 (d, *J* = 6 Hz, 2H), 3.75–3.68 (m, 1H), 3.57–3.48 (m, 1H), 2.63–2.40 (m, 2H), 2.28–1.98 (m, 2H), 1.75–1.57 (m, 1H), 1.49 (s, 9H), 0.85 (s, 9H), 0.02 (s, 6H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) δ 175.0, 150.2, 132.2, 126.4, 82.7, 59.1, 44.3, 43.5, 28.1, 27.9, 25.8, 23.5, 18.2, −5.2 ppm; IR (KBr) 2930, 2856, 1786, 1749, 1715, 1472, 1367, 1318, 1253, 1153, 1088, 837, 777 cm^{-1}; HRESI MS *m*/*z* calculated for $C_{19}H_{35}NO_4SiNa$ $(M+Na)^+$: 392.2233; found: 392.2229.

(*Z*)-2-(2-(Tert-butoxycarbonylamino)ethyl)-6-(tert-butyldimethylsilyloxy)hex-4-enoic Acid (4).[16] To a solution of 1.11 g (3 mmol) of lactam in a mixture of 20 mL of THF and 5 mL of water was added 0.21 g (9 mmol) of LiOH. After stirring overnight, the reaction solution was concentrated in vacuo, diluted with water, acidified with CH_3COOH to a pH = 4–5, and then extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate. The solvent was removed by distillation under reduced pressure to afford 1.03 g (89%) of the crude product. ^{1}H NMR ($CDCl_3$/300 MHz) δ 10.60–9.50 (bs, 1H), 6.50–6.09 (bs, 0.5H), 5.59 (m, 1H), 5.39 (m, 1H), 4.96–4.68 (bs, 0.5H), 4.21 (d, *J* = 6 Hz, 2H), 3.16 (m, 2H), 2.40 (m, 2H), 2.26 (m, 1H), 1.71 (m, 2H), 1.43 (s, 9H), 0.86 (s, 9H), 0.06 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 180.0, 132.4, 126.8, 79.6, 59.6, 43.0, 38.8, 32.0, 30.3, 28.6, 26.1, 25.8, 18.5, −4.9 ppm; IR (KBr) 3334, 2930, 2857, 1710, 1519, 1471, 1409, 1367, 1252, 1171, 1089, 837, 777, 667 cm^{-1}; HRESI MS *m*/*z* calcd for $C_{19}H_{38}NO_5Si$ $(M+H)^+$: 388.2519; found: 388.2506.

(*Z*)-2,5-Dioxopyrrolidin-1-yl-2-(2-(tert-butoxycarbonylamino)-ethyl)-6-(tertbutyldimethylsilyoxy) hex-4-enoate. A round-bottom flask was charged with 968 mg of **4** (2.5 mmol), 316 mg of *N*-hydroxyl succinimide (2.8 mmol), 619 mg of DCC (3 mmol), and 7 mL of DMF. After being stirred at room temperature overnight, the reaction

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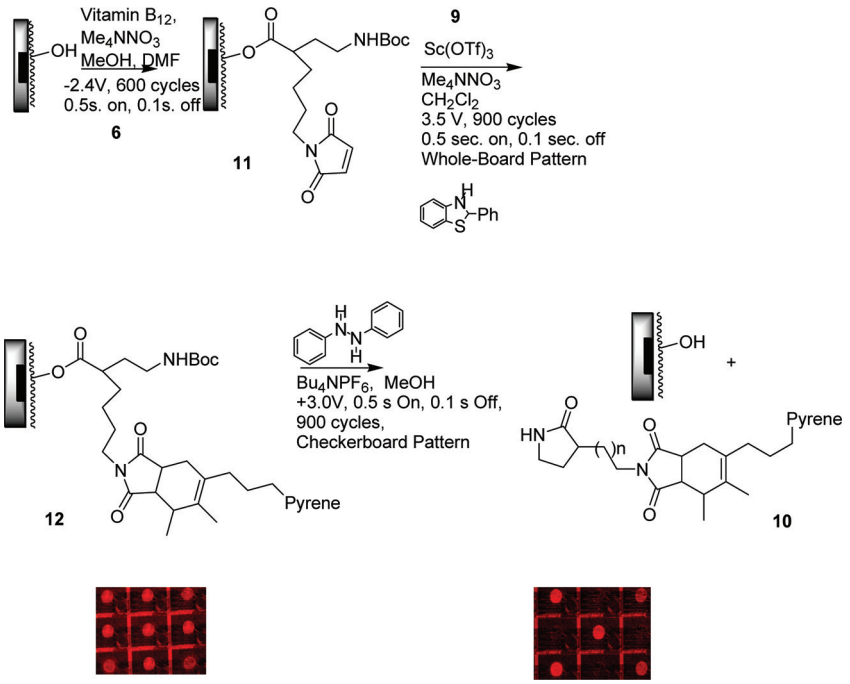
Scheme 8



