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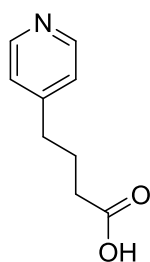
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## General

All the chemicals were obtained from Sigma-Aldrich and were all used without further purification. Ethylene glycol was distilled and stored over molecular sieves (0.5 nm), under nitrogen. DCM and DMF were distilled using an MBraun MB SPS-800 solvent purification system. Flash chromatography columns were performed on Silica gel (VWR Prolabo) particle size 40  $\mu\text{m}$  - 63  $\mu\text{m}$ , pH = 6.4. Thin layer chromatography (TLC) was performed on silica pre-coated aluminium plates (Merck Silica gel F254) and the spots were visualised with UV light or ninhydrine stain.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were obtained on a Bruker AVIII 400 Spectrometer; the chemical shifts ( $\delta$ ) are reported in ppm and coupling constants (J) in Hertz (Hz) are rounded up to the .5 or .0 value. Abbreviations for signals: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, b = broad. All  $^{13}\text{C}$  NMR chemical shifts are reported to one decimal place, except when, in order to discriminate, the second decimal figure is reported in brackets. UV-vis spectra were recorded on a Perkin Helmer Lambda35 spectrometer. LC-MS spectra were recorded on a Bruker Daltonics Esquire3000 plus. X-ray diffraction analyses were performed with a Kappa Apex II Duo diffractometer with dual Cu and Mo sources and Apex II CCD area detector. High-Res Mass spectra were recorded by the EPSRC UK National Mass Spectrometry Facility at Swansea University.

## Synthesis of compounds

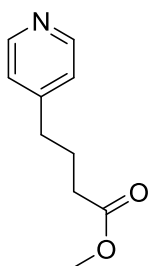
### 4-(4-pyridil)butyric acid (1)<sup>1</sup>



Sodium hydride (60% dispersion in mineral oil) (0.5 g, 22 mmol) was added portion-wise to dry ethanol (35 mL) under stirring. The solution was allowed to return to room temperature and diethylmalonate (7.1 mL, 47 mmol) was added. The mixture was stirred at 80°C for 1 hour, then cooled to room temperature. 4-vinylpyridine (2.2 mL, 20 mmol) was added and the solution was stirred at reflux for three hours. The ethanol was removed, the oily residue dissolved with 20% HCl (50 mL) and the solution refluxed overnight. The aqueous phase was extracted with ethyl acetate (3 x 30 mL) and the water removed. The solid residue was dissolved with the minimum volume of hot methanol, any insoluble salt was filtered and

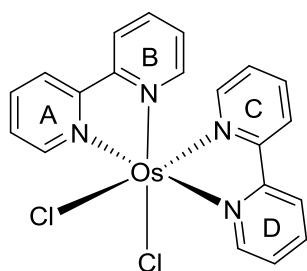
ethyl acetate was added to the filtrate to precipitate the product. The solid was filtered to obtain product **1** as a white solid in 60 % (1.9 g) yield. **m.p.** 111-114°C (from ethyl acetate). **IR**  $\nu_{\max}/\text{cm}^{-1}$  3248 (w), 2932 (w). 1573 (s), 1415 (m), 1018 (m), 1613 (m), 815 (m), 798 (m),  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 10.26 (1 H, bs, OH), 8.51 (2 H, d,  $J$  = 6, CHNCH), 7.25 (2 H, d,  $J$  = 6, CHC<sub>q</sub>CH), 2.75 (2 H, t,  $J$  = 7.5, CH<sub>2</sub>N), 2.40 (2 H, t,  $J$  = 7.3, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.01 (2 H, m, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO).  **$^{13}\text{C NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  = 176.6, 152.5, 147.9, 124.4, 34.5, 33.3, 25.2. **LR-MS ES+**  $m/z$  331.1 [2M+H]<sup>+</sup> (80%), 166.0 [M+H]<sup>+</sup> (20%).

**Methyl 4-(pyridin-4-yl)butanoate (2)**



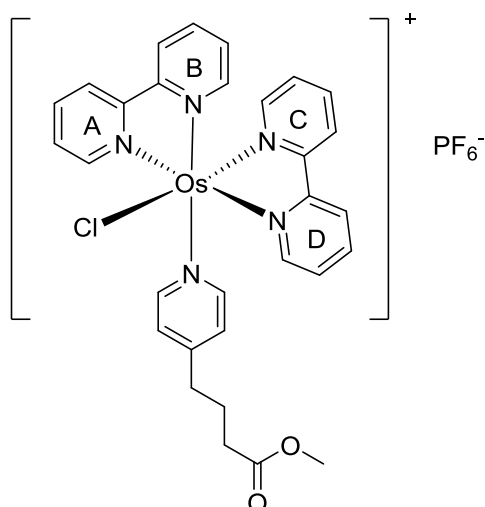
4-(4-pyridil)butyric acid (**1**) (0,4 g, 2.3 mmol) was dissolved in methanol (15 mL) and sulphuric acid (0.1 ml, 2.5 mmol) in methanol (5 mL) was added. The mixture was stirred at reflux for 3 hours. The solvent was removed and the oily residue dissolved with water (30 mL). The pH of the solution was adjusted to 9 by addition of a 1 M solution of sodium hydroxide and the aqueous phase extracted with ethyl acetate (3 x 25 mL). The organic phase was dried over magnesium sulphate and the solvent evaporated. The product was purified by flash chromatography over silica (DCM : MeOH = 95 : 5), to obtain a colourless oil in 47 % (0.2 g) yield. **IR**  $\nu_{\max}/\text{cm}^{-1}$  3055 (w), 2922 (w), 1731 (s), 1604 (m), 1415 (m), 1171 (m), 798 (m).  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.47 (2 H, d,  $J$  = 7.5, CHNCH), 7.08 (2 H, d,  $J$  = 7.5, CHC<sub>q</sub>CH), 3.64 (3 H, s, OCH<sub>3</sub>), 2.63 (2 H, t,  $J$  = 7.6, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.32 (2 H, t,  $J$  = 7.3, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 1.95 (2 H, m, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO).  **$^{13}\text{C NMR}$**  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  = 173.3, 151.8, 148.5, 124.2, 51.6, 34.4, 33.0, 25.2. **LR-MS ES+**  $m/z$  180.7 [M+H]<sup>+</sup> (100%).

*Cis-bis(2,2'-bipyridyl) osmium (II) chloride (3)<sup>2</sup>*



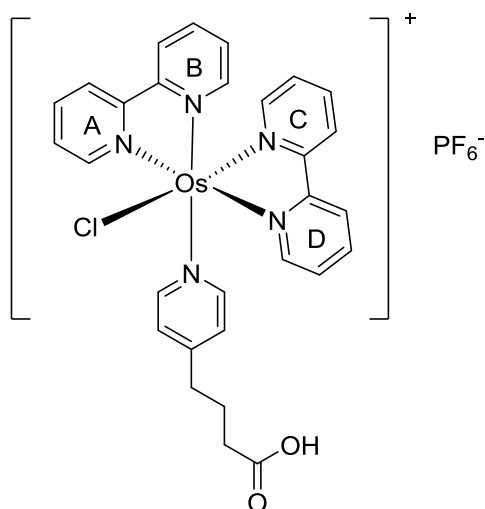
Ammonium hexachloroosmate(IV) ((NH<sub>4</sub>)<sub>2</sub>OsCl<sub>6</sub>) (0.5 g, 1.14 mmol) and 2-2'-bipyridyl (0.36 g, 2.30 mmol) were mixed with ethylene glycol (15 mL) and purged for 10 minutes with nitrogen. The mixture was refluxed for 1 hour under nitrogen, then allowed to cool at room temperature, before immersion in an ice-bath. Sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) (0.69 g, 3.98 mmol) was dissolved in 25 mL of deionised water and added drop wise to the stirred reaction mixture. The mixture left stirring for 1 h to allow the precipitation of the product. The solid was filtered and washed with 250 mL of water and 100 mL of diethyl ether. The product was a black-purple powder obtained in 86 % yield (0.565 g). **UV-vis:** λ<sub>max</sub> (CH<sub>3</sub>CN)/nm 381 (ε/dm<sup>3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 11976), 462 (ε/dm<sup>3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 10940), 554 (ε/dm<sup>3</sup>mol<sup>-1</sup>cm<sup>-1</sup> 12662). **IR:** ν<sub>max</sub>/cm<sup>-1</sup> 3041 (w), 1464 (m), 1412 (m), 1452 (m), 1249 (m), 1011 (m), 756 (s), 715 (m), 646 (m). **<sup>1</sup>H NMR** (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>): δ = 9.74 (2 H, d, *J* = 5.5, NCH-ring A), 8.09 (2 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring A), 7.89 (2 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring B), 7.40 (2 H, t with fine splitting, *J* = 8.0, C<sub>q</sub>CHCH-ring A), 7.33 (2 H, td, *J* = 5.5, 8.0) NCHCH-ring A), 7.28 (2 H, bd, *J* = 5.0, NCH-ring B), 7.07 (2 H, t, *J* = 8.0, C<sub>q</sub>CHCH-ring B), 6.57 (2 H, m, NCHCH-ring B). **LR-MS ES+** *m/z* 574.0 [M+H]<sup>+</sup> (100%). **XRD** Crystals of the complex were obtained by recrystallization from dichloromethane.

