

Improved Electrochemical Hydrogen Peroxide Detection Using a Nickel(II) Phthalimide-Substituted Porphyrazine Combined with Various Carbon Nanomaterials

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1. General procedures

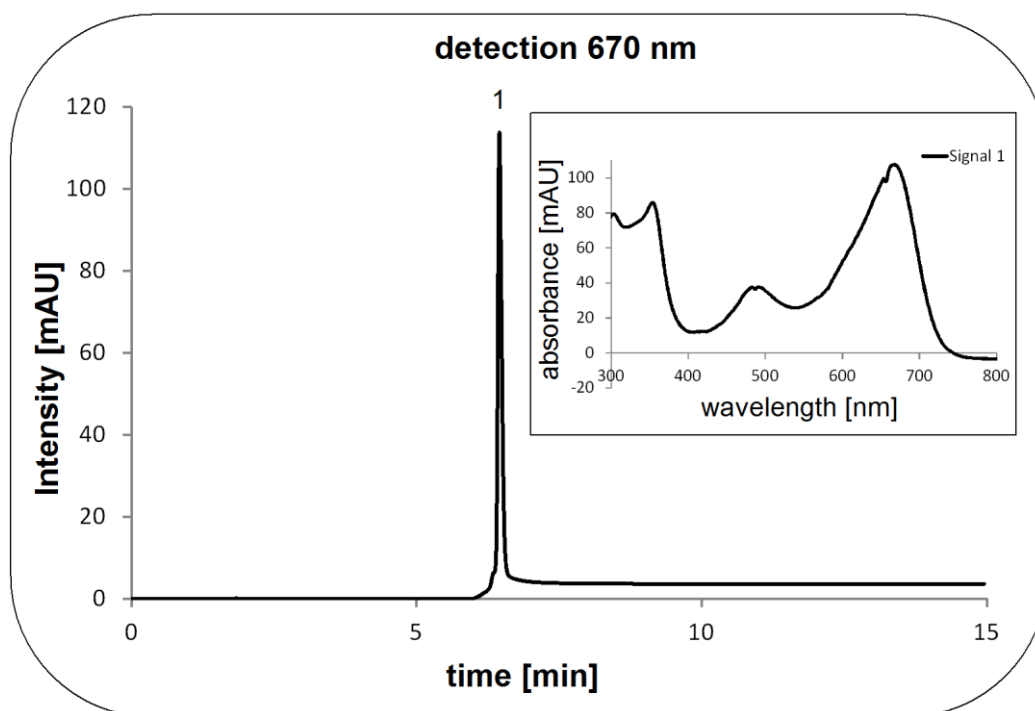
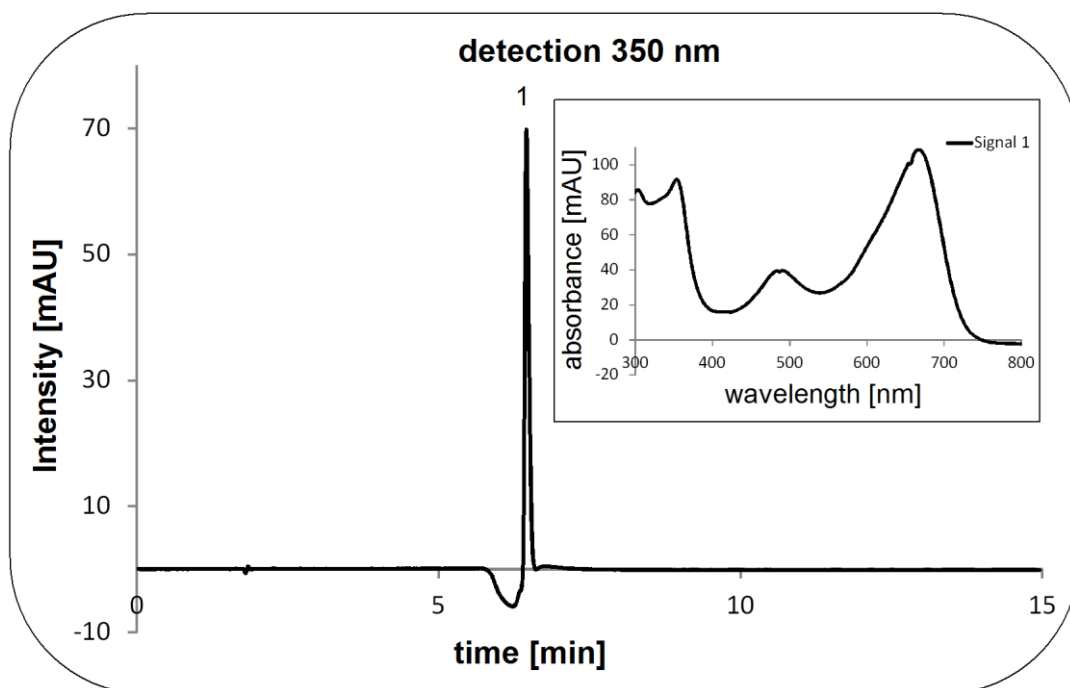
All reactions were conducted under argon atmosphere in glassware dried at 140 °C for at least 4 hours. All solvents were rotary evaporated at or below 60 °C under reduced pressure. The reported reaction temperatures refer to temperatures displayed by Radleys® Heat-On. Dry flash column chromatography was carried out on Merck silica gel 60, particle size of 40–63 µm and Aluminium oxide 90 active neutral (activity stage I) for column chromatography, 0.063-0.200 mm (Merck KGaA, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on silica gel Merck Kieselgel 60 F254 plates and visualized with UV light (λ_{max} = 254 or 365 nm). UV–VIS spectra were recorded on a Hitachi UV–VIS U-1900 spectrophotometer. The NMR spectra were acquired at 298 K on a full-fledged, two-channel 400 MHz Bruker AvanceCore NMR spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to a residual solvent peak – CDCl₃ (¹H NMR – 7.27 ppm; ¹³C NMR – 77.00 ppm). Coupling constants (*J*) are quoted in hertz (Hz) and are rounded to the nearest halves or whole numbers. The abbreviations dd and t refer to doublet of doublets and triplet, respectively. ¹H and ¹³C resonances were unambiguously assigned based on ¹H, ¹³C, ¹H–¹H COSY, ¹H–¹³C HSQC, and ¹H–¹³C HMBC spectra. Mass spectra (MALDI-TOF) were carried out by the Wielkopolska Center for Advanced Technologies at the Adam Mickiewicz University in Poznan. All solvents and reagents were of reagent-grade quality, purchased from commercial suppliers, and used without further purification unless otherwise stated.