mixture was poured into a 5% aqueous LiCl solution and extracted with EtOAc. The combined organic layer was washed with a saturated NaCl solution in order to remove most of the DMF, concentrated, and then chromatographed through silica gel using 30% EtOAc in hexane as the eluent to afford 981 mg (81%) of the pure product. ^{1}H NMR ($CDCl_3$/300 MHz) δ 5.64 (m, 1H), 5.40 (m, 1H), 4.98 (bs, 1H), 4.18 (m, 1H), 3.18 (m, 2H), 2.79 (s, 4H), 2.73 (m, 1H), 2.41 (m, 2H), 1.84 (m, 2H), 1.38 (s, 9H), 0.85 (s, 9H), 0.02 (s, 6H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 170.6, 169.2, 156.2, 133.2, 125.6, 79.4, 59.4, 40.8, 38.3, 32.1, 30.0, 28.6, 26.1, 25.8, 18.5, −5.0 ppm; IR (KBr) 3305, 2730, 2657, 1749, 1701, 1456, 1409, 1297, 1202, 1089, 839, 734, 657 cm^{-1}; HRESI MS m/z calcd for $C_{23}H_{40}N_2O_7SiNa$ $(M+Na)^+$: 507.2503; found: 507.2489.

2,5-Dioxopyrrolidin-1-yl 2-(2-(tert-butoxycarbonylamino)-ethyl)-6-hydroxyhexanoate (5).[17] A 25 mL pear-bottom flask was predried before being charged with 960 mg of the olefin substrate (2 mmol) and 10 mL of anhydrous methanol. Argon was bubbled through the solution for 5 min, and the resulting solution was transferred into a 25 mL round-bottom flask that contained 780 mg of 10% palladium (wt) on activated carbon. This solution was again degassed, and then the reaction was placed under a hydrogen atmosphere with the use of a balloon. The reaction was stirred for 5 h before being filtered through with Celite pad and washed with EtOAc. Chromotagraphy through a silica gel column using 50% EtOAc in hexane as the eluent led to the isolation of 566 mg (76%) of the pure product. ^{1}H NMR ($CDCl_3$/300 MHz) δ 4.97 (bs, 1H), 3.64 (t, J = 6 Hz, 2H), 3.36−3.08 (m, 2H), 2.83 (s, 4H), 2.75 (m, 1H), 1.97−1.46 (m, 9H), 1.42 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 171.3, 169.6, 156.3, 79.4, 62.3, 40.7, 38.3, 32.5, 32.3, 31.8, 28.6, 25.8, 23.2 ppm; IR (KBr) 3385, 2976, 2939, 2868, 1810, 1780, 1738, 1697, 1520, 1366, 1208, 1170, 1067, 733, 647 cm^{-1}; HRESI MS m/z calcd for $C_{17}H_{28}N_2O_7Na$ $(M+Na)^+$: 395.1794; found: 395.1784.

2,5-Dioxopyrrolidin-1-yl-2-(2-(tert-butoxycarbonylamino)-ethyl)-6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoate (6).[18] A 40% solution of diethyl azodicarboxylate in toluene (0.45 mL, 1 mmol) was added to 260 mg of PPh_3 in 10 of mL THF at room temperature. To this mixture was added 372 mg (1 mmol) of **5**. Maleimide (96 mg, 1 mmol) and 44.5 mg (0.5 mmol) of neopentyl alcohol were added; the solution was stirred overnight, and then, the reaction was concentrated under reduced pressure. The crude product was chromatographed through silica gel using 50% EtOAc in hexane as the eluent to afford 357 mg (79%) of **6**. ^{1}H NMR ($CDCl_3$/300 MHz) δ 6.68 (s, 2H), 4.92 (bs, 1H), 3.53 (t, J = 6 Hz, 2H), 3.33−3.12 (m, 2H), 2.83 (s, 4H), 2.71 (m, 1H), 1.94−1.56 (m, 7H), 1.43 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 171.1, 171.0, 169.0, 156.2, 134.2, 79.4, 40.8, 38.4, 37.6, 32.7, 31.7, 28.6, 28.4, 25.8, 24.2 ppm; IR (KBr) 3385, 2977, 2941, 2868, 1739, 1705, 1517, 1410, 1366, 1207, 1170, 1066, 829, 732, 695 cm^{-1}; HRESI MS m/z calcd for $C_{21}H_{29}N_3O_8Na$ $([M+Na)^+$: 474,1852; found: 474.1829.

