

1 Multi-functional imidazolium dendrimers based on thiacalix[4]arenes: self-
2 assembly, catalysis and DNA binding

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11

12 *Materials*

13 Chemicals were purchased from commercial suppliers and used as received. Solvents were
14 purified according to standard procedures. Melting points were measured using the OIptimelt
15 MPA100 melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA). ¹H and
16 ¹³C NMR spectra were recorded on Bruker Avance 400 Nanobay (Bruker Corporation, Billerica,
17 MA, USA) with signals from residual protons of DMSO-d₆ or CDCl₃ as internal standard. ATR-
18 IR spectra and IR spectra in KBr pellets were collected using a Infracpek 2202 spectrometer
19 (Infracpek Company, Saint Petersburg, Russia). High-resolution mass spectra with electrospray
20 ionization (HRESI MS) were obtained on an Agilent iFunnel 6550 Q-TOF LC/MS (Agilent
21 Technologies, Santa Clara, CA, USA). Carrier gas: nitrogen, temperature 300 °C, carrier flow
22 rate 12.1 × min⁻¹, nebulizer pressure 275 kPa, funnel voltage 3500 V, capillary voltage 500 V,
23 total ion current recording mode, 100–3200 m/z mass range, scanning speed 7 spectra × s⁻¹.
24 FTIR spectra were conducted using Bruker Vector-22 using KBr pellets or a thin film in the
25 frequency range of 4000–400 cm⁻¹. TLC was done using Merck UV 254 plates with Vilber
26 Lourmat VL-6.LC UV lamp (6W–254 nm tube). Column chromatography was performed on
27 Merck silica gel (70–230 mesh).

28 The UV/vis-spectra were recorded on a Shimadzu UV-2600 spectrophotometer (Shimadzu
29 Corporation, Kyoto, Japan) in an optical cell with 10 mm light pass at 298 K. Solubilization of the
30 dye (Orange OT) was performed by adding an excess of crystalline Orange OT to solutions of
31 compounds. These solutions were allowed to equilibrate for about 48 h at constant temperature
32 (25 °C), followed by filtration. Then the absorbance was measured at 495 nm.

33 Reduction of *p*-nitroaromatic compounds by Pd&Imd-TCA-Cn (n = 4, 14)/Pd&Imd-tetra-TCA
34 catalysts. In a quartz cuvette (*l* = 1 cm), an aqueous dispersion of Pd&Imd-TCA-Cn (n = 4,
35 14)/Pd&Imd-tetra-TCA (0.2 mM of Pd, 62.5 μl) was added to the 2.5 ml of an aqueous solution
36 containing 0.1 mM of *p*-nitrophenol and 5 mM NaBH₄. The reduction reactions were monitored
37 by a temporary change in absorption at 490 nm in the UV-Vis spectra at 20 °C.

38 The mean of micelles size and polydispersity index were determined by dynamic light scattering
39 (DLS) measurement using Malvern ZetaSizer Nano (Malvern Instruments, UK). The source of
40 laser radiation was a He-Ne gas laser with the power of 10 mW and the wavelength of 633 nm.
41 The light scattering angle is 173 °C. The pulse accumulation time is 5–8 min. The signals were
42 analyzed using a single-plate multichannel correlator coupled with IBM PC compatible computer
43 equipped with the software package for the evaluation of effective hydrodynamic radius of

44 dispersed particles. All samples were analyzed in triplicate, the average error of measurements
45 was approximately 4%.

46 Fluorescence experiments were performed in 10 mm quartz cuvettes and recorded on a Fluorolog
47 FL-221 spectrofluorimeter (HORIBA Jobin Yvon) in the range of 535–750 nm and excitation
48 wavelength 525 nm with 2.5 nm slit for ethidium bromide. All measurements were conducted in
49 1 cm cuvette for dendrimer/ctDNA solutions in Tris buffer (10 mM pH 7.4 ± 0.1). Solutions
50 (except stock EtBr/ctDNA) were prepared using a volume dilution method.

51 For atomic force microscopy (AFM) experiments 2 µL of dendrimers (0.1 mM) in water were
52 dropped on the silicon plates (1×1 cm) at 50°C and dried in an air. The surfaces of the silicon were
53 freshly cleaved before use. AFM images were recorded at 25°C using a Titanium atomic force
54 microscope (NT-MDT, Russia). Measurements were performed in air using tapping mode with
55 rate in range of 0.8-1 Hz and resolution of 512 points per line. The NSG 10 cantilevers (NT-MDT,
56 Russia) with resonance frequency of 140-390 kHz and force constant 3.1-37.6 N m⁻¹ were used.
57 The images were processed and analyzed using the Image Analysis software (NT-MDT, Russia).
58 TEM was performed on Hitachi HT7700 ExaLens (Hitachi High-Tech Corporation, Tokyo, Japan)
59 in Interdisciplinary Center for Analytical Microscopy of Kazan Federal University. The images
60 were acquired at an accelerating voltage of 100 kV. Samples were ultrasonicated in water for 10
61 min, dispersed on 200 mesh copper grids with continuous formvar support films and then dried
62 during 3 hours. Energy dispersive X-ray spectroscopy was performed using an Oxford Instruments
63 XMaxN 80T detector.

64 *General procedure for the synthesis of chlorinated derivatives 4-6*

65 0.1 g of thiacalixarene **1-3** (1 equiv) and dry CH₂Cl₂ (2 ml) were added in a glass autoclave.
66 When cooled to 0 °C, 150 µl of DMF was added to the solution and freshly distilled SOCl₂ was
67 added dropwise (12 equiv. for **1** and **2**, and 24 equiv. for **3**). The reaction mixture was left
68 stirring for 24 hours. To isolate, the mixture was dried *in vacuo* (5 Torr), distilling off the
69 remaining thionyl chloride and DMF

70 *Compound 4*. Yellow powder, M.P.126 °C, ¹H NMR (400 MHz, DMSO-*d*₆, 25 °C): δ 8.24 (s, 4H,
71 *m*-Trz), 8.05 (s, 2H, *p*-Trz), 7.98 (s, 2H, Trz), 7.40 (s, 4H, ArH_{Gal}), 7.37 (s, 4H, ArH_{Cal}), 7.31 (s,
72 4H, ArH_{Cal}), 5.19 (s, 8H, *m*-O-CH₂-Trz), 5.08 (s, 4H, *p*-O-CH₂-Trz), 4.53 – 4.34 (m, 24H, -
73 C(O)O-CH₂- + ArH_{cal}O-CH₂- + -O-CH₂-Trz + *m*-Trz-CH₂-CH₂-), 3.91 (s, 4H, -CH₂-CH₂-O-),
74 3.79 – 3.70 (m, 8H, Trz-CH₂-CH₂-O- + C(O)OCH₂-CH₂-), 3.60 – 3.50 (m, 20H, Trz-CH₂-CH₂-
75 O- + (-O-CH₂-CH₂-O-)₂), 3.07 (t, *J* = 7.2 Hz, 4H, ArH_{Cal}-OCH₂-), 2.28 (p, *J* = 6.4, 8H, *m*-Trz-
76 CH₂-CH₂-), 2.23 (p, *J* = 6.4, 4H, -*p*-Trz-CH₂-CH₂-), 1.23 (s, 18H, t-But), 1.22 (s, 18H, t-But),
77 1.13 – 0.95 (m, 8H, -(CH₂)₂-CH₃), 0.76 (t, *J* = 6.9 Hz, 6H, -CH₂-CH₃). ¹³C {¹H} NMR (101
78 MHz, DMSO-*d*₆, 25 °C) δ 165.1, 156.7, 155.9, 151.7, 145.6, 145.4, 143.6, 143.3, 142.5, 141.2,
79 127.9, 127.5, 127.4, 124.9, 124.7, 124.0, 108.9, 69.8, 69.7, 69.6, 69.5, 68.7, 68.4, 68.3, 67.5,
80 65.8, 65.5, 64.1, 64.0, 62.4, 49.3, 46.8, 46.7, 41.9, 41.8, 33.8, 32.4, 30.9, 30.8, 18.4, 13.8. IR
81 (KBr) ν_{max}, cm⁻¹: 2961, 2871, 1715, 1591, 1443, 1266, 1108. HRESI MS (m/z) [M + 2H]²⁺:
82 calculated for [C₁₂₄H₁₆₈Cl₆N₂₄O₂₂S₄]²⁺ 1343.4882, found 1343.4875.