2. HPLC purity

The purity of macrocycle **Pz3** was determined by HPLC analysis using an Agilent 1200 instrument equipped with UV-DAD detector. The chromatographic separation was obtained on an octadecylsilane-coated column, 150 mm × 4.6 mm, 5 µm (Eclipse XDB-C18, Agilent), using gradient elution conditions at a flow rate of 1.0 mL/min. Band dispersion, and additional peaks from aggregates significantly hampered HPLC analysis. The best conditions for each compound are shown below. Peaks of minor components were detected, but the impurity content never exceeded 5% of the total signal intensity.

Configuration 1

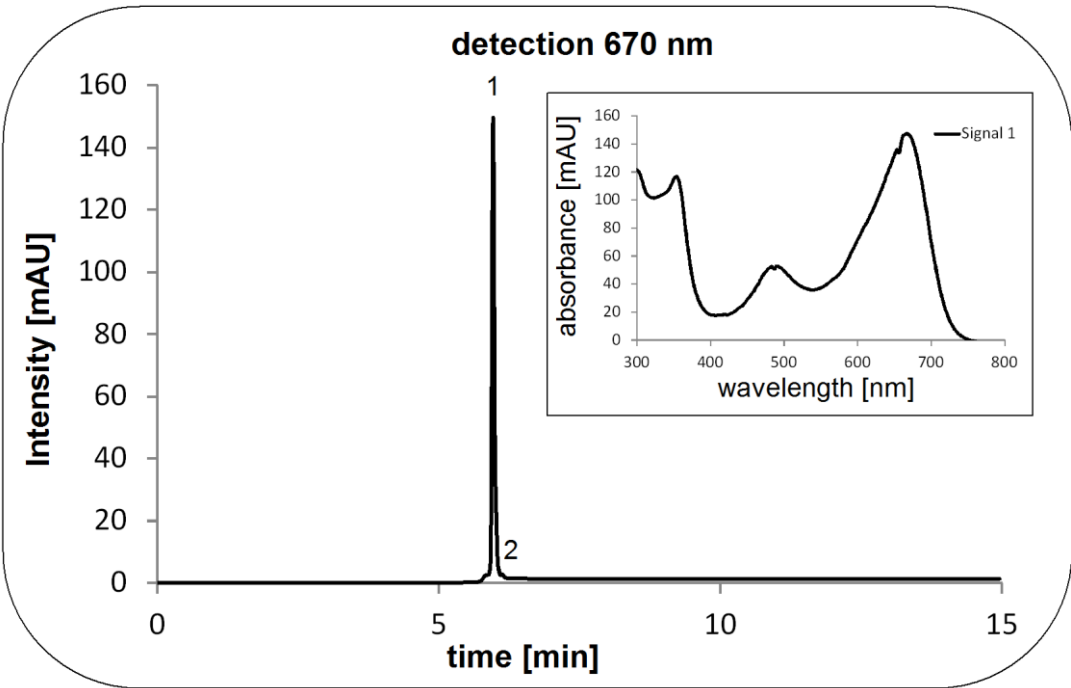
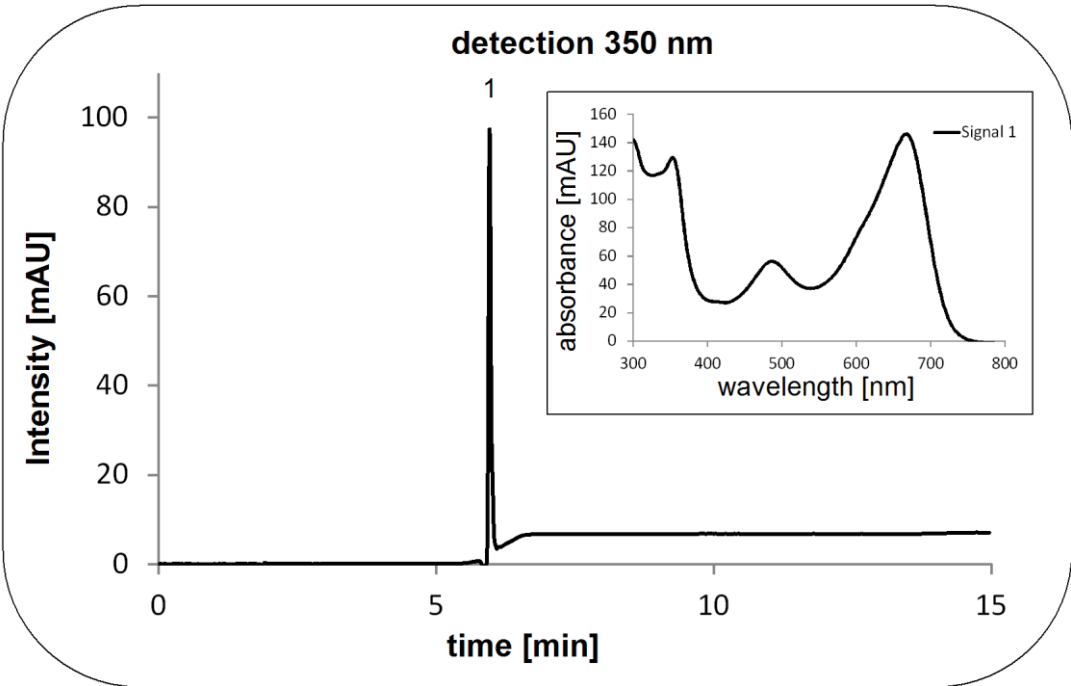
time [min]	phase		
	methanol	dichloro-	water
0	95	0	5
3	95	0	5
4	50	50	0
15	50	50	0



results			
signal	retention time [min]	area	purity [%]
detection 350 nm			
1	6.5	378.9	100.0
detection 670 nm			
1	6.5	584.5	100.0

