Electronic Supplementary Information

A ratiometric electrochemical sensor for selectively monitoring monoamine oxidase A in the live brain

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Contents

1. Experimental section

Reagents and Chemicals

4-Benzyloxy phenol, propargyl bromide, 1,3-dibromopropane, potassium phthalimide and hydrazine monohydrate were purchased from Adamas-Beta Co., Ltd. (Shanghai, China). Ethyl acetate, petroleum ether, acetone, tetrahydrofuran and N, N-dimethylformamide were bought from General Reagents Co., Ltd. (Shanghai, China). 3'-HC≡C-GGCGCGA(T)$_{13}$-MB-5' (DNA-MB) was bio-synthesized by Sangon Biotech Co., Ltd (Shanghai, China). NaCl, KCl, $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $ZnCl_2$, $MnCl_2 \cdot 4H_2O$, $CdCl_2 \cdot 2.5H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, K_2CO_3, KI and KO_2 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glutathione (GSH), L-cysteine(Cys), L-arginine (Arg), L-glutamine (Glu), L-histidine (His), glycine (Gly), L-isoleucine (Iso), Lysine (Lys), L-leucine (Leu), L-phenylalanine (Phe), L-methionine (Met), L-serine (Ser), L-valine (Val), L-threonine (Thr), bovine serum albumin (BSA), acetyl cholinesterase (AchE), butyrylcholinesterase (BuChE) were purchased from Aladdin Chemistry Co. Ltd. (China). Dopamine (DA), ascorbic Acid (AA), 5-hydroxytryptamine (5-HT), glucose, uric acid (UA), 5-hydroxyindoleacetic acid (5-HIAA), potassium superoxide (KO_2), clorgyline and 2-aminoethoxydiphenyl borate (APB) were purchased from Sigma-Aldrich (USA). Phosphate buffer solution (PBS, pH 7.4) was prepared from KH_2PO_4, $K_2HPO_4 \cdot 3H_2O$ and KCl. Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $CaCl_2$ (1.1 mM), $NaHCO_3$ (27.5 mM), and Na_2SO_4 (0.5 mM) into Milli-Q water, and the pH was adjusted to 7.4. All aqueous solutions were prepared with Milli-Q water (18.2 M Ω cm, Millipore) and all chemicals were used as purchase without further purification.

$O_2^{\bullet-}$ was provided by the enzymatic reaction between xanthine (XA) and xanthine oxidase (XOD).[R1-R3] The concentration of $O_2^{\bullet-}$ was determined by recording the reduction of ferricytochrome c spectrophotometrically and using the extinction coefficient (21.1 mM^{-1} cm^{-1}) of ferrocytochrome c at 550 nm. After the addition of 5 μM XA to the aqueous solution containing 10 μM ferricytochrome c and 0.01 U/mL XOD, an absorption peak at 550 nm was observed due to the reduction of cytochrome c^{3+} to cytochrome c^{2+} by the generated $O_2^{\bullet-}$. The rate of $O_2^{\bullet-}$ generation was determined by examining the reduction of ferrocytochrome c spectrophotometrically.[R2] Moreover, the absorbance at 550 nm increased with the increasing concentration of XA and became flattened after 40 μM XA, meaning about 25% of XA converted to superoxide.

SIN-1 was selected for generating $ONOO^-$ in this study [R4-R5]. In detail, SIN-1 was added to the oxygenated aCSF solution (pH 7.4) in concentrations of 1 mM. Based on the reported ESR of SIN-1 degradation and rate of $ONOO^-$ production, 1 mM SIN-1 forms $ONOO^-$ at a speed of 1 μM/min. [R6] Although $ONOO^-$ itself has a half-life of less than 1 s in aqueous solution, SIN-1 donor under this condition is relatively stable and has a half-life (t_{50}) of 14-26 min. [R4]

Synthesis of PA

4-Benzyloxy phenol (200 mg, 1 mmol), propargyl bromide (142 mg, 1.2 mmol) and potassium carbonate (276 mg, 2 mmol) were added into a 25 mL round-bottom flask in acetone (15 mL). The system was stirred and refluxed for 6 h. After the reaction was completed, the precipitate was moved under reduced pressure and the solution was evaporated for the further purification by column chromatography (petroleum ether: ethyl acetate=15: 1). The obtained Compound **2** was a white solid (202 mg, 85% yield). The corresponding ^1H NMR, ^{13}C NMR spectra were given in Figure S2-S3.

Compound **2** (238 mg, 1 mmol) was added into 15.0 mL anhydrous CH_2Cl_2. The system was cooled

at 0°C for 10 min, then boron tribromide (750 mg, 3 mmol) was added dropwise. The mixture was warmed to room temperature and evaporated after stirring for 6 h. The concentrate was purified by column chromatography (petroleum ether: ethyl acetate=20: 1) and then Compound **3** was collected as a brownish red oil (135 mg, 91% yield). The corresponding ^{1}H NMR, ^{13}C NMR spectra were given in Figure S4-S5.

Compound **3** (296mg, 2 mmol), 1,3-dibromopropane (606 mg, 3 mmol) and potassium carbonate (552 mg, 4 mmol) were added into a round-bottom flask and then 30 mL acetone was added in the system. After refluxing for 6 h, the solvent was removed by rotary evaporation, and the concentrate was loaded directly on silica gel for purification by silica chromatography (petroleum ether: ethyl acetate=15: 1) to produce Compound **4** (220 mg, 82% yield). The corresponding^{1}H NMR, ^{13}C NMR spectra were given in Figure S6-S7.

Compound **4** (268 mg, 1 mmol), potassium phthalimide (278 mg, 1.5 mmol) and potassium carbonate (276 mg, 2 mmol) were dissolved in N, N-dimethylformamide (DMF, 15 mL). The mixture was stirred at 90 °C overnight and concentrated. Purification by silica column chromatography (petroleum ether: ethyl acetate=30: 1) yielded Compound **5** (228 mg, 68% yield) as a white solid. The corresponding ^{1}H NMR, ^{13}C NMR spectra were given in Figure S8-S9.

Compound **5** (335 mg, 1 mmol) and hydrazine monohydrate (500 mg, 10 mmol) were added to a round-bottom flask in tetrahydrofuran (15 mL), and the reaction mixture was stirred and refluxed at 80 °C for 6 h. After 6 h, the mixture was concentrated and directly purified by column chromatography (petroleum ether: ethyl acetate=10: 1) to yield PA (165 mg, 80% yield) as a slight yellow oil. The corresponding ^{1}H NMR, ^{13}C NMR spectra and HR-MS were given in Figure S10-S12.

Preparation and Modification of CFME/Au/MB+PA Electrodes

According to our previous work,[R7] carbon fiber was firstly connected to a copper wire by silver conductive adhesive. After dried for 1 h, the above electrode was carefully moved into a 100 μm wide glass capillary and dried for 8 h at 60 °C. Then exposed carbon fiber was cut to a length of 500 μm under a microscope and carbon fiber microelectrode (CFME) was thus obtained. Before modification, the CFME was sequentially sonicated in acetone, 1.0 M HNO_3 and distilled water each for 3 min.

The Au nanostructures were modified onto the CFME electrodes by electrochemical deposition and the experimental conditions should be strictly controlled by applying a potential of -0.2 V (vs Ag/AgCl) for 100 s in the solution of 10 mL 4.0 mM $HAuCl_4$ at 25˚C. The as-prepared CFME/Au electrode was then immersed in a mixed ethanol solution of PA and HC≡C-DNA-MB (molar ratio 3:1) under N_2 atmosphere for 12 h at 60˚C and then 24 h at room temperature, resulting in a CFME/Au/MB+PA electrode via covalent Au–C bonds.

In addition, the real surface area (A_{real}) of CFME/Au electrode was obtained through CV methods in 10 mM H_2SO_4 solution with a potential window from -0.5 to 1.5 V at a scan rate of 50 mV s^{-1}. A reduction peak appeared at ca. 0.9 V (vs Ag/AgCl) was used for the calculation of charge consumed. A_{real} of the CFME/Au electrode was calculated by the equation of A_{real}=Q/386, where Q is the consumed charge obtained by integration of reduction peak of Au and 386 μC cm^{-2} is the consumed charge per unit area of a clean Au electrode. The current density was obtained by $j=i_p/A_{real}$, in which i_p represents the valve of peak current.

Electrochemical measurements

All electrochemical measurements were carried out on a CHI 660E electrochemical working station

(Chenhua Instrument Co., Ltd., Shanghai) in artificial cerebrospinal fluid (aCSF, pH 7.4) with a three-electrode system, in which Ag/AgCl (KCl-saturated) electrode was the reference electrode, platinum wire was employed as the counter electrode. All potentials mentioned in the text were versus Ag/AgCl electrode.