83

84 *Compound 5*. Yellow amorphous oil, ¹H NMR (400 MHz, DMSO-*d*₆, 25 °C): 8.24 (s, 4H, *m*-
85 Trz), 8.05 (s, 2H, *p*-Trz), 7.98 (s, 2H, Trz), 7.40 (s, 4H, ArH_{Gal}), 7.36 (s, 4H, ArH_{Cal}), 7.30 (s,
86 4H, ArH_{Cal}), 5.19 (s, 8H, *m*-O-CH₂-Trz), 5.08 (s, 4H, *p*-O-CH₂-Trz), 4.51 (t, *J* = 6.7 Hz, 4H, -
87 C(O)O-CH₂-), 4.48 – 4.33 (m, 20H, ArH_{cal}O-CH₂- + -O-CH₂-Trz + *m*-Trz-CH₂-CH₂- + *p*-Trz-

88 CH₂-CH₂-), 3.90 (s, 4H, -CH₂-CH₂-O-), 3.79 – 3.67 (m, 8H, Trz-CH₂-CH₂-O- + C(O)OCH₂-
89 CH₂-), 3.65 – 3.42 (m, 32H, Trz-CH₂-CH₂-O- + (-O-CH₂-CH₂-O-)₂ + *m*-CH₂-OH + *p*-CH₂-OH),
90 3.05 (s, 4H, ArH_{Cal}-OCH₂-), 2.33 – 2.20 (m, 12H, *p*-Trz-CH₂-CH₂- + *m*-Trz-CH₂-CH₂-), 1.21
91 (s, 100H, *t*-But + -(CH₂)₁₂-), 0.84 (t, *J* = 7.3 Hz, 6H, -(CH₂)₁₂-CH₃). ¹³C{¹H} NMR (101 MHz,
92 DMSO-*d*₆, 25 °C): 164.8, 156.8, 155.8, 150.8, 149.3, 145.8, 145.5, 145.2, 139.6, 138.9, 129.6,
93 128.4, 128.0, 127.9, 127.8, 127.6, 127.4, 126.7, 110.0, 74.1, 70.4, 69.3, 68.7, 68.3, 67.7, 66.6,
94 64.9, 62.1, 58.1, 51.8, 42.2, 40.1, 38.2, 32.9, 31.6, 31.0, 29.6, 29.4, 29.3, 29.0, 28.6, 25.4, 22.4,
95 13.8. IR (KBr) ν_{max}, cm⁻¹: 2924, 2854, 1714, 1590, 1444, 1266, 1108. HRESI MS (m/z) [M +
96 2H]²⁺, calculated for [C₁₄₄H₂₀₈Cl₆N₂₄O₂₂S₄]²⁺ 1483.6451, found 1483.6441

97 *Compound 6*. Yellow amorphous oil, ¹H NMR (400 MHz, DMSO-*d*₆, 25 °C): 8.24 (s, 8H, *m*-
98 Trz), 8.05 (s, 4H, Trz), 7.95 (s, 4H, Trz), 7.42 (s, 8H, ArH_{Cal}), 7.39 (s, 8H, ArH_{Gall}), 5.19 (s, 16H,
99 *m*-O-CH₂-Trz), 5.08 (s, 8H, *p*-O-CH₂-Trz), 4.55 – 4.41 (m, 40H, ArH_{cal}O-CH₂- + *m*-Trz-CH₂-
100 CH₂- + *p*-Trz-CH₂-CH₂-), 4.35 (s, 8H, -C(O)O-CH₂-), 3.97 (s, 8H, ArH_{cal}O-CH₂-CH₂-), 3.78 –
101 3.70 (m, 18H, -CH₂-), 3.63 – 3.44 (m, 56H, (TrzCH₂-CH₂-) + CH₂(_{TEG})), 3.34 (s, 8H, -OCH₂-),
102 2.33 – 2.20 (m, 24H, *p*-Trz-CH₂-CH₂- + *m*-Trz-CH₂-CH₂-), 1.18 (s, 36H, *t*-Bu). ¹³C{¹H} NMR
103 (101 MHz, DMSO-*d*₆, 25 °C): δ 165.0, 162.9, 162.3, 156.8, 151.6, 145.5, 143.7, 142.7, 141.1,
104 130.1, 127.6, 124.9, 124.4, 108.9, 79.3, 69.8, 69.7, 69.6, 69.5, 68.6, 68.3, 68.0, 67.6, 65.4, 64.1,
105 63.7, 62.4, 49.4, 46.9, 46.8, 42.0, 41.8, 35.8, 34.1, 32.4, 32.4, 30.8, 30.7. IR (KBr) ν_{max}, cm⁻¹:
106 2961, 2871, 1714, 1591, 1432, 1107. HRESI MS (m/z) [M + 2H]²⁺ calculated for
107 [C₁₉₂H₂₅₄Cl₁₂N₄₈O₄₀S₄]²⁺ 2213.7215, found 2213.7269.

108 *General procedure for the synthesis of imidazolium derivatives Imd-TCA-C_n (n = 4, 14) and*
109 *Imd-tetra-TCA*

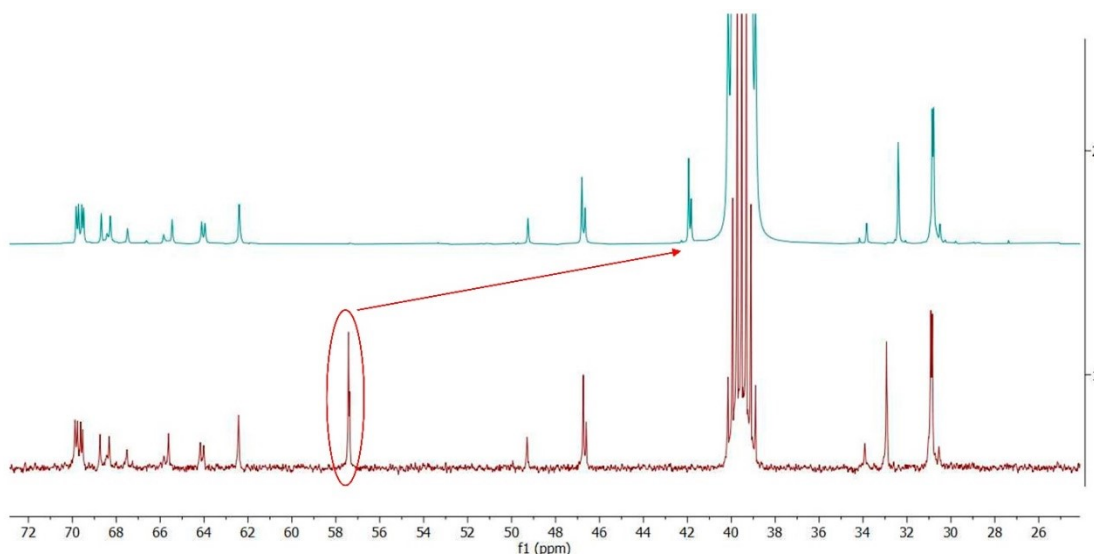
110 0.06 mmol of chlorinated thiacalixarene **4-6** was placed in a glass autoclave followed by N-
111 methylimidazole (at the rate of 0.06 mmol of dendrimer per 5 ml of N-methylimidazole) purged
112 with argon and left at 100 °C for 48 hours. At the end of the reaction, the solvent was evaporated
113 *in vacuo* (5 torr) and residue was washed with diethyl ester (2*10 ml). Regenerated
114 methylimidazole can be reused for next synthesis.

115 *Compound Imd-TCA-C4*. Yellow-brown amorphous powder, 94% yield. ¹H NMR (400 MHz,
116 DMSO-*d*₆/D₂O, 25 °C): 9.01 (s, 6H, Imdz), 8.22 (s, 4H, *m*-Trz), 8.08 (s, 2H, *p*-Trz), 7.97 (s, 2H,
117 Trz), 7.68 (s, 6H, Imdz), 7.62 (s, 6H, Imdz), 7.41 (s, 4H, ArH_{Gall}), 7.34 (s, 4H, ArH_{Cal}), 7.29 (s,
118 4H, ArH_{Cal}), 5.16 (s, , 8H, *m*-O-CH₂-Trz), 5.06 (s, , 4H, *p*-O-CH₂-Trz), 4.50 – 4.29 (m, 20H, -
119 C(O)O-CH₂- + ArH_{cal}O-CH₂- + -CH₂-), 4.21 (s, 8H, -CH₂-), 3.80 (s, 18H, Imdz-CH₃), 3.43 (s,
120 24H, *m*-CH₂-OH + *p*-CH₂-OH, -CH₂-), 3.05 (s, 4H, ArH_{Cal}-OCH₂-), 2.39 (s, 12H, *p*-Trz-CH₂-
121 CH₂- + *m*-Trz-CH₂-CH₂-), 1.20 (d, *J* = 5.3 Hz, 36H, *t*-But), 1.12 – 0.96 (m, 8H, -(CH₂)₂-CH₃),
122 0.73 (s, 6H, -CH₂-CH₃). ¹³C{¹H} NMR (101 MHz, CDCl₃, 25 °C): 165.8, 159.6, 157.3, 151.9,
123 145.7, 144.8, 142.9, 141.9, 141.0, 140.0, 137.0, 128.5, 128.1, 127.7, 125.4, 124.0, 123.6, 122.6,
124 122.5, 106.7, 70.5, 69.5, 68.7, 66.2, 64.7, 63.1, 50.3, 47.0, 43.8, 36.6, 36.6, 35.1, 34.3, 31.4,
125 31.3, 30.5, 29.7, 22.7, 20.7, 19.0, 14.1. IR (KBr) ν_{max}, cm⁻¹: 3147, 3111, 2960, 2870, 1741,
126 1676, 1578, 1449, 1105.

