

## Amine-Terminated Monolayers on Carbon: Preparation, Characterization, and Coupling Reactions

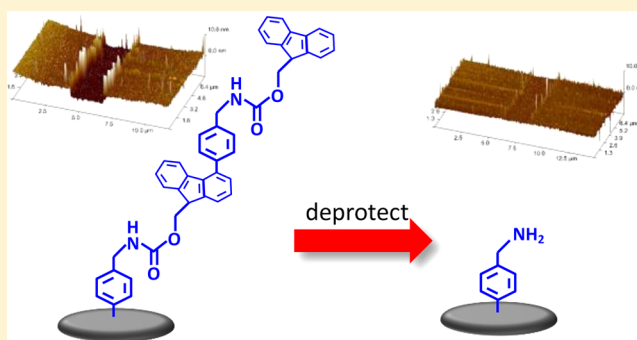
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### S Supporting Information

**ABSTRACT:** Aminophenyl and aminomethylphenyl monolayers have been electrografted to glassy carbon and pyrolyzed photoresist film from the corresponding diazonium ions using a protection–deprotection strategy based on Boc (*tert*-butoxycarbonyl) and Fmoc (fluorenylmethoxycarbonyl) groups. After grafting and then deprotecting films of Boc-NH-Ar, Fmoc-NH-Ar, and Fmoc-NH-CH<sub>2</sub>-Ar, depth profiling by atomic force microscopy confirmed that the resulting amine-terminated films were monolayers. In contrast, after deprotection, Boc-NH-CH<sub>2</sub>-Ar gave a multilayer film. Electroactive carboxylic acid derivatives were coupled to the monolayers through amide linkages. Electrochemical measurements revealed that the deprotected Fmoc-NH-CH<sub>2</sub>-Ar monolayer gave the highest surface concentration of coupled nitrophenyl and ferrocenyl groups and DFT calculations established that this monolayer has the highest theoretical surface concentration of those examined.



### ■ INTRODUCTION

Grafting nanoscale organic films through reduction of aryldiazonium ions is a well-established strategy for the preparation of surface modified substrates.<sup>1,2</sup> The method proceeds via generation of aryl radicals that attack the substrate surface;<sup>3</sup> for many materials including glassy carbon (GC), detailed analysis has demonstrated that the modifying groups are anchored to the surface via covalent bonds.<sup>1,2</sup> The strong bonding of the modifier to the surface is a major advantage of the method; however, through the same radical-attack mechanism, aryl groups can bind to already grafted groups resulting in a disordered multilayer film.<sup>4,5</sup> Such a film structure is incompatible with the need for well-defined interfaces for applications such as chemical- and bio-sensors and molecular electronics. Fully exploiting the stability advantages of films grafted from aryldiazonium salts requires reliable strategies for preparation of monolayers.

Our work focuses on the preparation of monolayers that have reactive terminal groups that can undergo further on-surface chemistry.<sup>6–8</sup> Monolayer tethers with a small number of carefully selected terminal groups can provide access to a broad range of coupling chemistries, constituting versatile platforms for the preparation of covalently attached monolayers of target species. For preparation of monolayers, Pedersen, Daasbjerg, and coworkers introduced the “formation–degradation” approach based on diazonium ions with a cleavable group in the position para to the diazonium moiety.<sup>9,10</sup> After grafting a

multilayer film (the formation step) followed by bond cleavage (degradation), film thickness measurements showed that most of the multilayer film had been removed, leaving a near monolayer on the surface. Thiophenolate<sup>10</sup> and benzaldehyde<sup>9</sup> terminated films were prepared by this route. Bartlett and coworkers adopted the same strategy using the *tert*-butoxycarbonyl- (Boc) protected aminobenzyl diazonium ion.<sup>11</sup> Coupling anthraquinone-2-carboxylic acid to the deprotected amine-terminated layer gave a surface concentration of anthraquinone groups consistent with a monolayer, but film thickness measurements were not reported. We have subsequently focused on the use of more bulky protecting groups in a “protection–deprotection” strategy.<sup>6–8</sup> Use of sufficiently bulky groups in the para position prevents radical attack at already-grafted phenyl groups and ensures that after deprotection only a monolayer film remains on the surface. Phenylethynylene (H-Eth-Ar)<sup>7,8</sup> and carboxyphenyl (COOH-Ar)<sup>6</sup> films have been prepared using silyl and 9-fluorenylmethyl protecting groups, respectively, and have been confirmed as monolayers through film thickness measurements.