The post-calibration curve was used for in vivo detection. The post-calibration curve was performed in aCSF solution (pH 7.4) with successive addition of MAO-A after the microelectrodes was in the rat brain for 1 h.

In Vivo Experiments

All animal care and in vivo experiments were performed according to Animal Care and Use Committee of East China Normal University (Shanghai, China). Mice had free access to water and food and were kept on a 12 h light-dark cycle. 13-month-old wild-type (WT) and APPswe/PSEN1dE9 (APP/PS1) mice (25-30 g) were purchased from Shanghai Biomodel Organism Science & Technology Development Co. Ltd (Shanghai, China). The detailed surgeries for WT and APP/PS1 mcie were performed as follows: mice were anesthetized by chloral hydrate solution with a dose of 30 mg/100 g and mounted in a stereotaxic frame. After exposing the skull and drilling a burr hole, the working electrode was implanted in the cortex (AP, 2.0 mm; ML, -1.2 mm from bregma; DV, -0.5 mm below the dura), and thalamus (AP, 1.5 mm; ML, -2.5 mm from bregma; DV, -3.0 mm below the dura). A heating pad was needed under the mouse to keep the body temperature at 37 °C.

The in vivo treatment with 2-aminoethoxydiphenyl borate (APB) via intraperitoneal injection was performed before injecting clorgyline for 1 h. And electrochemical measurements or brain removal was performed 40 min after injection of clorgyline for further studies.

Microdialysis was performed according to our previous work.[R8] In detail, the probe (CMA/110/111 Tub) was implanted in the cortex at the site of 2.0 mm from bregma, -1.2 mm from midline and -1.5 mm from dura. The microdialysis was collected at a rate of 10 μL min^{-1} for 30 min and the perfusion rate during the experiment was 2 μL min^{-1} for at least 60 min. The microdialysis probes used had a dialysis membrane with a molecular cutoff of 100 kDa. The tubes were kept on ice during collection and frozen immediately after sampling. Then a Micro Monoamine Oxidase Assay Kit was used for the detection of MAO-A activity according to the absorption peak at 360 nm obtained at a microplate reader.

Preparation and imaging of mouse brain tissue slices

WT and APP/PS1 mice were directly decapitated and coronal slices with a thickness of 300 μm were prepared by using vibrating-blade microtome. The obtained tissue slices were incubated with 20 μM Ca^{2+} probe in aCSF solution by bubbling the mixed gas (5% CO_2, 95% O_2) for 90 min at 37°C. [R9] Before confocal imaging, slices were washed three times by aCSF solution.

2. Synthetic route of PA

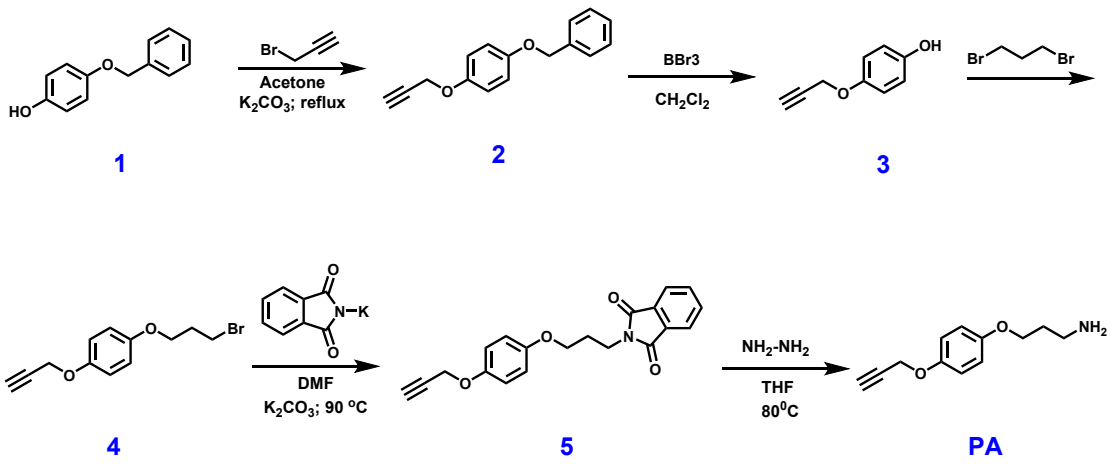

Figure S1. Synthetic route of PA.

3. NMR spectra and HR-MS data of PA

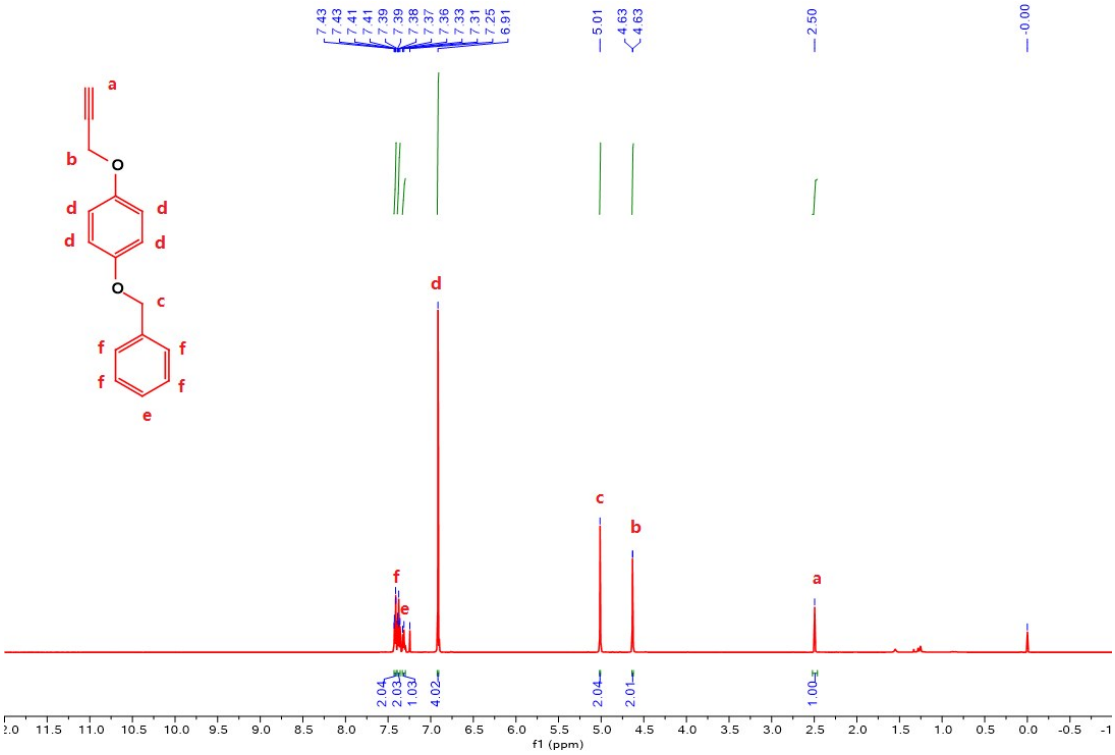

Figure S2. ^{1}H NMR spectrum of Compound 2.