127 *Compound Imd-TCA-C14*. Yellow-brown amorphous powder, 91% yield. ¹H NMR (400 MHz,
128 DMSO-*d*₆/D₂O, 25 °C): 9.34 (s, 6H, Imdz), 8.36 (s, 4H, *m*-Trz), 8.23 (s, 2H, *p*-Trz), 8.01 (s, , 2H,
129 Trz), 7.82 (s, 6H, Imdz), 7.73 (s, 6H, Imdz), 7.43 (s, 4H, ArH_{Gall}), 7.37 (s, 4H, ArH_{Cal}), 7.31 (s,
130 4H, ArH_{Cal}), 5.21 (s, 8H, *m*-O-CH₂-Trz), 5.08 (s, 4H, *p*-O-CH₂-Trz), 3.85 (s, Imdz-CH₃), 3.57 (s,
131 4H, ArH_{Cal}-OCH₂-), 3.46 – 3.39 (m, 16H, -CH₂-), 3.39 – 3.32 (m, 18H, -CH₂-), 3.32 – 3.25 (m,
132 16H, -CH₂-), 2.43 (s, 12H, *p*-Trz-CH₂-CH₂- + *m*-Trz-CH₂-CH₂-), 1.32 – 0.98 (m, 100H, *t*-But +

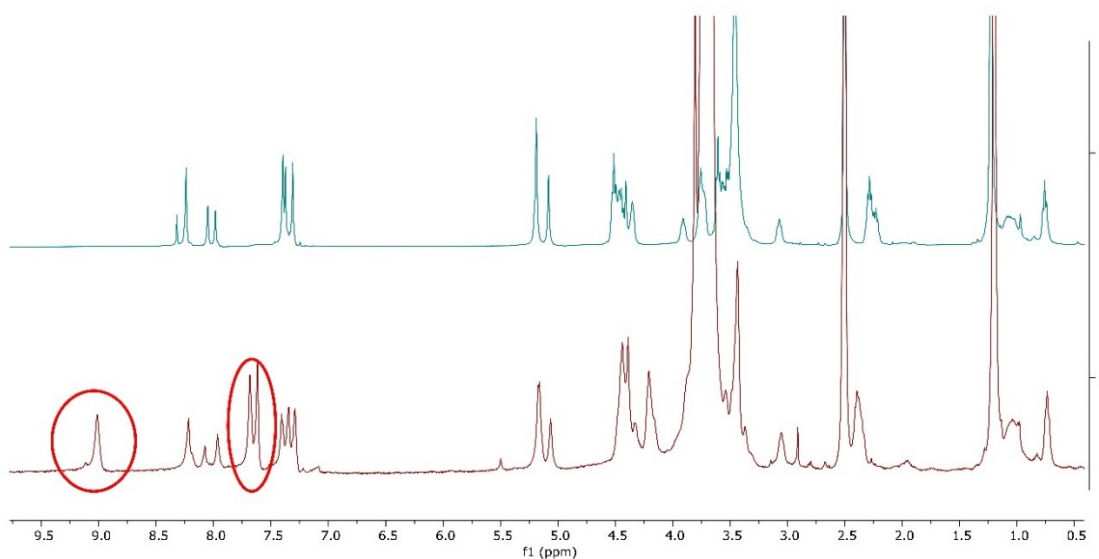
133 $-(\text{CH}_2)_2\text{-CH}_3$), 0.84 (t, $J = 7.1$ Hz, 6H, $-(\text{CH}_2)_2\text{-CH}_3$). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3 , 25 °C):
134 166.1, 156.7, 151.9, 145.8, 145.6, 145.5, 144.9, 143.0, 136.7, 132.1, 128.5, 128.1, 126.8, 125.4,
135 123.9, 123.0, 122.5, 109.3, 72.6, 70.5, 69.4, 68.6, 66.4, 64.8, 63.1, 61.5, 50.2, 47.3, 46.9, 36.4,
136 34.3, 34.2, 31.9, 31.4, 31.3, 31.1, 29.9, 29.7, 29.7, 29.4, 29.0, 25.8, 22.7, 14.1. IR (KBr) ν_{max} , cm^{-1} :
137 3148, 3111, 2921, 2852, 1741, 1665, 1585, 1449, 1100.

138 *Compound Imd-tetra-TCA*. Yellow-brown amorphous powder, 88% yield. ^1H NMR (400 MHz,
139 $\text{DMSO-}d_6$, 25 °C): 9.22 (s, 12H, Imdz), 8.32 (s, 12H, *m*-Trz + *p*-Trz), 8.04 (s, 4H, Trz), 7.81 –
140 7.67 (m, 24H, Imdz) 7.42 (s, 16H, $\text{ArH}_{\text{Cal}} + \text{ArH}_{\text{Gall}}$), 5.19 (s, 16H, *m*- $\text{O-CH}_2\text{-Trz}$), 5.09 (s, 8H, *p*-
141 $\text{O-CH}_2\text{-Trz}$), 4.55 – 4.07 (m, 48H, $-\text{CH}_2-$), 3.97 (s, 8H, $-\text{CH}_2-$), 3.89 – 3.70 (m, 54H, $-\text{CH}_2-$ +
142 Imdz- CH_3), 3.61 – 3.23 (m, 56H, $-\text{CH}_2-$), 2.39 (s, 24H, *p*-Trz- $\text{CH}_2\text{-CH}_2-$ + *m*-Trz- $\text{CH}_2\text{-CH}_2-$),
143 1.18 (s, 36H, *t*-Bu). IR (KBr) ν_{max} , cm^{-1} : 3148, 3113, 2959, 2871, 1742, 1666, 1581, 1451, 1105.



144

145 Figure S1. Comparison of $^{13}\text{C}\{^1\text{H}\}$ spectra of compounds **1** (lower spectrum) and **4**
146 (upper spectrum), $\text{DMSO-}d_6$, 101 MHz, 25 °C.

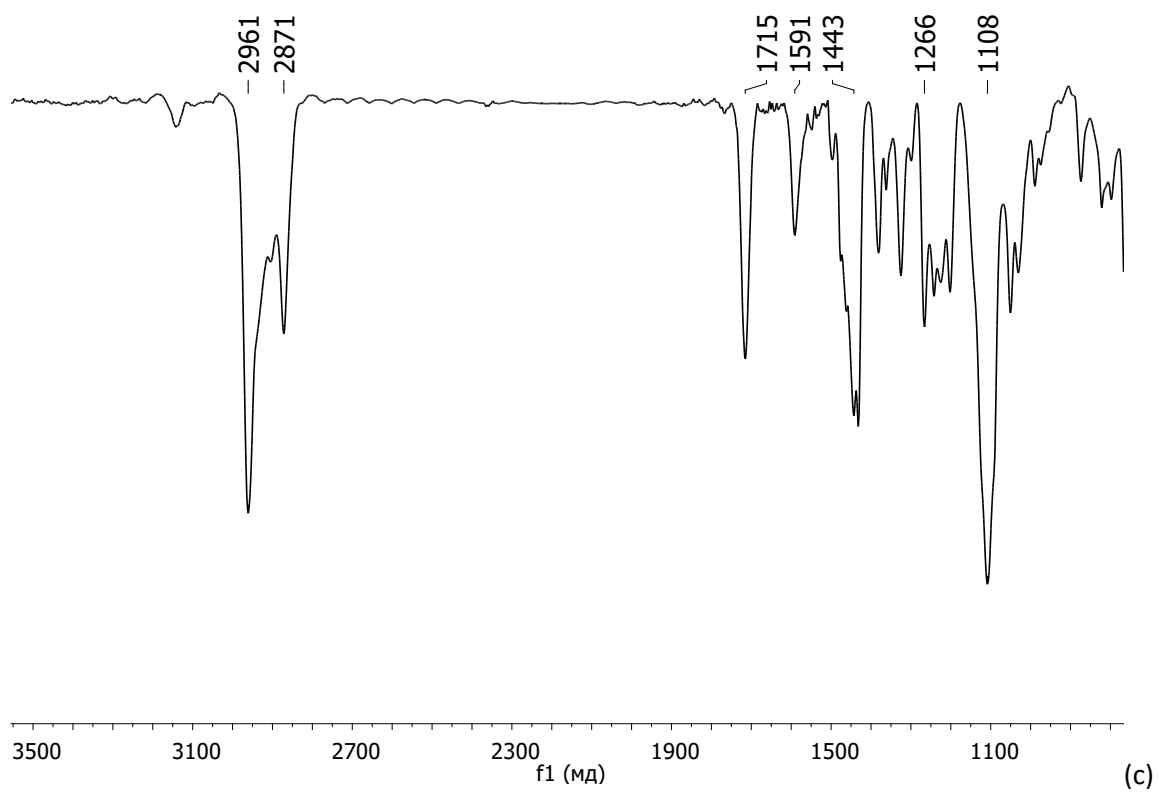


147

148 Figure S2. Comparison of $^{13}\text{C}\{^1\text{H}\}$ spectra of compounds **1** (lower spectrum) and **4**
149 (upper spectrum), $\text{DMSO-}d_6$, 400 MHz, 25 °C.