**bis-(2,2'-bipyridyl) (Methyl-4-(pyridin-4-yl)butanoate)chloride osmium(II) hexafluorophosphate (4)<sup>2</sup>**



Compound **3** (0.1 g, 0.2 mmol) was dissolved in ethylene glycol (5 mL) under nitrogen and methyl 4-(pyridin-4-yl)butanoate (**2**) (0.03 g, 0.2 mmol) was added. The mixture was stirred at 100°C for 2 hours and allowed to reach room temperature. A solution of ammonium hexafluorophosphate (0.1 g, 0.5 mmol) in water (15 mL) was added drop-wise to the stirring reaction mixture to precipitate the product. The solid was filtered, washed with water (200 mL) and dried with diethyl ether (100 mL). The product was a red-black solid obtained in 80 % (0.12 g) yield. **m.p.** 152-158°C (from dichloromethane). **UV-vis:**  $\lambda_{\text{max}}$  (CH<sub>3</sub>CN)/nm 360 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  14563), 429 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  13140), 500 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  13057). **IR:**  $\nu_{\text{max}}/\text{cm}^{-1}$  3073 (w), 2924 (w), 1727 (m), 1458 (m), 1418 (m), 1255 (m), 1062 (m), 831 (s), 758 (s), 723 (m), 658 (m). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 9.62 (1 H, bd, *J* = 5.5, NCH-ring A), 8.74 (1 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring D), 8.60 (2 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring A and ring B), 8.48 (1 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring C), 8.30 (3 H, bd, *J* = 5.5, CHNCH-ring E, NCH-ring D), 7.84-7.73 (3 H, m, NCHCHCH-ring A, C<sub>q</sub>CHCH-ring D), 7.71 (1 H, bd, *J* = 5.5, NCH-ring C), 7.64 (1 H, t with fine splitting, C<sub>q</sub>CHCH-ring B), 7.59 (1 H, t with fine splitting, NCHCH-ring D), 7.52 (1 H, t with fine splitting, C<sub>q</sub>CHCH-ring C), 7.37 (1 H, bd, *J* = 5.5, NCH-ring B), 7.17 (2 H, bd, *J* = 5.5, CHC<sub>q</sub>CH-ring E), 7.10 (2 H, m, NCHCH-ring B and C), 3.52 (3H, s, OCH<sub>3</sub>), 2.54 (2H, t, *J* = 7.5, C<sub>q</sub>CH<sub>2</sub>), 2.30 (2H, t, *J* = 7.3, CH<sub>2</sub>CO), 1.77 (2H, m, C<sub>q</sub>CH<sub>2</sub>CH<sub>2</sub>). **<sup>13</sup>C NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 172.8, 162.5, 160.8, 159.6, 151.9, 151.7, 150.6, 135.9, 135.0, 133.7, 135.5, 127.9, 127.5, 126.9, 125.8, 124.2, 123.6, 123.2, 123.1, 33.1, 32.6, 24.1. **LR-MS ES+** *m/z*: 718.0 [M-PF<sub>6</sub>]<sup>+</sup> (100%). **HR-MS** *m/z*: [M-PF<sub>6</sub>]<sup>+</sup> calculated for C<sub>30</sub>H<sub>29</sub>ClN<sub>5</sub>O<sub>2</sub>Os: 718.1609, observed: 718.1594.

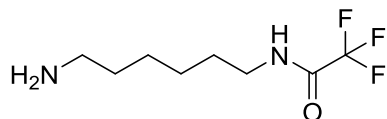
*bis-(2,2'-bipyridyl) (4-(pyridin-4-yl)butanoic acid) chloride osmium (II) hexafluorophosphate*  
(5)<sup>3</sup>



Compound **4** (0.1g, 0.1 mmol) was dissolved in a 2M solution of potassium hydroxide (1 g, 20 mmol) in methanol/water 1:1 (10 mL) and the mixture was refluxed for 3 hours. The organic solvent was removed and water was added. The aqueous phase was extracted with dichloromethane (3 x 15 mL) in order to remove any starting material left. The pH of the aqueous phase was adjusted to 2 by addition of 1M hydrochloric acid and the resulting solution extracted with dichloromethane (3 x 30 mL). The organic phase was dried over magnesium sulphate and the solvent removed to obtain the product as a red-black solid in 83% (0.08 g) yield. **m.p.** 172-175°C (from dichloromethane/ methanol) **UV-vis:**  $\lambda_{\max}$  (CH<sub>3</sub>CN)/nm 359 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  14876), 430 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  13417), 505 ( $\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$  13325). **IR:**  $\nu_{\max}/\text{cm}^{-1}$  3069 (w), 1720 (m), 1499 (m), 1476 (m), 1319 (m), 1017 (m), 828 (s), 761 (s), 723 (m), 658 (m). **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 12.04 (1H, bs, OH), 9.62 (1 H, bd, *J* = 5.5, NCH-ring A), 8.74 (1 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring D), 8.60 (2 H, bd, *J* = 8.0, C<sub>q</sub>CH-ring A and ring B), 8.48 (1 H, bd, *J* = 8.0, C<sub>q</sub>CH -ring C), 8.29 (3 H, bd, *J* = 5.5, CHNCH-ring E, NCH-ring D), 7.84-7.73 (3 H, m, NCHCHCH-ring A, C<sub>q</sub>CHCH-ring D), 7.70 (1 H, bd, *J* = 5.5, NCH-ring C), 7.64 (1 H, t with fine splitting, C<sub>q</sub>CHCH-ring B), 7.59 (1 H, t with fine splitting, NCHCH-ring D), 7.52 (1 H, t with fine splitting, C<sub>q</sub>CHCH-ring C), 7.36 (1 H, bd, *J* = 5.5, NCH-ring B), 7.17 (2 H, bd, *J* = 5.5, CHC<sub>q</sub>CH-ring E), 7.10 (2 H, m, NCHCH-ring B and C), 2.54 (2H, t, *J* = 7.5, C<sub>q</sub>CH<sub>2</sub>), 2.20 (2H, t, *J* = 7.3, CH<sub>2</sub>CO), 1.74 (2H, m, C<sub>q</sub>CH<sub>2</sub>CH<sub>2</sub>). **<sup>13</sup>C NMR** (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 173.9, 162.5, 160.8, 159.6, 151.9, 151.7, 150.6, 135.9, 135.0, 133.7, 135.5, 127.9, 127.5, 126.9, 125.8, 124.2, 123.6, 123.2, 123.1, 33.1, 30.6, 24.3. **LR-MS ES+** *m/z*: 704.1 [M-PF<sub>6</sub>]<sup>+</sup> (100%). **HR-MS** *m/z*: [M-PF<sub>6</sub>]<sup>+</sup>

calculated for  $C_{29}H_{27}ClN_5O_2Os$ : 704.1461, observed: 704.1442. **XRD** Crystals of the complex were obtained by recrystallization from dichloromethane/methanol.