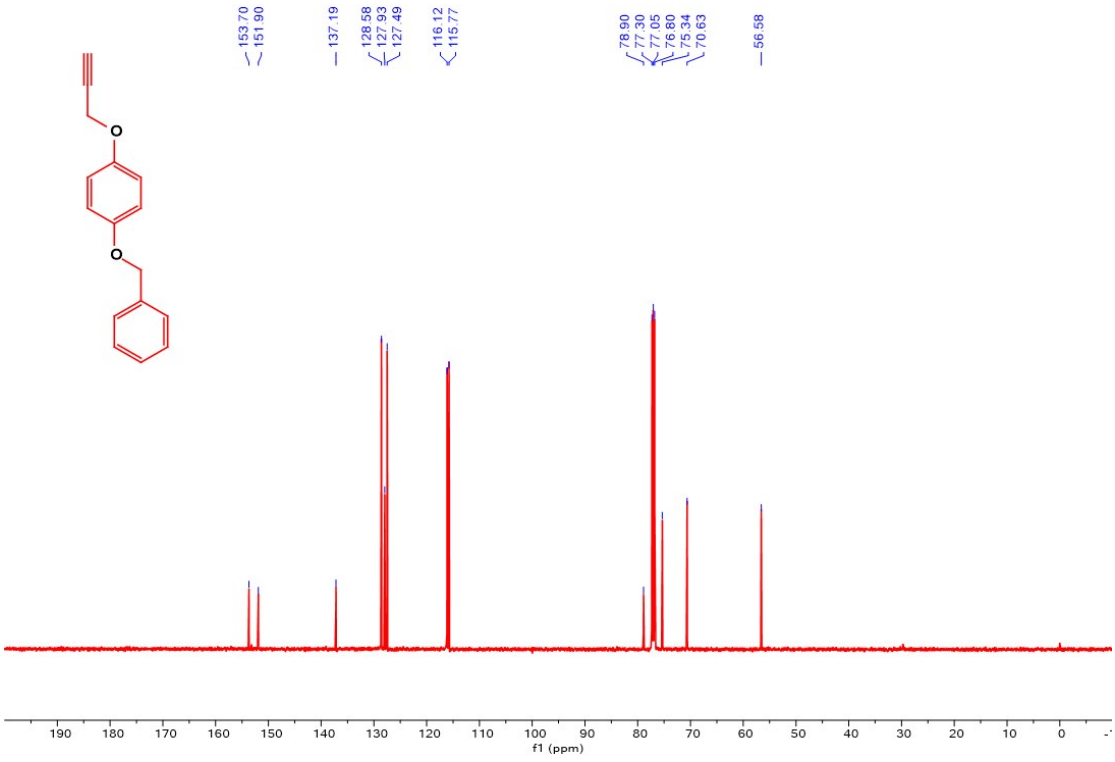

Figure S3. ^{13}C NMR spectrum of Compound 2.

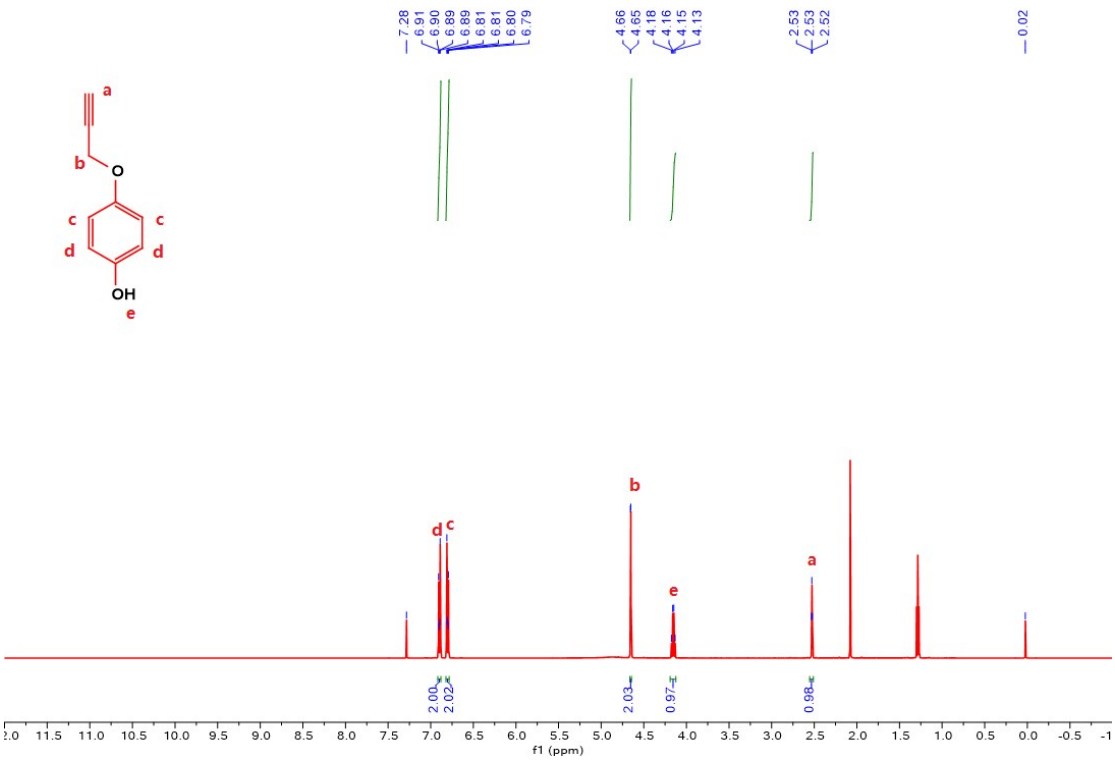

Figure S4. ^{1}H NMR spectrum of Compound 3.

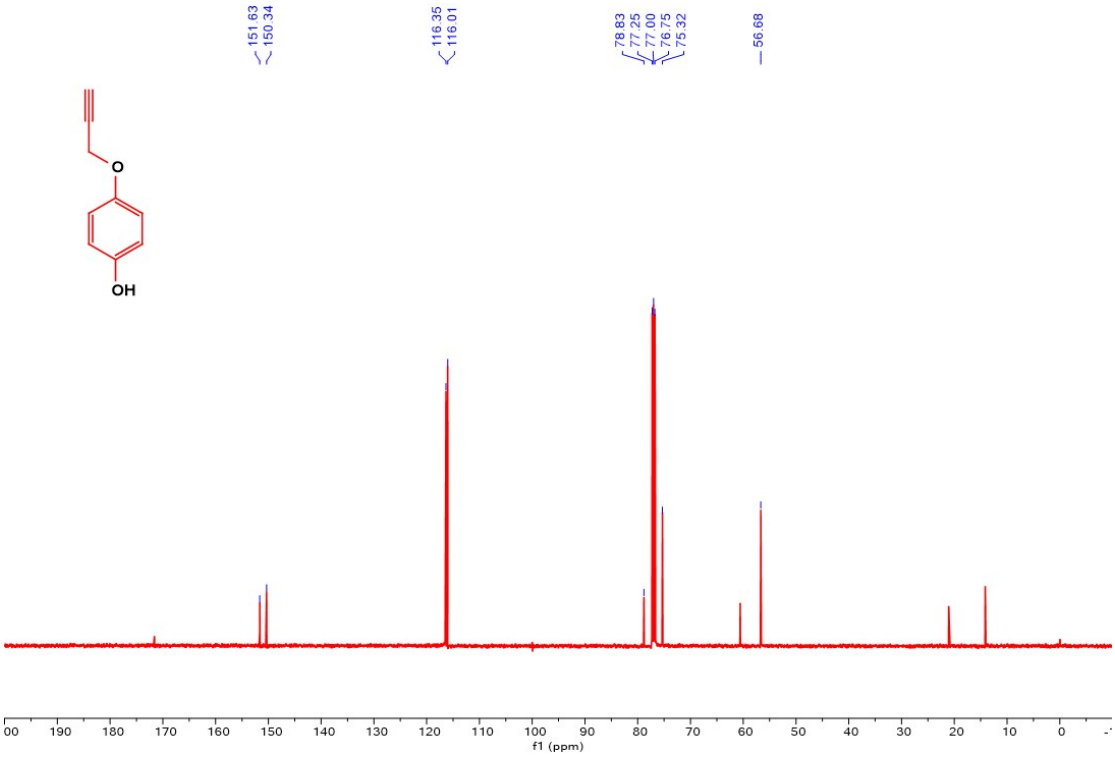

Figure S5. ^{13}C NMR spectrum of Compound 3.

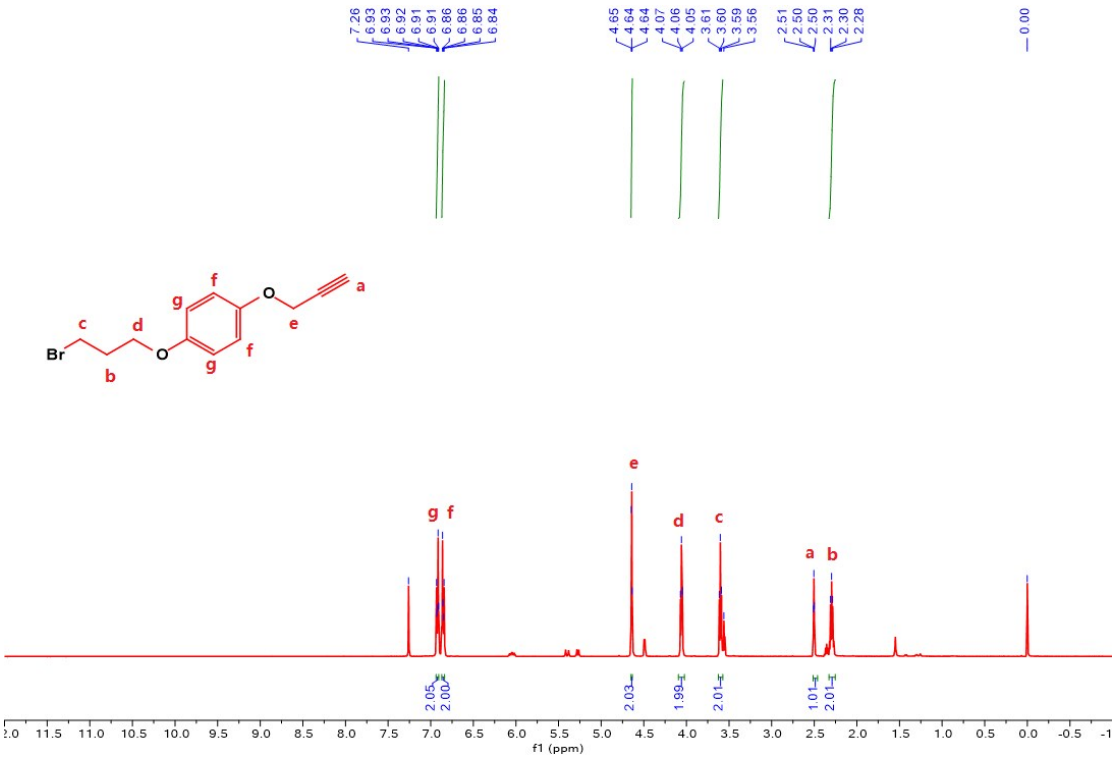

Figure S6. ^{1}H NMR spectrum of Compound 4.

