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Electrochemically Active Cross-Linking Reaction for Fluorescent Labeling of Aliphatic Alkenes

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Abstract: Although cross-linking reactions serve as a valuable tool for the integration of two or more functionalities or properties, the application of electrochemical synthesis to cross-linking reactions is restricted due to the difficulty of mass transfer. Thus, the primary purpose of this research is to explore electrochemical cross-linking systems to construct a fluorescent probe, triggered by the formation of a covalent linkage. The second purpose is to apply the probe to insoluble targets. Towards these goals, a combination of electrochemically active phenol derivatives and aliphatic alkenes were employed to form polycyclic compounds. Several of the dihydrobenzofuran derivatives formed through [3+2] cyclization reactions exhibited fluorescence. Furthermore, this approach allowed the effective modification of alkene-modified silica gel with electrochemically active species, which enables the construction of fluorescent probes that are triggered by C–C bond formation.

Keywords: C–C coupling · electrochemistry · luminescence · polycycles · solid-phase synthesis

Introduction

Cross-linking is a valuable method for the integration of two or more functionalities or properties to facilitate the effective design and fabrication of bioconjugates and nanomaterials.[1] In particular, cross-linking reactions that use photoactive linkers[2] or click-chemistry[3] for fluorescent labeling offer versatile strategies to manufacture biomolecular probes, which allow surface modification through stable covalent bonds. Although electro-organic synthetic methods have produced a wide variety of useful intermediate species under mild conditions,[4] their application to practical cross-linking is restricted due to the difficulty of mass transfer, that is, the unstable reactive intermediate generated from the electrode must be transported to another location. To date, attempts in solid-phase electro-organic synthesis have been limited mostly to immobilization of substrates on an electrode[5] or indirect electro-organic synthesis.[6] Furthermore, solid-phase syntheses often require excess amounts of reagents to complete the target reactions due to low reactivity, therefore, typical electro-organic approaches suffer from in situ generation of unstable intermediates trapped within the solid supports. Therefore, the development of electrochemically active cross-linkers for the modification of insoluble targets, nonconductive samples, and biomolecules remains a significant challenge.

We have recently accomplished intermolecular carbon–carbon bond-formation reactions with aliphatic alkenes via anodically generated cation intermediates to afford a variety of polycyclic systems.[7] Furthermore, less reactive alkenes could be coupled with electrochemically active substrates, which can then be trapped in a thermomorphic solution phase, spatially separated from the electrode, by using cyclohexane/nitromethane as a medium.[8] These solution-phase reaction systems effectively accelerated intermolecular interactions between the less reactive olefins and unstable cation intermediates. We hypothesized that this approach would allow selective, direct, and stable elaboration of alkene-modified insoluble targets with electrochemically active species. The electrochemically active cross-linking system could also enable the construction of a fluorescent probe, triggered by the formation of a covalent linkage (Figure 1). This strategy addresses the time-consuming, multistep post-processing problems associated with fluorescent labeling, such

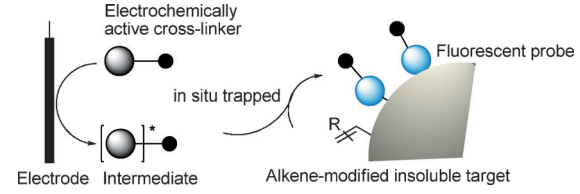

Figure 1. Schematic view of an electrochemically active cross-linking reaction for the attachment of fluorescent labels to aliphatic alkenes.

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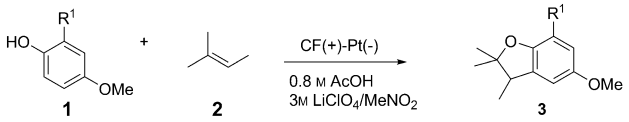
as washing and purification, and reduces false signals that arise from nonspecific adsorption or undesired cleavage of a fluorophore in the detection environment.