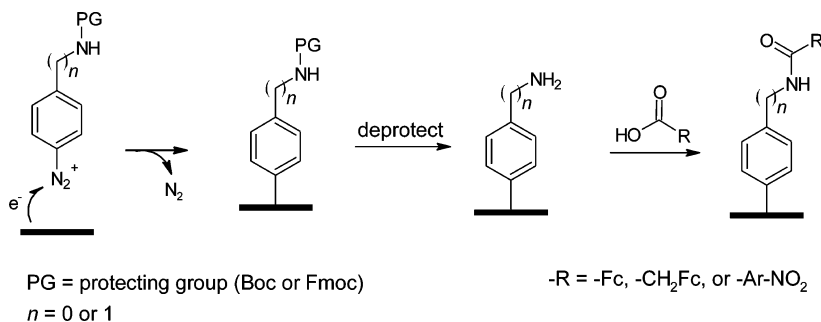
H-Eth-Ar and COOH-Ar monolayers on GC have been shown to be robust and reactive tethers for subsequent click reactions with azide derivatives<sup>7,8</sup> and amide coupling with

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Scheme 1. Strategy for Preparation of Amine-Terminated Monolayers and for Coupling Fc and NP Groups to the Layer



amines,<sup>6</sup> respectively. Access to amine-terminated monolayers offers further chemical versatility for coupling target molecules to surfaces, especially through amide linkages with carboxylic acid derivatives. Amines also react with aldehydes and ketones to form imines, with isocyanates (or isothiocyanates) to give ureas (or thioureas), and an arylamine-terminated surface can be used as a precursor for the formation of a diazonium ion-terminated monolayer.<sup>12–14</sup> Collectively, protected H-Eth-Ar, COOH-Ar, and arylamine diazonium ion derivatives offer opportunities for the preparation of mixed monolayers with components with orthogonal reactivity for selective reaction with more than one target molecule.

Here we expand on Bartlett's work<sup>11</sup> to prepare amine-terminated monolayers through the protection–deprotection strategy. We describe the synthesis and electrografting of aminobenzene- and aminomethylbenzenediazonium salts protected with Boc and fluorenylmethyloxycarbonyl (Fmoc) groups. The effectiveness of Boc and Fmoc as protecting groups for monolayer formation and the reactivities of the resulting amino- and aminomethyl-terminated films for subsequent amide coupling reactions are compared. We investigate both oxalyl chloride ( $\text{COCl}_2$ ) and [benzotriazol-1-yloxy(dimethylamino)methylidene]-dimethylazanium hexafluorophosphate (HBTU) as activating agents for amide bond formation and compare the yields of coupling reactions involving  $\text{FcCH}_2\text{COOH}$  (Fc is ferrocenyl) and  $\text{FcCOOH}$ .

## EXPERIMENTAL SECTION

**Materials and Reagents.** Unless stated otherwise, all reagents were used as received. Acetonitrile (ACN) from VWR BDH was HPLC grade; Millipore Milli-Q water (resistivity  $>18 \text{ M}\Omega \text{ cm}$ ) was used for all aqueous solutions and washing. Tetrabutylammonium tetrafluoroborate ( $\text{TBABF}_4$ ) and pyrolyzed photoresist film (PPF) were prepared by literature methods.<sup>15</sup>

The syntheses and characterization of  $[\text{Boc-NH-Ar-N}_2]\text{BF}_4$ ,  $[\text{Boc-NH-CH}_2\text{-Ar-N}_2]\text{BF}_4$ ,  $[\text{Fmoc-NH-Ar-N}_2]\text{BF}_4$ , and  $[\text{Fmoc-NH-CH}_2\text{-Ar-N}_2]\text{BF}_4$  are described in the Supporting Information. All diazonium salts were isolated as microcrystalline powders.

**Electrochemistry, Surface Modification, and Coupling Reactions.** All electrochemical measurements were performed using an Ecochemie Autolab PGSTAT302 potentiostat/galvanostat. Glassy carbon (GC) working electrodes (3 mm diameter) were polished with a slurry of  $1 \mu\text{m}$  alumina powder in water, followed by ultrasonication in water and drying with  $\text{N}_2(\text{g})$ . PPF plates were clamped to the base of an electrochemical cell with a hole in the base; a Kalrez O-ring defined the area of the PPF working electrode. The auxiliary electrode was a Pt mesh, and the reference electrode was a saturated calomel electrode (SCE) for measurements in aqueous solutions and a calomel electrode with  $1 \text{ M LiCl}_{(\text{aq})}$  (CE) for nonaqueous solutions.