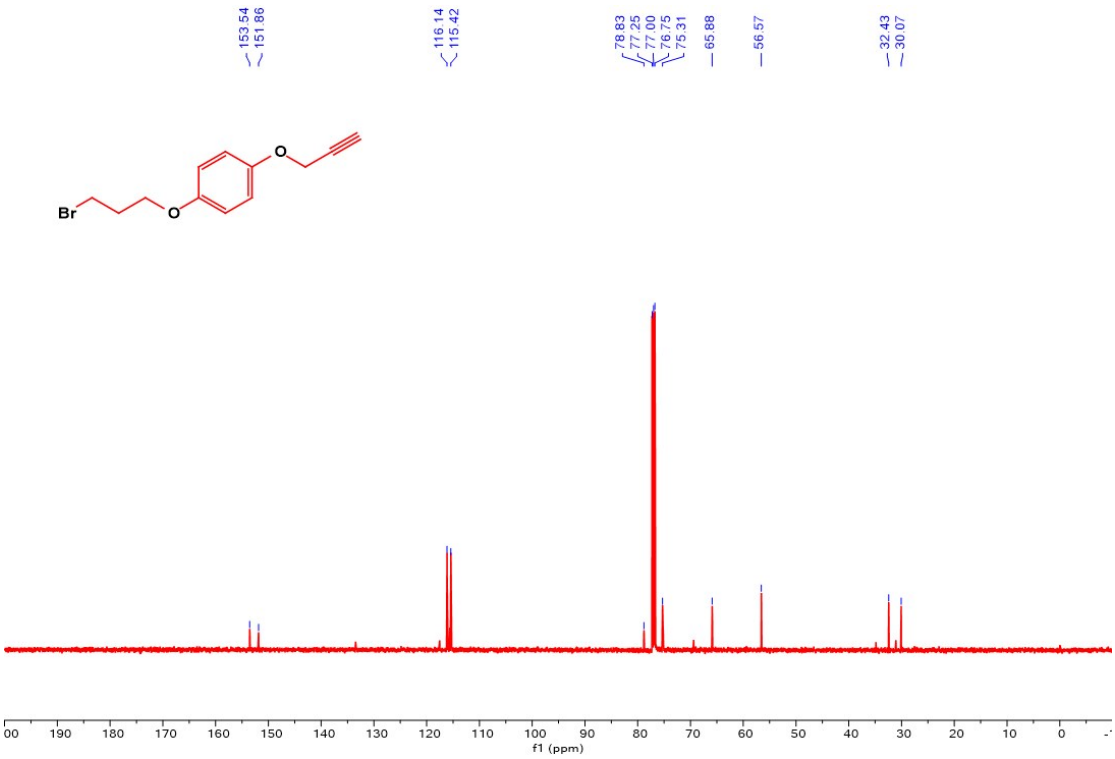

Figure S7. ^{13}C NMR spectrum of Compound 4.

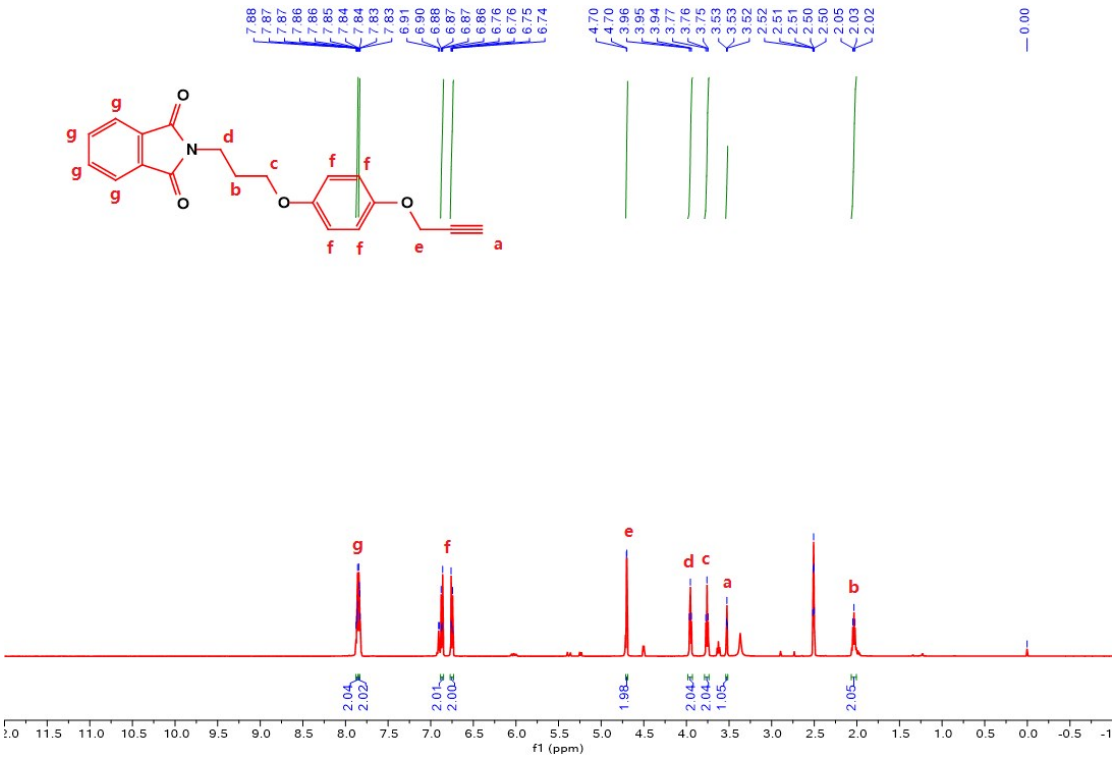

Figure S8. ^{1}H NMR spectrum of Compound 5.

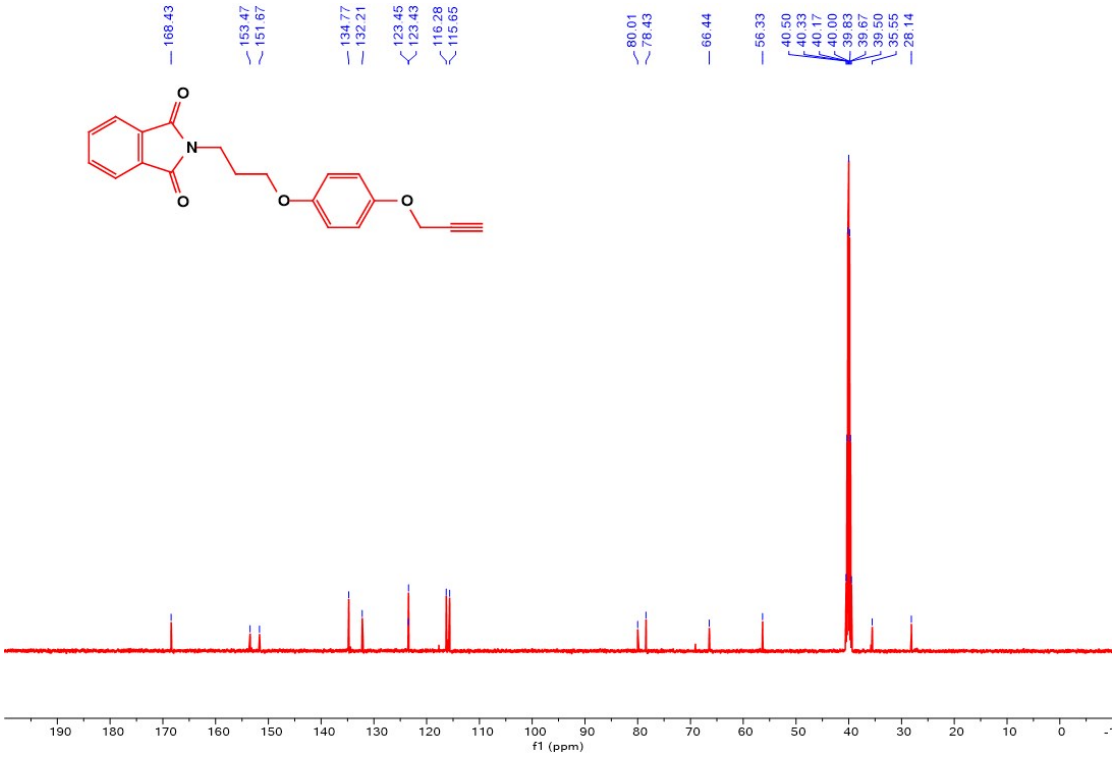

Figure S9. ^{13}C NMR spectrum of Compound 5.