Configuration 2

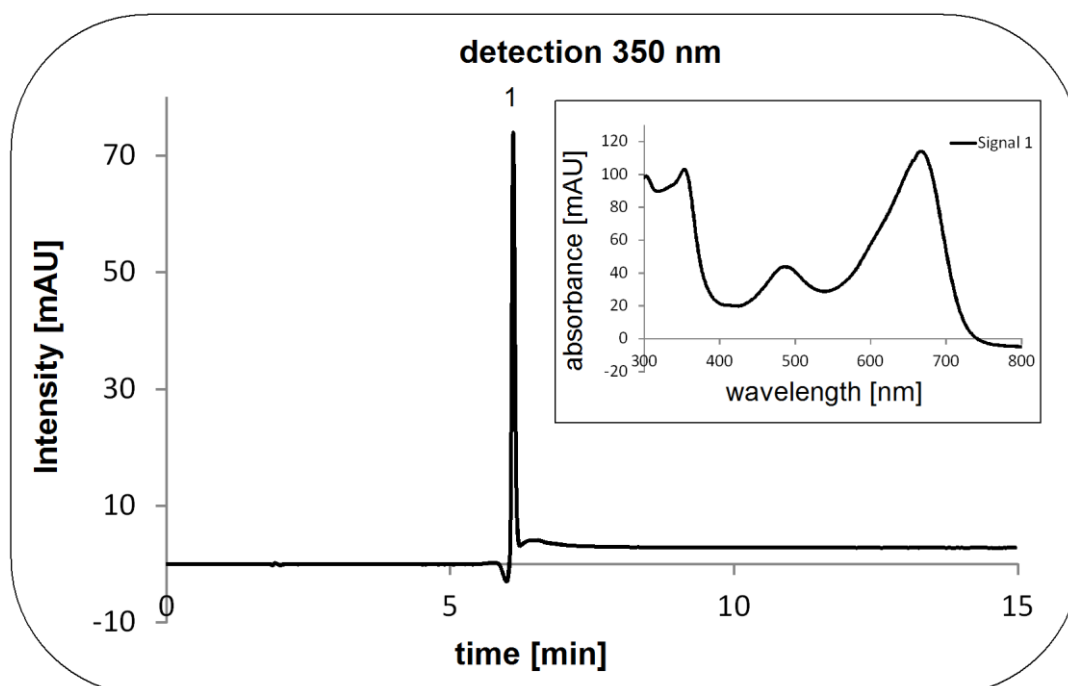
time [min]	phase			
	acetonitrile	methanol	water	tetrahydrofu-
0	60	25	10	5
3	60	25	10	5
4	10	5	0	85
15	10	5	0	85

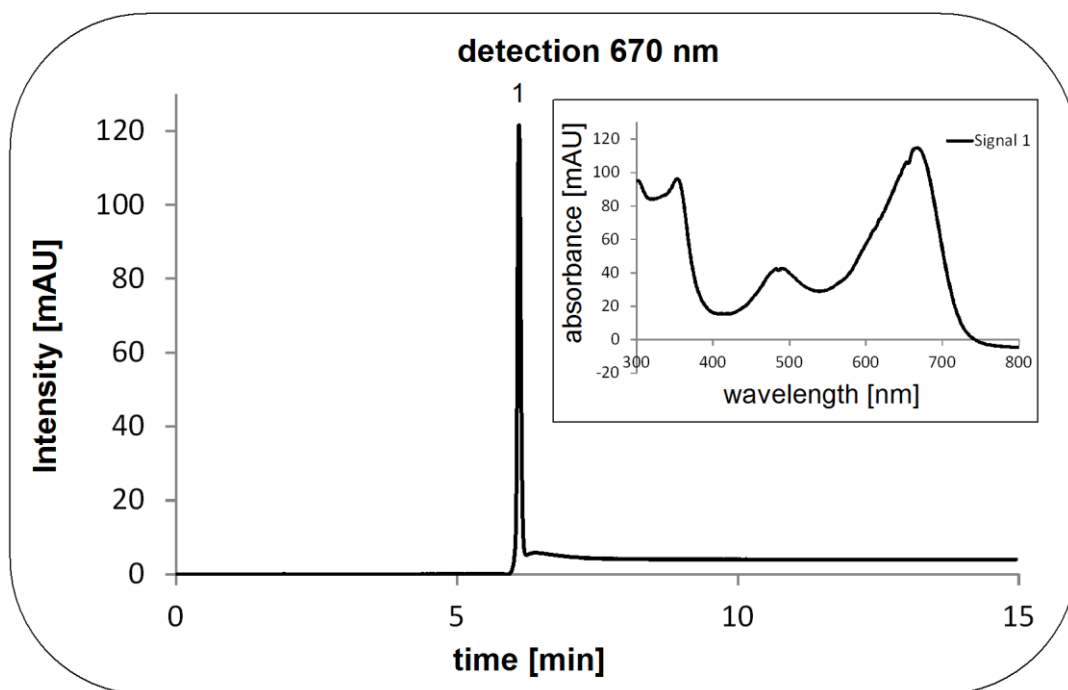


results			
signal	retention time [min]	area	purity [%]
detection 350 nm			
1	6.0	334.7	100.0
detection 670 nm			
1	6.0	503.2	98.5
2	6.1	7.7	1.5