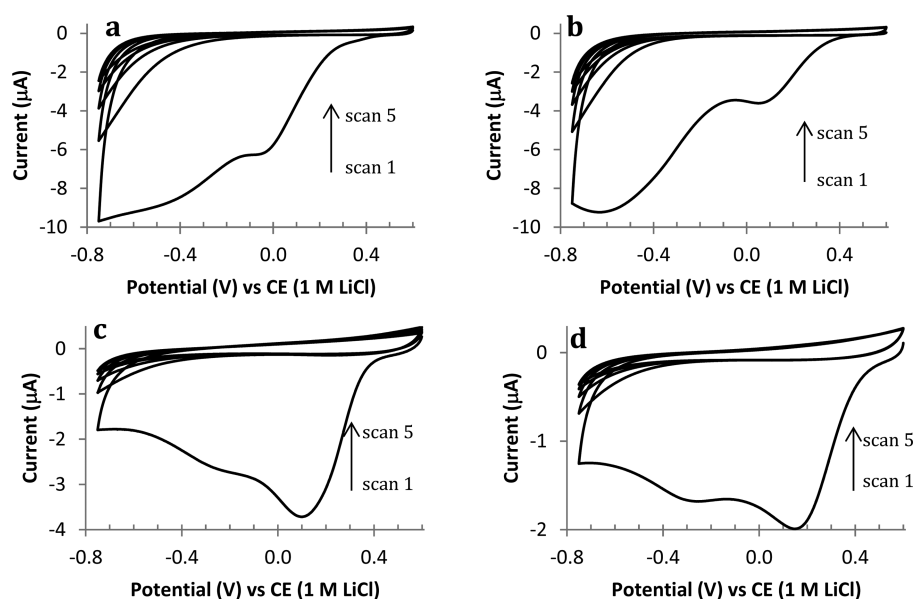
Films were electrografted to GC and PPF from deaerated  $5 \text{ mM}$  solutions of the diazonium salt in ACN containing  $0.1 \text{ M TBABF}_4$  using five potential cycles between  $0.60$  and  $-0.75 \text{ V}$  at a scan rate of

$50 \text{ mV s}^{-1}$ . The modified electrodes were rinsed with acetone, sonicated in ACN for  $5 \text{ min}$ , and dried with  $\text{N}_2(\text{g})$ . Boc and Fmoc protecting groups were cleaved from the layers by immersing the modified electrode in a stirred solution of  $4 \text{ M HCl-MeOH}$  and  $20\%$  piperidine-DMF, respectively. The standard deprotection times were  $90$  and  $120 \text{ min}$  for Boc- and Fmoc-protected layers, respectively. Electrodes were rinsed with acetone and  $\text{H}_2\text{O}$  after deprotection.

Ferrocenyl (Fc) and nitrophenyl (NP) groups were immobilized on the deprotected layers using  $(\text{COCl})_2$ <sup>16</sup> and HBTU-activated coupling of  $\text{FcCOOH}$ ,  $\text{FcCH}_2\text{COOH}$ , and  $4\text{-nitrobenzoic acid}$ . After reaction, the modified electrodes were rinsed with acetone and sonicated in ACN for  $5 \text{ min}$ . For  $(\text{COCl})_2$ -activated coupling, the carboxylic acid derivative ( $10 \text{ mg}$ ) was placed in a sealed reaction vessel under  $\text{N}_2(\text{g})$ . Anhydrous dichloromethane (DCM,  $6 \text{ mL}$ ) was introduced, followed by  $(\text{COCl})_2$  ( $25 \mu\text{L}$ ) and pyridine ( $8 \mu\text{L}$ ). After refluxing for  $1 \text{ h}$ , all volatile compounds were removed under vacuum. Anhydrous DCM ( $6 \text{ mL}$ ) was introduced into the reaction vessel to redissolve the activated compound. Amine-modified or polished GC electrodes were then inserted into the reaction vessel, followed by the addition of  $\text{Et}_3\text{N}$  ( $\sim 50 \mu\text{L}$ ). The reaction was stirred in an ice bath for  $5 \text{ min}$  and then at room temperature overnight. A  $\text{N}_2(\text{g})$  atmosphere was maintained throughout the procedure. For HBTU-activated coupling, the amine-modified or polished GC electrode was immersed overnight at room temperature under  $\text{N}_2(\text{g})$  in a stirred  $5 \text{ mL}$  solution of DMF containing  $12 \text{ mM}$  of HBTU,  $20 \text{ mM}$  of  $N,N$ -diisopropylethylamine (DIPEA), and  $10 \text{ mM}$  of the carboxylic acid compound.