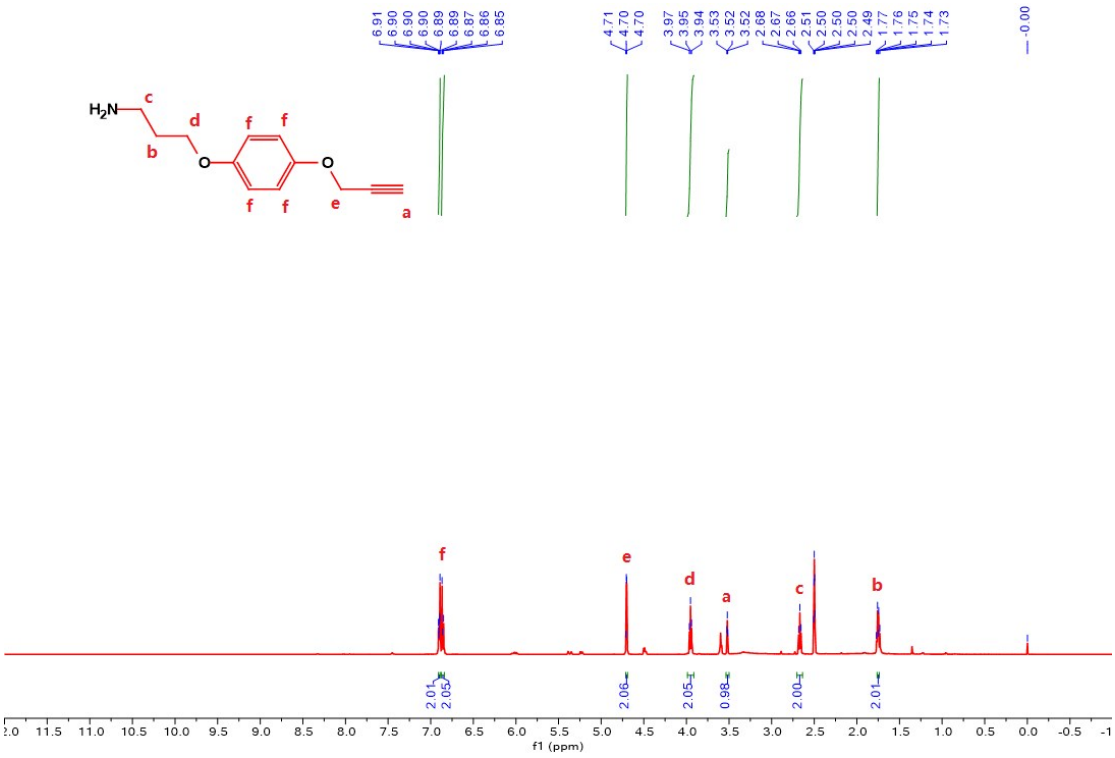

Figure S10. ^{1}H NMR spectrum of PA.

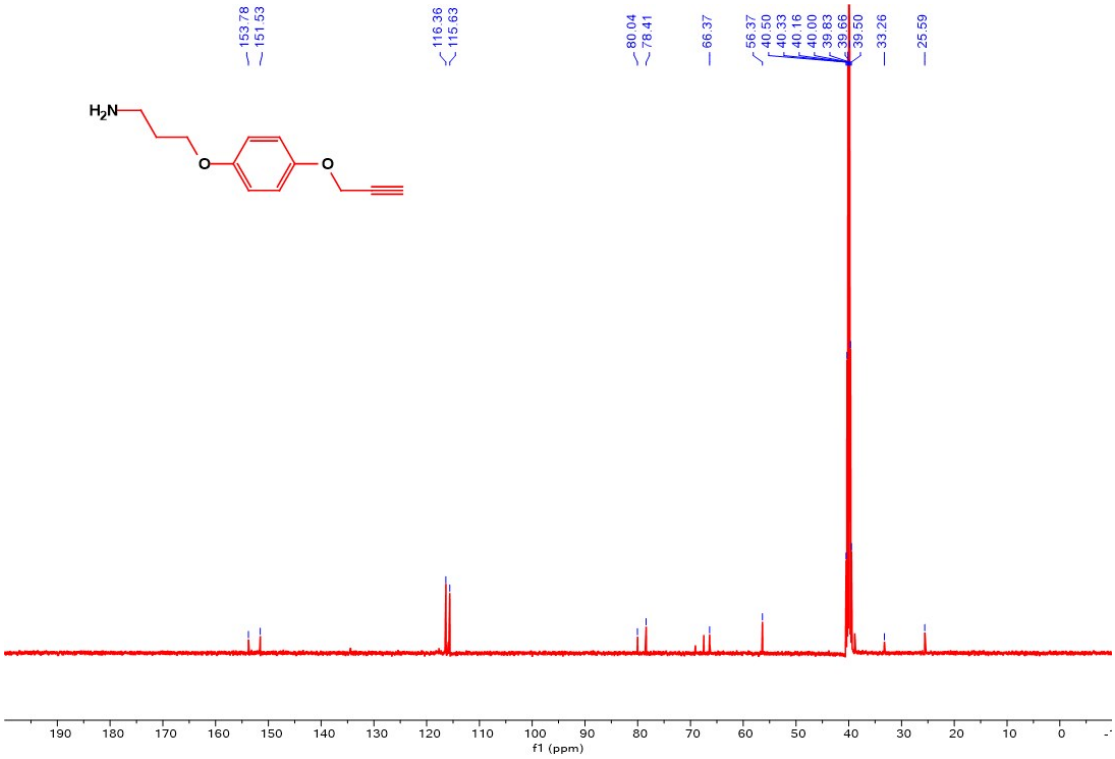

Figure S11. ^{13}C NMR spectrum of PA.

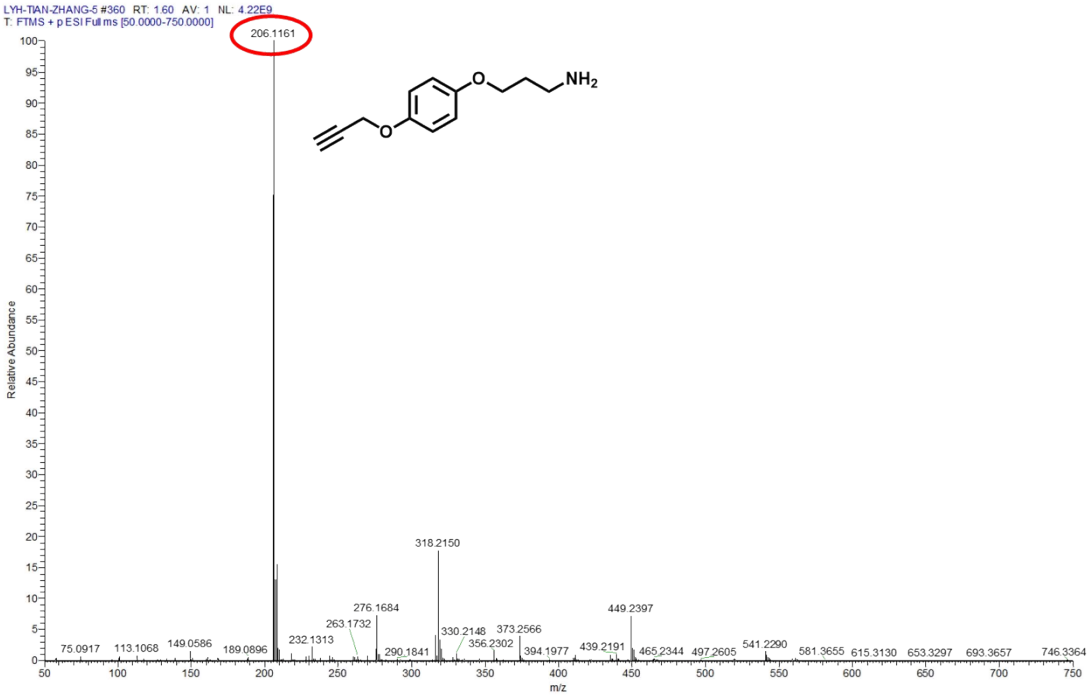

Figure S12. HR-MS spectrum of PA.