Configuration 3

phase				
time [min]	methanol	water	dichloro-	tetrahydrofu-
0	80	10	5	5
3	80	10	5	5
4	20	0	40	40
15	20	0	40	40





results			
signal	retention time [min]	area	purity [%]
detection 350 nm			
1	6.1	321.2	100.0
detection 670 nm			
1	6.1	507.1	100.0

3. NMR study

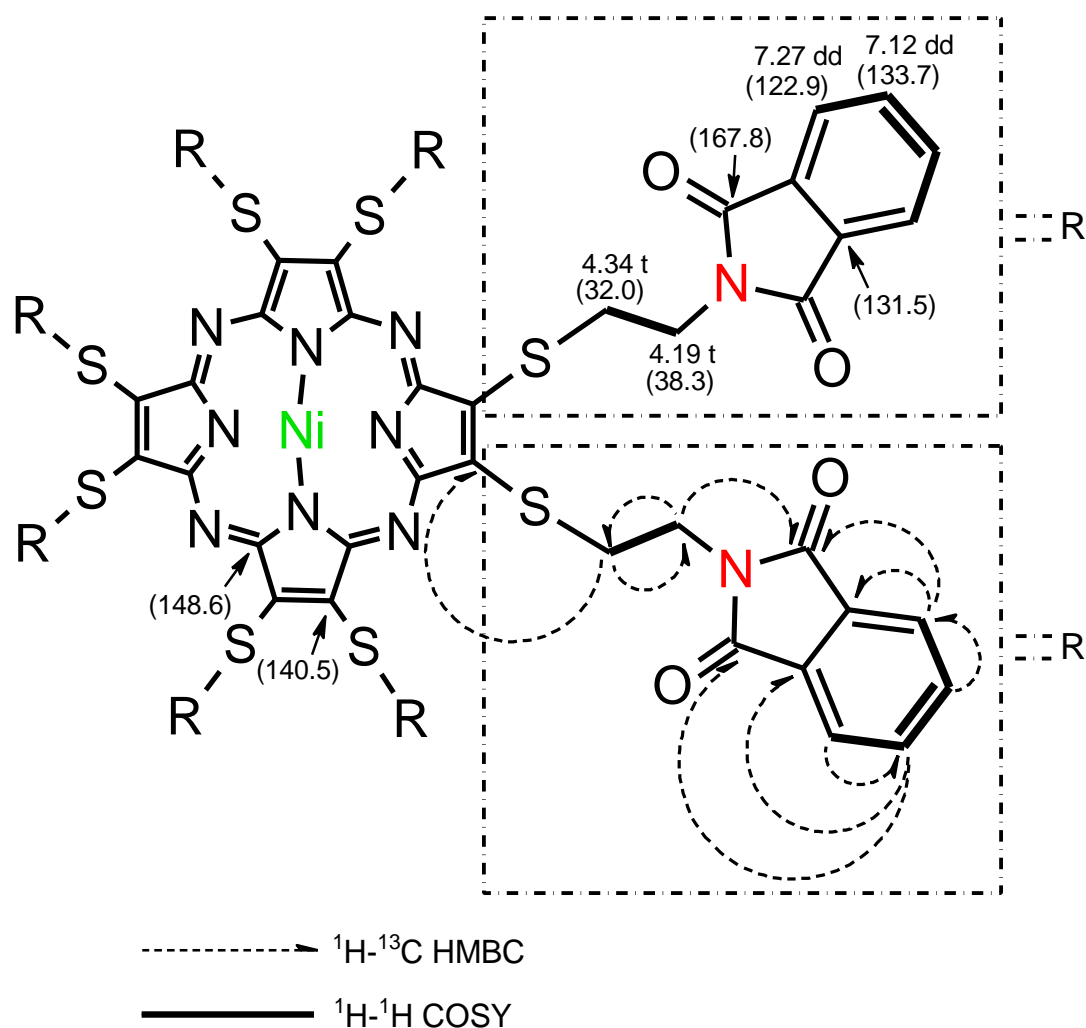


Figure S1. NMR data of **Pz3**: ^1H and ^{13}C chemical shift values [ppm] and key correlations observed in NMR spectra. Bold lines: ^1H - ^1H COSY; Dashed arrows: ^1H - ^{13}C HMBC.

Table S1. ^1H and ^{13}C NMR data obtained for **Pz3** including key correlations determined from ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC spectra.