Cyclic voltammograms of immobilized Fc groups were obtained in  $0.1 \text{ M LiClO}_4\text{-EtOH}$  at a scan rate of  $200 \text{ mV s}^{-1}$ . The surface concentration of immobilized Fc was determined from the third voltammetric cycle by averaging the areas under the anodic and cathodic peaks. The third scan was used because the background currents changed between the first and second and sometimes third potential cycle. Cyclic voltammograms of NP groups were obtained in  $0.1 \text{ M H}_2\text{SO}_4$  at a scan rate of  $100 \text{ mV s}^{-1}$ . The surface concentration of immobilized NP groups was estimated from the first voltammetric cycle, using the areas of the irreversible NP reduction peak and the oxidation peak of the reversible hydroxyaminophenyl/nitrosophenyl couple and the number of electrons involved in each redox process.<sup>17</sup> Peak areas were calculated after correcting the baseline using a third-order polynomial. Uncertainties derived from the experimental data are indicated in the text. For sample sizes  $>2$ , uncertainties are the standard deviation of the indicated number of samples ( $n$ ). For sample size  $= 2$ , the uncertainty indicates the range of values obtained. There are estimated additional uncertainties of  $20$  and  $10\%$  for absolute surface concentrations of NP and Fc, respectively, reflecting the difficulty of defining appropriate peak baselines. The geometric working electrode area was assumed for all surface concentration calculations.

Theoretical surface coverage values ( $\Gamma_{\text{theo}}$ , expressed in  $\text{mol cm}^{-2}$ ) for aminophenyl and aminomethylphenyl monolayers were estimated using eq 1, which assumes that the deposited protected monolayers are arranged in a hexagonal close packing lattice and that  $d$  is expressed in angstroms.



**Figure 1.** Five consecutive scans at polished GC electrodes in solutions of 5 mM: (a) [Boc-NH-Ar-N<sub>2</sub>]<sup>+</sup>BF<sub>4</sub><sup>−</sup>, (b) [Boc-NH-CH<sub>2</sub>-Ar-N<sub>2</sub>]<sup>+</sup>BF<sub>4</sub><sup>−</sup>, (c) [Fmoc-NH-Ar-N<sub>2</sub>]<sup>+</sup>BF<sub>4</sub><sup>−</sup>, and (d) [Fmoc-NH-CH<sub>2</sub>-Ar-N<sub>2</sub>]<sup>+</sup>BF<sub>4</sub><sup>−</sup> in 0.1 M TBABF<sub>4</sub>-ACN. Scan rate = 50 mV s<sup>−1</sup>.

$$\Gamma_{\text{theo}} = \frac{1.9210^{-8}}{d^2} \quad (1)$$

The diameter  $d$  of the disk representing the protecting group was evaluated by computing the conformation of the molecule (Supporting Information) and assuming a rotation of the protecting group along the aryl amine axis.

**Atomic Force Microscopy.** Measurements were made with a Dimension 3100 and Nanoscope IIIa controller (Digital Instruments, Veeco, USA). Film thicknesses were measured on modified PPF working electrodes by profiling across a scratch in the film.<sup>15</sup> As previously described,<sup>6</sup> the reported film thicknesses are the means of at least 16 values obtained from three scratches, and the uncertainty is the standard deviation of the mean.

**Computational Methods.** The conformations of Boc-NH-Ar, Boc-NH-CH<sub>2</sub>-Ar, Fmoc-NH-Ar, and Fmoc-NH-CH<sub>2</sub>-Ar groups were optimized using DFT calculations at the B3LYP/6-31G\* level. All calculations were performed using the Gaussian 03 package with the default parameters.<sup>18</sup> The xyz coordinates of the optimized geometries are given in Tables S1–S4 (Supporting Information).

## RESULTS AND DISCUSSION

Syntheses of the four protected aryldiazonium salts are described in the Supporting Information.

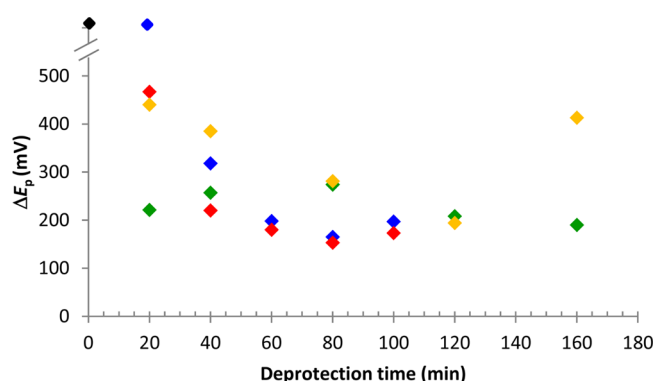
Scheme 1 shows the sequence of steps for electrografting the protected diazonium salts, cleavage of the protecting groups, and subsequent coupling reactions with carboxylic acid derivatives.