150

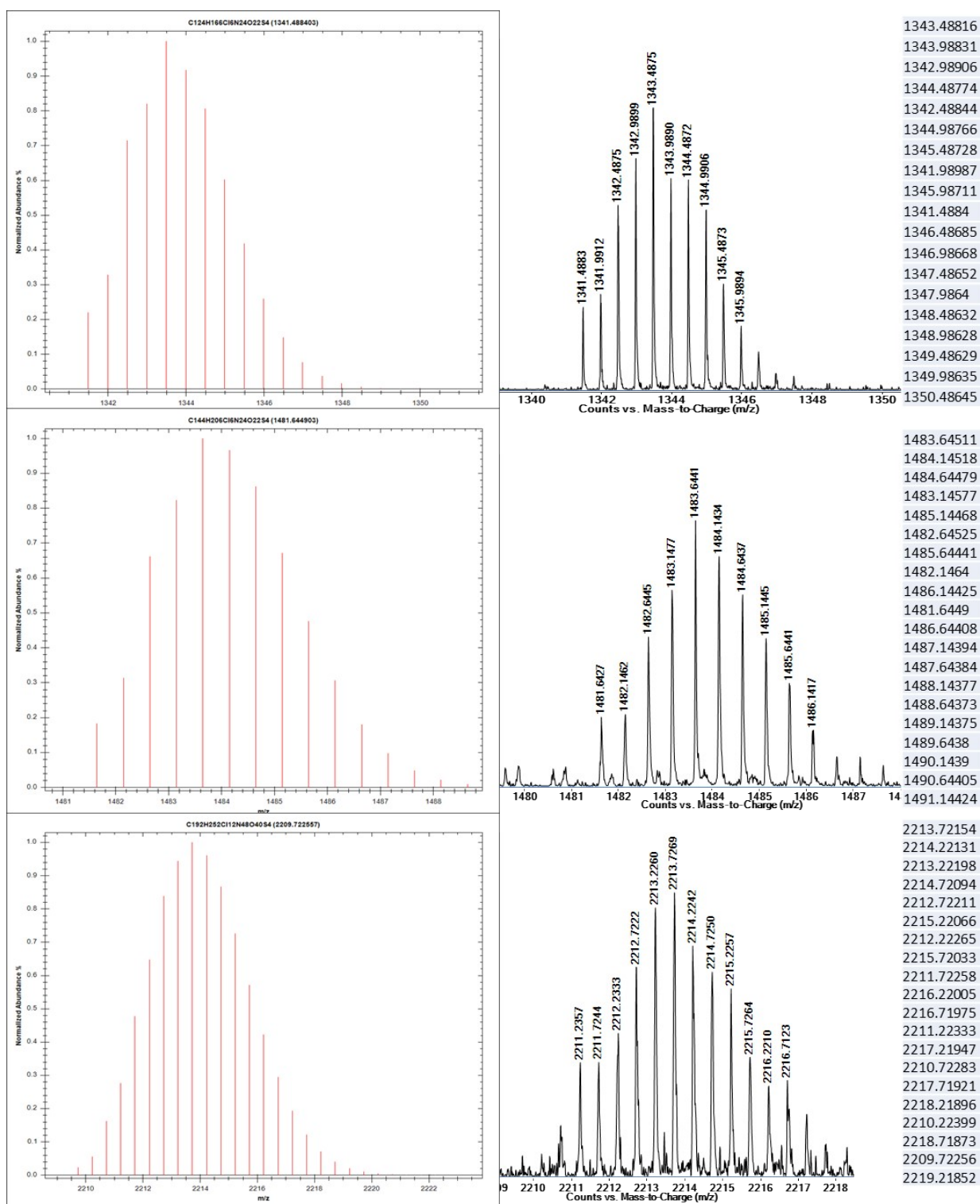
151



152

153 Figure S3. FT IR spectra of *compound (4)*.

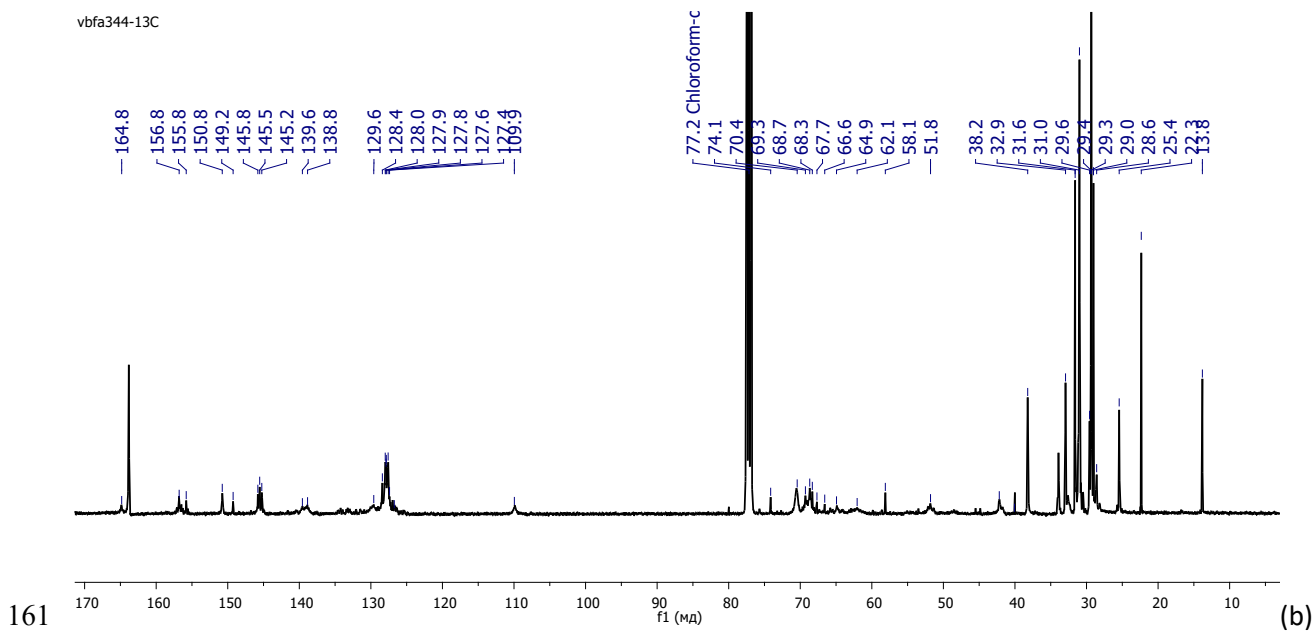
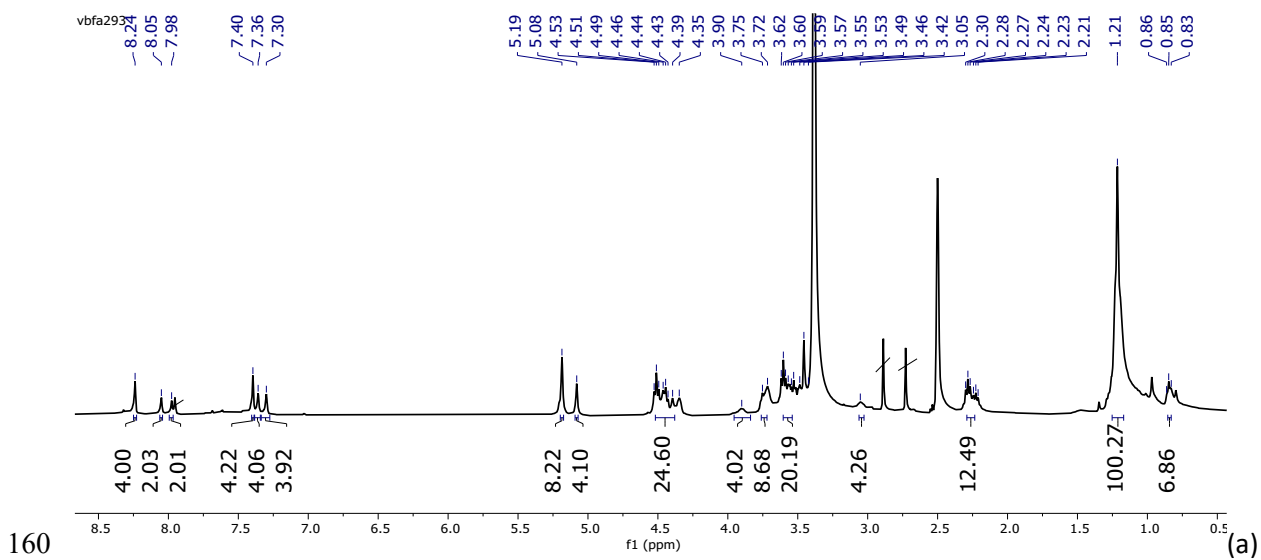
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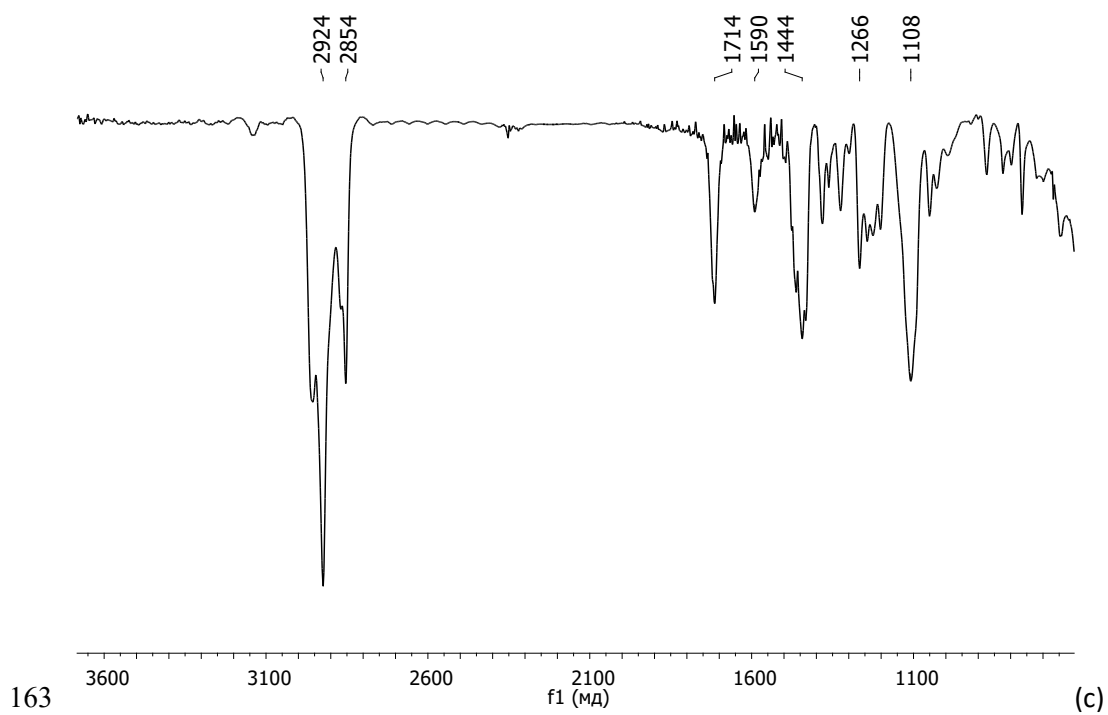


