

Electronic Supplementary Information

Application of PAMAM dendrimers in electrochemical aptasensor for semi-quantitative determination of heart fatty acid-binding protein

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1. General

^1H NMR and ^{13}C NMR spectra were obtained on the Bruker Avance-400 spectrometer (Bruker Corp., USA) ($^{13}\text{C}\{^1\text{H}\}$ 100 MHz and ^1H 400 MHz). Chemical shifts were determined against the signals of residual protons of deuterated solvent (CD_3OD). Concentrations of the compounds were equal to 3–5 mass. %. FTIR ATR spectra were recorded on the Spectrum 400 FT-IR spectrometer (Perkin–Elmer, UK) with the Diamond KRS-5 attenuated total internal reflectance attachment (resolution 0.5 cm^{-1} , accumulation of 64 scans, recording time 16 s in the wavelength range $400\text{--}4000\text{ cm}^{-1}$). Melting points were determined using the Boetius Block apparatus (VEB Kombinat Nagema, Germany). ESI HRMS experiments were performed at Agilent 6550 iFunnel Q-TOF LC/MS (Agilent Technologies, USA), equipped with Agilent 1290 Infinity II LC.

PAMAM-calix-dendrimers **G2** were synthesized by the previously described protocol [31].

1.1. 5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis[*N*-(6-(*N,N*-di(*N*-(2-(*N,N*-di(*N*-(2-aminoethyl)carbamoylethyl)amino)ethyl)carbamoylethyl)amino)hexyl)carbamoylmethoxy]-2,8,14,20-tetrathiacalix[4]arene, [**G2-cone**]. White Solid Foam, m.p. $68\text{ }^\circ\text{C}$, Yield: 1.01 g (90 %)

^1H NMR (CD_3OD , δ , ppm, J/Hz): 1.14 (s, 36H, $(\text{CH}_3)_3\text{C}$), 1.35 (m, 16H, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.49 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.59 (m, 8H, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.30–2.42 (m, 48H, $\text{NCH}_2\text{CH}_2\text{C}(\text{O})$), 2.44–2.51 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.57 (m, 16H, $\text{NHCH}_2\text{CH}_2\text{N}$), 2.72 (t, 32H, $\text{NHCH}_2\text{CH}_2\text{NH}_2$, $^3J_{\text{HH}}=6.3$), 2.75–2.84 (m, 48H, $\text{NCH}_2\text{CH}_2\text{C}(\text{O})$), 3.25 (t, 16H, $\text{NHCH}_2\text{CH}_2\text{N}$, $^3J_{\text{HH}}=6.3$), 3.33 (m, 8H, $\text{NHCH}_2\text{CH}_2\text{CH}_2$), 4.12 (s, 8H, $\text{OCH}_2\text{C}(\text{O})$), 7.45 (s, 8H, ArH).

^{13}C NMR (CD_3OD , δ , ppm): 27.88, 28.17, 28.46, 30.74, 31.67, 34.38, 34.79, 38.63, 40.40, 41.99, 42.79, 50.83, 51.17, 53.50, 54.48, 75.39, 129.81, 136.07, 148.88, 159.37, 164.13, 170.54, 174.78, 175.19.

FTIR ATR (ν , cm^{-1}): 3278 (N-H), 3061 (N-H), 1635 ($\text{C}(\text{O})\text{NH}$, amide I), 1539 ($\text{C}(\text{O})\text{NH}$, amide II), 1094 ($\text{C}_{\text{Ph}}\text{OCH}_2$).

ESI, Calculated $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3007$, $[\text{M} + 11\text{H} + \text{Na}]^{12+}$ $m/z = 343.2273$, $[\text{M} + 17\text{H} + \text{Na}]^{18+}$ $m/z = 229.1540$. Found $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3228$, $[\text{M} + 11\text{H} + \text{Na}]^{12+}$ $m/z = 343.2446$, $[\text{M} + 17\text{H} + \text{Na}]^{18+}$ $m/z = 229.1657$.

1.2. 5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis[*N*-(6-(*N,N*-di(*N*-(2-(*N,N*-di(*N*-(2-aminoethyl)carbamoylethyl)amino)ethyl)carbamoylethyl)amino)hexyl)carbamoylmethoxy]-2,8,14,20-tetrathiacalix[4]arene, [**G2-paco**]. White Solid Foam, m.p. $69\text{ }^\circ\text{C}$, Yield: 0.96 g (85 %)

^1H NMR (CD_3OD , δ , ppm, J/Hz): 1.09 (s, 18H, $(\text{CH}_3)_3\text{C}$), 1.20–1.73 (m, 32H, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$, $\text{CH}_2\text{CH}_2\text{N}$), 1.35 (s, 18H, $(\text{CH}_3)_3\text{C}$), 2.28–2.43 (m, 48H, $\text{NCH}_2\text{CH}_2\text{C}(\text{O})$), 2.47 (m, 8H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 2.57 (m, 16H, $\text{NHCH}_2\text{CH}_2\text{N}$), 2.67–2.90 (m, 80H, $\text{NHCH}_2\text{CH}_2\text{NH}_2$, $\text{NCH}_2\text{CH}_2\text{CO}$), 3.14 (m, 2H, $\text{C}(\text{O})\text{NHCH}_2$), 3.25 (m, 48H, $\text{NHCH}_2\text{CH}_2\text{N}$), 3.28–3.42 (m, 6H, $\text{C}(\text{O})\text{NHCH}_2$), 4.26 (br.s, 2H, $\text{OCH}_2\text{C}(\text{O})$), 4.60 (s, 2H, $\text{OCH}_2\text{C}(\text{O})$), 4.84 (s, 2H, $\text{OCH}_2\text{C}(\text{O})$), 4.98 (br.s, 2H, $\text{OCH}_2\text{C}(\text{O})$), 7.12 (s, 2H, ArH), 7.62 (s, 2H, ArH), 7.66 (s, 2H, ArH), 7.84 (s, 2H, ArH).

^{13}C NMR (CD_3OD , δ , ppm): 27.90, 28.16, 28.32, 28.44, 30.59, 30.75, 31.73, 31.82, 31.93, 34.46, 34.85, 35.21, 38.66, 40.06, 40.13, 40.42, 42.05, 42.95, 50.85, 51.21, 53.56, 54.52, 54.65, 70.87, 74.18, 74.55, 128.02, 129.53, 129.97, 134.73, 135.62, 135.99, 137.51, 147.64, 148.86, 157.91, 159.41, 160.91, 170.12, 170.48, 170.82, 174.84, 175.19.

FTIR ATR (ν , cm^{-1}): 3279 (N-H), 3061 (N-H), 1635 ($\text{C}(\text{O})\text{NH}$, amide I), 1539 ($\text{C}(\text{O})\text{NH}$, amide II), 1090 ($\text{C}_{\text{Ph}}\text{OCH}_2$).