Cyclic voltammograms obtained during electrografting of the protected aryldiazonium salts at GC are shown in Figure 1 and are typical of those reported for many aryldiazonium salts. The first cathodic scan leads to deposition of a nonconducting film that in subsequent scans significantly blocks electron transfer to aryldiazonium ions in solution. As frequently observed, each aryldiazonium ion exhibits two reduction processes on the first scan. On gold, the origin of multiple peaks has been assigned to reduction on different crystalline facets.<sup>19</sup> A similar explanation has been tentatively proposed for carbon<sup>19,20</sup> or alternatively that the generation of an aryl radical in the first reduction process is followed by its reduction to the corresponding anion.<sup>21</sup> The cyclic voltammograms show that the Boc-

protected species are reduced at lower potentials than the corresponding Fmoc derivatives, consistent with the less electron-withdrawing character of Boc.

Boc and Fmoc protecting groups were removed from the grafted layers by immersing the modified electrodes in 4 M HCl-MeOH and 20% piperidine-DMF,<sup>22</sup> respectively. The progress of the deprotection treatments was monitored by transferring the modified electrodes to a solution of 1 mM dopamine in 0.1 M H<sub>2</sub>SO<sub>4</sub> and recording cyclic voltammograms. Dopamine is an inner sphere redox probe that depends on interactions with the GC surface to catalyze its electron-transfer reaction.<sup>23,24</sup> Hence the rate of electron transfer, qualitatively indicated by cyclic voltammetric peak-to-peak separation ( $\Delta E_p$ ) values, can be used to infer the access of dopamine to the GC surface. Figure 2 shows  $\Delta E_p$  values versus immersion time in the deprotection solutions for voltammograms obtained at scan rate = 100 mV s<sup>−1</sup> at the modified electrodes.

At the initially grafted films, no peaks were observed for dopamine redox chemistry over the potential range 0.1 to 0.9 V versus SCE ( $E_{1/2} \approx 0.5$  V for dopamine at polished GC) consistent with a lack of accessible catalytic surface sites for dopamine electron transfer. Figure 2 shows that on immersion in deprotection solution,  $\Delta E_p$  values at the Boc-NH-Ar and Boc-NH-CH<sub>2</sub>-Ar films decreased with immersion time reaching a constant  $\Delta E_p$  of  $\sim 180$  mV after 80 min, indicating that changes to the films are complete after that time. A minimum  $\Delta E_p$  of  $\sim 200$  mV was obtained at the Fmoc-NH-CH<sub>2</sub>-Ar film after 120 min immersion in deprotection solution, after which time the  $\Delta E_p$  value increased. The reason for the increase is not understood and was not investigated further. For the Fmoc-NH-Ar film,  $\Delta E_p$  was  $\sim 200$  mV after 20 min of immersion in 20% piperidine-DMF, and there was no significant change on longer immersion time. On the basis of these observations, the standard deprotection times for Boc and Fmoc films were 90 and 120 min, respectively. Further investigations, using redox probe voltammetry, of the modified surfaces in each stage of preparation are described in the Supporting Information (Figure S1).



**Figure 2.** Plot of  $\Delta E_p$  for dopamine cyclic voltammograms versus deprotection time, obtained at electrodes modified with blue: Boc-NH-Ar-GC; red: Boc-NH-CH<sub>2</sub>-Ar-GC; green: Fmoc-NH-Ar-GC; yellow: Fmoc-NH-CH<sub>2</sub>-Ar-GC. The black point represents all modified surfaces before deprotection. Cyclic voltammograms were recorded in 1 mM dopamine in 0.1 M H<sub>2</sub>SO<sub>4</sub> at scan rate = 100 mV s<sup>-1</sup>.

To unambiguously confirm that the deprotection treatment yields monolayer films, film thicknesses were directly measured by atomic force microscopy (AFM) depth profiling.<sup>15</sup> For these experiments, films were electrografted to PPF, a very smooth GC-like material.<sup>25,26</sup> Table 1 lists the average thicknesses of

**Table 1.** AFM Depth Profiling Measurements of Film Thickness on PPF before and after Deprotection

film	before deprotection		after deprotection	
	average film thickness (nm)	calculated height (nm) <sup>a</sup>	average film thickness (nm)	calculated height (nm) <sup>a</sup>
Boc-NH-Ar	1.0 ± 0.2	1.1	0.4 ± 0.3	0.6
Boc-NH-CH <sub>2</sub> -Ar	1.9 ± 0.4	1.2	1.0 ± 0.3	0.7
Fmoc-NH-Ar	2.8 ± 0.5	1.4	0.4 ± 0.3	0.6
Fmoc-NH-CH <sub>2</sub> -Ar	2.1 ± 0.4	1.5	0.4 ± 0.3	0.7

<sup>a</sup>Molecular height for a monolayer was estimated from optimized structures from DFT calculations.

the grafted films before and after removal of the protecting groups and also the calculated theoretical film thicknesses for monolayers of the protected and deprotected films. As can be seen from the data, with the exception of the Boc-NH-Ar film, the average thicknesses of all films before deprotection are consistent with a multilayer structure with an average of 1.5 to 2 layers. The Boc-NH-Ar film appears to be a monolayer. After deprotection, with the exception of the Boc-NH-CH<sub>2</sub>-Ar film, the average thickness of all films is 0.4 ± 0.3 nm, consistent with sparse monolayers. After deprotection of the Boc-NH-CH<sub>2</sub>-Ar film, an average film thickness of 1.0 ± 0.3 nm was obtained. Considering the experimental uncertainty, the measured thickness could correspond to a monolayer or multilayer; however, experiments described later indicate that this is a multilayer film.

