**Synthesis and deposition of Tröger base polymer on electrode surface for potentiometric detection of neuroblastoma tumor marker metabolite**

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**Scheme 1** Preparation of coumarin Tröger’s base derivative 6

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**Experimental Section**

**Measurements and Materials**

All chemicals were purchased from commercial suppliers and used without further purification. The NMR spectra were recorded on a 500 MHz (11.75 T) instrument at room temperature. The chemical shifts (δ) are presented in ppm, and coupling constants (J) are presented in Hz; the residual solvent signals were used as the standard. Mass spectra were obtained using electrospray ionisation (ESI) with a LTQ Orbitrap spectrometer. Silica (40-63 D, 60 Å) was used to separate the compounds via column chromatography.

**Amine 2**: Amine 1 (3.0 g, 19.7 mmol) was heated at reflux with Boc₂O (12.9 g, 59.1 mmol) and DMAP (0.2 g, 1.6 mmol) in tetrahydrofuran (80 mL) overnight. The reaction mixture was evaporated to dryness *in vacuo*, and the residue (Boc₂N derivative) was dissolved in dichloromethane (150 mL). TFA (5.0 mL) was then added. The mixture was stirred at room temperature (the formation of amine 2 was followed by TLC analysis eluting with toluene/ethyl acetate, 5:1). The mixture was made basic using concentrated aqueous ammonia, and then extracted with water. The organic portion was dried with Na₂SO₄, and the solvents were evaporated to dryness *in vacuo*. Pure amine 2 (3.5 g, 70.4 %) was obtained by crystallisation from dichloromethane/petroleum ethere. $^1$H NMR ([D₆]DMSO): δ = 9.07 (1H, s), 7.65 (1H, dd, 8.1, 0.9), 7.61 (1H, dd, 8.1, 0.9), 7.38 (1H, t, 8.1), 2.23 (3H, s), 1.47 (9H, s) ppm. $^{13}$C APT NMR ([D₆]DMSO): δ = 153.45 (C), 150.96 (C), 138.64 (C), 129.37 (CH), 126.57 (CH), 126.04 (C),
Bromide 3: Amine 2 (1.0 g, 4.0 mmol) was treated with N-bromosuccinimide (0.8 g, 4.5 mmol) and \( \alpha,\alpha \)-azobis(isobutyronitrile) (0.1 g) in tetrachloromethane (60 mL) under irradiation with an IR lamp (250 W) for 8 h. The reaction mixture was evaporated to dryness in vacuo, and the residue was purified by column chromatography (toluene/ethyl acetate, 25:1) to give bromide 3 (1.1 g, 83.8 %).

1H NMR (DMSO-\( d_6 \)): \( \delta = 9.17 \) (1H, br s), 7.75 (1H, dd, 8.2, 1.3), 7.74 (1H, dd, 8.2, 1.3), 7.56 (1H, t, 8.2), 4.83 (2H, s), 1.48 (9H, s) ppm.

13C APT NMR (DMSO-\( d_6 \)): \( \delta = 153.54 \) (C), 149.90 (C), 138.76 (C), 130.44 (CH), 129.65 (CH), 124.52 (C), 120.88 (CH), 79.91 (C), 28.05 (CH\( _3 \)), 23.95 (CH\( _2 \)) ppm. HRMS (ESI-): calcd. for \( \text{C}_{12}\text{H}_{15}\text{N}_{2}\text{O}_4 \) [M - H] - found 251.10397.

Diamine 4: Bromide 3 (1.0 g, 3.0 mmol) was treated with 7-amino-4-methylcoumarin (7, 0.7 g, 4.0 mmol) and \( \text{K}_2\text{CO}_3 \) (0.5 g, 3.6 mmol) in N,N-dimethylformamide (40 mL) at 50 °C for 8 hours. The reaction mixture was evaporated to dryness in vacuo. The residue was purified by column chromatography (dichloromethane/methanol, 90:10) to give 650 mg (50.6 %) of diamine 4.

1H NMR (DMSO-\( d_6 \)): \( \delta = 9.42 \) (1H br s), 7.62 (1H, dd, 8.0, 1.3), 7.61 (1H, dd, 8.1, 1.3), 7.48 (1H, t, 8.1), 7.37 (1H, d, 8.8), 6.97 (1H, br t, 5.4), 6.51 (1H, dd, 8.8, 2.3), 6.20 (1H, d, 2.3), 5.93 (1H, q, 1.2), 4.37 (2H, d, 5.4), 2.28 (3H, d, 1.2), 1.44 (9H, s) ppm.

13C APT NMR (DMSO-\( d_6 \)): \( \delta = 160.55 \) (C), 155.30 (C), 153.72 (C), 153.62 (C), 151.96 (C), 150.56 (C), 138.37 (C), 129.28 (CH), 128.36 (CH), 127.13 (C), 125.88 (CH), 120.93 (CH), 110.22 (CH), 109.48 (C), 108.07 (CH), 96.25 (CH), 79.84 (C), 39.49 (CH\( _2 \)), 27.95 (CH3), 17.98 (CH\( _3 \)) ppm. HRMS (ESI+): calcd. for \( \text{C}_{22}\text{H}_{23}\text{N}_{3}\text{O}_6\text{Na} \) [M + Na]+; found 448.14835.