4. X-ray photoelectron spectroscopy (XPS) spectra of the modified electrodes

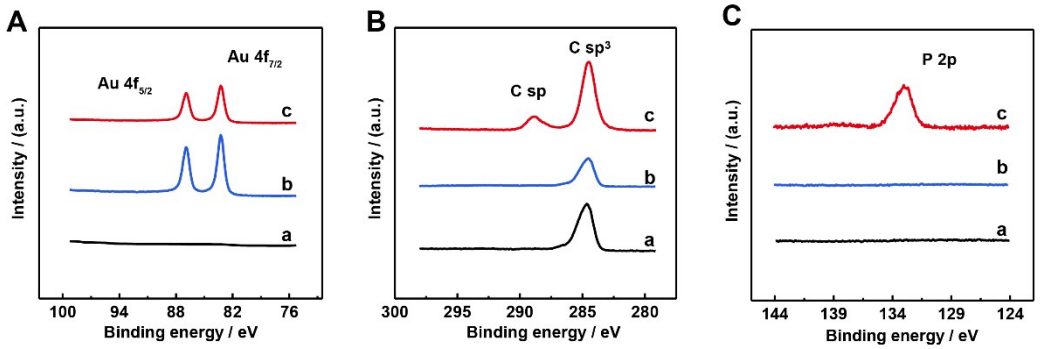

Figure S13. XPS spectra of Au, C and P elements on (a) CFME, (b) CFME/Au and (c) CFME/Au/MB+PA electrodes.

5. Proposed reaction mechanism of PA on the electrode

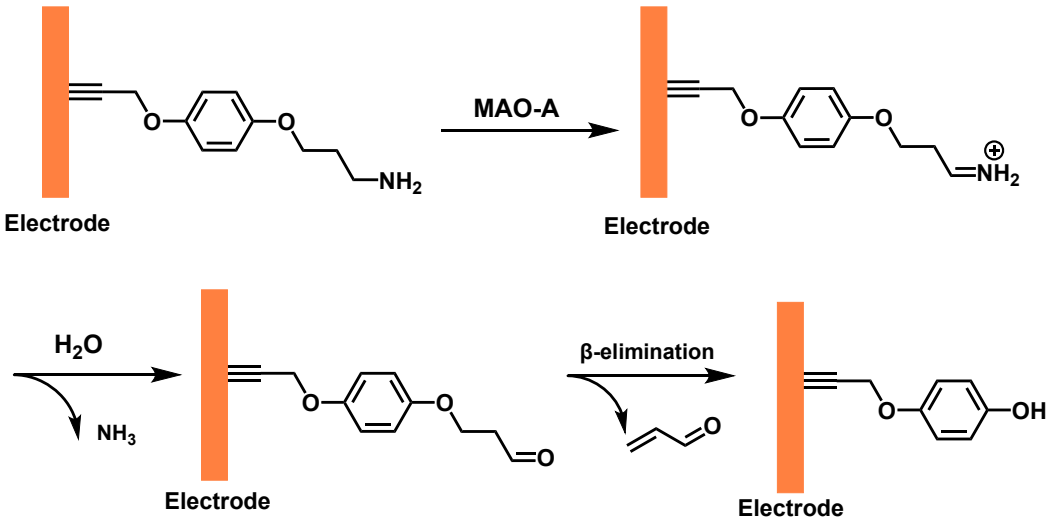

Figure S14. Proposed reaction mechanism of PA.

6. HPLC-MS spectra of the reaction system

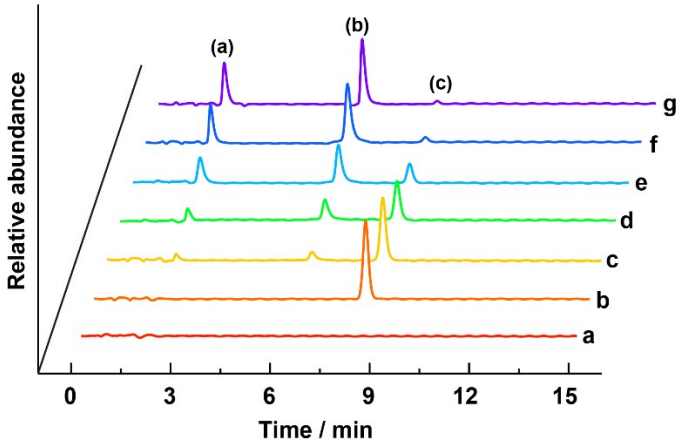

Figure S15. HPLC kinetic profiles of PA molecules (5 mM) reacting with 5 mg mL^{-1} MAO-A. a) Blank; b) PA molecules; c–g) the reaction between PA molecules and MAO-A for 6, 12, 18, 24, 30 min, respectively.

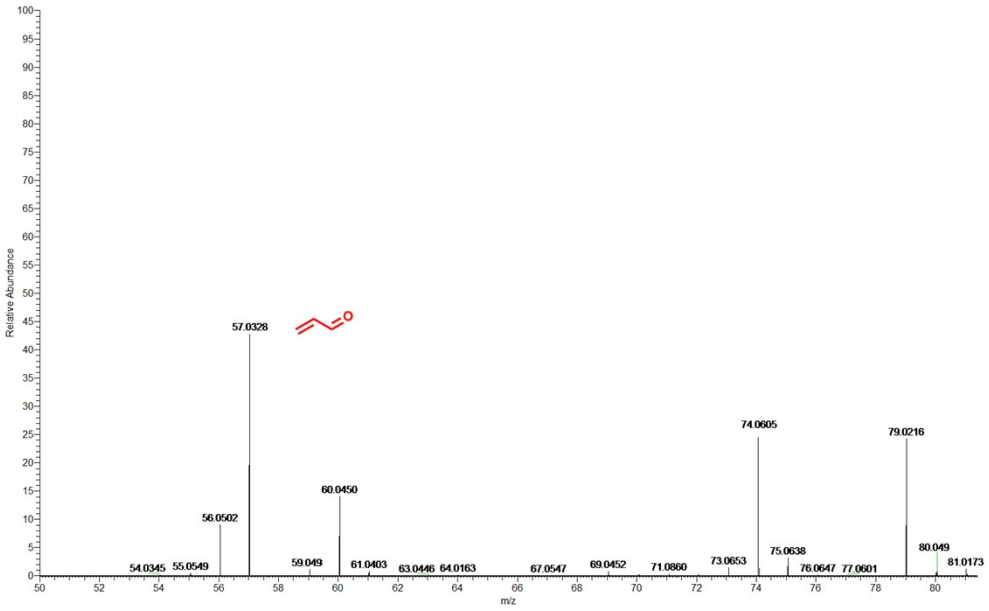

Figure S16. Mass spectrum of peak (a) shown in Figure S15.

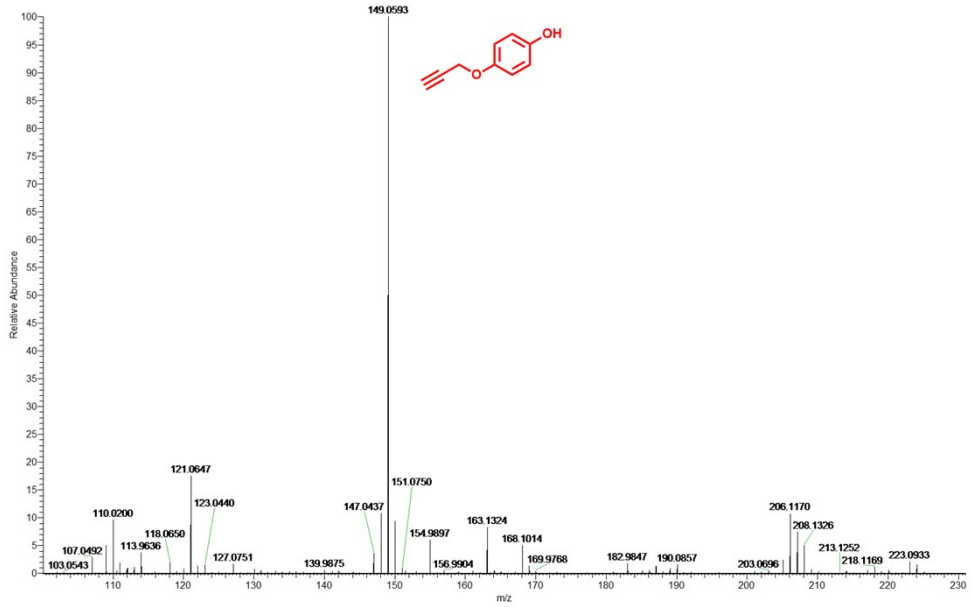

Figure S17. Mass spectrum of peak (b) shown in Figure S15.

