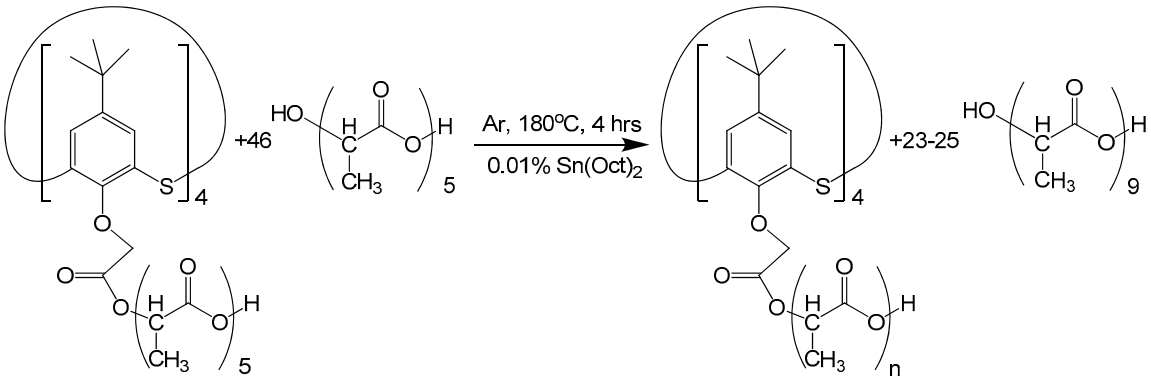
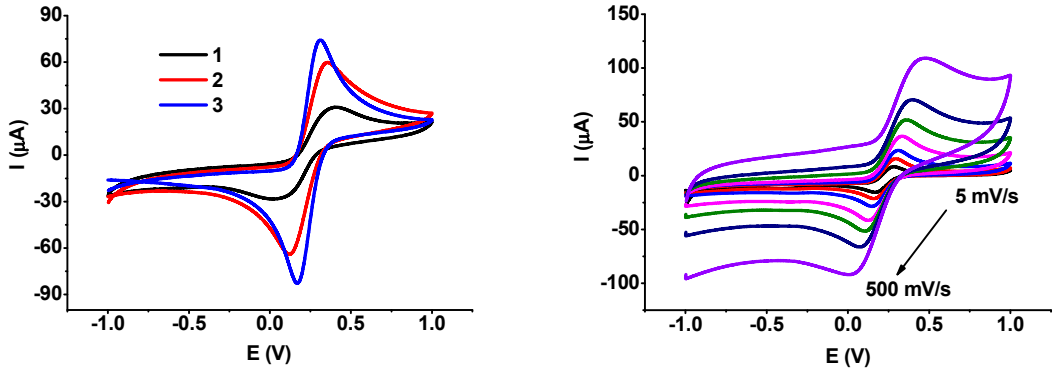


Scheme S1. General scheme of the synthesis of thiacalix[4]arene bearing oligolactide fragments (OLA-*cone*).



(**a**) (**b**)

Figure S1. Cyclic voltammograms recorded with the glassy carbon electrode (GCE) covered with the carbon black (CB), OLA-*cone*, and aptamers. Measurements in phosphate buffer (PB), pH = 7.0, in the presence of 0.01 M $K_3[Fe(CN)_6]$ and 0.01 M $K_4[Fe(CN)_6]$ at the scan rate of 100 mV/s. (**a**) 1: CB in chitosan matrix; 2: CB in chitosan matrix mixed with the OLA-*cone*/aptamer 1:1 mixture; 3: the same aptasensor after 20 min incubation in 1.0 nM KANA; (**b**) CB in chitosan matrix mixed with the OLA-*cone*/aptamer 1:2 mixture, depending on the scan rate.

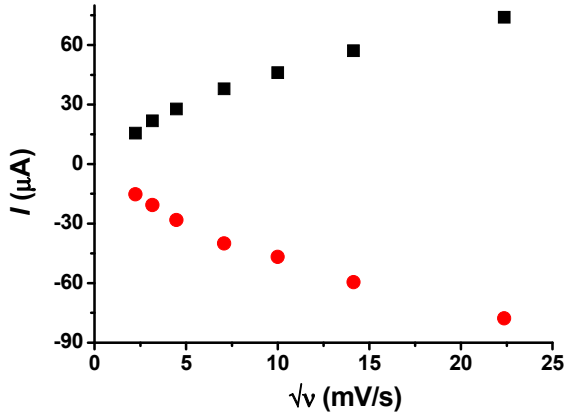


Figure S2. The dependence of the cathodic (black squares) and anodic (red circles) peak currents of cyclic voltammograms recorded with the GCE covered with the CB/chitosan and OLA-*cone*/aptamer (1:2) suspension on the square root from the scan rate, mV/s. Measurements in PB, pH = 7.0, in the presence of 0.01 M $K_3[Fe(CN)_6]$ and 0.01 M $K_4[Fe(CN)_6]$.

Table S1. The dependence of the relative increase of the working surface area of the electrode (n) calculated from the Randles–Sevcik equation on the pH and concentration of the PB. Deposition of 2 μL of 1 mg/mL suspension of the CB in 0.275% chitosan.

PB Concentration (mM)	pH	Real Electrode Surface (mm²)	n
25	6.0	2.50	1.3
25	7.0	2.54	1.3
25	7.4	2.53	1.3
25	8.0	2.55	1.3
10	7.0	2.53	1.3
35	7.0	2.54	1.3
50	7.0	2.56	1.3

Table S2. The dependence of the electrochemical impedance spectroscopy (EIS) parameters ($(R_{et})_1$, CPE_1, and n_1) on the period of incubation in 1.0 nM Kanamycin A (KANA) solution. Aptasensor with the CB/chitosan and the OLA-*cone*/aptamer 1:2 mixture. Electrode–layer interface, average ± standard deviation for three measurements with individual aptasensors. $(R_{et})_1$ is the charge transfer resistance, CPE_1 is the constant phase element and n_1 is exponent in Equation (6) of the main text.

Incubation Period (min)	$(R_{et})_1$ (kΩ)	CPE_1 (μF)	n_1
10	0.52 ± 0.02	1.50 ± 0.10	0.95
20	0.43 ± 0.03	1.41 ± 0.22	0.93
30	0.42 ± 0.01	1.32 ± 0.11	0.02
40	0.41 ± 0.02	1.44 ± 0.09	0.93