ESI, Calculated $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3007$, $[\text{M} + 12\text{H}]^{12+}$ $m/z = 341.3119$, $[\text{M} + 17\text{H} + \text{Na}]^{18+}$ $m/z = 229.1540$. Found $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3228$, $[\text{M} + 12\text{H}]^{12+}$ $m/z = 341.2290$, $[\text{M} + 17\text{H} + \text{Na}]^{18+}$ $m/z = 229.1658$.

1.3. 5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis[*N*-(6-(*N,N*-di(*N*-(2-(*N,N*-di(*N*-(2-aminoethyl)carbamoylethyl)amino)ethyl)carbamoylethyl)amino)hexyl)carbamoylmethoxy]-2,8,14,20-tetrathiacalix[4]arene [**G2-alt**]. White Solid Foam, m.p. 75 °C, Yield: 0.99 g (88 %)

^1H NMR (CD_3OD , δ , ppm, J/Hz): 1.28 (s, 36H, $(\text{CH}_3)_3\text{C}$), 1.34 (m, 16H, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.42-1.61 (m, 16H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$, $\text{C}(\text{O})\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.31-2.42 (m, 48H, $\text{NCH}_2\text{CH}_2\text{C}(\text{O})$), 2.44-2.52 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.57 (m, 16H, $\text{NHCH}_2\text{CH}_2\text{N}$), 2.72 (t, 32H, $\text{NHCH}_2\text{CH}_2\text{NH}_2$, $^3J_{\text{HH}}=6.3$), 2.75-2.84 (m, 48H, $\text{NCH}_2\text{CH}_2\text{C}(\text{O})$), 3.17-3.29 (m, 54H, NHCH_2), 4.12 (br.s, 8H, $\text{OCH}_2\text{C}(\text{O})$), 7.60 (s, 8H, ArH).

^{13}C NMR (CD_3OD , δ , ppm): 27.85, 28.22, 28.39, 30.77, 31.76, 34.37, 34.79, 38.63, 40.72, 42.03, 42.95, 50.80, 51.16, 53.50, 54.46, 71.53, 129.21, 133.20, 148.86, 157.88, 164.13, 169.89, 174.79, 175.16.

FTIR ATR (ν , cm^{-1}): 3282 (N-H), 3061 (N-H), 1636 ($\text{C}(\text{O})\text{NH}$, amide I), 1539 ($\text{C}(\text{O})\text{NH}$, amide II), 1086 ($\text{C}_{\text{Ph}}\text{OCH}_2$).

ESI, Calculated $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3007$, $[\text{M} + 11\text{H} + \text{Na}]^{12+}$ $m/z = 343.2273$. Found $[\text{M} + 8\text{H} + \text{Na}]^{9+}$ $m/z = 457.3231$, $[\text{M} + 11\text{H} + \text{Na}]^{12+}$ $m/z = 343.2446$.