155

156 Figure S4. Calculated and experimental peaks (HRESI-MS) of quasi-molecular ions of
 157 compounds **Imd-TCA-C_n (n = 4, 14)**, **Imd-tetra-TCA** and the exact calculated masses of
 158 isotopes in order of decreasing relative intensity.

159

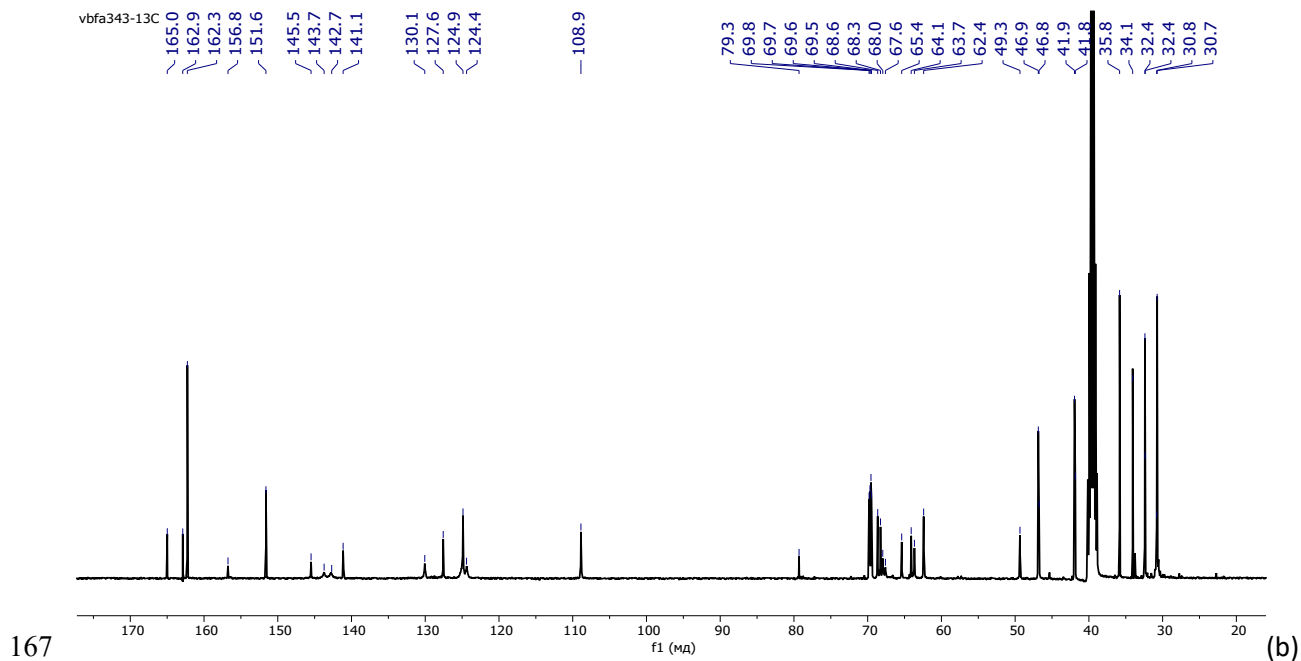
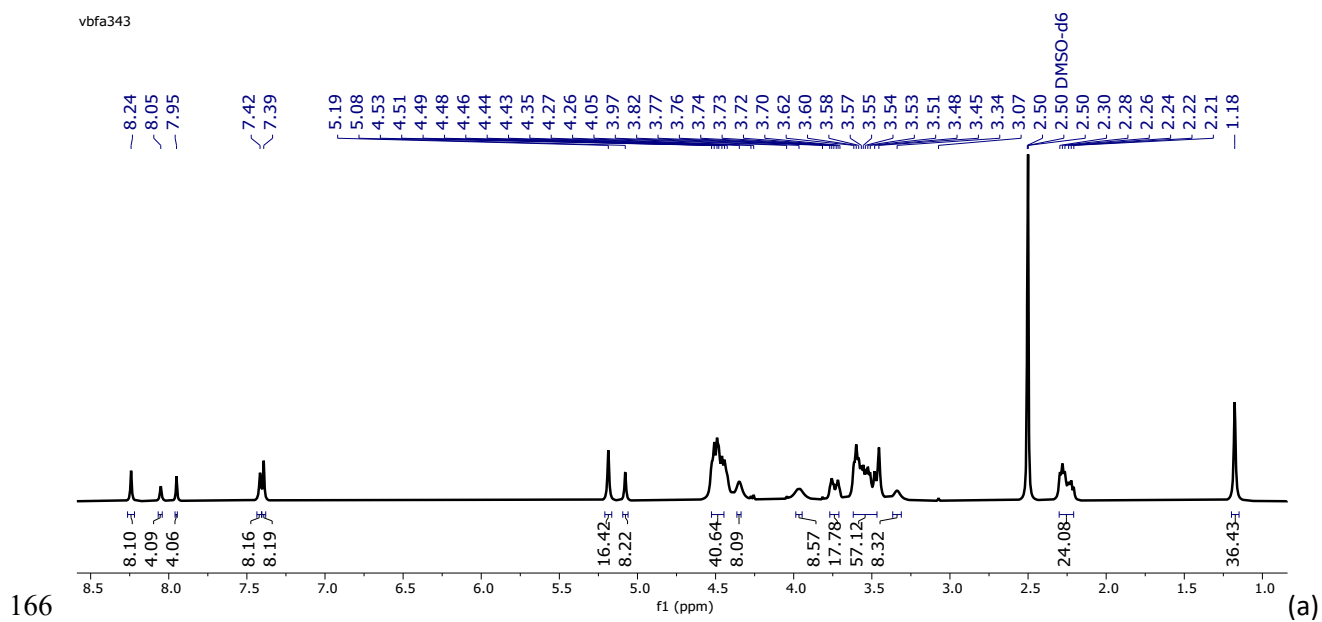