***N*-(6-aminohexyl)-2,2,2-trifluoroacetamide (6)**<sup>4</sup>



1,6-hexamethylenediamine (0.4 g, 4 mmol) was dissolved in methanol (20 mL) and the solution was cooled at  $-70^{\circ}\text{C}$ . Ethyltrifluoroacetate (0.5 mL, 4 mmol) was dissolved in methanol (50 mL) and added drop-wise to the amine solution. The mixture was stirred for 2 hours, then allowed to reach  $0^{\circ}\text{C}$  and stirred for 1 hour. The solvent was evaporated to obtain and the residue dissolved with water (30 mL). The product was extracted with ethyl acetate (3 x 25 mL), the organic phase was dried with magnesium sulphate and the solvent removed. The product was a white solid obtained in 62 % (0.5 g) yield. **m.p.**  $53\text{--}55^{\circ}\text{C}$  (from methanol) **IR**  $\nu_{\text{max}}/\text{cm}^{-1}$  3306 (m), 2944 (m), 1702 (s), 1568 (m), 1147 (s), 722 (m).  **$^1\text{H}$  NMR** (poorly resolved) (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.26 (2 H, bs,  $\text{NHCO}$ ), 3.35 (2 H, bs,  $\text{CH}_2\text{NHCO}$ ), 2.70 (2 H, t,  $J$  = 6.5,  $\text{CH}_2\text{NH}_2$ ), 1.40 (8 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ).  **$^{13}\text{C}$  NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  = 157.4, 117.3, 42.0, 39.7, 33.5, 28.7, 26.6, 25.8. **LR-MS ES+**  $m/z$  213.6  $[\text{M}+\text{H}]^+$  (100%).



### ***Glassy carbon surface modification***

Electrochemical experiments for single electrodes were recorded on an Autolab PGSTAT30 Potentiostat/Galvanostat and data were analysed with Origin 7.0 software. Electrochemical experiments were performed using a three electrodes electrochemical system: the counter electrode was a 1 cm<sup>2</sup> platinum gauze, the reference electrode was a homemade saturated calomel electrode (SCE) or silver/silver chloride (Ag/AgCl) electrode. The working electrode was a 3 mm diameter (geometrical area of 0.071 cm<sup>2</sup>) glassy carbon disc (HTW, Hochtemperatur-Werkstoffe GmbH, Germany) sealed in a glassy tube and wired up with copper wire by using melted indium. Prior to modification, the working GC electrodes were dry polished on silicon carbide coated abrasive paper grade P800, followed by silicon carbide coated abrasive paper grade P1200 and Aluminium Oxide Fibre Optic Lapping Film grade 5µm. The electrodes were rinsed with Milli-Q distilled using an Elga Purelab Ultra purification system. The electrodes were then polished successively with Buehler alumina (grade 1 µm and 0.3 µm) slurries with Milli-Q water on Buehler polishing pads, rinsed with Milli-Q water between each step and stored in HPLC grade acetonitrile. Unless otherwise stated, the electrochemical characterisation of the modified electrodes was performed in 0.1 M phosphate buffer pH 7.

### ***General procedure for electrochemical attachment of mixtures of amines at GC electrodes***

The attachment of mixtures of amine linkers was carried out in a 20 mM solution of the two components in acetonitrile (5 mL) and TBATFB (0.1 M, 0.165 g), varying the amount of each according to their fraction, by chronoamperometry, holding the potential from at 2.1 V vs. Ag/AgCl for 3 minutes. The electrode was rinsed with distilled water (5 mL) and acetonitrile (5 mL) and dried with air for 10 seconds.

### ***General procedure for the Boc-deprotection of modified GC electrodes<sup>5</sup>***

The electrode was suspended in a 4 M solution of HCl (10 mL) in 1,4-dioxane for 1 hour at room temperature. The deprotected electrode was then rinsed with deionised water (5 mL), acetonitrile (5 mL) and dried in air for 10 seconds.

### ***General procedure for the tfa-deprotection of modified GC electrodes<sup>6</sup>***

The electrode was suspended in a 10% solution of K<sub>2</sub>CO<sub>3</sub> (10 mL) in methanol/water (7:3) for 6 hours at room temperature. The deprotected electrode was then rinsed with deionised water (5 mL), acetonitrile (5 mL) and dried in air for 10 seconds.

#### ***Attachment of complex 5 to the amine modified GC electrodes***

A 3 mL solution containing complex **8** (10 mM) (0.025 g, 0.03 mmol), EDC (0.054 mL, 0.3 mmol) and NHS (0.007 g, 0.2 mmol) in dry DMF was stirred for 1 hour at room temperature. The electrode was dipped in the solution for 16 hours at room temperature. The electrode was rinsed with deionised H<sub>2</sub>O (5 mL), CH<sub>3</sub>CN (5 mL) and left to dry in air for 5 minutes. After a first check by cyclic voltammetry, the electrode was left in stirring CH<sub>3</sub>CN for 24 hours and checked by cyclic voltammetry.

#### ***Attachment of anthraquinone-2-carboxylic acid at amine modified GC electrodes***

A 3 mL solution containing anthraquinone-2-carboxylic acid (0.012 g, 0.05 mmol), EDC (0.088 mL, 0.5 mmol) and NHS (0.034 g, 0.3 mmol) in dry DMF was stirred for 1 hour at room temperature. The electrode was dipped in the solution for 16 hours at room temperature. The electrode was rinsed with deionised H<sub>2</sub>O (5 mL), CH<sub>3</sub>CN (5 mL) and left to dry in air for 5 minutes. After a first check by cyclic voltammetry, the electrode was left in stirring CH<sub>3</sub>CN for 45 hours and checked by cyclic voltammetry.

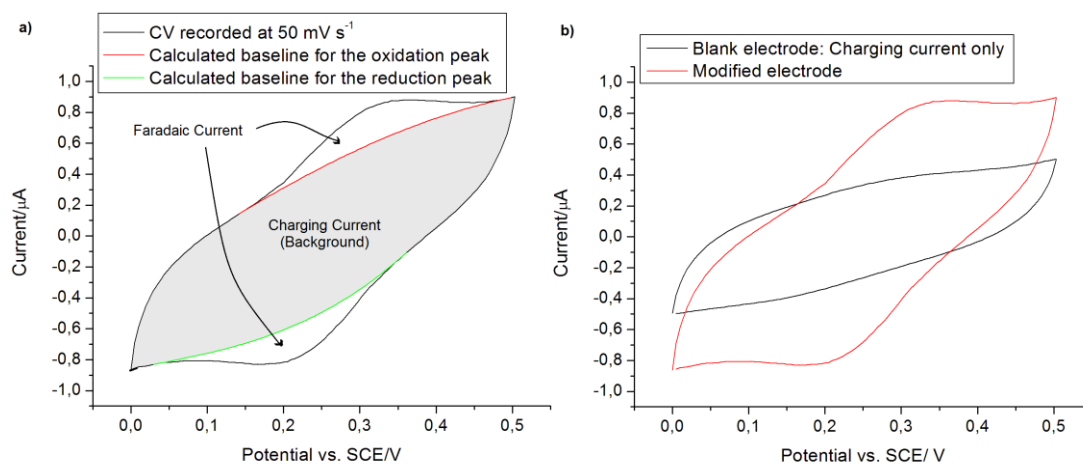
#### ***General procedure for the capping of the residual free amines on the modified GC electrodes***

The electrode was dipped in a 10 mM solution of acylating agent (Ac<sub>2</sub>O (0.005 mL; 0.05 mmol) or AcCl (0.004 mL; 0.05 mmol)) in dry DCM (5 mL) with Et<sub>3</sub>N (0.008 mL; 0.055 mmol) and DMAP (0.6 mg; 0.005 mmol), under nitrogen and left stirring overnight at room temperature. The electrodes were rinsed with HPLC grade CH<sub>3</sub>CN and dried with air for 30 seconds.