2. NMR, IR and mass spectra of the compounds

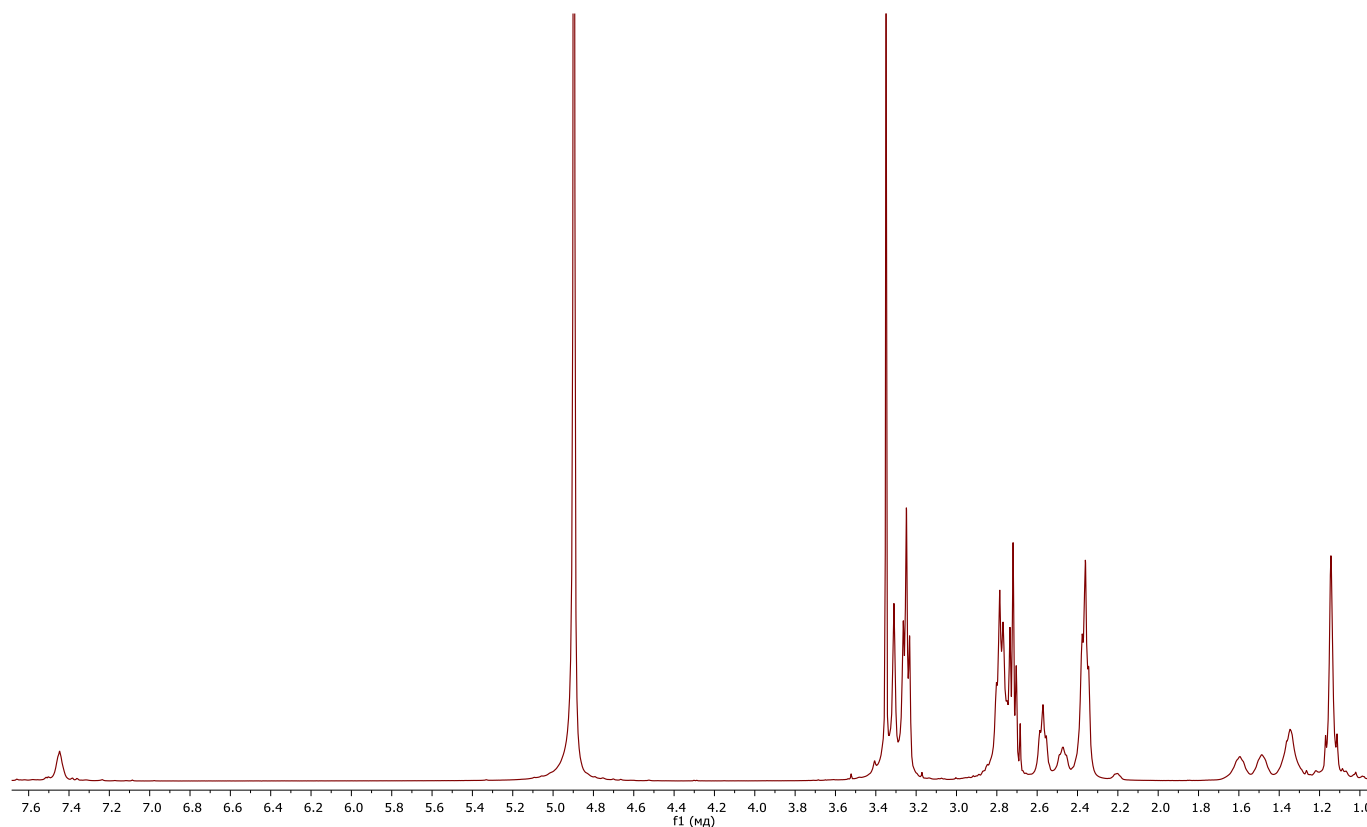


Fig. S1. ^1H NMR spectrum of **G2-cone**, CD_3OD , 298 K, 400 MHz

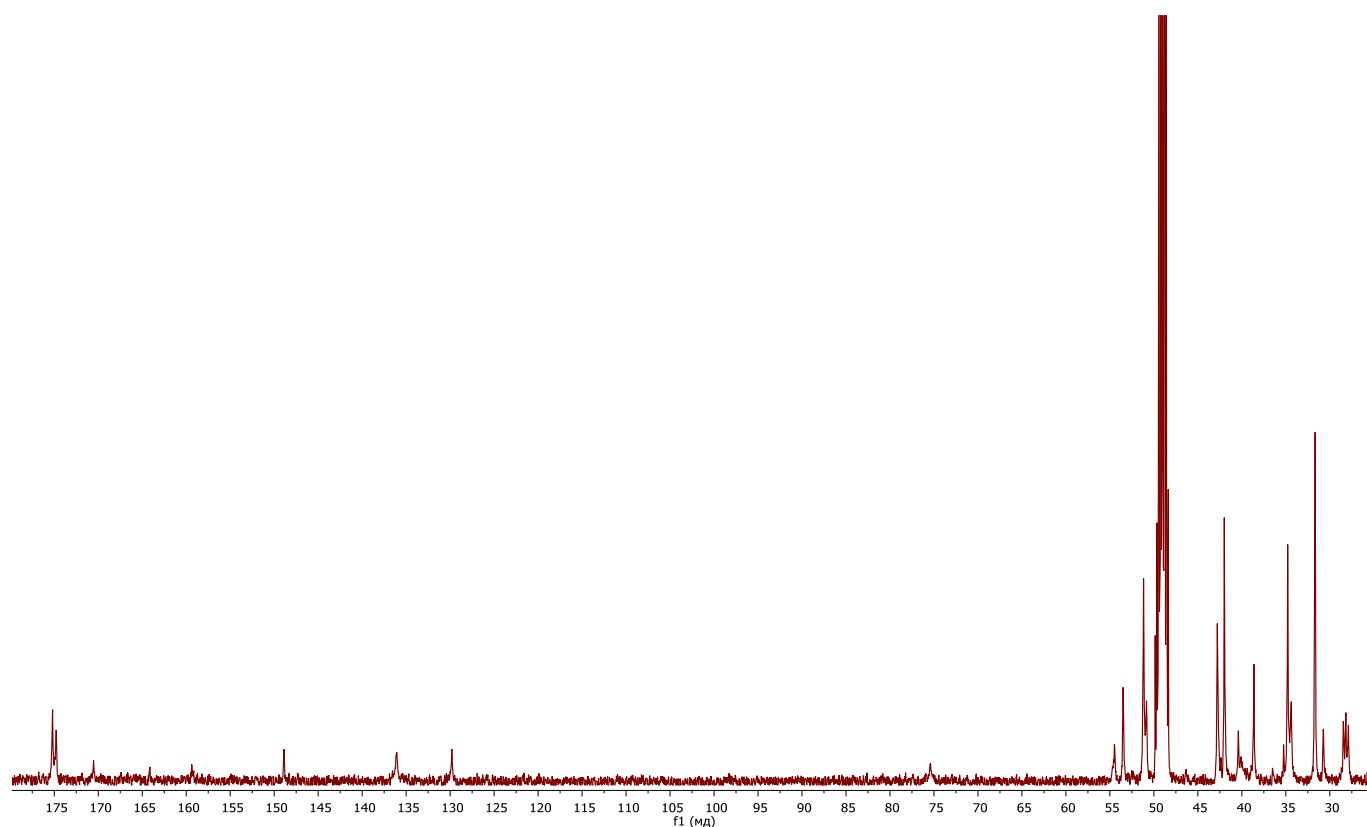


Fig. S2. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **G2-cone**, CD_3OD , 298 K, 100 MHz