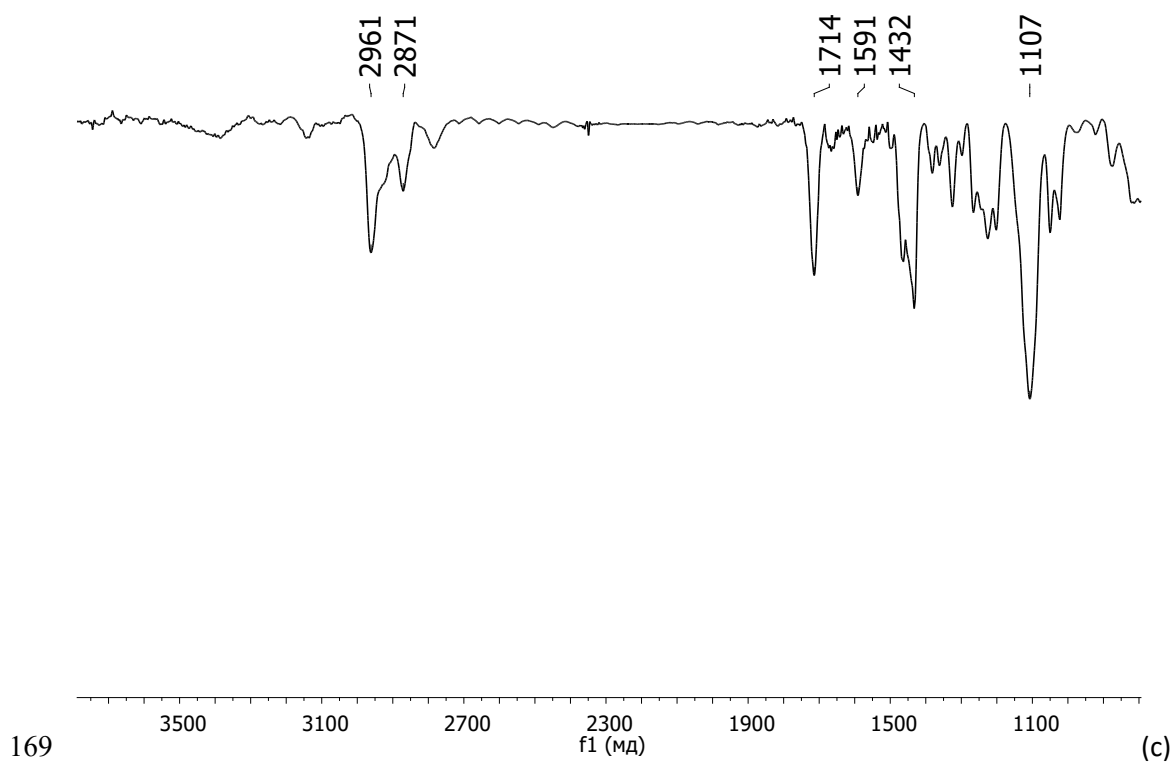


164 Figure S5. NMR ¹H (a), ¹³C (b), and FT IR (c) spectra of *compound (5)*.

165

vbfa343

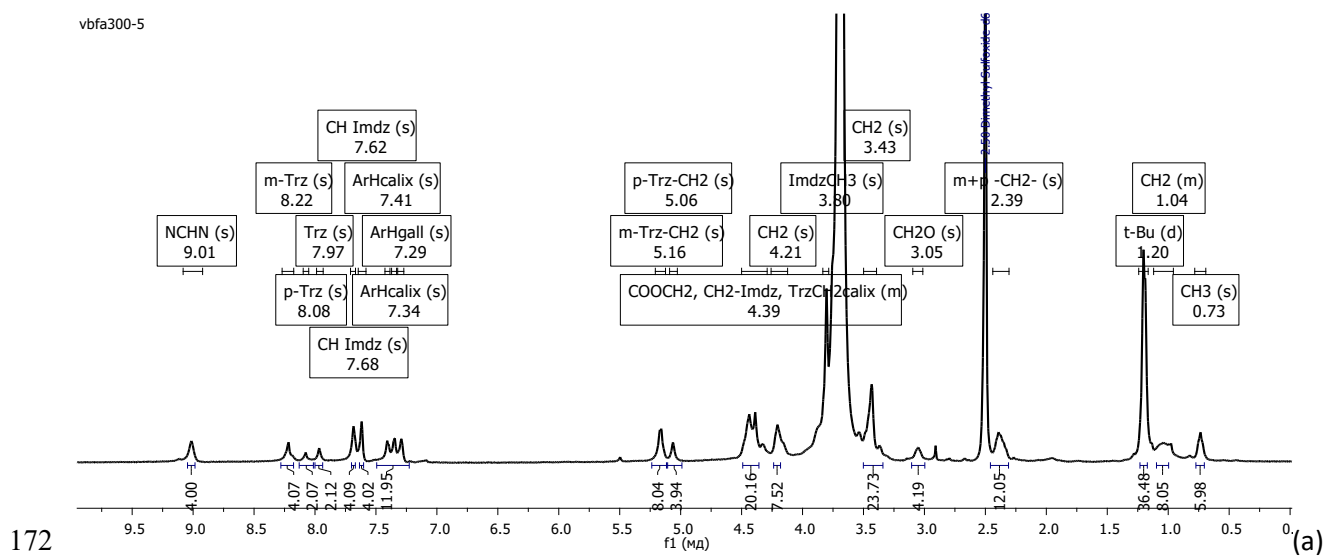




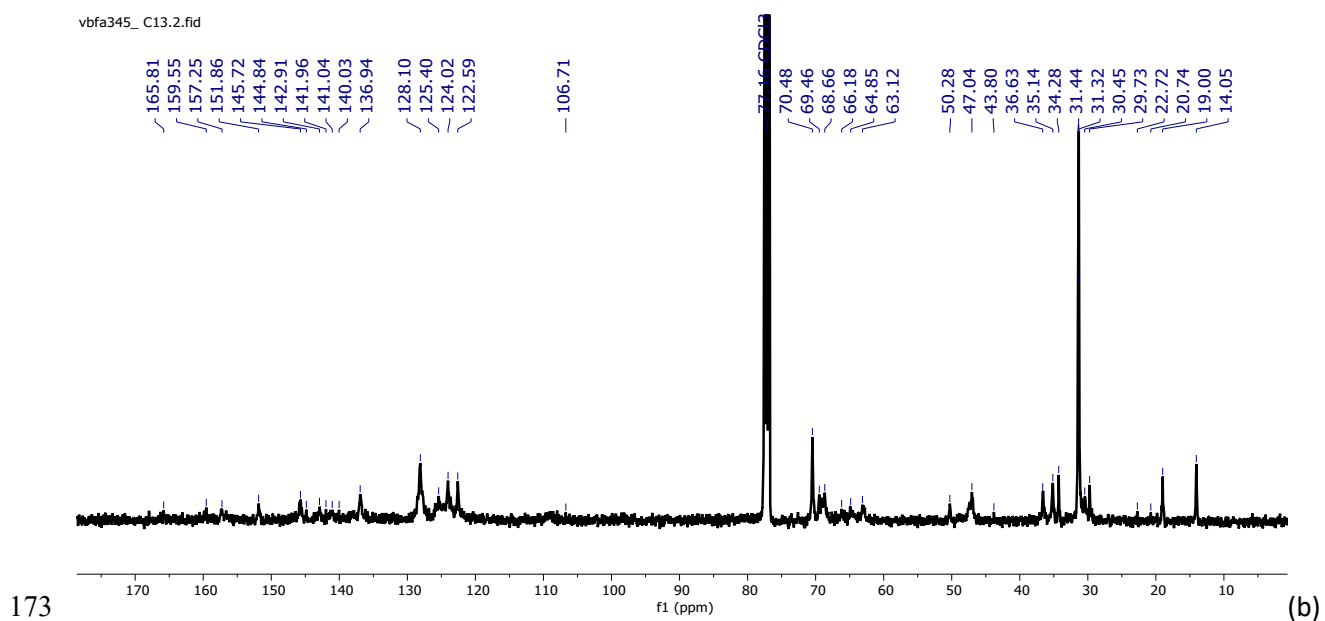
169 Figure S6. NMR ¹H (a), ¹³C (b), and FT IR (c) spectra of *compound (6)*.

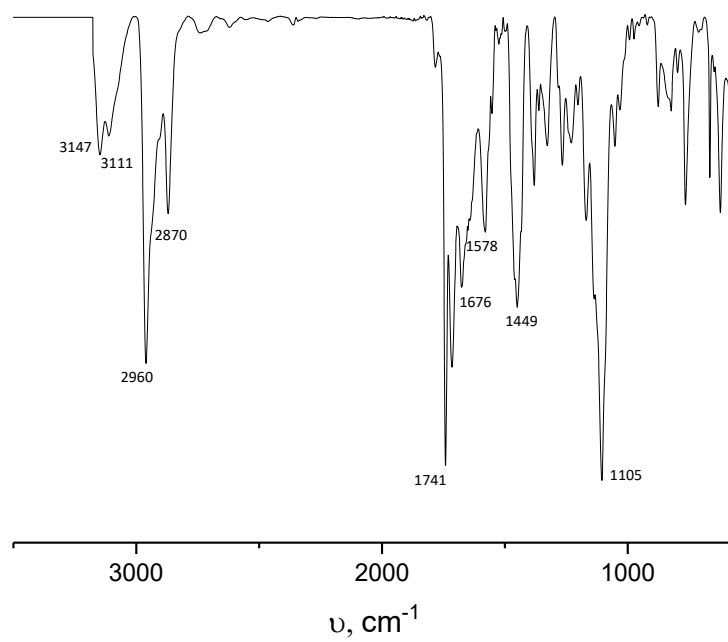
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vbfa300-5



vbfa345_ C13.2.fid





174

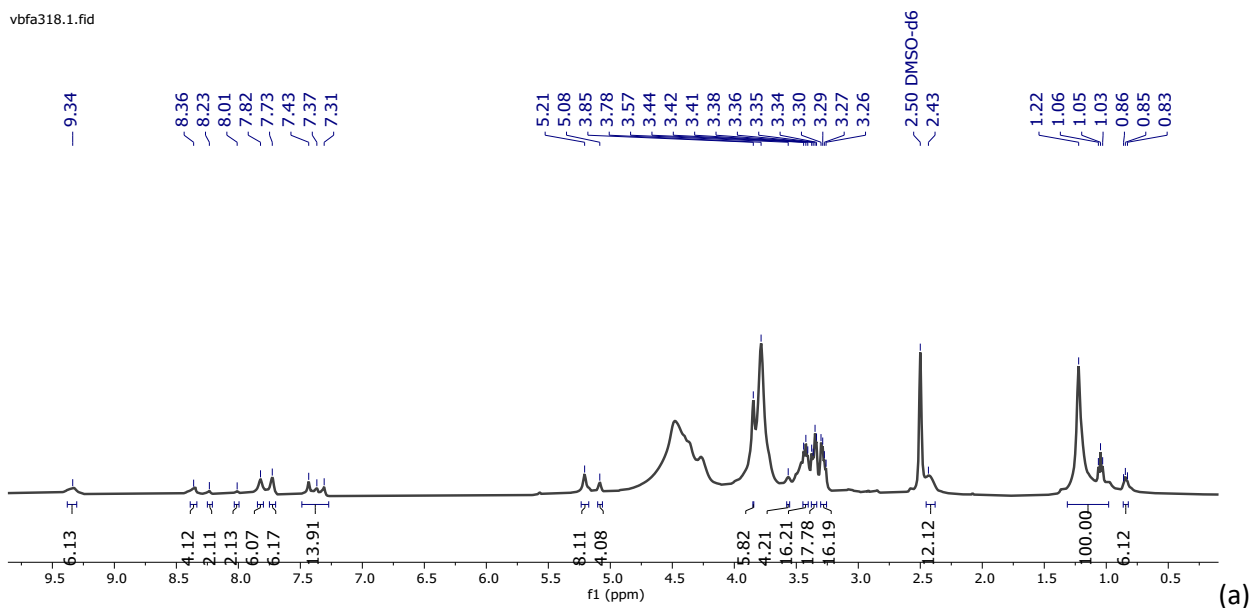
(c)