#### ***Determination of surface coverage***

The charge associated with the surface redox process was calculated by analysing the recorded CVs using Origin 7.0. To subtract the background current a rigorous procedure of baseline creation and subtraction was used, this has been described in our earlier work.<sup>7</sup> For each cathodic and anodic scan 100 anchoring points were automatically placed on the curve over the full potential range. Then those points falling within 200 mV of the Faradaic peak were removed and the remaining anchoring points interpolated using the B-spline function to generate the baseline.

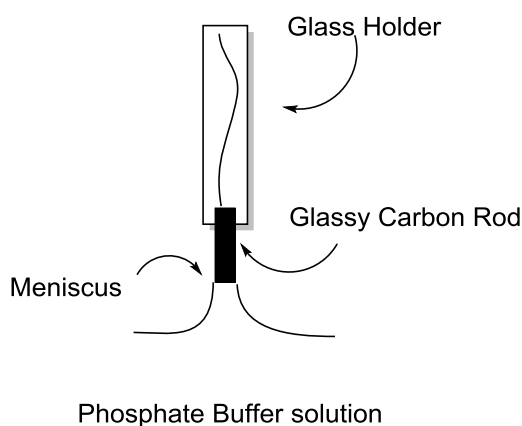
Figure S1 illustrates the procedure and Figures S6b to S11b below show examples of the results of the background subtraction.



**Figure S1.** CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for complex 5: a) elaborated with Origin 7.0 to show the simulated peak baselines and the current components, b) comparison between modified and blank electrode.

### *Use of the meniscus configuration*

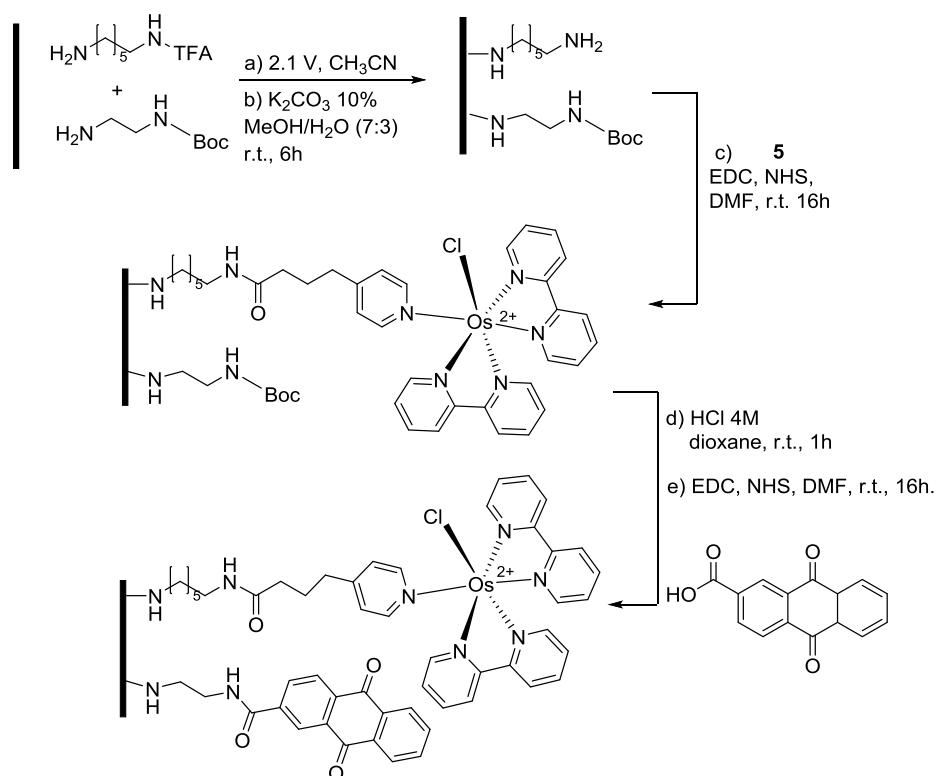
During the course of the work we found that it was difficult to obtain very reproducible results for replicate surface modification experiments when glassy carbon electrodes sealed in glass in the conventional way using casting araldite. Reproducible cleaning of the electrodes after each set of experiments proved difficult, requiring the oxidation of the surface in phosphate buffer at high potentials and various rounds of polishing on abrasive paper. Even after these rigorous treatments non-covalent adsorption of anthraquinone in particular increased as more experiments were performed on the same electrode. Suspecting that that the problem arose from material trapped in the araldite or around the sides of the GC electrodes we moved to the use of a meniscus configuration (Figure S2) in which only the end of the GC rod contacts the solution during electrochemical measurements. This arrangement mimics that more frequently used in studies of single crystal electrodes.<sup>8,9</sup> Using this approach repeatability of surface modification experiments was significantly improved.



**Figure S2.** Working electrode setup for the electrochemical experiments.

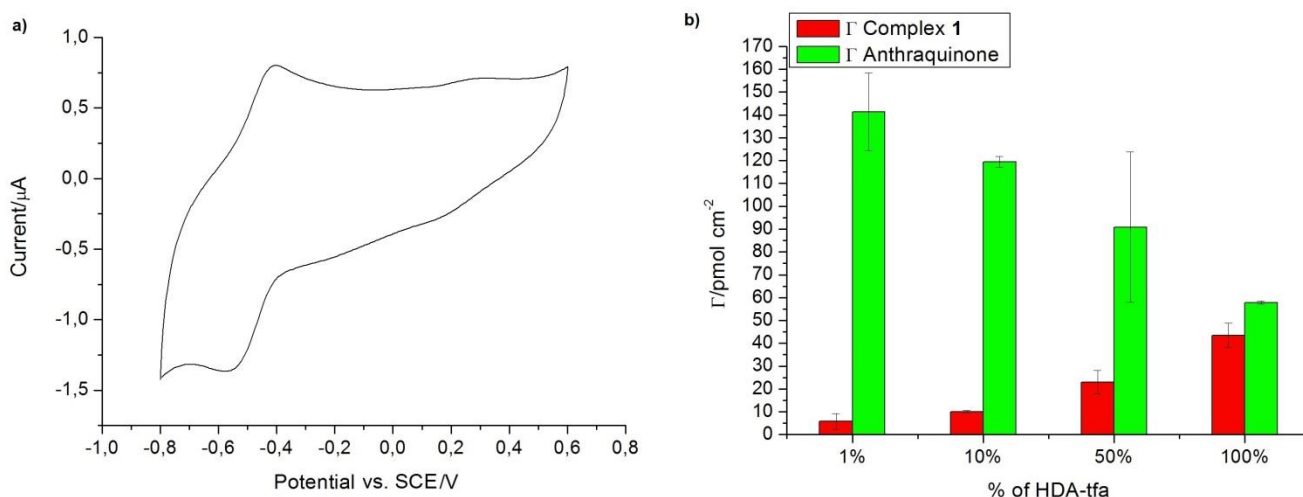
### ***Attachment of complex 5 and anthraquinone-2-carboxylic acid***

A step-wise approach was used to attach complex **5** and anthraquinone to the same surface. Two mono protected diamines were chosen: mono-*N*-Boc-ethylenediamine (EDA-Boc) and mono-*N*-(6-aminohexyl)-2,2,2-trifluoroacetamide (HDA-tfa). The Boc group can be easily removed in acidic conditions, while tfa requires mild basic conditions. Scheme **S1** shows the synthetic steps performed on the electrodes surface: as described above, the grafting of the linkers to form a monolayer was performed by chronoamperometry from 20 mM solutions of the two components in different ratios in CH<sub>3</sub>CN, starting from 100% of HDA-tfa and increasing the fraction of EDA-Boc up to 99%. After the removal of tfa, leaving the EDA-Boc unaltered, complex **5** was coupled to the free amine groups on the HDA linkers, controls were prepared by dipping the amine modified electrodes in a solution of complex **5** in DMF with no coupling agents added. CVs were recorded and the corresponding surface coverage for the osmium complex calculated. After removal of the Boc group, the free EDA was then coupled to anthraquinone-2-carboxylic acid, in this case the controls were obtained by dipping the electrodes in a solution of anthraquinone in DMF with no coupling agents. CVs were recorded for anthraquinone and the surface coverage of the two mediators was calculated.