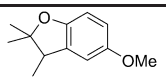
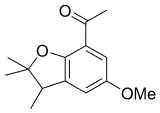
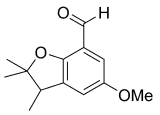
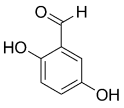
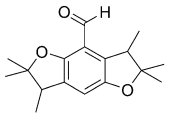
Herein, we describe an electrochemically active cross-linking reaction for fluorescent labeling of aliphatic alkenes. We are particularly interested in the possibility that the electrochemically active species could react with less reactive aliphatic alkenes bound to an insoluble target to form fluorescent molecules.

Results and Discussion

We have previously developed intermolecular [4+2], [3+2], and [2+2] cyclization reactions with unactivated alkenes to provide polycyclic compounds. Initially, we examined the fluorescent properties of the polycyclic products, consistent with the concept described above. As a result, several dihydrobenzofuran derivatives produced by the [3+2] cyclization reaction were found to exhibit fluorescence; these results are summarized in Table 1. The reactivity of various phenol derivatives was investigated by using 2-methyl-2-butene (**2**) to synthesize dihydrobenzofurans **3**. In the case of *p*-methoxyphenol (**1a**) and 2-hydroxy-5-methoxyacetophenone (**1b**), the expected cycloaddition reactions proceeded to afford dihydrobenzofurans **3a** and **3b** in excellent yields (Table 1, entries 1 and 2). In contrast, when aldehydes **1c** and **1d** were used, the desired products **3c** and **3d** were obtained in lower yields (Table 1, entries 3 and 4). It was assumed that the lower amount of phenoxonium cation generated was due to oxidation of the aldehyde group. Moreover, the aldehyde group could destabilize the phenoxonium cation because the carbonyl carbon atom does not neutralize a partial positive charge as effectively as the acetyl group. With the exception of compound **3a**, all compounds exhibited fluorescence characteristics; compound **3d** provided a particularly high quantum yield ($\Phi = 0.65$). The increased conjugation of compound **3d** was assumed to be caused by a structure in which two alkene molecules are united with the two hydroxyl groups at the *para* positions of benzaldehyde. Next, we investigated the reactions of various alkenes with **1b** (Table 2). No reaction was observed when allylbenzene was

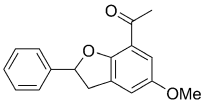
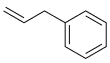
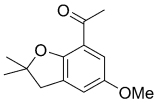
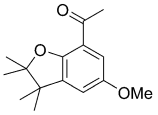
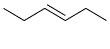
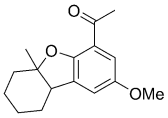
Table 1. Anodic intermolecular [3+2] cycloaddition reactions between various phenols **1** and 2-methyl-2-butene (**2**).[a]



	Phenol	Product	Yield [%][b]	λ_{max} abs [nm][c]	λ_{max} fl [nm][c]	Φ_f[d]
1	**1a** (R^1=H)	**3a** 	85	306	405	0.04
2	**1b** (R^1=Ac)	**3b** 	90	349	430	0.25
3	**1c** (R^1=CHO)	**3c** 	52	367	454	0.26
4	**1d** 	**3d** 	71	395	495	0.65

[a] All reactions were performed in a lithium perchlorate/nitromethane electrolyte solution. [b] Yields were determined by ^{1}H NMR spectroscopy. [c] Measured in chloroform, abs = absorbance, fl = fluorescence. [d] Calculated figures relative to the quantum yield of quinine sulfate dehydrate.

Table 2. Anodic intermolecular [3+2] cycloaddition reactions between various alkenes and 2-hydroxy-5-methoxyacetophenone.[a]