δ_{H} (ppm)	Multiplicity ($J_{\text{H-H}}$ in Hz)	^1H - ^{13}C HSQC δ_{C} (ppm)	^1H - ^{13}C HMBC δ_{C} (ppm)		
7.27	dd (5.5, 3.0)	122.9	167.8	133.7	131.5
7.12	dd (5.5, 3.0)	133.7	167.8	131.5	122.9
4.34	t (6.5)	32.0	140.5	38.3	
4.19	t (6.5)	38.3	167.8	32.0	
Other quaternary carbon atoms: 148.6 (ppm)					

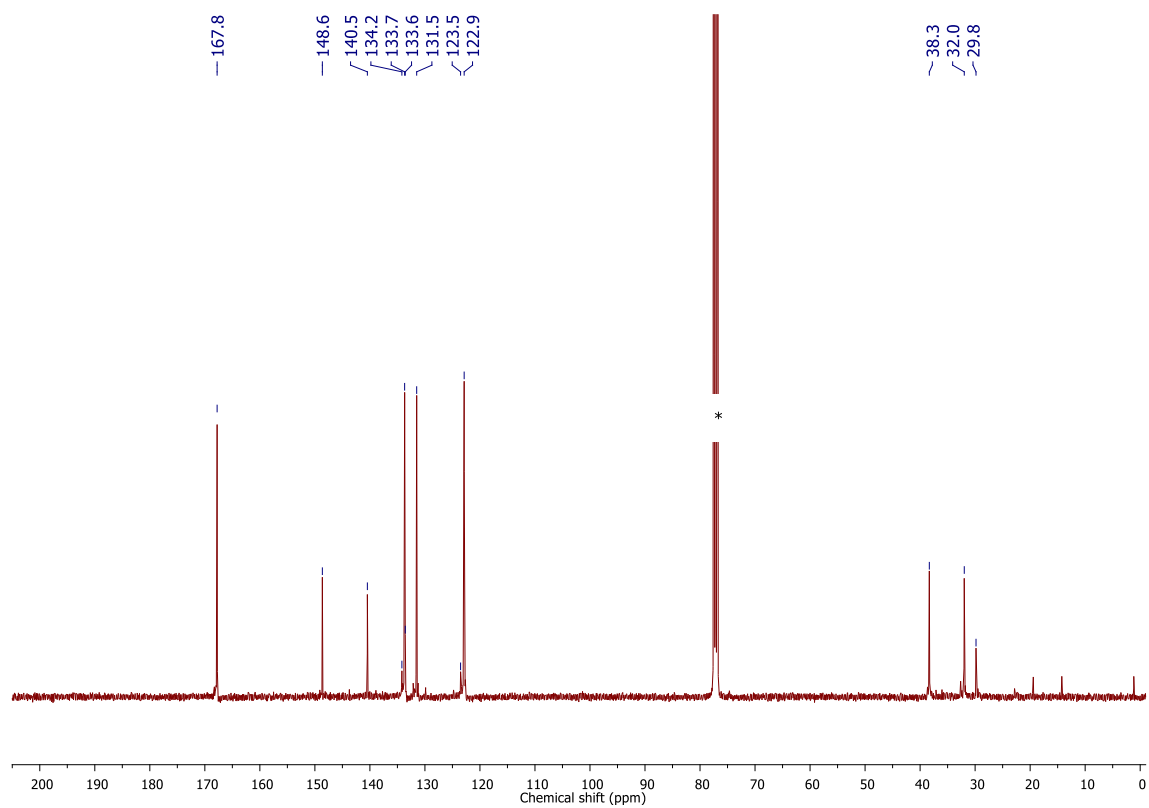
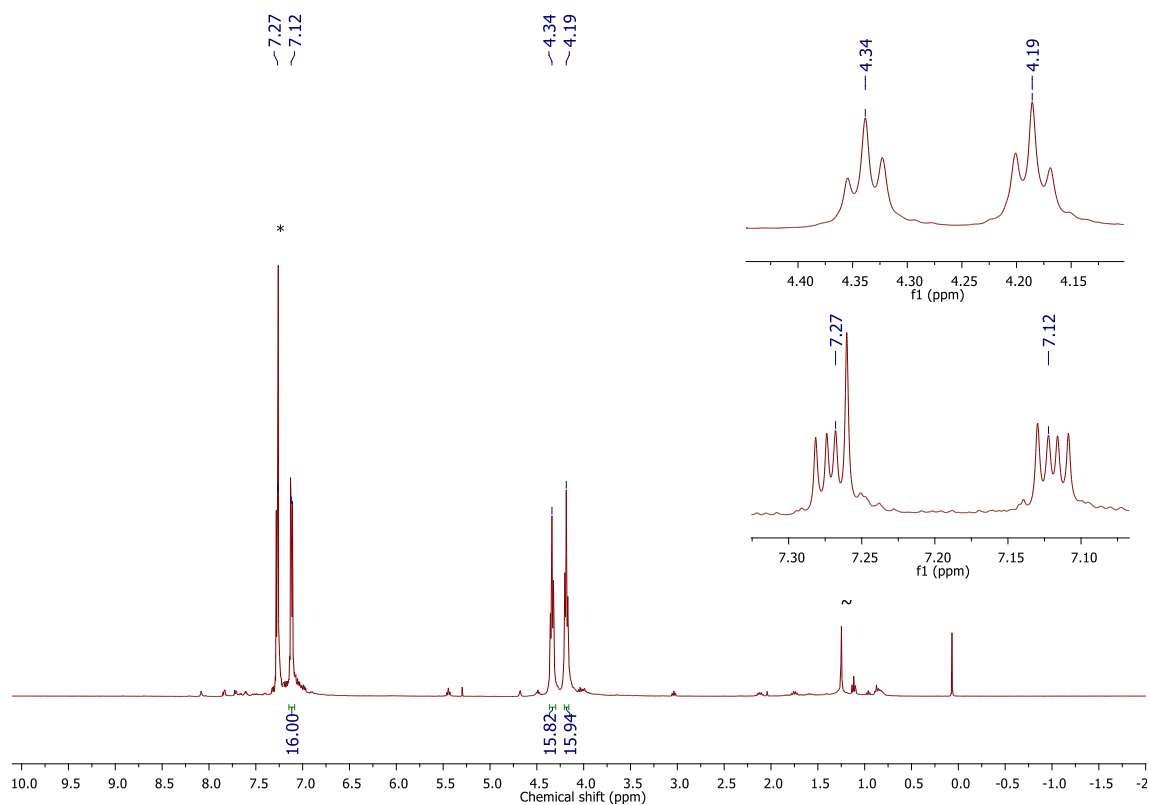