TB 5: Diamine 4 (600 mg, 1.4 mmol) was treated with paraformaldehyde (271 mg, 5.6 mmol, 4 equiv.) in trifluoroacetic acid (40 mL) at room temperature overnight. The reaction mixture was diluted with ice-water and made basic using concentrated aqueous ammonia. The crude product was extracted into dichloromethane. The organic solution was washed sequentially with water and brine, then it was dried with \( \text{Na}_2\text{SO}_4 \), and the solvent was evaporated to dryness in vacuo. The residue was purified by column chromatography (dichloromethane/methanol, 95:5) to give 440 mg (89.3 %) of TB 5.

1H NMR (CDCl\( _3 \)): \( \delta = 7.86 \) (1H, dd, 8.1, 1.3), 7.57 (1H, dd, 8.1, 1.3), 7.47 (1H, d, 8.6), 7.39 (1H, t, 8.1), 7.17 (1H, d, 8.6), 6.17 (1H, q, 1.3), 5.10 (1H, dd, 18.0, 0.7), 4.77 (1H, dd, 17.4, 0.8), 4.61 (1H, br d, covered), 4.58 (1H, br d, covered), 4.41 (2H, t, 1.3), 2.37 (3H, d, 1.3) ppm.

13C APT NMR (CDCl\( _3 \)): \( \delta = 160.30 \) (C), 152.71 (C), 151.17 (C), 148.95 (C), 148.60 (C), 147.78 (C), 131.61 (CH), 128.49 (CH), 124.17 (CH), 123.66 (C), 122.10 (CH), 120.89 (CH), 116.76 (C), 115.21 (C), 113.91 (CH), 65.65 (CH\( _3 \)), 57.47 (CH\( _2 \)).
TB 6: TB 5 (350 mg, 1.0 mmol) was treated with iron (350 mg, 6.3 mmol) in the concentrated aqueous hydrochloride acid (50 mL) at 100°C for 5 hours. The reaction mixture was diluted with ice-water and made basic using concentrated aqueous ammonia. The crude product was extracted into dichloromethane. The organic solution was washed sequentially with water and brine, then it was dried with Na₂SO₄, and the solvent was evaporated to dryness in vacuo. The residue was purified by column chromatography (dichloromethane/methanol, 93:7) to give 273 mg (85.3 %) of TB 6.

¹H NMR ([D₆]DMSO): δ = 7.48 (1H, d, 8.6), 7.08 (1H, d, 8.6), 6.83 (1H, t, 7.9), 6.39 (1H, dd, 7.9, 0.9), 6.25 (1H, dd, 7.9, 0.9), 6.18 (1H, q, 1.2), 4.82 (2H, bs), 4.57 (1H, d, 16.7), 4.34 (1H, d, 16.7), 4.28 (1H, d, 16.7), 4.22 (1H, d, 13.2), 4.19 (1H, d, 13.2), 4.02 (1H, d, 16.7), 2.30 (3H, d, 1.2) ppm. ¹³C APT NMR ([D₆]DMSO): δ = 159.69 (C), 153.45 (C), 152.57 (C), 150.60 (C), 148.07 (C), 145.58 (C), 127.28 (CH), 123.35 (CH), 120.70 (CH), 116.00 (C), 114.59 (C), 112.58 (CH), 111.96 (C), 111.94 (CH), 109.26 (CH), 65.35 (CH₃), 55.49 (CH₃), 53.64 (CH₃), 18.09 (CH₃) ppm. HRMS (ESI⁺): calcd. 320.13935 for C₁₉H₁₈N₃O₂ [M + H]⁺; found 320.13953.

Electrochemical polymerization of Tröger base on platinum electrode

The electrochemical polymerization of 6 was carried out in a three-electrode electrochemical cell. A platinum wire (diameter 0.4 mm, length 7 mm) was used as the working electrode. Prior to polymerization, the polymerization solution was purged with N₂ for 10 min. The electrochemical polymerization was achieved by cycling the potential of the working electrode from 0.0 to +1.2 V versus Ag/AgCl in acetonitrile in the presence of 1x10⁻³ mol L⁻¹ derivate 6, 0.2 mol L⁻¹ tetrabutylammonium fluoroborate (TBAFB) and 1x10⁻³ mol L⁻¹ HCl. A scan rate was 50 mV s⁻¹, number of scans was 10. Before potentiometric measurements, the electrode surface was rinsed with water and then soaked in 0.1 mol L⁻¹ phosphate buffer at pH=7.

Potentiometric measurements

The potentiometric measurements were performed with an AUTOLAB PGSTAT. The electrode assembly consisted of a platinum electrode coated with the polymer derived from 6 (section “Electrochemical polymerization of Tröger base on platinum electrode”) as the indicator electrode and Ag/AgCl (3 M KCl) as the reference electrode. Calibration solutions were prepared by adding various volumes of a 1·10⁻² mol L⁻¹ solution of the corresponding TM to 10 mL of 0.1 mol L⁻¹ phosphate buffer at pH=7. The pH was monitored using a type SEUJ 212 glass electrode (Monokrystaly Turnov, Czech Republic) with a PHI 04 MG pH meter (Labio, Czech Republic). After
each potentiometric measurement, the electrodes were regenerated in 0.1 mol L⁻¹ phosphate buffer at pH=7 for 10 min.

The potentiometric selectivity coefficient (log $K^{pot.}_{VMA, HVA} = -0.9$) was obtained using the fixed interference method with concentration of HVA $1 \times 10^{-3}$ mol L⁻¹ [Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda, and S. Amemiya, Pure Appl. Chem., 2000, 72, 1851].

The determination of VMA in an artificial urine was carried out using the standard addition method.