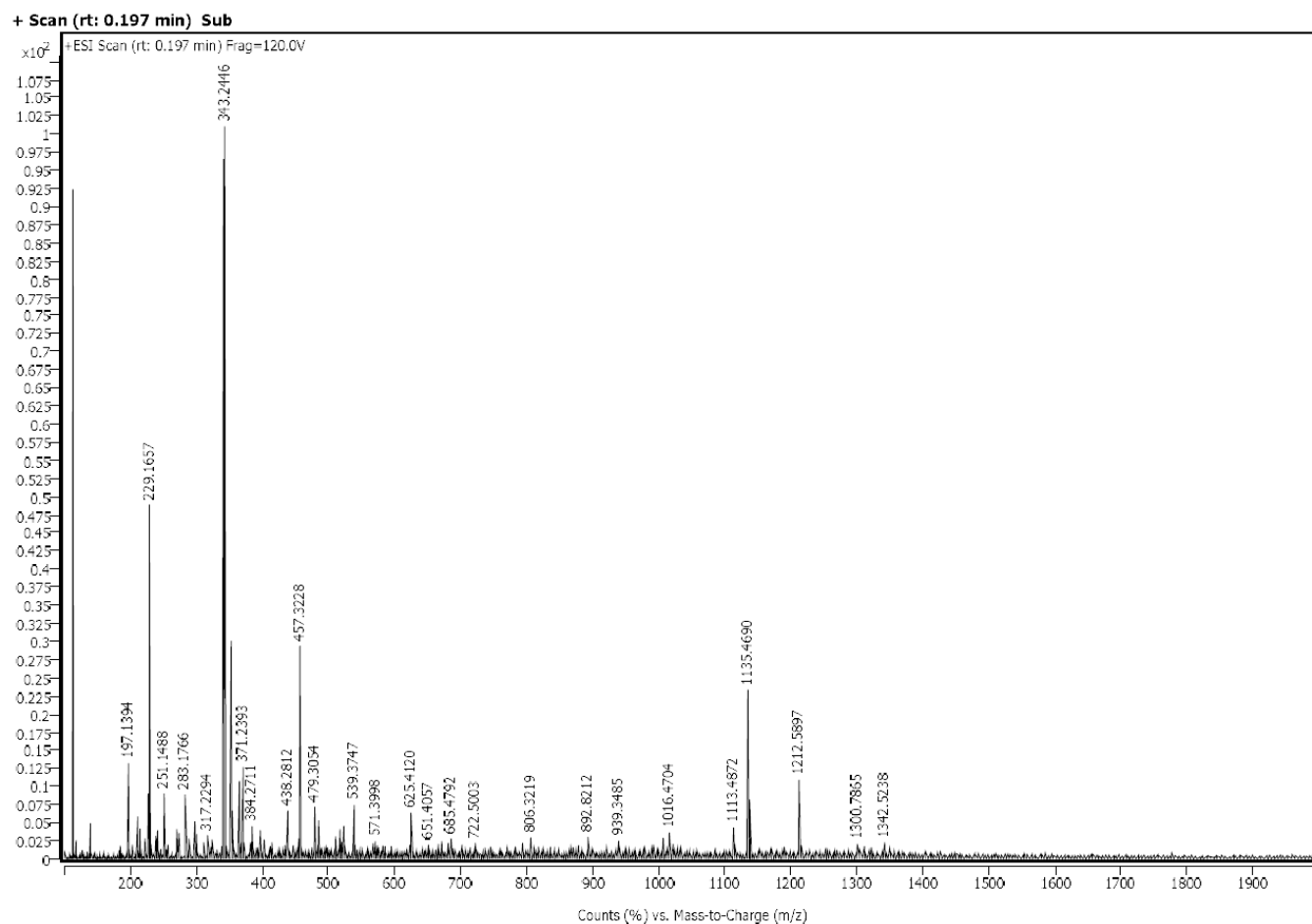


Fig. S3. Mass spectrum (HRESI) of **G2-cone**

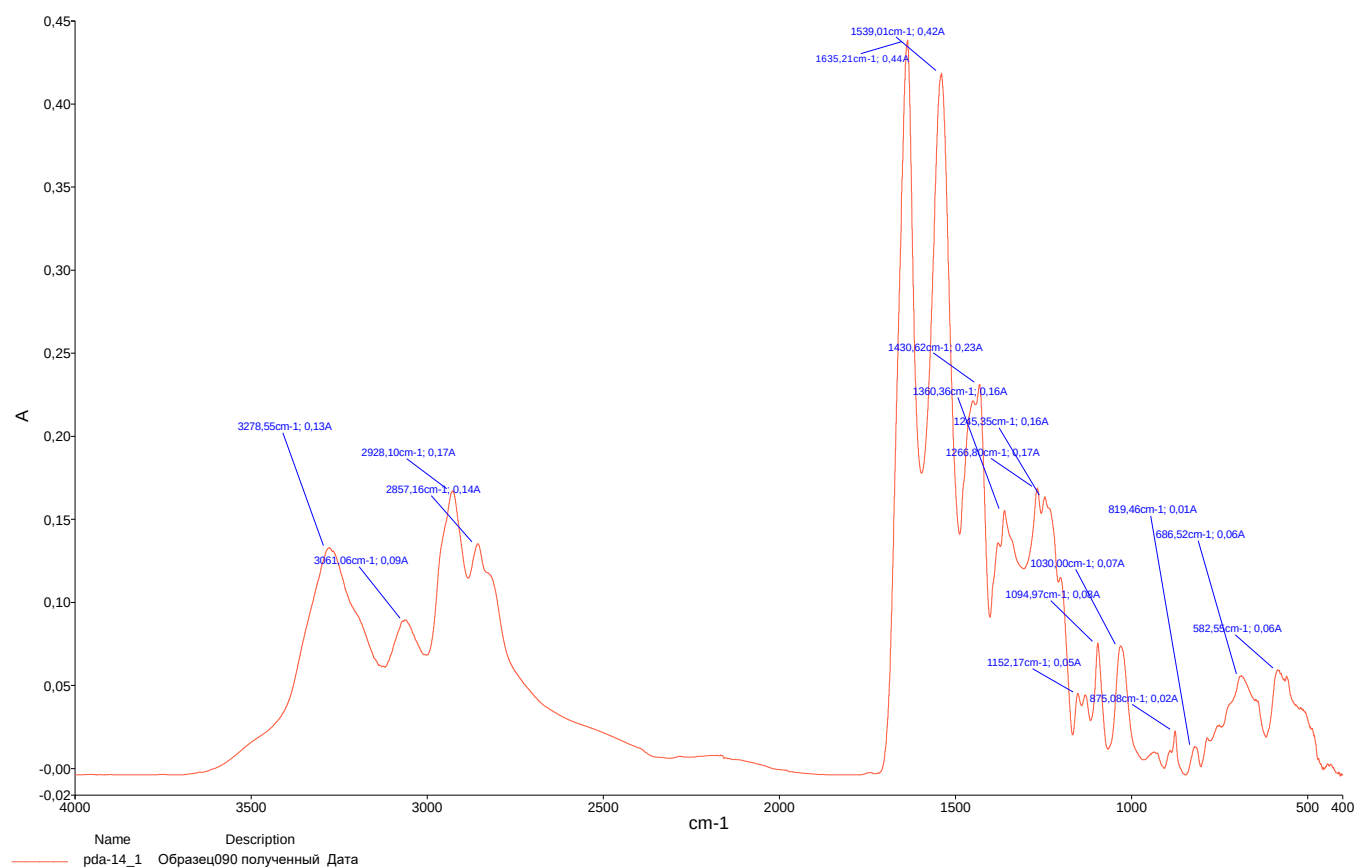


Fig. S4. FTIR-ATR spectrum of **G2-cone**

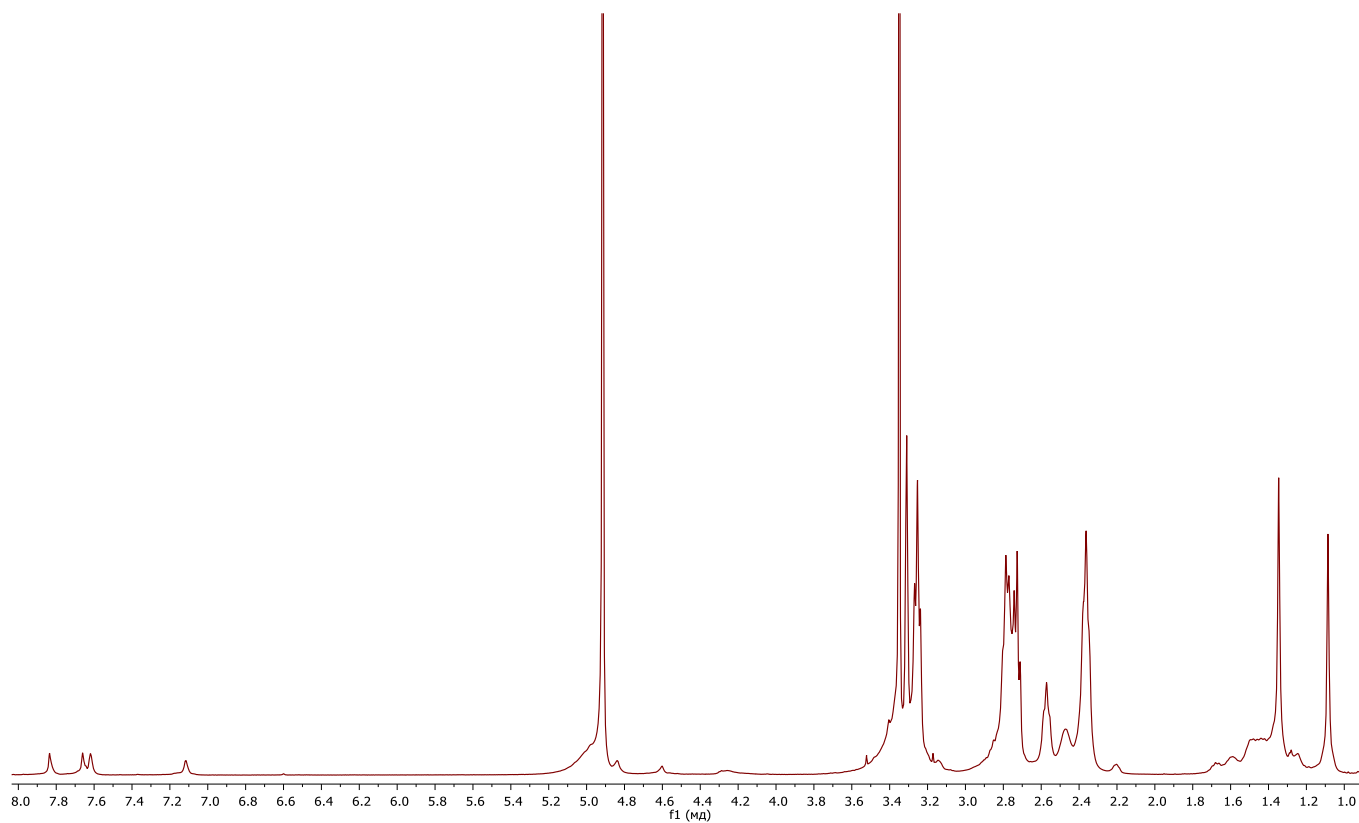