Figure S3. ¹³C NMR spectrum recorded for **Pz3** (100 MHz, chloroform-*d*₁, 298 K). The symbol * indicates chloroform-*d*₁ residual peak.

4. Photochemical study

Table S2. UV–VIS absorption maxima (λ_{Abs}) and logarithms of molar absorption coefficients ($\log \epsilon$) of **Pz3** in selected organic solvents.

Solvent	$\lambda_{1\text{Abs}}$ ($\log \epsilon$)	$\lambda_{2\text{Abs}}$ ($\log \epsilon$)	$\lambda_{3\text{Abs}}$ ($\log \epsilon$)
Dichloromethane	303 (4.54)	352 (4.56)	667 (4.58)
<i>N,N</i> -Dimethylformamide	304 (4.52)	352 (4.52)	668 (4.57)
Dimethylsulfoxide	303 (4.56)	352 (4.55)	667 (4.58)

5. Electrochemical study

Multi-walled carbon nanotubes (MWCNTs, with an average diameter of 10 nm and an average length of 1.5 μm) were delivered by Metrohm DropSens (Oviedo, Spain). Single-walled carbon nanotubes (SWCNTs, with an average diameter of 2 nm and an average length of 3 μm), dichloromethane (DCM, puriss. p.a., $\geq 99.9\%$), *N,N*-dimethylformamide (anhydrous 99.8%), and hydrogen peroxide stock solution 30 wt. % were provided by Merck (Darmstadt, Germany). Graphene oxide (GO, water dispersion at a concentration of 4 mg/mL) for the preparation of reduced graphene oxide (rGO) were purchased from Nanopoz (Poznan, Poland). Glucose, fructose, lactose, maltose, and saccharose were delivered by Warchem (Warszawa, Poland). Caffeine was delivered by Fluka (Buchs, Switzerland). Monosodium and disodium phosphates for the preparation of phosphate buffer (PB, pH 7.4) were supplied by POCH (Gliwice, Poland). Sodium chloride, potassium hexacyanoferrate(II) trihydrate, and potassium hexacyanoferrate(III) were provided also by POCH (Gliwice, Poland). All of these compounds were reagent grade, thus they did not need to be purified further.