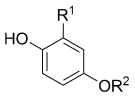
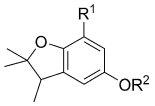
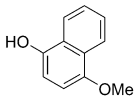
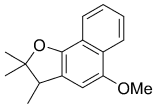
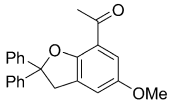
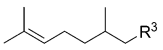
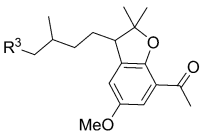
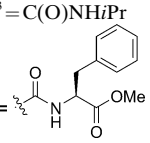
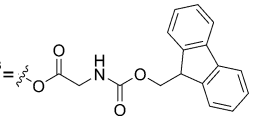
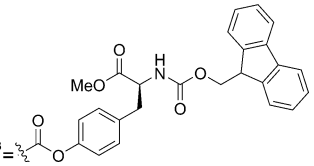
	Alkene	Product	Yield [%][b]	λ_{max} abs [nm][c]	λ_{max} fl [nm][c]	Φ_f[d]
1		**4** 	>99	350	430	0.19
2		–	n.d.[e]	–	–	–
3		**5** 	84	351	432	0.25
4		**6** 	92	348	433	0.31
5		–	n.d.	–	–	–
6		**7** 	80	350	432	0.22

[a] All reactions were performed in a lithium perchlorate/nitromethane electrolyte solution. [b] Yields were determined by ^{1}H NMR spectroscopy. [c] Measured in chloroform. [d] Calculated figures relative to the quantum yield of quinine sulfate dehydrate. [e] None detected.

used, whereas a high yield of 99% was obtained when styrene was used (Table 2, entries 1 and 2). A causal relationship between the reaction yield and conjugation of the electron-rich aromatic ring with the unsaturated moiety was observed. In addition, it was confirmed that one of the carbon atoms of the carbon–carbon double bond must be fully substituted (Table 2, entries 3–6) to stabilize the reaction intermediate. The phenoxonium cation derived from the phenol was subjected to nucleophilic attack of the alkene, which generated a bi- or trivalent cation. However, the stability of the bivalent cation was low and the reaction did not proceed. The compounds produced exhibited similar levels of fluorescence.

The utility of this protocol was evaluated by testing a variety of phenols and aliphatic alkenes. As shown in Table 3, the [3+2] cyclization reactions proceed effectively to afford the desired products in good to high yields, without side reactions or serious decomposition of the functional groups, even when *p*-alkoxyphenols substituted with electron-withdrawing groups ($-NO_2$, $-CO_2H$, $-CO_2Me$ $-C(O)NHiPr$) at the 2-position were used. However, the naphthalene-1-ol derivative **9f** was produced in moderate yield, although the cation species derived from **8f** is more stable than those derived from phenols **1a–d** and **8a–e**. Interestingly, the highly hydrophobic alkenes **11a–e**, which were derived from citronellol or citroneic acid, were successfully coupled with phenol **1b** in high yields in a polar electrolyte solution.

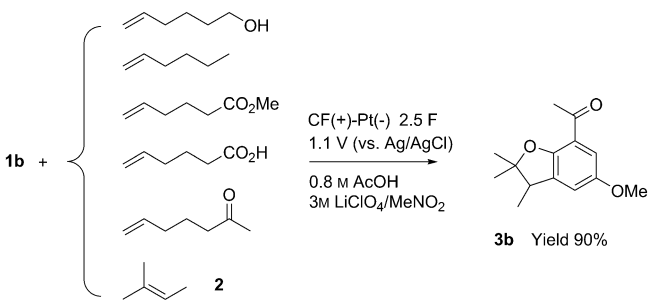
Table 3. Anodic intermolecular [3+2] cycloaddition reactions between various phenols and alkenes.[a]

	Phenol	Alkene	Product	Yield [%][b]
				
1	**8a** $R^1=NO_2$, $R^2=Me$	**2**	**9a** $R^1=NO_2$, $R^2=Me$	70
2	**8b** $R^1=H$, $R^2=Ph$	**2**	**9b** $R^1=H$, $R^2=Ph$	92
3	**8c** $R^1=CO_2H$, $R^2=Me$	**2**	**9c** $R^1=CO_2H$, $R^2=Me$	85
4	**8d** $R^1=CO_2Me$, $R^2=Me$	**2**	**9d** $R^1=CO_2Me$, $R^2=Me$	70
5	**8e** $R^1=C(O)NHiPr$, $R^2=Me$	**2**	**9e** $R^1=C(O)NHiPr$, $R^2=Me$	91
6	**8f** 	**2**	**9f** 	54
7	**1b**		**10** 	>99
				