**Scheme S1.** Synthetic steps for the difunctionalisation of the electrode surface with complex **5** and anthraquinone.

The results obtained are summarised in Figure S3. The CV reported shows the redox peaks for the two species present at the same time on the surface. The peak associated with anthraquinone is present at  $\sim -0.48$  V vs. SCE, while the peak for Os<sup>2+/3+</sup> is at  $\sim 0.25$  V vs. SCE as expected. The barplot shows the variation of surface coverage of the two redox centres plotted against the fraction of HDA-tfa in the starting solutions corrected by the corresponding controls. The trend of the bars clearly represents the results expected: a decrease of the osmium coverage corresponding to a decrease of HDA on the surface, however surface coverages calculated for anthraquinone were higher than expected.

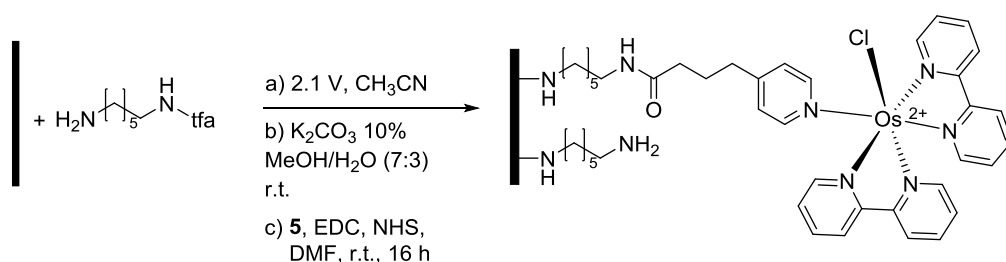


**Figure S3.** a) CV recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for 100% HDA-tfa linker showing both redox centres present on the surface. b) Barplot comparing the variation of  $\Gamma$  of complex **5** and anthraquinone according to the ratio of HDA-tfa linker in solution corrected by the corresponding controls  $\Gamma$ .

It is worth noting the result for 100% HDA-tfa: here, although only osmium complex should have been present, a significant amount of anthraquinone was detected. We explain this result by considering the dimensions and shape of the two redox molecules: the osmium complex has a bulky structure, while anthraquinone is flat and relatively small. During the coupling step, the osmium complexes already present on the surface hinder the approach of other similar molecules, leaving free amine groups available for coupling later with anthraquinone, able to fill the gaps. This said, it is important to underline that the total surface coverage stays reasonably constant for all the different concentrations, suggesting that the methodology developed was broadly successful. The background subtracted CVs suggested a shift of the redox potential of the species depending on the amount of the linkers present at the surface. The calculation of the middle-peak potential ( $E_{mp}$ ) showed a clear shift for complex **5** to lower potentials with the decrease of the amount of HDA-tfa linker with  $\Delta E_{mp} = 42$  mV, while the effect for anthraquinone was more contained with  $\Delta E_{mp} = 12$  mV to lower potentials with a decrease of the linker for anthraquinone, EDA-Boc. Since here we focused on the development of a methodology for the control of the creation of mixed monolayers, no further investigation was dedicated to the study of the factors affecting the potential of the redox probes. In the literature, several factors that can influence the redox potential of molecules are reported, such as pH of the buffer, environment at the surface and interactions between the molecules themselves.<sup>10</sup> The redox potential of osmium complexes with similar structure to complex **5** are not affected by changes in the pH of the buffer solution, hence the effect measured could have been due either to the distance of the redox centre from the

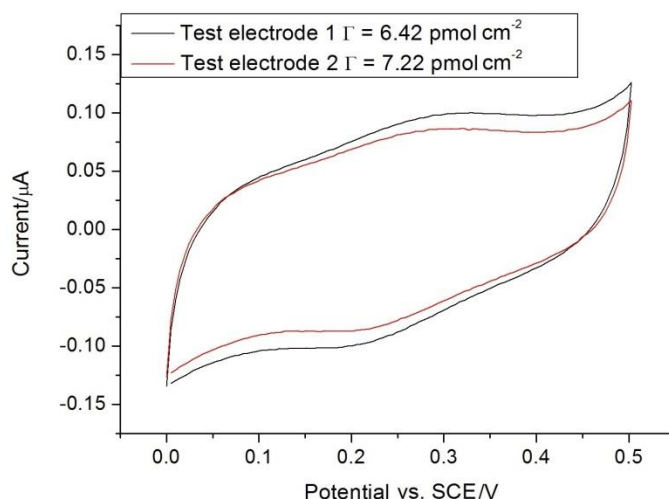
underlying amine monolayer, by the protonation state of the amines surrounding it or by the interaction between osmium complexes, whose intensity decreases when the molecules are diluted at the surface. None of these hypotheses could be discarded without more experiments.

### Optimisation of deprotection steps for HDA-tfa



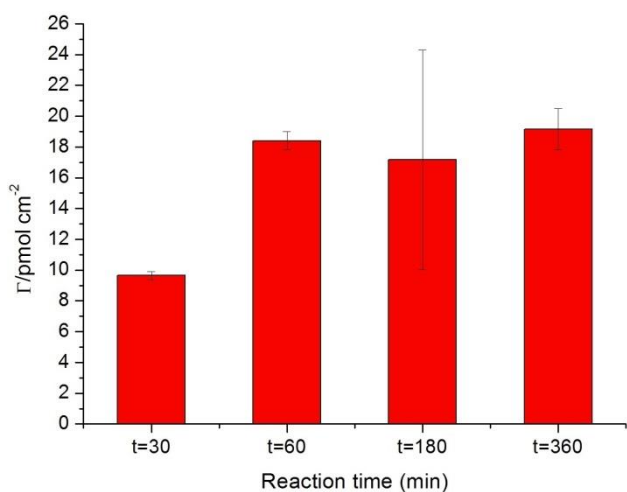
**Scheme S2.** Synthetic steps for the different deprotection times of HDA-tfa.

A set of electrodes were modified with amine grafting solutions containing 0.1% and 0.01% HDA-tfa and complex **5** was coupled to the surface after removal of the tfa protecting group, Scheme **S2**. The Results showed that the same surface coverages of **5** was obtained independent of the percentage of HDA-tfa originally used, suggesting that the deprotection conditions for tfa caused the removal of a small number of Boc groups, although this was an unexpected result. When a set of electrodes was modified with a monolayer of EDA-Boc, exposed to the removal conditions for tfa and dipped in a coupling solution of complex **5** an average surface coverage  $\Gamma = 6.82 \pm 0.4 \text{ pmol cm}^{-2}$  was detected confirming this hypothesis (figure **S4**). Assuming that the 6 h reaction time was longer than necessary to remove the tfa group and a shorter reaction time could remove the tfa but minimise the unwanted Boc removal, a screening of deprotection times was run on a set of electrodes modified with a monolayer of HDA-tfa, followed by coupling of complex **5** (Scheme **S2**).



**Figure S4.** Test tfa removal conditions on Boc protected amine. Comparison of the CVs recorded in 0.1 M PBS solution pH 7, electrode area  $0.071 \text{ cm}^2$ , at  $50 \text{ mV s}^{-1}$  scan rate for two replicates.

The surface coverages calculated (Figure S5) showed that a deprotection time of 1 h was sufficient.