Fig. S5. ¹H NMR spectrum of **G2-paco**, CD₃OD, 298 K, 400 MHz

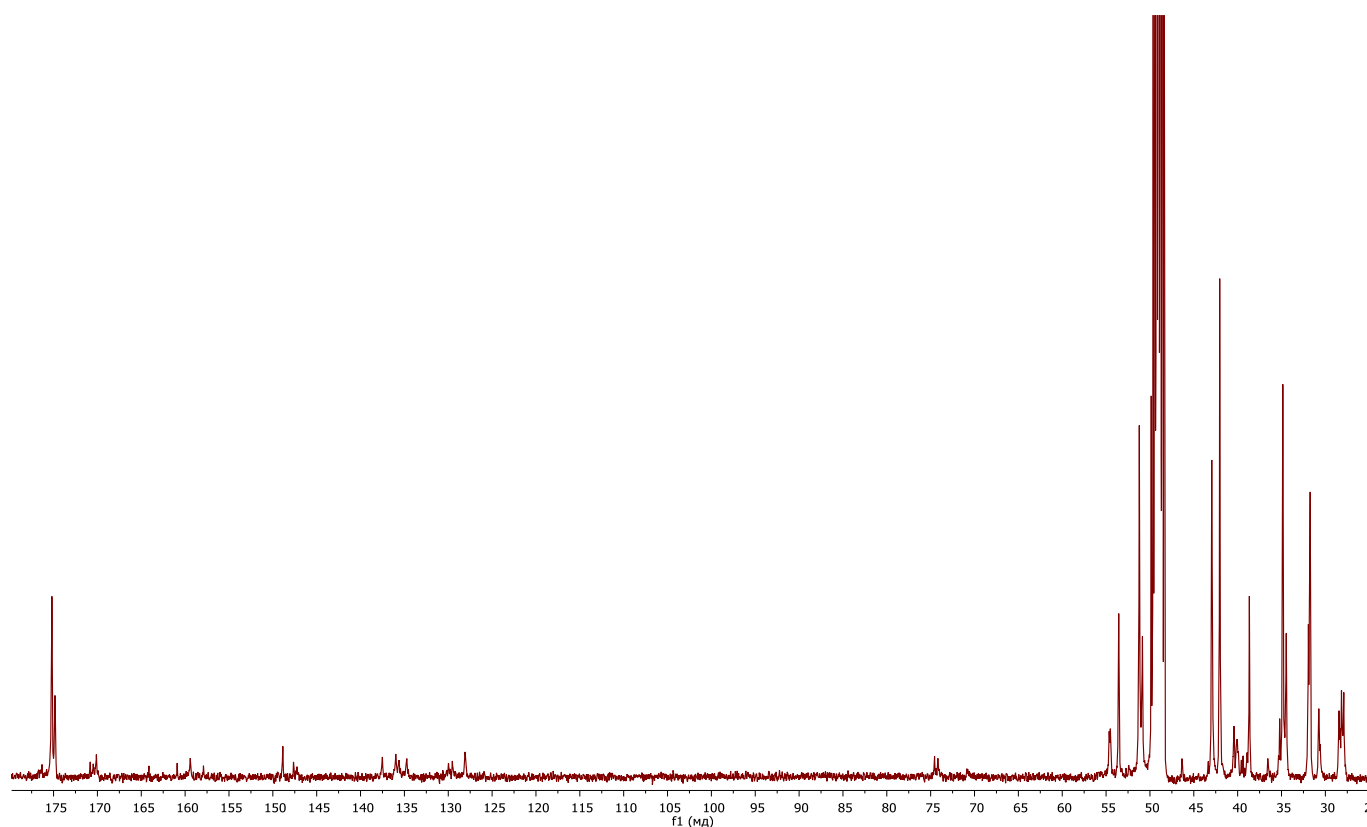


Fig. S6. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **G2-paco**, CD_3OD , 298 K, 100 MHz