175 Figure S7. NMR ^1H (a), ^{13}C (b) and FT IR (c) spectra of compound (Imd-TCA-C4).

176

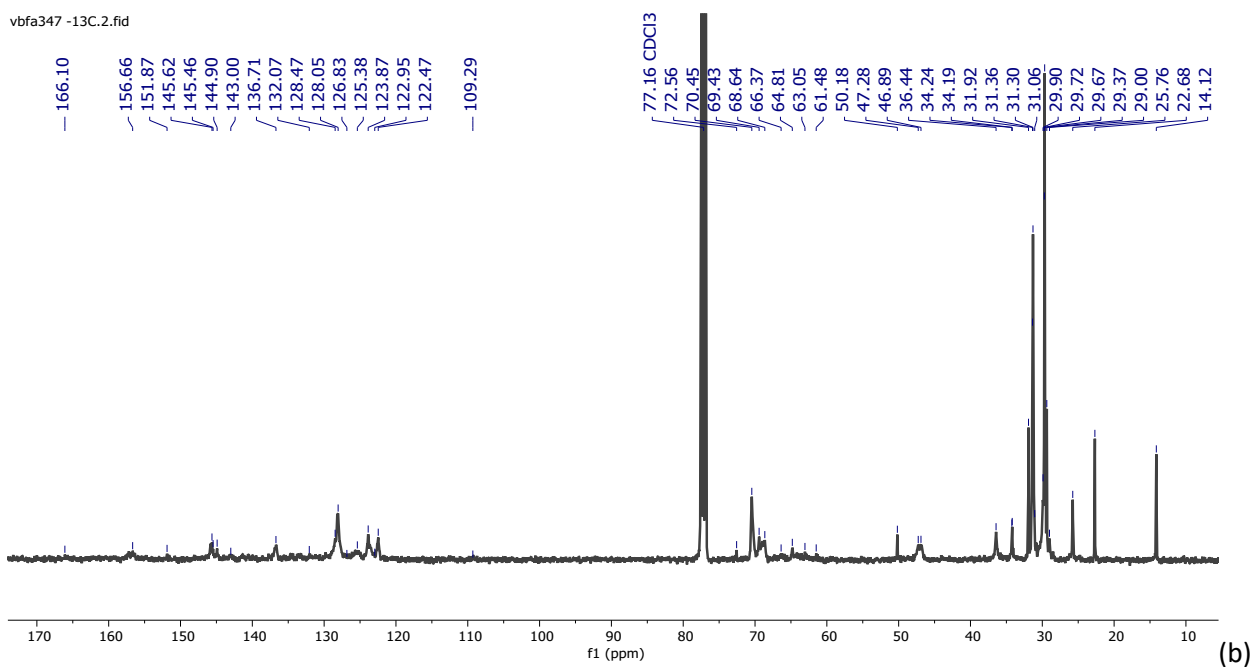
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vbfa318.1.fid

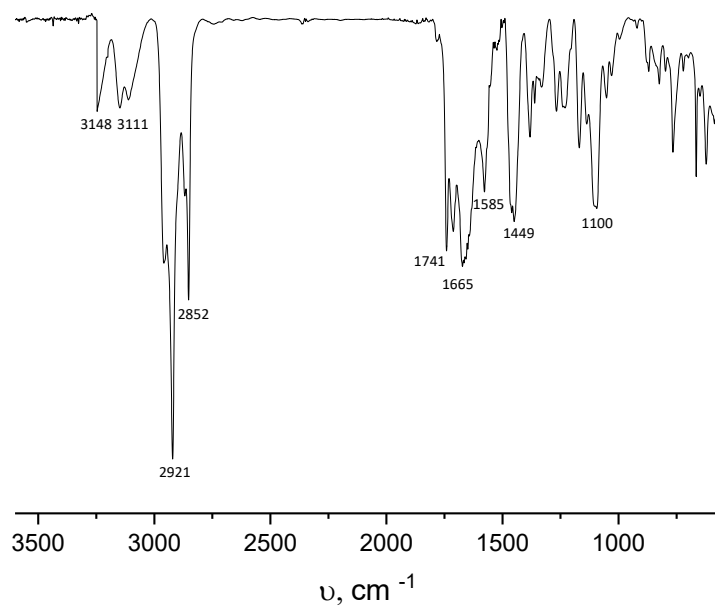


178

vbfa347 -13C.2.fid



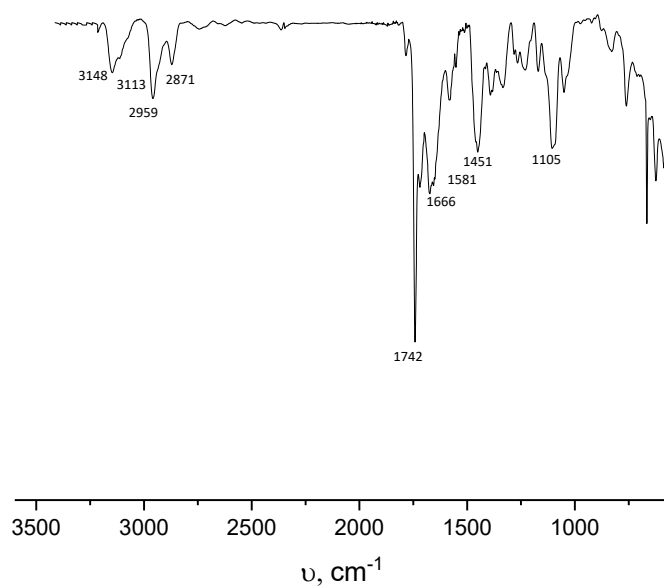
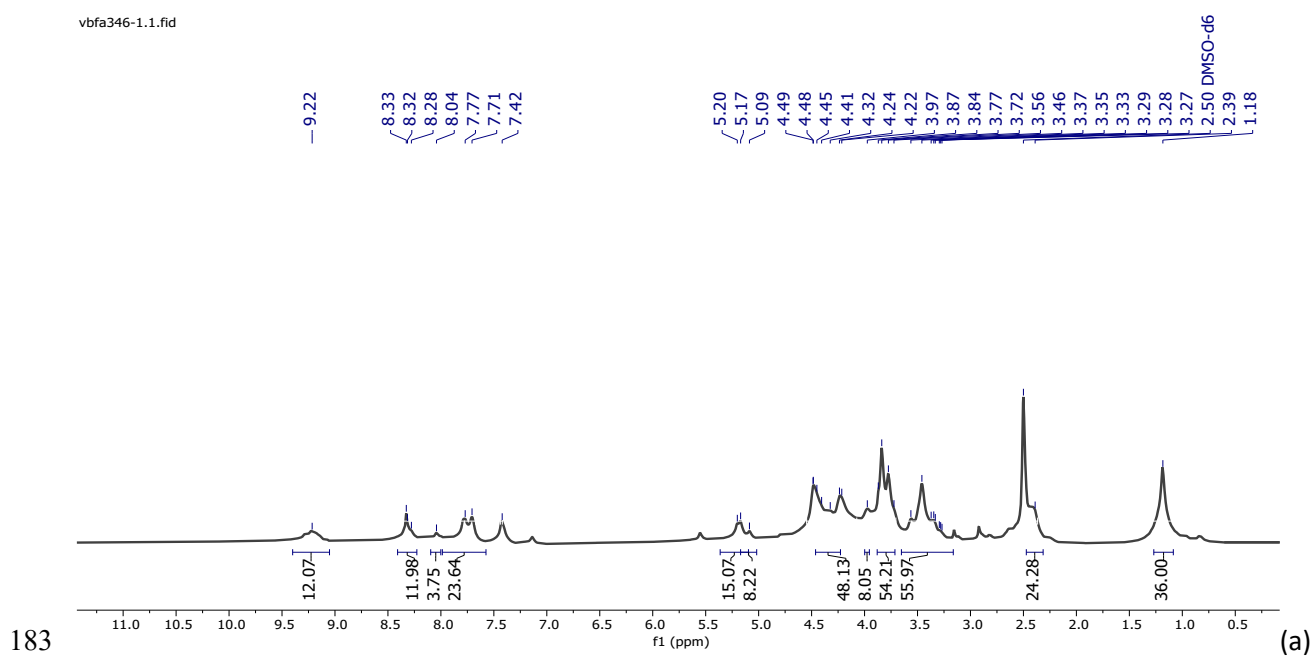
179