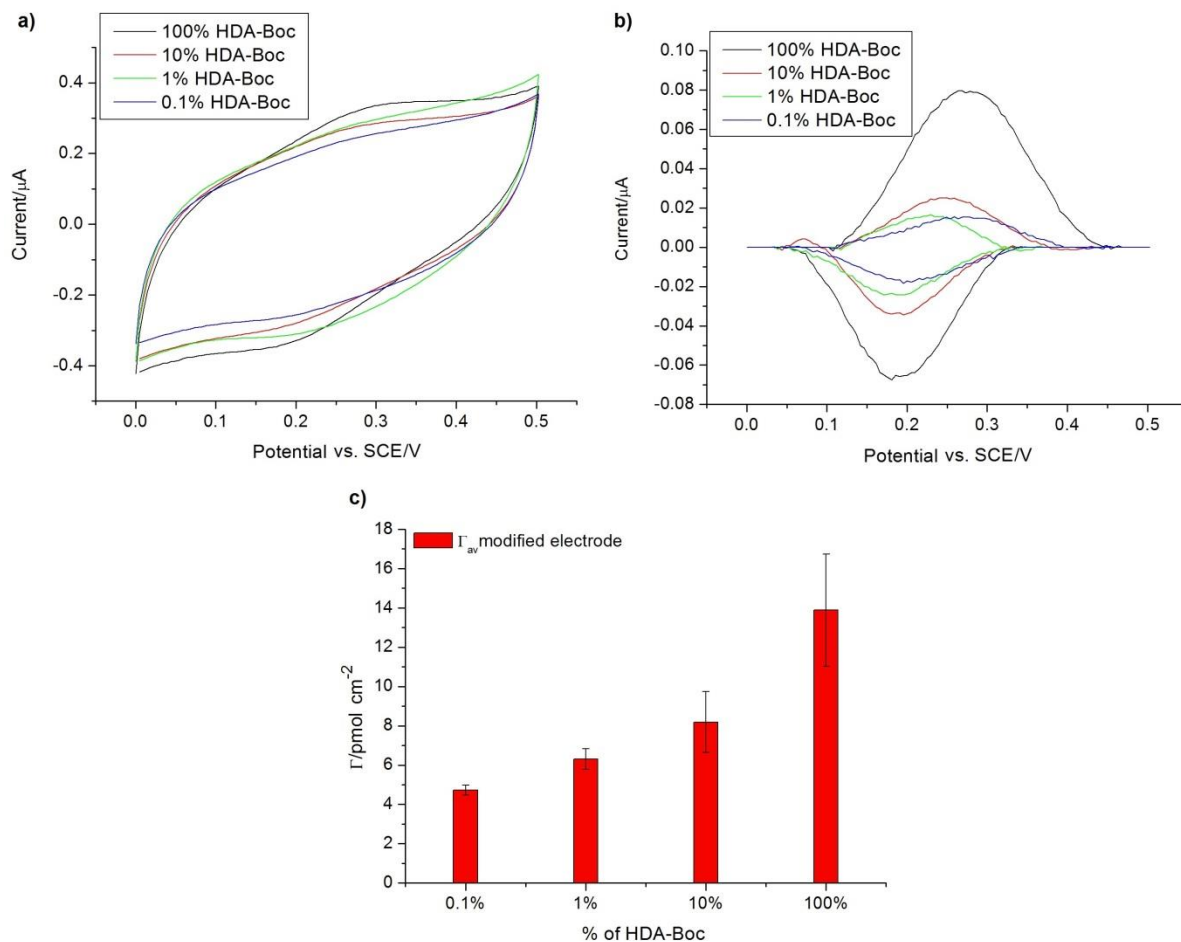
**Figure S5.** Barplot for the variation of  $\Gamma$  of complex **5** according to the deprotection time.

To verify that the new reaction time did not affect the Boc group, a set of EDA-Boc modified electrodes were exposed to a 10 %  $\text{K}_2\text{CO}_3$  solution for 1 h: after the required washing, the signal of complex **5** was not detected on such electrodes.



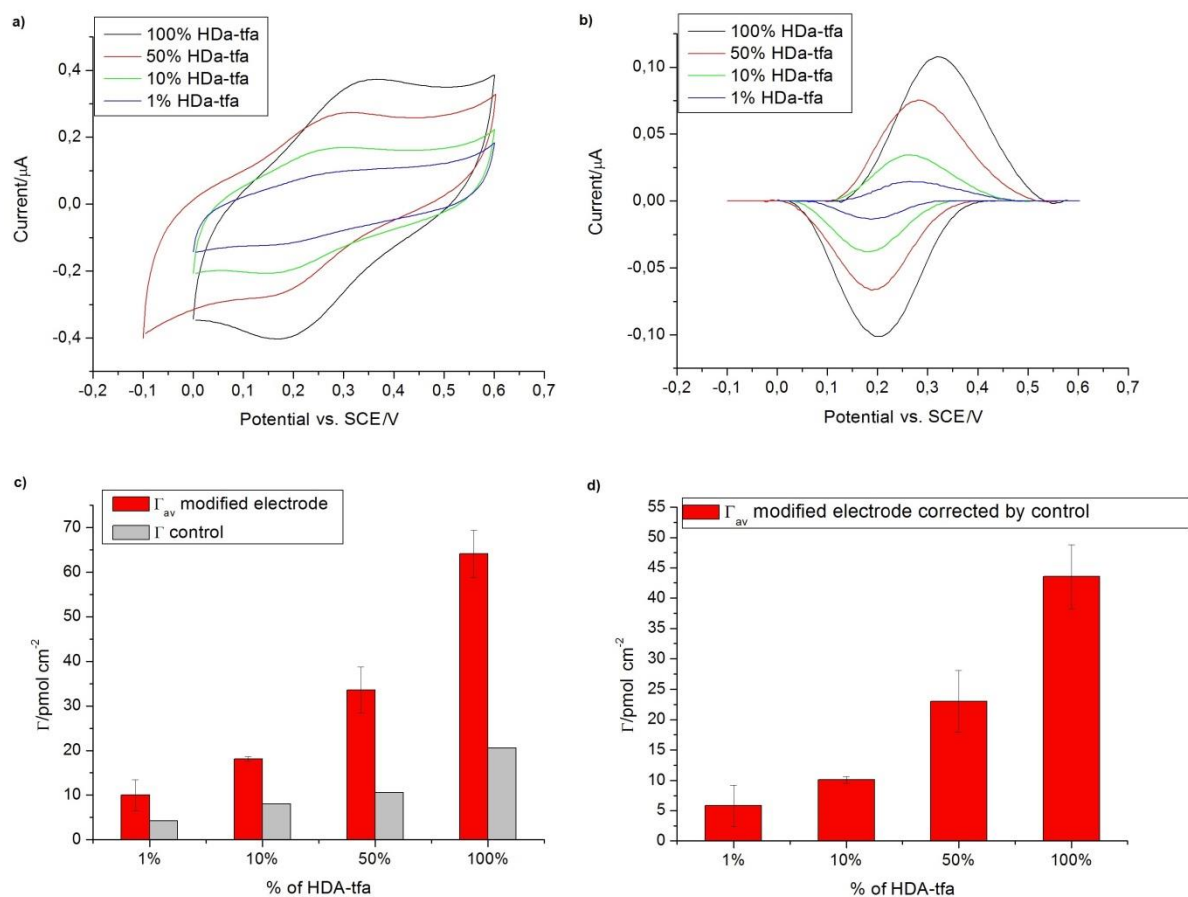
## Electrochemical results.