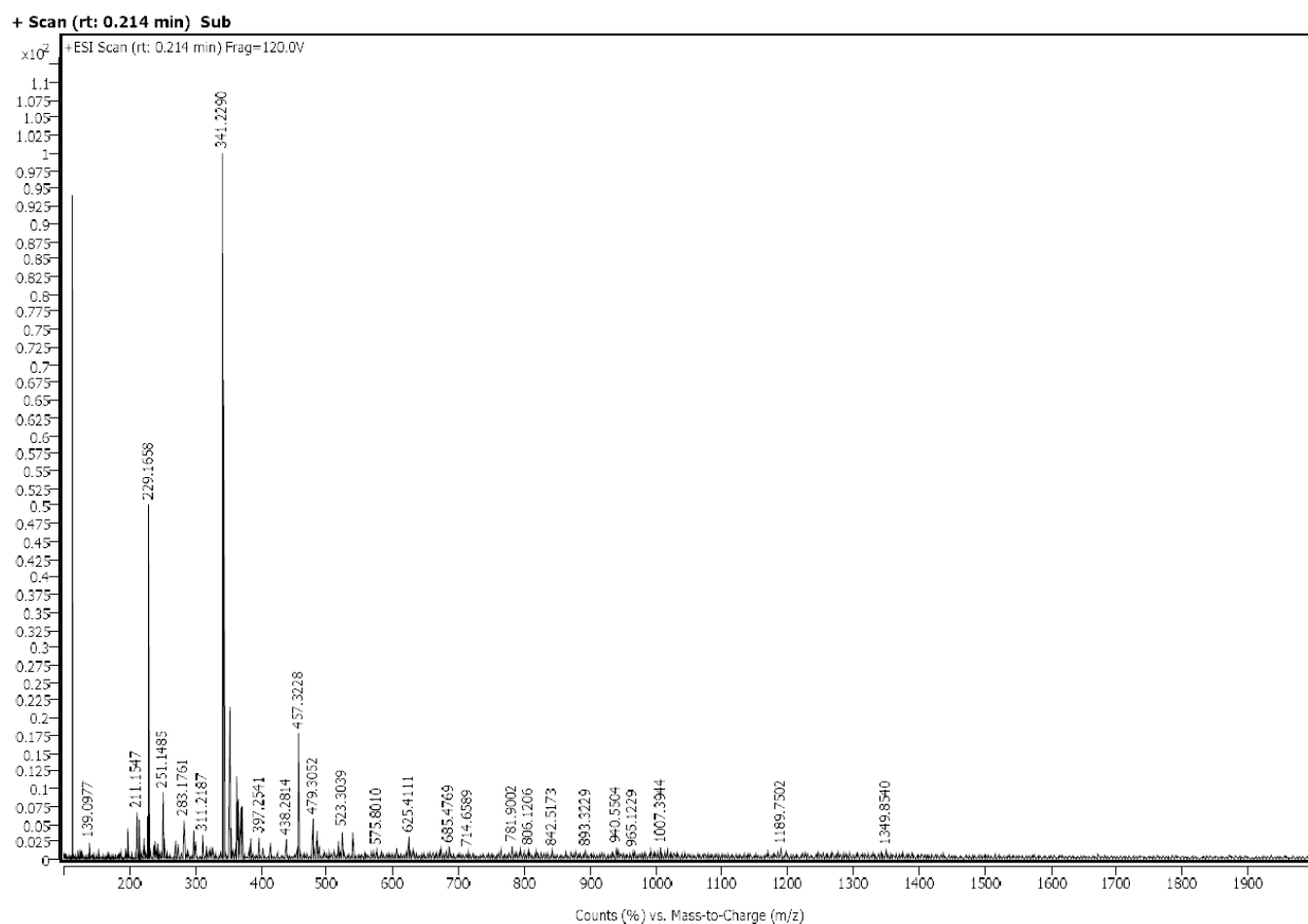


Fig. S7. Mass spectrum (HRESI) of **G2-paco**

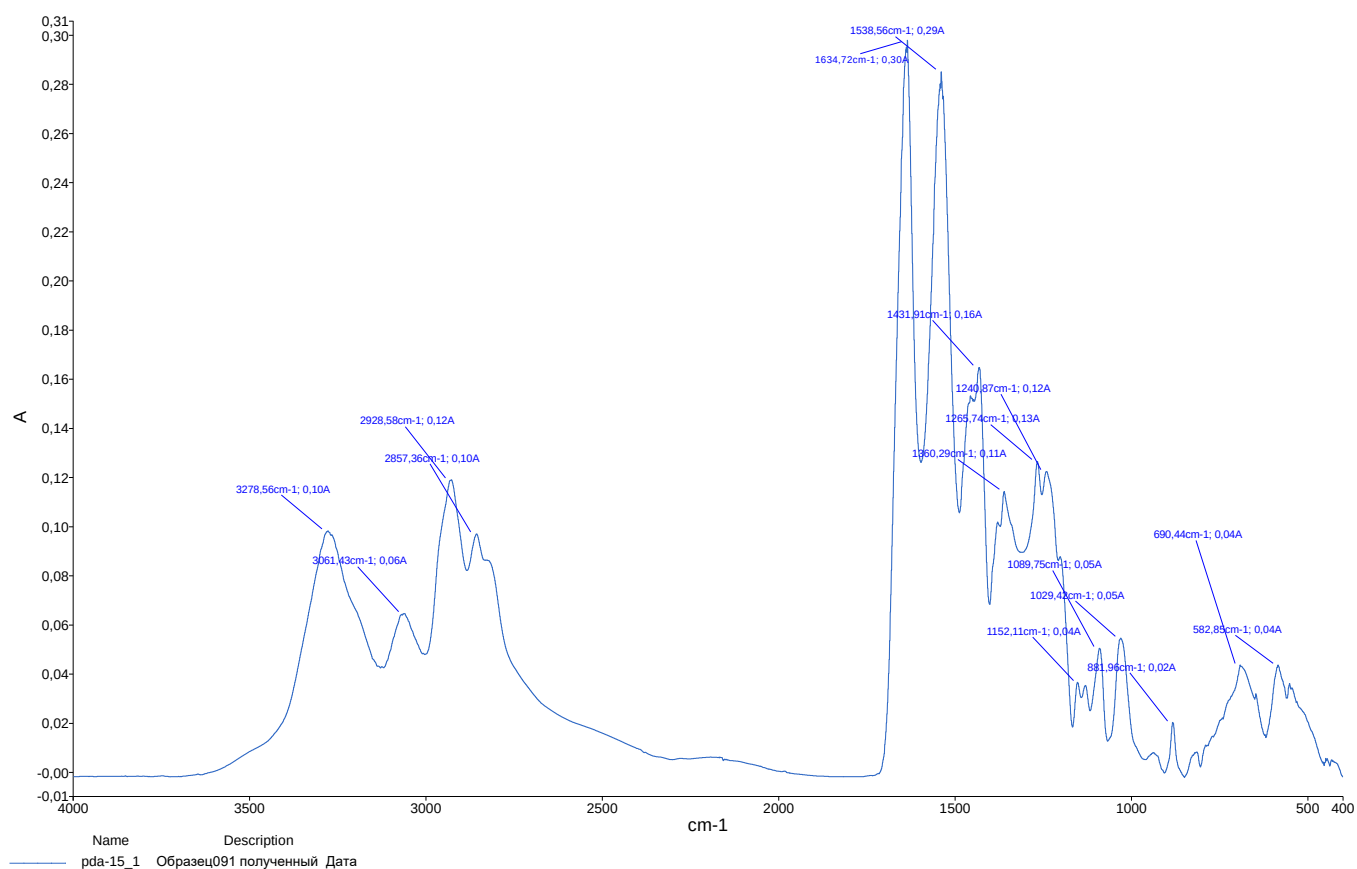


Fig. S8. FTIR-ATR spectrum of **G2-paco**

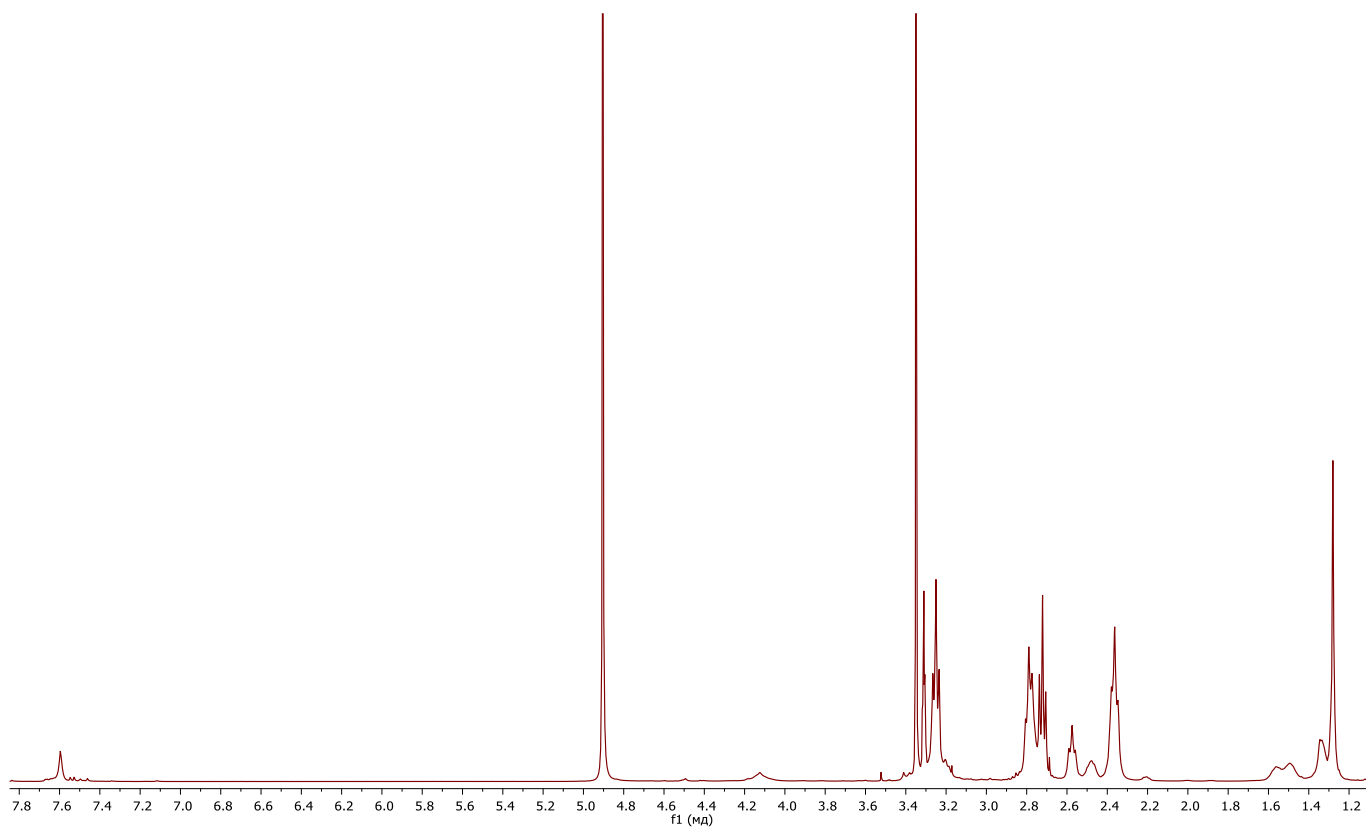