Multilayer films are formed by the attack of aryl radicals on the already grafted modifiers. For the Fmoc-protected derivatives, multilayer formation is assumed to result from attack of radicals on the aromatic fluorenyl protecting groups. Removal of these groups during deprotection leaves an amine-terminated monolayer. In contrast, for the Boc-protected derivatives, multilayer formation is assumed to include radical attack at the aryl rings of already grafted modifiers. In this case, removal of the protecting groups will not leave a monolayer film. This behavior is exemplified by the Boc-NH-CH<sub>2</sub>-Ar film. It appears that the relatively small protecting group attached to the amine via a flexible linker does not fully protect the aryl ring from radical attack, leading to multilayer growth that is not completely removed in the deprotection step. In contrast, multilayer growth is prevented in the Boc-NH-Ar film, presumably because radical attack does not occur at the Boc group and there is adequate protection of the aryl ring by the more closely positioned protecting group.

The surface concentrations of the amine-terminated monolayers were investigated by coupling electroactive species to the four deprotected layers and estimating surface concentrations from cyclic voltammograms of the modified surfaces. The carboxylic acid derivatives NO<sub>2</sub>-Ar-COOH, FcCOOH, and FcCH<sub>2</sub>COOH were reacted with the layers using (COCl)<sub>2</sub> and HBTU as amide bond activating agents. The results of these experiments were also analyzed to establish the relative reactivities toward amide coupling of the NH<sub>2</sub>-CH<sub>2</sub>-Ar and NH<sub>2</sub>-Ar films and the two ferrocene derivatives and hence to identify the most useful tether monolayer for on-

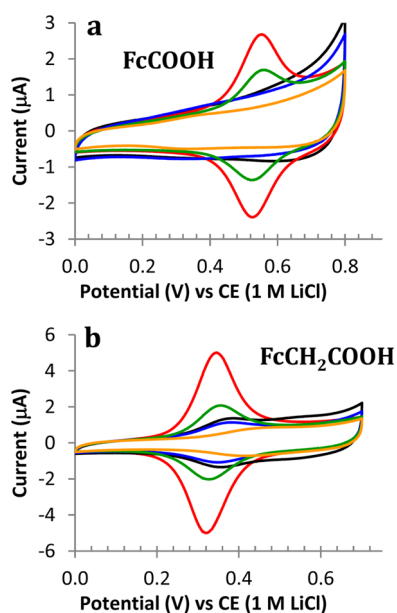
**Table 2.** Electrochemical Data for Fc and NP Groups Coupled to Deprotected Amine Functionalized Layers, And the Calculated Theoretical Surface Concentrations

grafted film	amide coupling method	$\Gamma_{NP}$ (10 <sup>-10</sup> mol cm <sup>-2</sup> ) <sup>a</sup>	$\Gamma_{Fc}$ (10 <sup>-10</sup> mol cm <sup>-2</sup> ) <sup>a</sup>	$E_{1/2}(\text{FcCOOH})$ (V)	$\Gamma_{Fc}(\text{FcCH}_2\text{COOH})$ (10 <sup>-10</sup> mol cm <sup>-2</sup> ) <sup>a</sup>	$E_{1/2}(\text{FcCH}_2\text{COOH})$ (V)	$\Gamma_{theo}$ (10 <sup>-10</sup> mol cm <sup>-2</sup> ) <sup>d</sup>
Boc-NH-Ar	(COCl) <sub>2</sub>	3.0 ± 0.1	3.3 ± 0.1	0.60	2.5 ± 0.2	0.36	1.1
	HBTU	1.2 ± 0.1	trace		0.9 ± 0.1	0.37	
Boc-NH-CH <sub>2</sub> -Ar	(COCl) <sub>2</sub>	5.4 ± 0.3	4.5 ± 0.1	0.54	4.1 ± 0.4 <sup>c</sup>	0.35	n/a (multilayer)
	HBTU	3.8 ± 0.3	1.5 ± 0.1	0.54	3.9 ± 0.1	0.33	
Fmoc-NH-Ar	(COCl) <sub>2</sub>	3.0 ± 0.2	2.9 ± 0.2	0.61	2.3 ± 0.2	0.36	0.5
	HBTU	0.8 ± 0.1	trace		0.5 ± 0.1	0.36	
Fmoc-NH-CH <sub>2</sub> -Ar	(COCl) <sub>2</sub>	3.2 ± 0.6	2.5 ± 0.2	0.64	1.7 ± 0.3	0.37	1.2
	HBTU	2.1 ± 0.2	0.6 ± 0.3 <sup>b</sup>	0.54	1.4 ± 0.1	0.34	
Polished GC (blank)	(COCl) <sub>2</sub>	2.4 ± 0.2	1.6 ± 0.2	0.66	2.4 ± 0.4 <sup>b</sup>	0.40	n/a
	HBTU	trace	trace		0.5 ± 0.3	0.44	