4-(Pyren-4-yl)butanal. To a solution of pyridinium chlorochromate (0.646 g, 1.5 equiv.) suspended in 20 mL of dichlormethane was added 0.558 g (2 mmol) of 1-pyrenebutanol in 20 mL of dichloromethane at RT. The reaction was monitored by TLC. After no starting material remained, the black reaction mixture was diluted with ether. The ether was decanted off of the black solid, and then, the procedure was repeated two more times. The crude product was isolated by passing the ether washings through a short plug of silica gel followed by concentration of the filtrate under reduced pressure. The crude oil obtained was chromatographed through a silica gel column with 10% ethyl acetate in hexane as the eluent to afford 0.52 g (95%) of desired product as a pale yellow, needle-like crystal. The spectral data of the aldehyde matched the data previously reported.[11]

(*E*)-3-Methyl-7-(pyren-1-yl)hept-2-en-4-ol (8). A solution of 270 mg (2 mmol) of (*E*)-2-bromobut-2-ene dissolved in 10 mL of THF was placed under an argon atmosphere and cooled to −78 °C. *t*-BuLi in pentane (2.4 mL, 4 mmol) was then added in a dropwise fashion. The mixture was stirred for 30 min followed by the addition of the aldehyde synthesized above in 2 mL of THF. The reaction was held at −78 °C for 10 additional min and then warmed to room temperature. After 3 h, several drops of water were added to the reaction in order to quench any excess *t*-BuLi. The resulting mixture was then quenched with a 5% aqueous $NaHCO_3$ solution. The layers were separated, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were dried with Na_2SO_4, the solution concentrated under reduced pressure, and the product carried on to the next step without further purification.

(*E*)-3-Methyl-7-(pyren-1-yl)hept-2-en-4-one. The crude product synthesized above was dissolved in 5 mL of CH_2Cl_2. To this mixture was added 12 mL of Dess−Martin periodinane (0.3 mmol) in CH_2Cl_2 at RT. The reaction mixture was stirred for 2 h before being quenched with 5% $Na_2S_2O_3$ and 5% $NaHCO_3$ aqueous solutions. The reaction was then poured into a separatory funnel and extracted with

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EtOAc. The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo. The crude product was purified with a short silica gel column with 5% EtOAc in hexane as the eluent to afford 449 mg (68%) of the desired enone product. ^{1}H NMR ($CDCl_3$/300 MHz) δ 8.40−7.84 (m, 9H), 6.66 (q, J = 6 Hz, 1H), 3.37 (t, J = 9 Hz, 2H), 2.78 (t, J =6 Hz, 2H), 2.19 (m, 2H), 1.81 (d, J = 9 Hz, 3H), 1.79 (s, 3H); ^{13}C NMR ($CDCl_3$/300 MHz) 202.1, 144.8, 136.5, 131.2, 130.8, 130.2, 129.1, 128.3, 127.8, 127.7, 127.6, 127.0, 126.1, 125.3, 125.2, 125.1, 125.0, 124.8, 123.8, 37.1, 33.4, 26.6, 17.9, 17.7; IR (KBr) 3056, 2950, 2922, 1681, 1602, 1508, 1428, 1310, 1090, 918, 848, 756.0 cm^{-1}; HRESI MS m/z calcd for $C_{24}H_{23}O$ $(M+H)^+$: 327.1749; found: 327.1666.