### Mixed monolayer HDA-Boc/1-BA

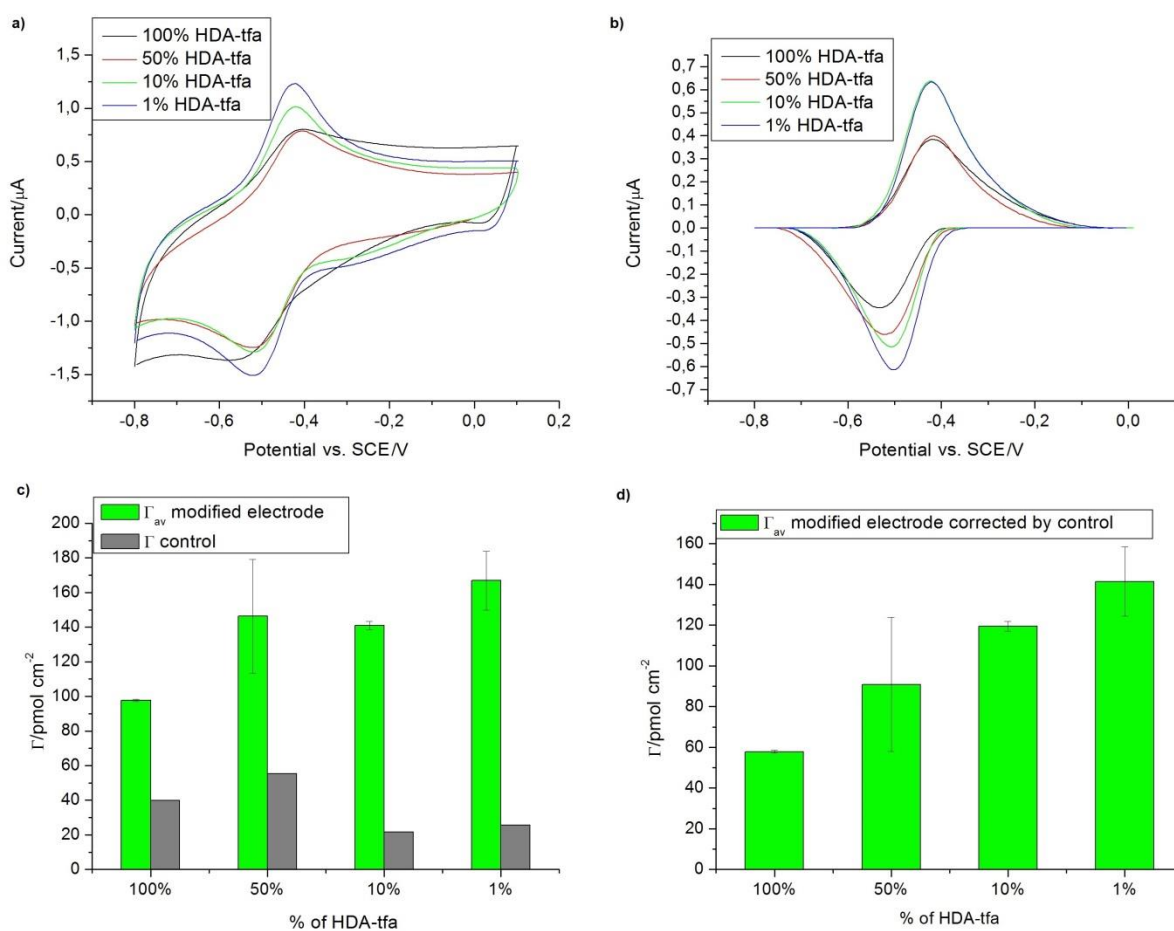


**Figure S6.** a) Comparison of the CVs recorded in 0.1M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for different HDA-Boc linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of complex **5** according to the ratio of HDA-Boc linker in solution, calculated by averaging the values obtained for two replicates; the control was obtained by dipping amine modified electrodes in a neat 10 mM solution of complex **5** in DMF.

### Mixed monolayer HDA-tfa/EDA-Boc

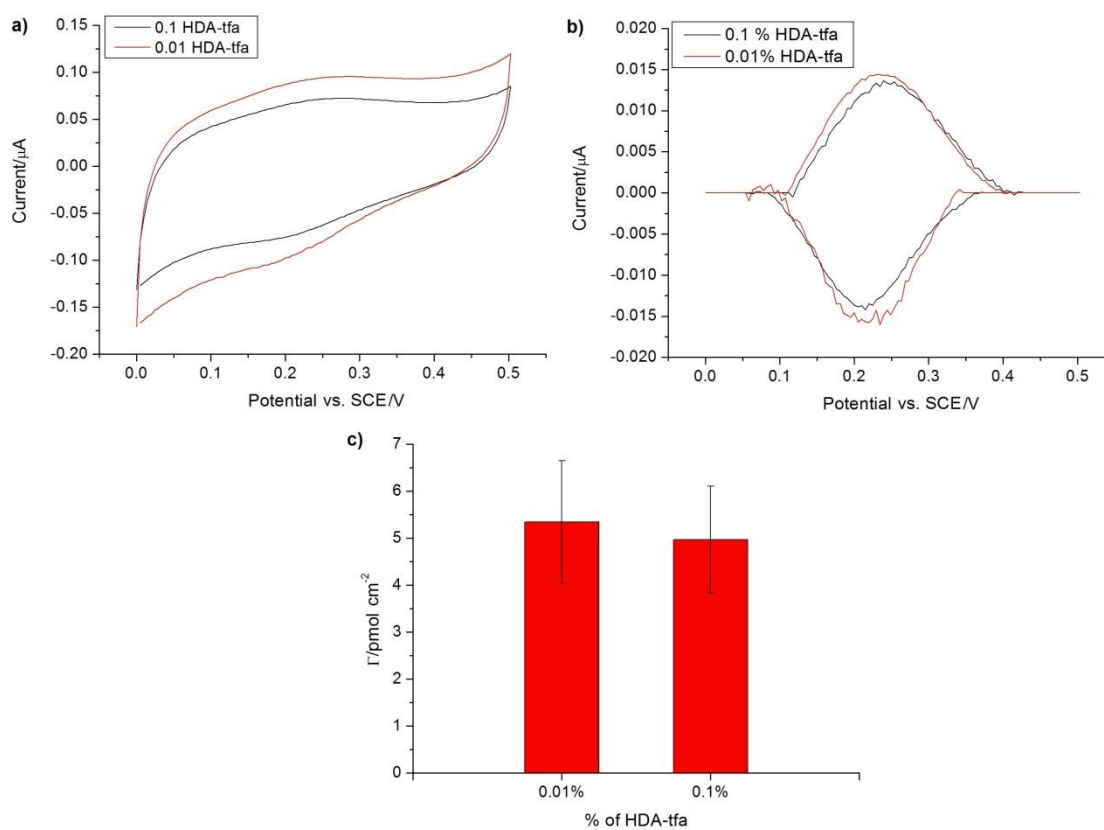


**Figure S7.** a) Comparison of the CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mVs<sup>-1</sup> scan rate for different HDA-tfa linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of complex **5** according to the ratio of HDA-tfa linker in solution, calculated by averaging the values obtained for two replicates; the control was obtained by dipping amine modified electrodes in a neat 10 mM solution of complex **5** in DMF. d) Barplot for the variation of  $\Gamma$  of complex **5** according to the ratio of HDA-tfa linker in solution corrected by the corresponding controls  $\Gamma$ .