<sup>a</sup>Unless indicated otherwise, the number of repeat samples, *n*, was 2. <sup>b</sup>*n* = 3. <sup>c</sup>*n* = 4. <sup>d</sup>Calculated for a monolayer of grafted groups based on optimized structures from DFT calculations using van der Waals radii and assuming hexagonal closest packing on a flat surface.



surface chemistry and the optimum ferrocene derivative for preparation of ferrocene-modified surfaces. All surface concentration data obtained from cyclic voltammetry of the modified surfaces are listed in Table 2. Figure 3 shows typical cyclic



**Figure 3.** Cyclic voltammograms obtained in 0.1 M LiClO<sub>4</sub>-EtOH at a scan rate of 200 mV s<sup>-1</sup> after reaction of deprotected amine-modified and polished GC with (a) FcCOOH and (b) FcCH<sub>2</sub>COOH using HBTU as the activating agent. Black line: Boc-NH-Ar-GC; red line: Boc-NH-CH<sub>2</sub>-Ar-GC; blue line: Fmoc-NH-Ar-GC; green line: Fmoc-NH-CH<sub>2</sub>-Ar-GC; yellow line: polished GC.

voltammograms obtained in 0.1 M LiClO<sub>4</sub>-EtOH at GC modified with deprotected films that were subsequently reacted with FcCOOH and FcCH<sub>2</sub>COOH using HBTU as the activating agent.

Scrutiny of the data in Table 2 shows that higher surface concentrations of immobilized electroactive species are obtained when reactions of the carboxylic acid derivatives with amine modified surfaces are activated by (COCl)<sub>2</sub> than by HBTU. Furthermore, when reactions are performed under the same conditions but at polished GC, significant amounts of electroactive species are coupled to the surface using the (COCl)<sub>2</sub>-activated carboxylic acid derivatives, whereas HBTU activation gives only very low or trace amounts of immobilized species (Table 2). Immobilization of Fc and NP groups at GC by reaction of the acid chloride derivatives is consistent with our recently reported studies in which we tentatively proposed the formation of ester linkages to the surface.<sup>27</sup> Hence we conclude that at grafted surfaces, (COCl)<sub>2</sub>-activated carboxylic acid derivatives couple both to the grafted films and directly to GC. Measured surface concentrations of coupled groups are therefore expected to exceed the surface concentrations of amine groups. Cyclic voltammograms of the grafted surfaces obtained using (COCl)<sub>2</sub> as the activating agent are presented and discussed in the Supporting Information (Figure S2).

In the following discussion, considering only the data in Table 2 for HBTU-activated coupling, the surface concentrations of immobilized redox groups at the deprotected Boc-NH-CH<sub>2</sub>-Ar tether layer are at least twice those coupled to the other layers. This supports the suggestion from film thickness measurements that after deprotection Boc-NH-CH<sub>2</sub>-Ar is a

multilayer. Comparing the measured surface concentrations of coupled groups at each monolayer, in all cases the order is NP > FcCH<sub>2</sub>COOH > FcCOOH groups. Close-packed monolayers of NP and Fc groups on flat surfaces have calculated surface concentrations of ~12 and (4 to 5) × 10<sup>-10</sup> mol cm<sup>-2</sup>, respectively,<sup>28,29</sup> and hence the sizes of the NP and Fc moieties do not limit the amounts coupled. The lower reactivity of FcCOOH than FcCH<sub>2</sub>COOH toward HBTU-activated amide coupling is attributed to both steric and electronic effects, and it is clear that FcCOOH does not react with a significant proportion of surface amine groups. Considering the coupling of NP and FcCH<sub>2</sub>COOH to the tether layers, it is tentatively suggested that the voltammetry of Fc groups gives a more reliable estimate of surface concentration than does voltammetry of NP groups due to the irreversible reduction of NP moieties and the difficulties of baseline correction.