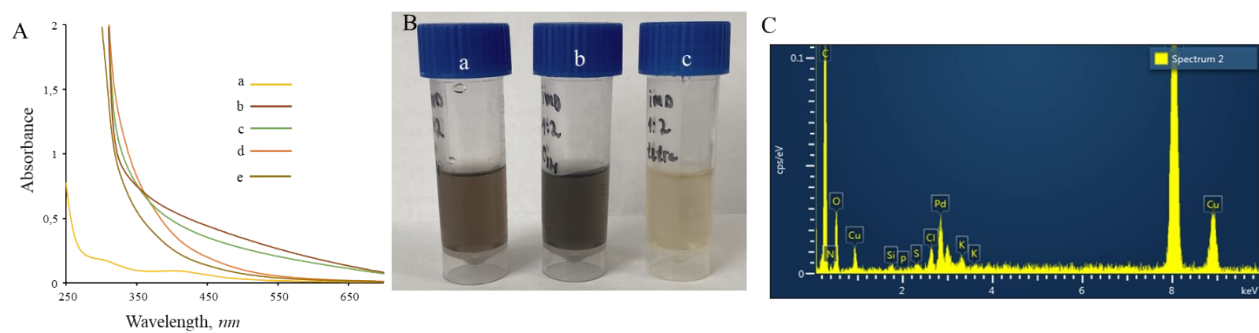
180

(c)

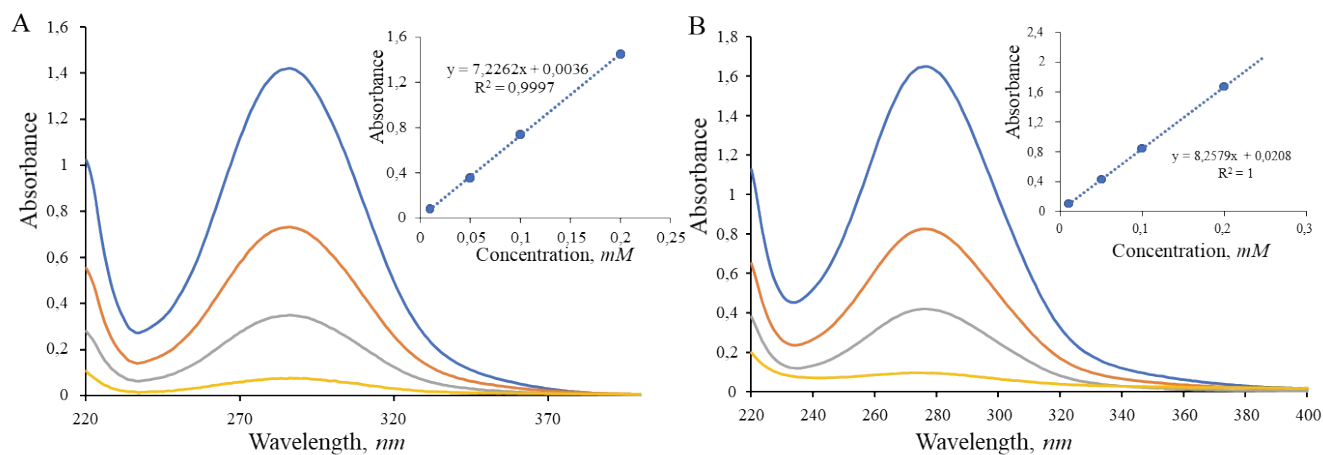
181 Figure S8. NMR ^1H (a), ^{13}C (b), and FT IR (c) spectra of compound (Imd-TCA-C14).



185 Figure S9. NMR ^1H (a), and FT IR (b) spectra of compound (Imd-TCA-Tetra).



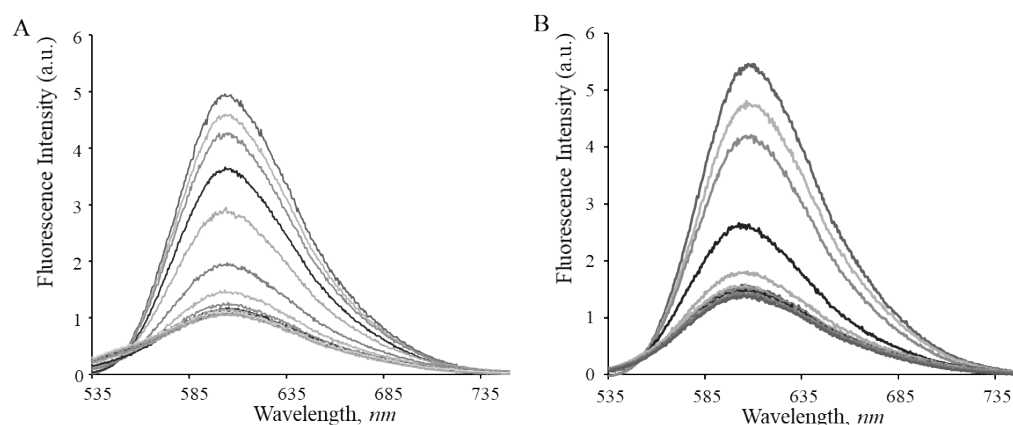
187 Figure S10. A) UV-vis spectrum of (a) K_2PdCl_4 , (b) Pd&Imd-TCA-C4, (c) Pd&Imd-TCA-C14
 188 (d) Pd&Imd-tetra-TCA (e) Pd^0 and B) Photos of (a) Pd&Imd-TCA-C4, (b) Pd&Imd-TCA-C14
 189 (c) Pd&Imd-tetra-TCA, C) EDX spectrum of Pd&Imd-TCA-C14, H_2O , $C(\text{dendrimers})=0.1 \text{ mM}$,
 190 $C(K_2PdCl_4)=0.2 \text{ mM}$, $l = 1 \text{ cm}^{-1}$.



191

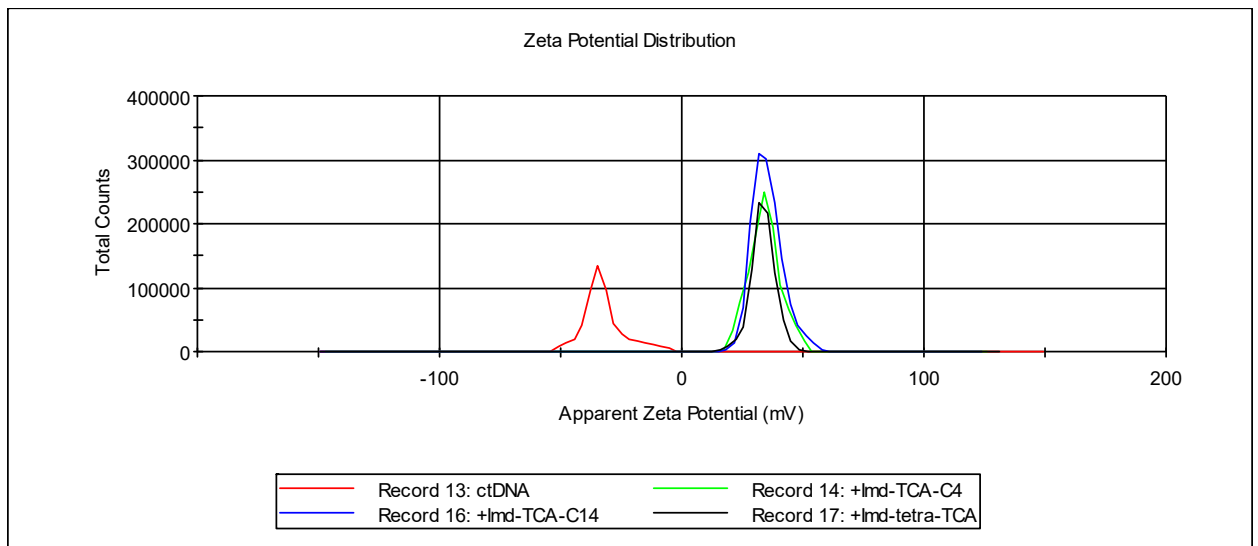
192 Figure S11. UV-vis spectrum of A) 4-ethylnitrobenzene, B) 4-nitrobenzyl alcohol, $C(R-Ph-$
 193 $NO_2)=0.01-0.2 \text{ mM}$, H_2O , $l = 1 \text{ cm}^{-1}$.

194



195

196 Figure S12. Emission spectra of the EtBr – ctDNA system in the absence and presence of various
 197 concentrations of the B) Imd-TCA-C14, C) Imd-tetra-TCA, $C(\text{ctDNA}) = 0.04 \text{ mM}$, $C(\text{EtBr}) = 0.008 \text{ mM}$,
 198 $C(\text{dendrimers}) = 0 - 0.4 \text{ mM}$, Tris-HCl buffer solution (10 mM) with pH 7.4.



199

200 Figure S13. Zeta potential of ctDNA and after adding 0.01 mM of dendrimers.