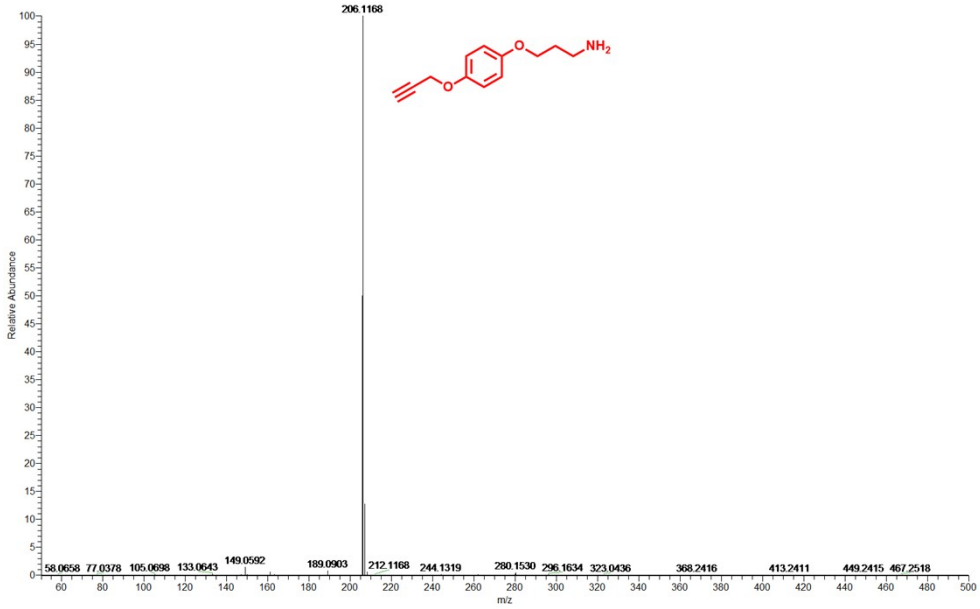

Figure S18. Mass spectrum of peak (c) shown in Figure S15.

7. Chemical process and electrochemical redox process on the electrode

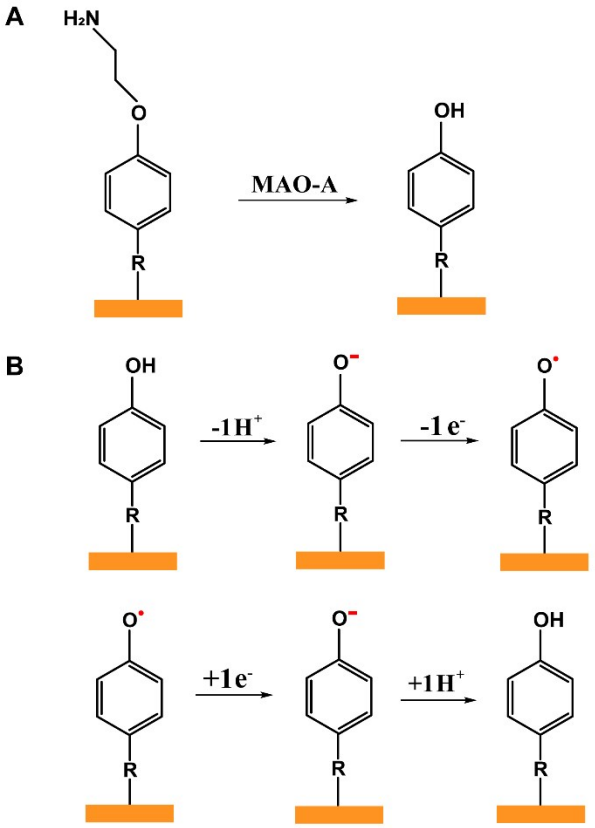

Figure S19. (A) Chemical process (A) and electrochemical redox process (B) occurred on CFME/Au/MB+PA electrode in MAO-A containing aCSF solution (pH 7.4).

8. Response time of the electrode for detection of MAO-A

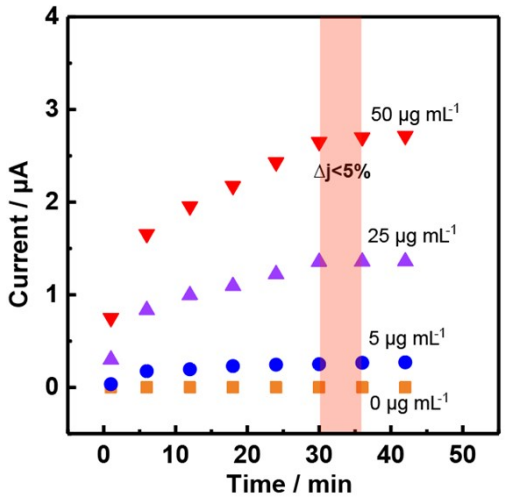

Figure S20. Time-dependent peak current obtained by CVs at CFME/Au/MB+PA electrode in aCSF solution (pH 7.4) in the presence of 0, 5, 25, 50 μg mL^{-1} MAO-A.

9. Cyclic voltammograms of PA at GCE/Au/MB+PA electrode for 300 cycles

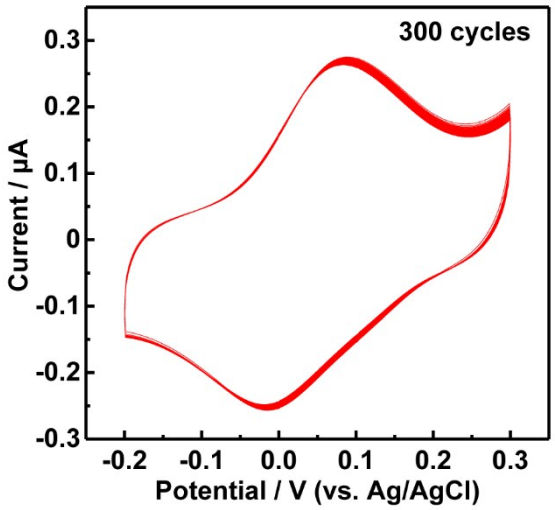

Figure S21. CVs obtained at GCE/Au/MB+PA electrode for 300 cycles in aCSF solution (pH 7.4) containing 4 mU mL^{-1} MAO-A.

10. Interference from GSH

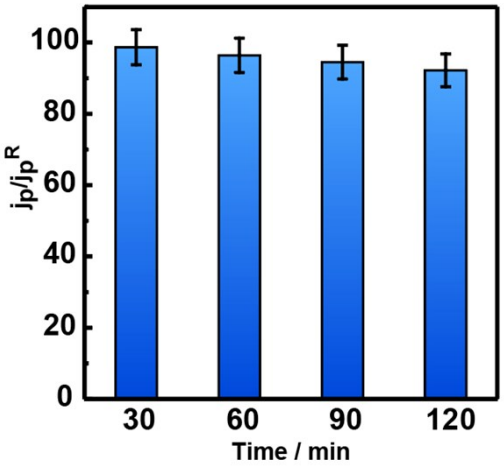

Figure S22. DPVs obtained at CFME/Au/MB+PA electrode in aCSF solution containing 5 mM GSH for different time.

11. Anti-biofouling capability

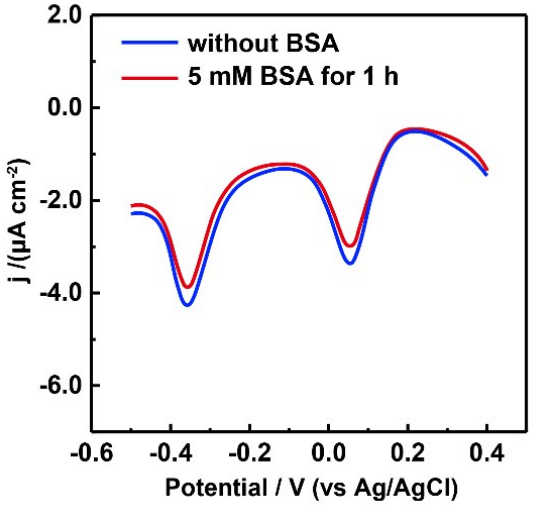

Figure S23. DPVs obtained at CFME/Au/MB+PA electrode in aCSF solution without BSA and with 5 mM BSA for 1 h.