For all electroactive groups coupled to the monolayers, Table 2 shows that the order of surface concentrations is Fmoc-NH-CH<sub>2</sub>-Ar > Boc-NH-Ar > Fmoc-NH-Ar. This is the same order as the theoretical surface concentrations (Γ<sub>theo</sub>) for hexagonally close-packed monolayers of the protected derivatives on an ideally flat surface. These values (Table 2: 1.2, 1.1, and 0.5 × 10<sup>-10</sup> mol cm<sup>-2</sup> for Fmoc-NH-CH<sub>2</sub>-Ar, Boc-NH-Ar, and Fmoc-NH-Ar, respectively) were calculated assuming optimized structures from DFT calculations (Supporting Information, Figures S3–S6 and Tables S1–S4).<sup>18</sup> Comparing these values with the measured surface concentrations of Fc groups immobilized by reaction of FcCH<sub>2</sub>COOH using HBTU as the activating agent and assuming a typical surface roughness factor of 2 for GC surfaces,<sup>30,31</sup> the surface concentration of amine groups in each layer is estimated to be approximately half that of a close-packed monolayer; however, noting that coupling reactions using HBTU are typically less than quantitative (yields of peptide coupling using uronium reagents can range from ~30 to 96%<sup>32,33</sup>), the surface concentrations of the grafted monolayers might be significantly higher than those of immobilized Fc groups. This is particularly true for the NH<sub>2</sub>-Ar monolayers for which comparison of the experimentally and theoretically determined surface concentrations at each monolayer indicates a somewhat lower coupling yield than that at the NH<sub>2</sub>-CH<sub>2</sub>-Ar monolayer.

As a final note concerning the theoretical surface concentrations, it is interesting that the greater flexibility introduced by the -CH<sub>2</sub> group decreases the protective character of the Fmoc substituent in Fmoc-NH-CH<sub>2</sub>-Ar relative to that in Fmoc-NH-Ar, leading to a greater theoretical (and experimental) surface concentration for the former modifier. That flexibility combined with the relatively small Boc protecting group can also account for the formation of multilayer amine films from the Boc-NH-CH<sub>2</sub>-Ar modifier.

## CONCLUSIONS

Amine-terminated monolayers can be prepared by deprotection of Fmoc-NH-CH<sub>2</sub>-Ar, Fmoc-NH-Ar, and Boc-NH-Ar films, whereas Boc-NH-CH<sub>2</sub>-Ar films yield multilayers. The theoretical surface concentrations of close-packed layers of Fmoc-NH-CH<sub>2</sub>-Ar and Boc-NH-Ar groups are very similar and are approximately twice that for the Fmoc-NH-Ar monolayer. Experimentally determined surface concentrations follow the same order and are estimated to be at least half those of close-packed monolayers. The NH<sub>2</sub>-CH<sub>2</sub>-Ar monolayer appears to have higher reactivity for HBTU-activated coupling than do the NH<sub>2</sub>-Ar tether layers, and HBTU-activated coupling of

FcCH<sub>2</sub>COOH gives higher yields of immobilized Fc groups than does FcCOOH. When carboxylic acid derivatives are activated using (COCl)<sub>2</sub>, they react with both the amine-terminated layers and directly with the GC surface. Thus, HBTU-activated coupling to the deprotected Fmoc-NH-CH<sub>2</sub>-Ar monolayer gives the highest yield of amide coupled species without interference from reactions directly at GC, whereas to obtain the maximum total concentration of species in a monolayer on the surface (that is, coupled to the tether layer and directly to GC), reaction of (COCl)<sub>2</sub>-activated carboxylic acid derivatives at the deprotected Boc-NH-Ar layer is the best approach. Future work will utilize the amine-terminated monolayers for building up stable functional surfaces.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Syntheses of [Boc-NH-Ar-N<sub>2</sub>]<sub>2</sub>BF<sub>4</sub>, [Boc-NH-CH<sub>2</sub>-Ar-N<sub>2</sub>]<sub>2</sub>BF<sub>4</sub>, [Fmoc-NH-Ar-N<sub>2</sub>]<sub>2</sub>BF<sub>4</sub>, and [Fmoc-NH-CH<sub>2</sub>-Ar-N<sub>2</sub>]<sub>2</sub>BF<sub>4</sub>. Cyclic voltammograms and discussion of redox probes at modified electrodes before and after deprotection and after immersion in blank deprotection solution. Cyclic voltammograms and discussion of modified electrodes after reaction with FcCOOH and FcCH<sub>2</sub>COOH using (COCl)<sub>2</sub> as the activating agent. Figures of optimized geometries of Boc-NH-Ar, Boc-NH-CH<sub>2</sub>-Ar, Fmoc-NH-Ar, and Fmoc-NH-CH<sub>2</sub>-Ar modifiers and tables of corresponding xyz coordinates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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