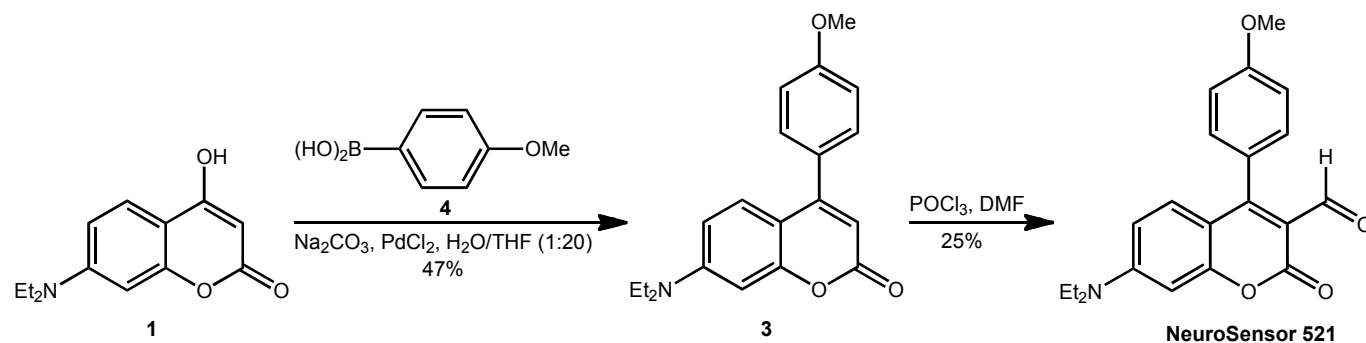


# Selective Catecholamine Recognition with NeuroSensor 521: A Fluorescent Sensor for the Visualization of Norepinephrine in Fixed and Live Cells

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## Synthetic Procedures



**7-diethylamino-4-(4'-methoxyphenyl)coumarin (3).** Compound **1** (250.0 mg, 1.072 mmol), *p*-toluenesulfonyl chloride (224.8 mg, 1.179 mmol), and  $\text{Na}_2\text{CO}_3$  (340.8 mg, 3.215 mmol) were added to a flame-dried round bottom and degassed with  $\text{N}_2$  for 15 minutes. Degassed  $\text{H}_2\text{O}/\text{THF}$  (1:20, 15.0 mL) was added and the mixture stirred at 50 °C for 30 minutes. The mixture was allowed to cool to room temperature. 4-Methoxyphenylboronic acid **4** (179.2 mg, 1.179 mmol) was added and the mixture was allowed to stir at room temperature for 5 minutes. Palladium chloride (9.5 mg, 0.054 mmol) was added and the mixture stirred at 50 °C for 6 hours. The mixture was filtered and the solvent removed *in vacuo*. The remaining residue was purified by chromatography (100%  $\text{CH}_2\text{Cl}_2 \rightarrow 95:5 \text{ CH}_2\text{Cl}_2/\text{EtOAc}$ ) to yield compound **3** (172 mg, 47%) as a pale-yellow oil:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.40 (dd, 2H,  $J = 6.0, 1.0$ ), 7.32 (dd, 1H,  $J = 8.8, 1.0$ ), 7.02 (dd, 2H,  $J = 7.8, 1.5$ ), 6.57 (d, 1H,  $J = 2.0$ ), 6.53 (dd, 1H,  $J = 9.3, 2.0$ ), 6.00 (d, 1H,  $J = 1.0$ ), 3.88 (s, 3H), 3.42 (q, 4H,  $J = 7.0$ ), 1.21 (t, 6H,  $J = 7.0$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  162.3, 160.5, 156.8, 150.5, 129.8, 128.5, 127.9, 114.0, 108.4, 108.0, 107.9, 97.8, 55.4, 44.7, 12.4; IR (neat,  $\text{cm}^{-1}$ ) 2970, 1712, 1614, 1524, 1417, 1246, 1115, 829; HRMS calculated for  $\text{C}_{20}\text{H}_{21}\text{NO}_3\text{Na}^+$  ( $M + \text{Na}^+$ ): 346.1414. Found: 346.1415.

**7-diethylamino-3-formyl-4-(4'-methoxyphenyl)coumarin (NeuroSensor 521).** POCl<sub>3</sub> (5.2 mL, 56.1 mmol) was added to DMF (10.8 mL, 139.5 mmol) at 0°C in a flame-dried round bottom flask. The Vilsmeier reagent was stirred at ambient temperature for 45 minutes. The Vilsmeier reagent (5 mL) was added to a solution of compound **3** (171.8 mg, 0.532 mmol) in DMF (1 mL). The solution was stirred at ambient temperature for 12 hours. The resulting red solution was poured onto cold H<sub>2</sub>O (100 mL), basified with saturated NaHCO<sub>3</sub> (50 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL x 3). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed *in vacuo*. The residue was purified by chromatography (80:20 hexanes:EtOAc → 50:50 hexanes:EtOAc) to yield NeuroSensor 521 (46.1 mg, 25%) as a yellow oil: <sup>1</sup>H NMR (500 MHz, d-acetone) δ 9.80 (s, 1H), 7.27 (dd, 2H, *J* = 2.0, 6.5), 7.08 (dd, 2H, *J* = 2.0, 6.5), 6.99 (d, 1H, *J* = 9.5), 6.71 (dd, 1H, *J* = 2.5, 9.5), 6.55 (d, 1H, *J* = 2.5), 3.89 (s, 3H), 3.56 (q, 4H, *J* = 7.0), 1.22 (t, 6H, *J* = 7.0); <sup>13</sup>C NMR (125 MHz, d-acetone) δ 188.0, 161.8, 161.2, 160.0, 158.7, 153.9, 131.7, 131.0, 126.2, 114.5, 113.2, 110.6, 109.8, 97.5, 55.7, 45.5, 12.7; IR (neat, cm<sup>-1</sup>) 2970, 2919, 2846, 1742, 1615, 1495, 1418, 1356, 1248, 1132; HRMS calculated for C<sub>21</sub>H<sub>21</sub>NO<sub>4</sub>Na<sup>+</sup> (M + Na<sup>+</sup>): 374.1363. Found: 374.1364.