(*E*)-1-(5-Methyl-4-methylenehept-5-en-1-yl)pyrene (9). Methyltriphenylphosphonium bromide (1.07 g, 3 mmol) under an atmosphere of argon was taken up in 3 mL of a 1 M sodium bis(trimethylsilyl)amide (3 mmol) in THF. After stirring for 30 min, the clear orange solution was treated with 326 mg (1.0 mmol) of the enone synthesized above in 10 mL of THF. The reaction as stirred for 4 h before being quenched with 25 mL of a saturated aqueous ammonium chloride solution. The mixture was placed in a separatory funnel, the layers separated, and the aqueous phase extracted three times with dichloromethane. The combined organic layers were dried over $MgSO_4$, concentrated in vacuo, and chromatographed through a silica gel column with hexane as the eluent to afford 230 mg (71%) of a pale orange oil. ^{1}H NMR ($CDCl_3$/300 MHz) δ 8.30−7.80 (m, 9H), 5.66 (q, J = 6 Hz, 1H), 5.04 (s, 1H), 4.92 (s, 1H), 3.34 (t, J = 9 Hz, 2H), 2.47 (t, J = 9 Hz, 2H), 2.02 (m, 2H), 1.81 (s, 3H), 1.71 (d, J = 6 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 149.6, 137.3, 135.3, 134.1, 133.9, 131.7, 131.2, 130.0, 129.0, 128.7, 127.8, 127.3, 126.8, 126.0, 125.3, 125.0, 124.9, 123.7, 121.9, 110.3, 34.2, 33.6, 31.1, 14.4, 14.2 ppm; IR (KBr) 3435, 3042, 2936, 2862, 1602, 1586, 1433, 1181, 842, 743, 696, 501 cm^{-1}; HRESI MS m/z calcd for $C_{25}H_{25}$ $(M+H)^+$: 325.1956; found: 325.1937.

4,5-Dimethyl-2-(4-(2-oxopyrrolidin-3-yl)butyl)-6-(3-(pyren-1-yl)propyl)-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3-(2*H*)-dione (10). *(a). Sc(OTf)$_3$-Catalyzed Reaction.* Dienophile **6** (45.2 mg, 0.1 mmol), 32.4 mg (0.1 mmol) of diene **9**, and 14.8 mg of $Sc(OTf)_3$ were dissolved in 7 mL of dichloromethane in a 25 mL round-bottom flask. The reaction was stirred at RT overnight and then quenched with water. The reaction was poured into a separatory funnel, the layers separated, and the aqueous phase extracted three times with dichloromethane. The combined organic layers were then dried with sodium sulfate, concentrated in vacuo, and then redissolved in a 7:3 mixture of CH_2Cl_2/CF_3COOH. The resulting mixture was stirred for 30 min at RT followed by the addition of excess triethylamine. The solution was stirred for 1 h before being quenched with a mixture of saturated ammonium chloride and ethyl acetate. The pH of the solution was found to be approximately 5−6. The layers were separated and the aqueous phase extracted with dicholormethane. The combined organic layers were dried with $NaSO_4$, concentrated in vacuo, and chromatographed through silica gel using 10% methanol in ethyl acetate as the eluent to afford 39.8 mg (71%) of the desired product.

(b). Noncatalyzed, Thermal Reaction. In this case, 45.2 mg (0.1 mmol) of dienophile **6** and 32.4 mg (0.1 mmol) of diene **9** were dissolved in 4 mL of toluene, and then, the mixture was heated to reflux and stirred overnight. The reaction was then cooled to RT, and the majority of toluene was removed under reduced pressure. The resulting crude product was diluted with water and the mixture extracted three times with dichloromethane. The combined organic layers were dried with sodium sulfate, concentrated in vacuo, and then redissolved in a 7:3 mixutre of CH_2Cl_2/CF_3COOH. The resulting solution was stirred for 30 min at RT followed by the addition of an excess of triethylamine. The mixture was stirred for 1 h and then quenched with a mixture of saturated ammonium chloride and ethyl acetate. The pH of the solution was again found to be approximately 5−6. The layers were separated, and the aqueous phase wsa extracted with dicholormethane. The combined organic layers were dried with $NaSO_4$, concentrated in vacuo, and chromatographed through silica gel using 10% methanol in ethyl acetate as the eluent to afford 38.1 mg (68%) of the desired Diels−Alder product. ^{1}H NMR ($CDCl_3$/300 MHz) δ 8.22−7.84 (m, 9H), 3.38 (t, J = 6 Hz, 2H), 3.28 (t, J = 6 Hz, 2H), 3.18, (m, 2H), 2.98 (m, 1H), 2.89 (m, 1H), 2.49 (m, 2H), 2.30−2.16 (m, 4H), 2.11 (m, 1H), 1.87 (m, 2H), 1.73 (m, 2H), 1.65 (s, 3H), 1.58 (m, 1H), 1.45 (m, 2H), 1.25−1.21 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$/300 MHz) 182.9, 182.8, 181.3, 139.2, 134.7, 134.0, 133.5, 133.2, 133.1, 132.4, 131.2, 130.2, 129.9, 129.7, 129.2, 128.4, 127.7, 127.6, 127.5, 127.3, 125.9, 47.4, 43.2, 42.8, 42.6, 41.0, 40.9, 37.8, 36.2, 35.9, 32.9, 32.8, 31.1, 30.4, 30.1, 30.0, 27.2 ppm; IR (KBr) 3368, 2934, 2911, 1810, 1790, 1693, 1434, 1401, 1243, 1161, 846, 724 cm^{-1}; HRESI MS m/z calcd for $C_{37}H_{41}N_2O_3$ $(M+H)^+$: 561.3117; found: 561.3119. COSY, HMQC, and TOCSY experiments were used to confirm the structure, and a NOESY experiment was used to assign the stereochemistry of the endoproduct.