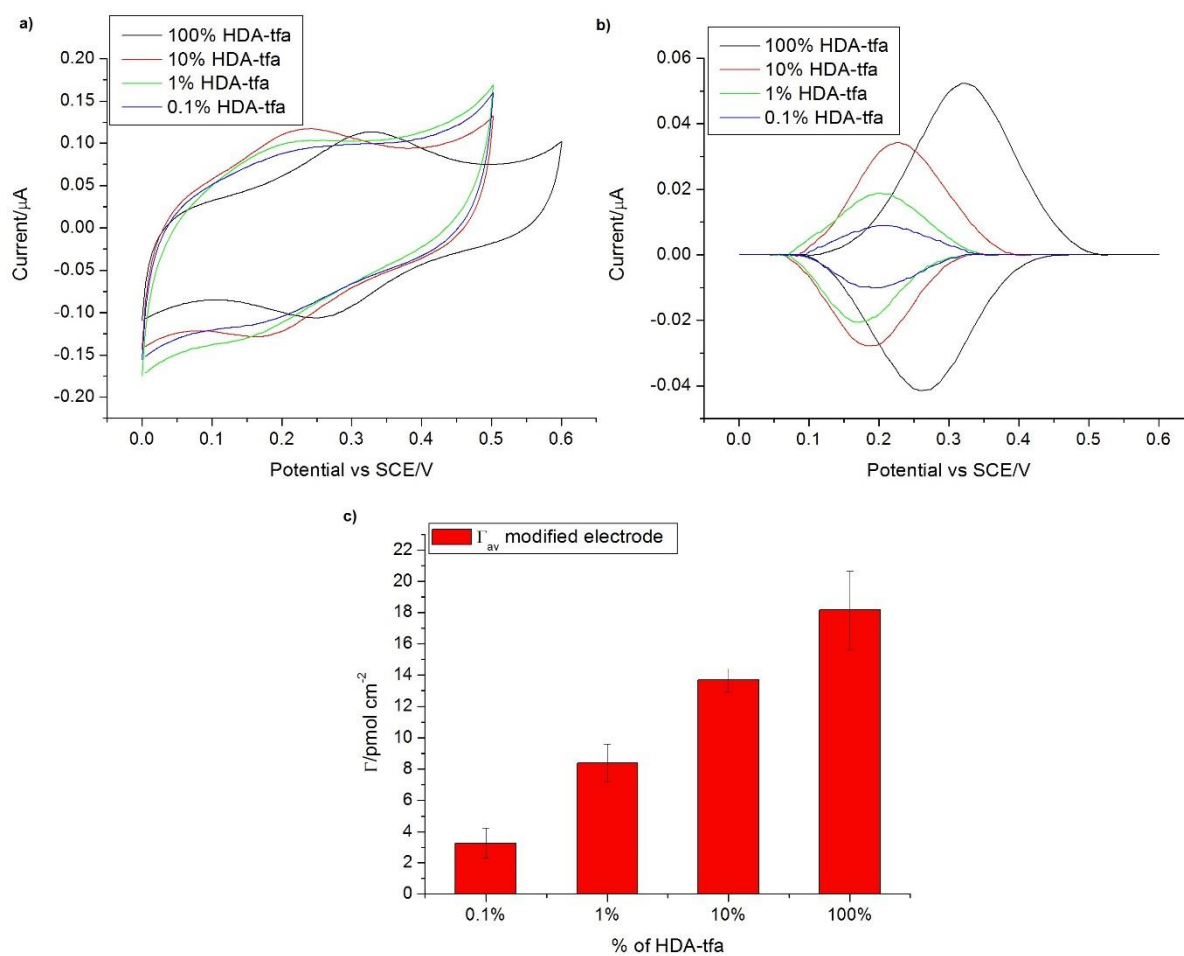
**Figure S8.** a) Comparison of the CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071  $\text{cm}^2$ , at 50  $\text{mV s}^{-1}$  scan rate for different HDA-tfa linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of anthraquinone according to the ratio of HDA-tfa linker in solution, calculated by averaging the values obtained for two replicates; the control was obtained by dipping amine modified electrodes in a neat 50 mM solution of anthraquinone in DMF. d) Barplot for the variation of  $\Gamma$  of anthraquinone according to the ratio of HDA-tfa linker in solution corrected by the corresponding controls  $\Gamma$ .

### Mixed monolayer HDA-tfa/EDA-Boc low concentrations

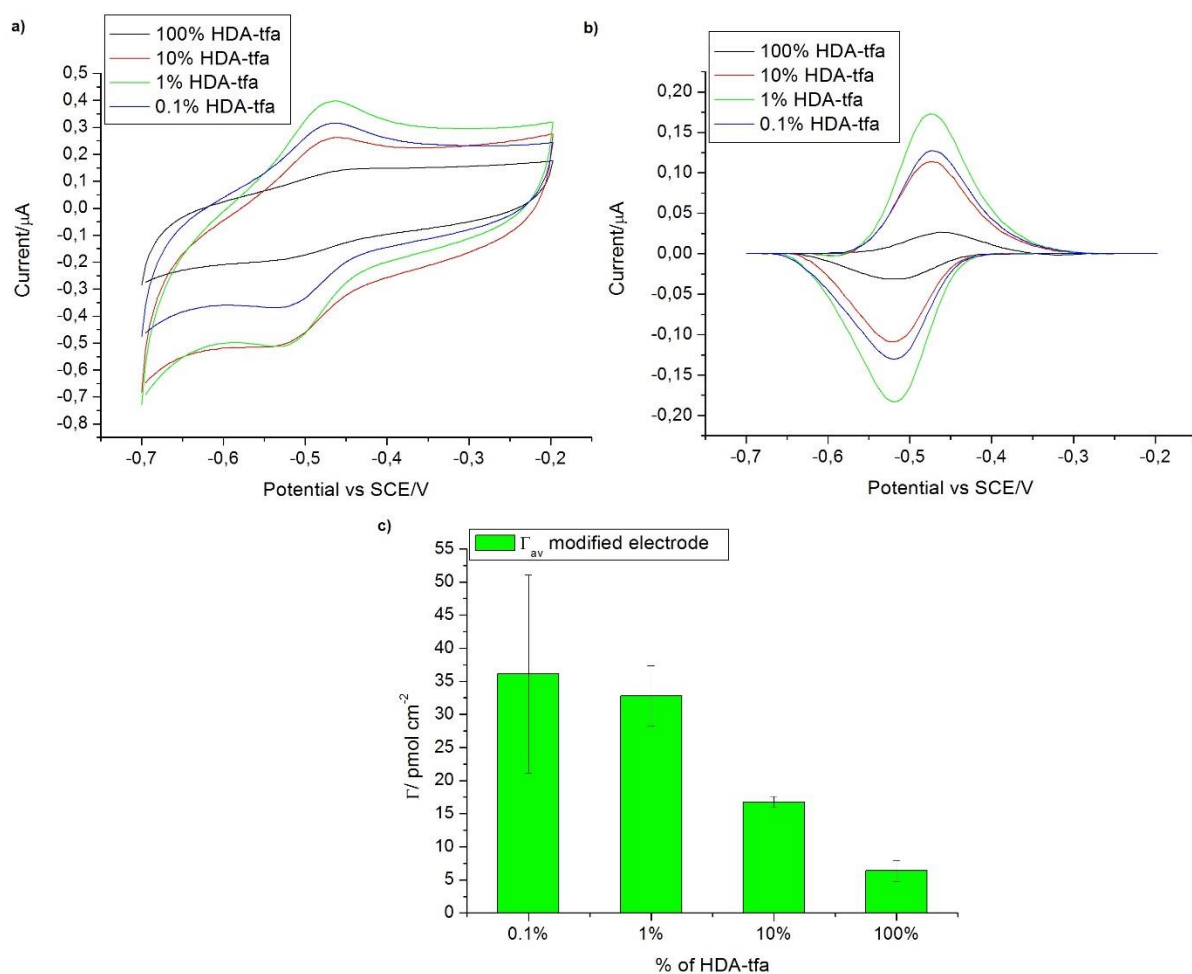


**Figure S9.** a) Comparison of the CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for different HDA-tfa linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of complex 5 according to the ratio of HDA-tfa linker in solution, calculated by averaging the values obtained for two replicates.

### Mixed monolayer HDA-tfa/EDA-Boc after optimisation



**Figure S10.** a) Comparison of the CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for different HDA-tfa linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of complex 5 according to the ratio of HDA-tfa linker in solution.



**Figure S11.** a) Comparison of the CVs recorded in 0.1 M PBS solution pH 7 vs. SCE, electrode area 0.071 cm<sup>2</sup>, at 50 mV s<sup>-1</sup> scan rate for different HDA-tfa linker solution ratios. b) Background-subtracted CVs obtained by analysing the data with Origin 7.0. c) Barplot for the variation of  $\Gamma$  of anthraquinone according to the ratio of HDA-tfa linker in solution corrected by the corresponding controls  $\Gamma$ .

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