8	**1b**	**11a** $R^3=CH_2OH$	**12a** $R^3=CH_2OH$	94
9	**1b**	**11b** $R^3=CO_2H$	**12b** $R^3=CO_2H$	91
10	**1b**	**11c** $R^3=OAc$	**12c** $R^3=OAc$	88
11	**1b**	**11d** $R^3=CH_2OC(O)C_5H_{11}$	**12d** $R^3=CH_2OC(O)C_5H_{11}$	88
12	**1b**	**11e** $R^3=C(O)NHiPr$	**12e** $R^3=C(O)NHiPr$	80
13	**1b**	**11f** $R^3=$ 	**12f** $R^3=C(O)-Phe-OMe$	88
14	**1b**	**11g** $R^3=$ 	**12g** $R^3=Fmoc-Gly-O-$	87
15	**1b**	**11h** $R^3=$ 	**12h** $R^3=Fmoc-Tyr[O-C(O)-]-OMe$	83

[a] All reactions were performed in a lithium perchlorate/nitromethane electrolyte solution. [b] Yields were determined by ^1H NMR spectroscopy.

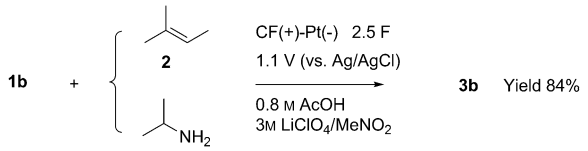
Furthermore, the amino acid derivatives **11f–h**, which were modified with lipophilic chains at each N-terminal, C-terminal, and side-chain position, also reacted to give products **12f–h**. It was expected that these cross-linking reaction systems could be used for the effective modification of lipophilic biomaterials with olefin regions, especially for the modification of lipophilic peptide targets.

To study the selectivity of the functional groups in this reaction system, electrochemical coupling reactions were conducted with phenol **1b** in the presence of an assortment of terminal monosubstituted olefins and trialkyl-substituted olefin **2** (Scheme 1). As expected, phenol **1b** did not couple



Scheme 1. Alkene selectivity in the $LiClO_4/MeNO_2$ reaction system.

with the terminal olefins and provided product **3b** exclusively. In addition, the product of coupling with the amino group of isopropyl amine was not detected in a competition experiment with **2** under similar conditions (Scheme 2). These results indicate that the phenoxonium cations anodically generated in the $LiClO_4/MeNO_2$ reaction system can be selectively trapped with trialkyl-substituted olefins to afford the cycloadduct as a fluorescent unit in high yields.



Scheme 2. Functional group selectivity in the $LiClO_4/MeNO_2$ reaction system.

We next turned our attention to the determination of suitable combinations of phenol and cyclized product consistent with the concept that the formation of a covalent linkage can trigger fluorescence (Table 4). Thus, it is required that the cycloadduct exhibit fluorescence but the phenol derivative does not. Neither **8a** nor the respective cyclization product **9a** exhibit fluorescent properties (Table 4, entry 4), whereas both starting material **8f** and product **9f** fluoresce (Table 4, entry 7). On the other hand, phenols **1b–d** and **8d** could act fluorescent probe precursors; the construction of the fluorescent unit was triggered by the formation of a covalent linkage (Table 4, entries 1–3 and 6). In addition, the presence of an ester bond (Table 4, entry 6) can allow for

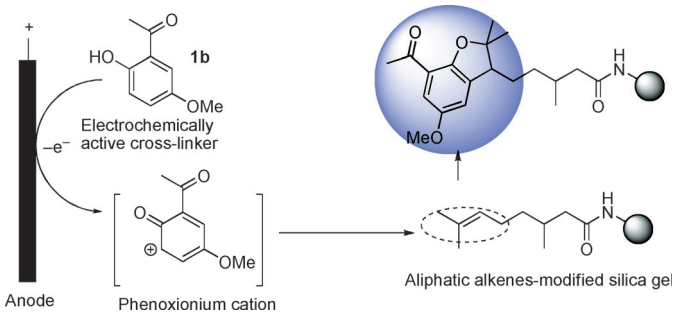
Table 4. Luminescence images of phenols and their respective dihydrobenzofuran derivatives.[a]