12. Cyclic voltammograms of PA obtained at different scan rates

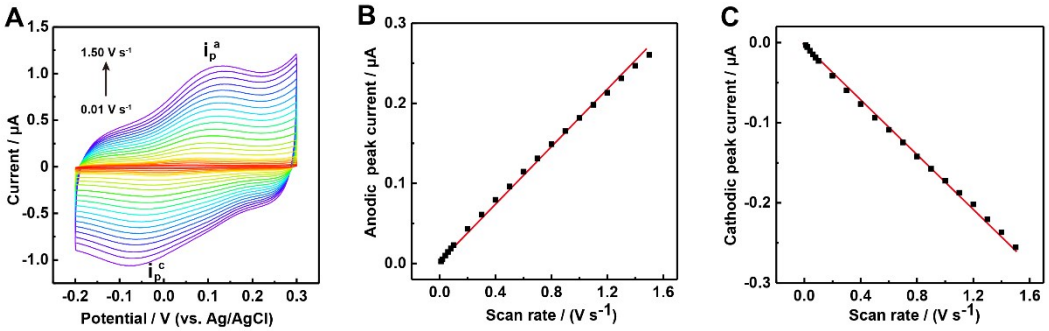

Figure S24. (A) CVs obtained at GCE/Au/MB+PA electrode at the scan rates of 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 and 1500 mV s^{-1} (from inner to outer) in aCSF solution (pH 7.4) containing 4 mU mL^{-1} MAO-A, (B) Plots of anodic peak current versus scan rate, (C) Plots of cathodic peak current versus scan rate.

13. pH effect

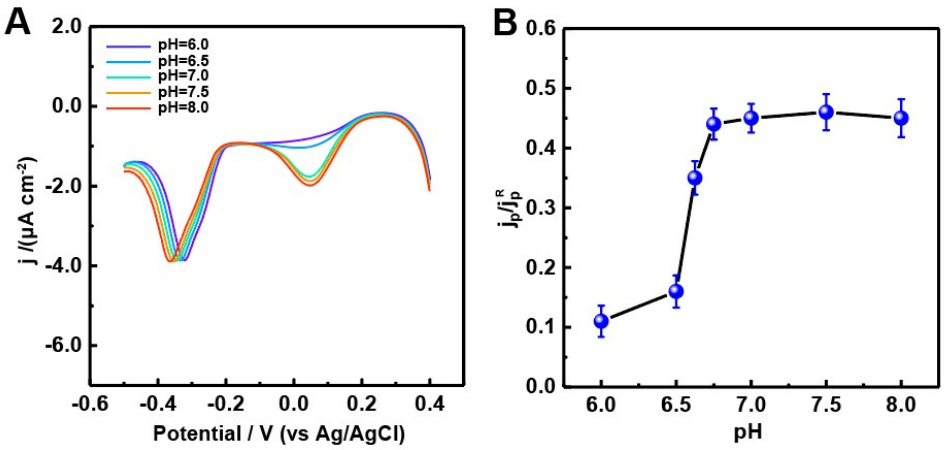

Figure S25. The obtained DPVs (A) and j_p/j_p^R values (B) after CFME/Au/MB+PA electrode was immersed into aCSF solution with pH range from 6.0 to 8.0 for 30 min.

14. Selectivity

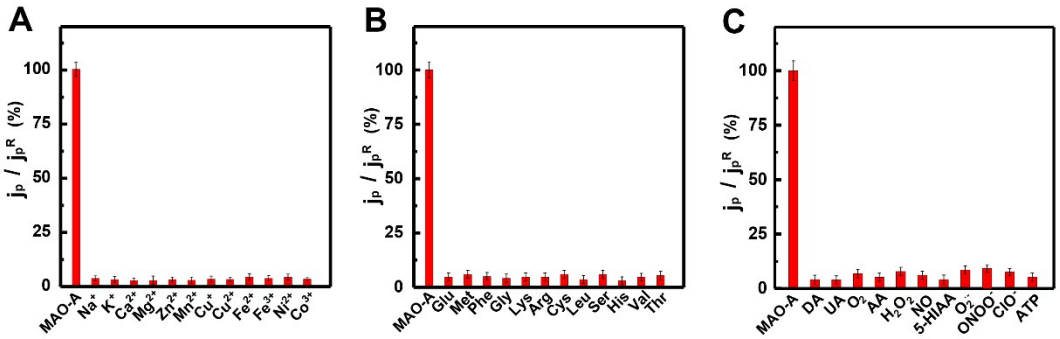

Figure S26. (A) Selectivity tests for MAO-A in the presence of (A) metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^+, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+} and Co^{3+}, respectively. The concentration was 100 mM for Na^+ and K^+, 10 mM for Ca^{2+} and 10 μM for other anions), (B) amino acids (Glu, Met, Phe, Gly, Lys, Arg, Cys, Leu, Ser, His, Val and Thr, respectively. Concentrations: 10 mM), (C) biomolecules and neurotransmitters (DA, UA, O_2, AA, H_2O_2, NO, 5-HIAA, $O_2^{\cdot-}$, $ONOO^-$, ClO^- and ATP, respectively. Concentrations: 10 μM for DA, UA, AA, 5-HIAA and ATP, 1 μM for all the others).

15. Tissue damage and neuron disturbance

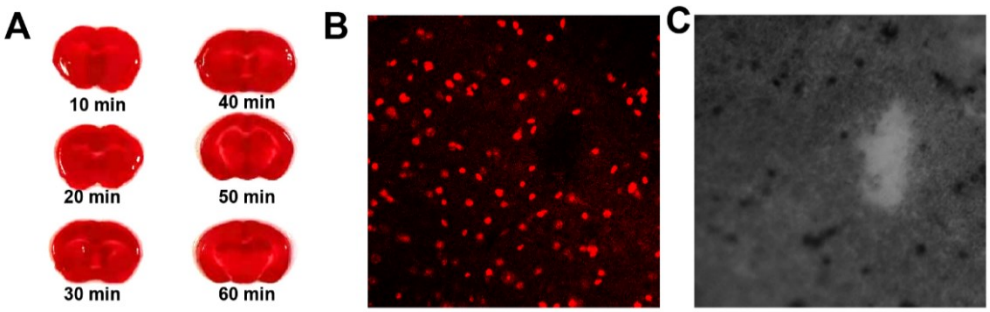

Figure S27. (A) TTC staining of brain tissue slices after the CFME/Au/MB+PA was implanted into live brain for 10 to 60 min. (B-C) confocal image (B) and bright-field image (C) of brain tissue slices after implanting CFME/Au/MB+PA electrode for 1 h.

16. Post-calibration curve of CFME/Au/MB+PA electrode

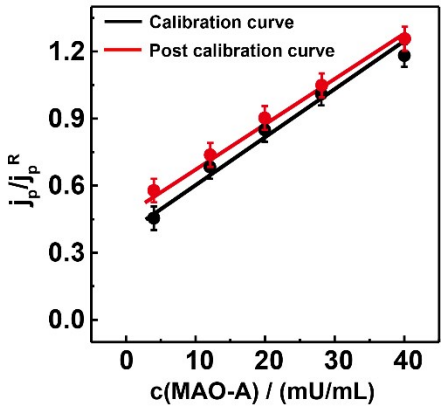

Figure S28. The post-calibration plots of j_p/j_p^R with activities of MAO-A.

17. The obtained MAO-A activity by the developed sensor and Assay Kit

Table S1. The obtained MAO-A activity by the developed sensor and Assay Kit

		CFME/Au/MB+PA	Assay Kit
Normal	Cortex	3.0±0.8 mU mL^{-1}	2.8±0.5 mU mL^{-1}
	Thalamus	2.2±0.9 mU mL^{-1}	2.1±0.6 mU mL^{-1}
AD	Cortex	6.8±1.0 mU mL^{-1}	6.9±0.9 mU mL^{-1}
	Thalamus	6.1±1.2 mU mL^{-1}	5.9±0.8 mU mL^{-1}

The value of t obtained in cortex of normal mouse was calculated to be 0.37. Because $t_{0.05,\,4}$=2.78, the mean value (n=3) of MAO-A activity obtained by CFME/Au/MB+PA electrode are not significant different from that obtained by MAO-A Assay Kit. The t values in other regions were also less than 2.78.

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