Preparation of Agarose-Coated 1K Microelectrode Arrays. A microelectrode array containing 1024 electrodes cm^{-1} was spin-coated at 2000 rpm for 45 s with 4% agarose in 90% *N*,*N*-dimethylformamide and 10% water. The array was then allowed to dry in air for 1 h, after which it was plugged into a circuit board that allowed for control of the electrodes with a PC.[2] The circuit was also outfitted with a Pt wire counter electrode.

Preparation of the Dienophile Functionalized Array 11. An agarose-coated microelectrode array was inserted along with a Pt wire counter electrode into a 1.5 mL Eppendorf tube that contained 3 mg of vitamin B_{12}, 6 mg of tetramethylammonium nitrate, and 8 mg of activated ester **6** in 1.5 mL of methanol. A whole-board pattern was programmed with 600 potential cycles that turned each electrode on at a potential of −2.4 V relative to the Pt wire counter electrode for 0.5 s and then off again (0 V) for 0.1 s. After the 600 cycles were complete, the array was removed from the solution and then washed with deionized water and 95% ethanol.

Preparation of the Diels−Alder Product Functionalized Array 12. The dienophile-functionalized array (**11**) and a remote Pt wire counter electrode were immersed in a solution that contained 7.5 mg of $Sc(OTf)_3$, 6.6 mg of 2-phenylbenzthiazole (the confining agent), and 10 mg of diene **9** in 2.0 mL of dichloromethane. The reaction solution had been premixed and let stand for 30 min in order to make sure that the Sc(III) reagent was completely reduced by the 2-phenylbenzthiazole. A whole-board pattern of electrodes was then activated by cycling the electrodes at a potential of +3.5 V relative to the remote Pt wire cathode for 0.5 s and then off again for 0.1 s. The reaction was conducted for 900 such cycles. When complete, the microelectrode array was washed with deionized water and 95% ethanol and then the success of the reaction determined by imaging the array with the use of a fluorescence microscope.

Sample Experimental for the Site-Selective Cleavage of the Safety-Catch Linker. The Diels−Alder product functionalized microelectrode array and a remote Pt wire counter electrode were inserted into an Eppendorf tube that contained a solution comprised of 50 mg of 1,2-diphenylhydrazine and 100 mg of tetrabutylammonium hexafluorophosphate in 1.5 mL of methanol. In this case, excess 1,2-diphenylhydrazine served as the confining agent to scavenge acid generated at the electrodes. A checkerboard pattern of electrodes was then activated for 900 potential cycles by setting them at a potential of +3.0 V relative to the remote Pt wire electrode for a period of 0.5 s and then turning them off again for 0.1 s. After the 900 cycles were complete, the array was removed from the solution and washed with deionized water and 95% ethanol. An image of the array was then obtained with a fluorescence microscope to determine the success of the reaction. The reaction solution in the Eppendorf tube was collected and analyzed by LC-MS.

Analysis by LC-MS. Samples were diluted with 85% methanol/15% water and then chromatographed through a C18 column (Gemini C18, 5 μm, 110 A, 4.6 mm × 250 mm) using an isocratic elution with the 85% methanol/15% water solution at a flow rate of 1 mL/min. The flow was split for a final flow rate of 200 μL/min prior to the ESI source used for mass spectrometry. The MS experiment was conducted with a spray voltage of 3.5 kV, a sheath gas flow rate of 8 arb, a capillary temperature of 275 °C, a capillary voltage of 35 V, and a tube lens of 110 V.

■ ASSOCIATED CONTENT

Supporting Information

A picture of the 1K array setup, proton and carbon NMR spectra for the key synthetic intermediates, and COSY, HMQC, TOCSY, and NOESY spectrum for endoproduct **10** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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