All of the electrochemical experiments were performed using an EmStat4S electrochemical analyzer (Eindhoven, Netherlands). A three-electrode system was used, with a reference electrode of Ag/AgCl (3 M KCl) and a counter electrode of a platinum wire. The working electrode was a glassy carbon electrode (GC) with a diameter of 3 mm (BASi, USA).

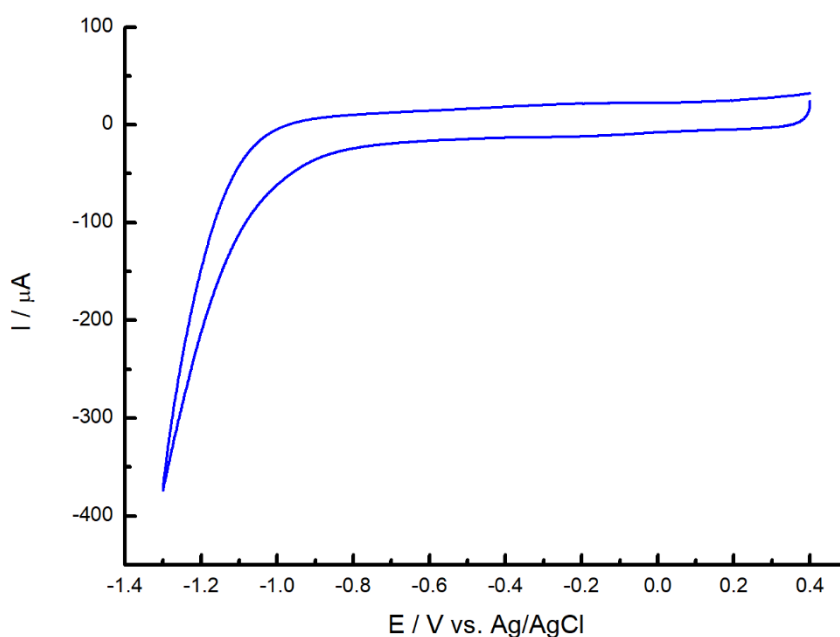


Figure S4. Electrochemical reduction of GC/GO to GC/rGO in phosphate buffer (PB, pH 7.4) in the potential range from 0.4 to -1.3 V (scanning rate 50 mV s^{-1}).

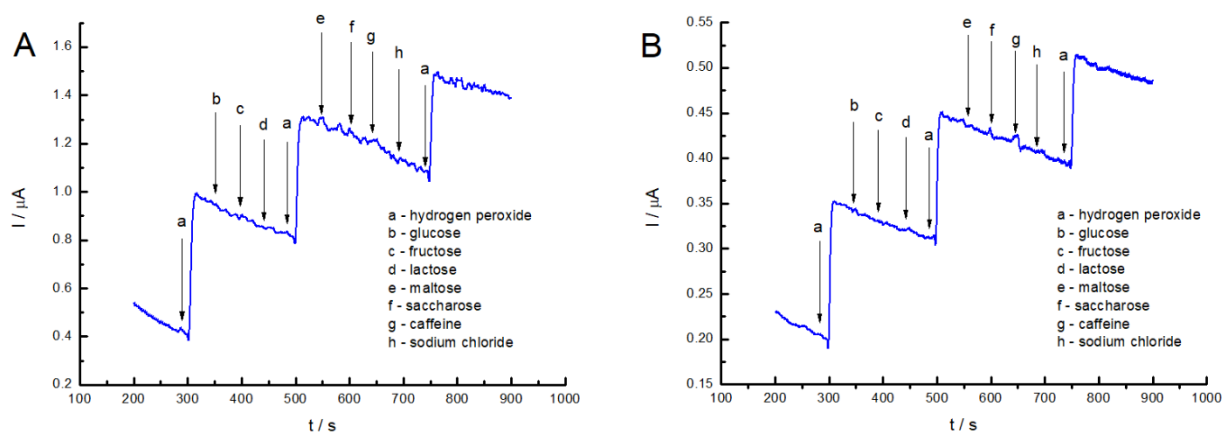


Figure S5. Chronoamperometric responses recorded at (A) GC/SWCNTs/Pz3 and (B) GC/rGO/Pz3 sensor upon addition of $100 \mu\text{M H}_2\text{O}_2$ and selected interferents. The applied potential was $+0.6$ V. The supporting electrolyte was PB (pH 7.4).