Fig. S9. ¹H NMR spectrum of **G2-alt**, CD₃OD, 298 K, 400 MHz

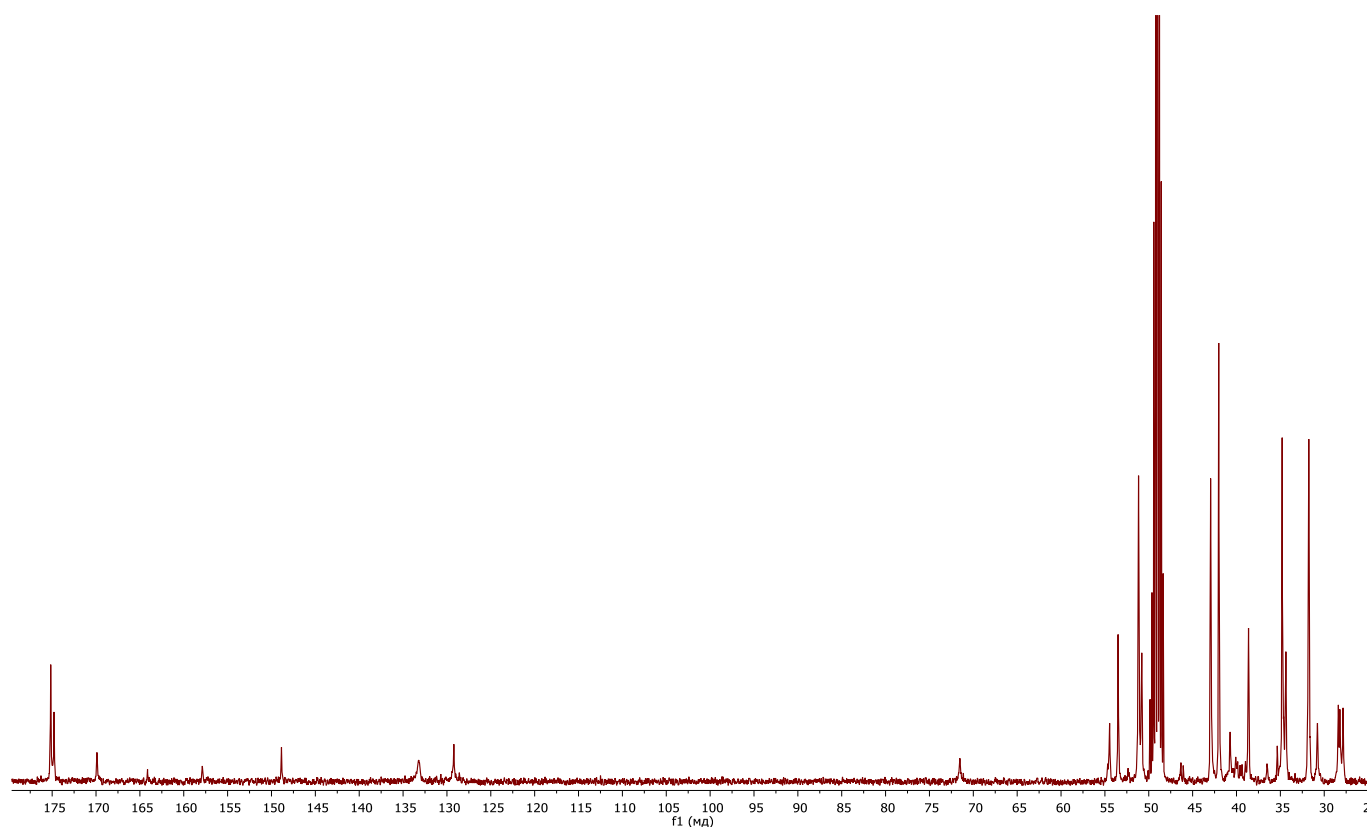


Fig. S10. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **G2-alt**, CD_3OD , 298 K, 100 MHz