	Phenol	Dihydrobenzofuran		Phenol	Dihydrobenzofuran
1	**1b**	**3b**	5	**8c**	**9c**
2	**1c**	**3c**	6	**8d**	**9d**
3	**1d**	**3d**	7	**8f**	**9f**
4	**8a**	**9a**			

[a] Photograph of each compound dissolved in chloroform taken under black light ($\lambda = 330$–350 nm).

further modification and integration of target functionalities or properties. These results indicate that this strategy can allow effective opportunities to label fluorescent probes for reducing false signals that arise from nonspecific adsorption or undesired cleavage of a fluorophore in the detection environment.

Finally, based on these results, we attempted to synthesize a fluorescent probe, constructed from unactivated 3,7-dimethyl-6-octenoic acid and amino-modified silica, to confirm the lifetime of the phenoxonium cation (Scheme 3). The size of the amino-silica particles was approximately 0.6–0.7 μm and the amino group content was 0.4 mmol g^{-1}. After the reaction, the mixture was washed in water and the solid phase was collected by filtration. Fluorescence on the silica was observed by fluorescent microscopy (Figure 2). The reactive site of the alkene cannot approach the electrode because the silica molecule is too large, thus, it was assumed



Scheme 3. Anodic cycloaddition between **1b** and 3,7-dimethyl-6-octenoic acid tethered to the silica surface.

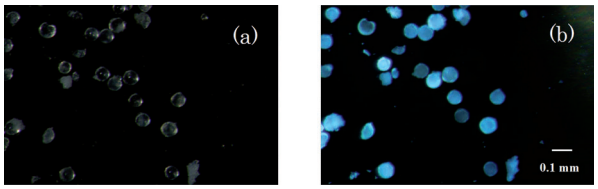


Figure 2. Luminescence images of the silica surface obtained by fluorescent microscopy under a) incandescent and b) black light ($\lambda = 330$–350 nm).

that the phenoxonium cation was stable until reaction on the silica surface. We have previously examined the lifetime of the phenoxonium cation in the presence of aliphatic alkenes or styrene by using laser Raman spectroscopy on an Au electrode. The microscopic laser Raman spectrum showed a typical signal for the anodically generated phenoxonium cation at $\tilde{\nu} = 1664\ cm^{-1}$ (assigned to C=O) for more than 30 s after the oxidation current was stopped, even in the presence of methylenecyclohexane as an unactivated nucleophilic alkene. Thus, this phenomenon could be visually monitored.

Conclusion

An electrochemically active cross-linking reaction was developed to attach fluorescent probes to aliphatic alkenes. Several dihydrobenzofuran derivatives formed by [3+2] cyclization reactions exhibited fluorescence properties. Furthermore, this approach allowed the effective and stable elaboration of alkene-modified silica gel with electrochemically active species, which enables the construction of fluorescent probes, triggered by the formation of a covalent linkage.

Experimental Section

General procedure: Phenol (0.1 mmol), alkene (0.3 mmol), and AcOH (17.5 mmol) were added to $LiClO_4/CH_3NO_2$ (3.0 M, 20 mL). The reaction cell was capped with a septum equipped with a carbon felt anode (20 × 30 mm) and Pt cathode (10 × 10 mm). The electrolysis was performed at the peak oxidation potentials of the substrates. After the reaction was complete (2.5–3.0 F), the reaction mixture was poured into EtOAc and the solution was washed with a 5% aqueous solution of $NaHCO_3$, then brine. The organic layer was dried over anhydrous $MgSO_4$. After filtration and evaporation under reduced pressure, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give the cycloadduct. The products yields were determined by ^1H NMR spectroscopy.

Acknowledgements

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