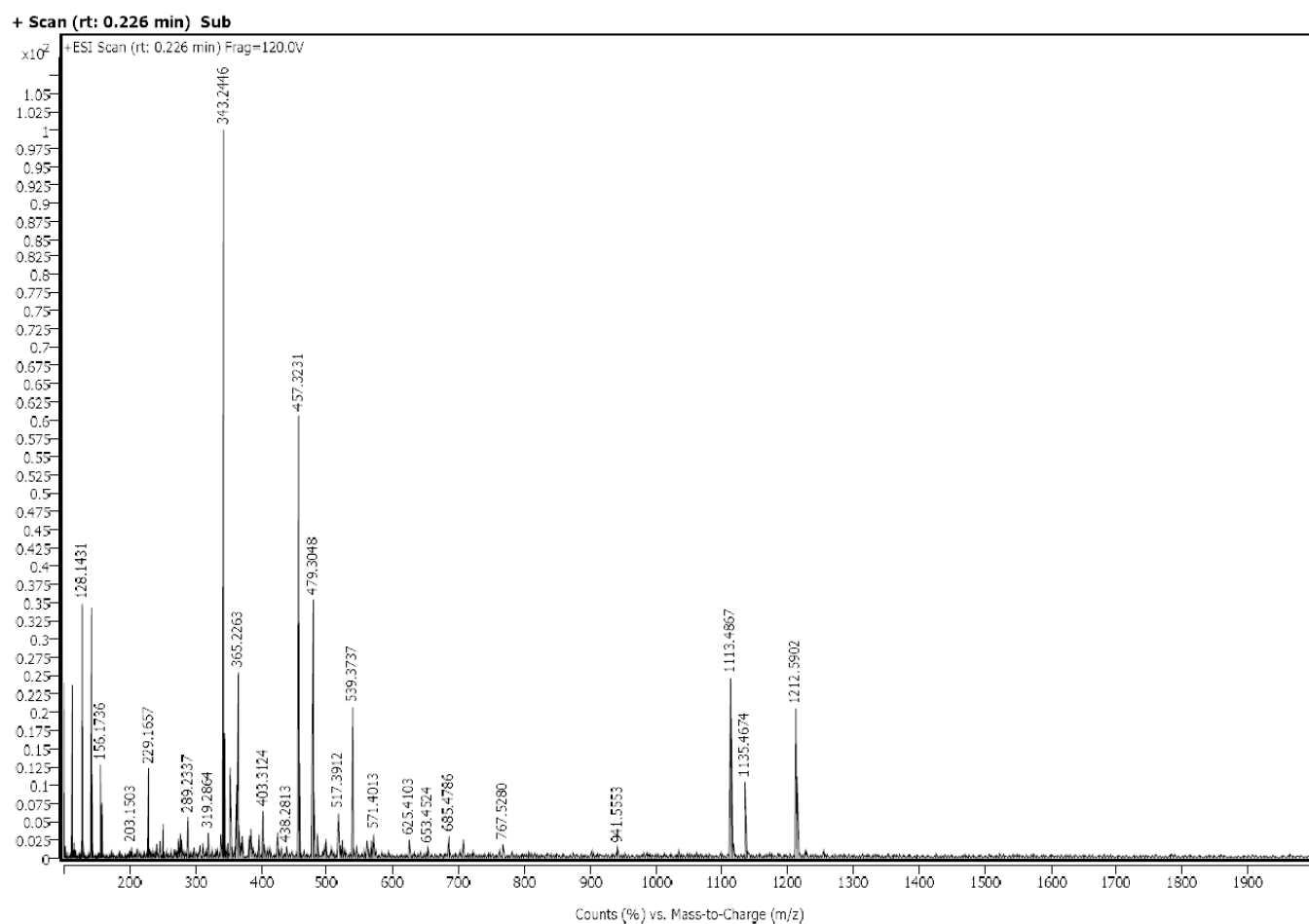


Fig. S11. Mass spectrum (HRESI) of **G2-alt**

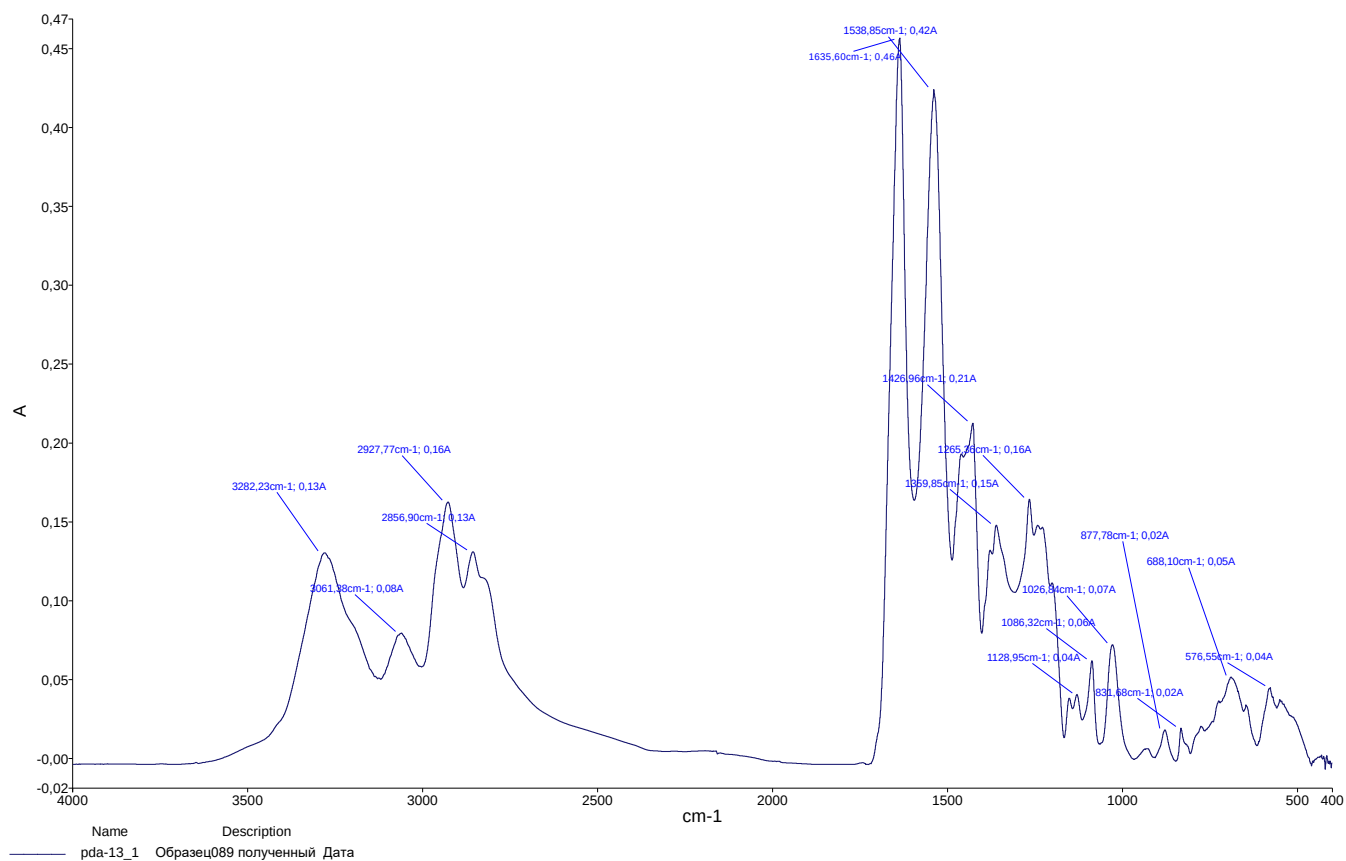


Fig. S12. FTIR-ATR spectrum of G2-alt

Table S1. The electrochemical impedance spectroscopy parameters determined for the various modified GCEs. Average value $\pm$ S.D. for six individual sensors

Sensor content	Electrochemical impedance parameters			
	R_1, Ω	$C_1, \mu F$	R_2, Ω	$C_2, \mu F$
GCE	930 ± 160	11 ± 3	1100 ± 100	540 ± 90
GCE/CB	580 ± 120	9 ± 3	1900 ± 200	740 ± 120
GCE/CB/aptamer	1370 ± 90	11 ± 3	4100 ± 800	106 ± 27
GCE/CB/aptamer-G2-alt	430 ± 80	39 ± 7	4000 ± 300	850 ± 50