Electronic Supplementary Material (ESI) for Nanoscale.

^{19}F NMR ON/OFF Nanoparticles: A Universal Approach for the Specific Detection of DNA-binding Cancer Biomarkers

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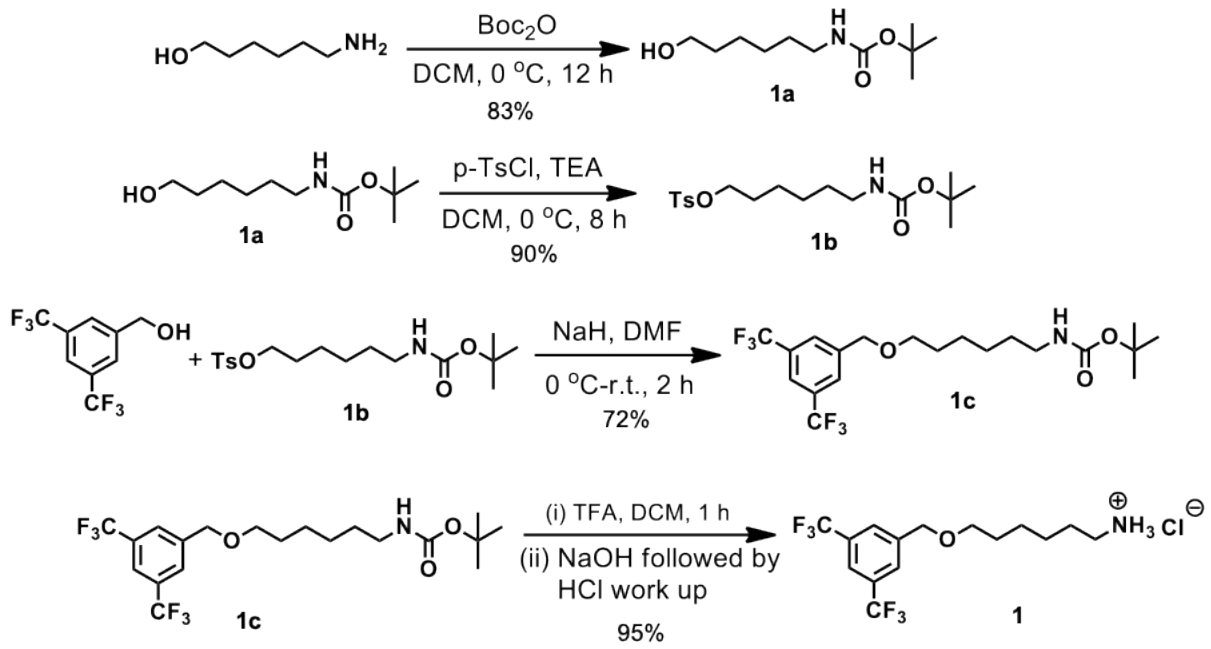
Experimental materials and methods

All chemicals used for the organic syntheses were purchased from Sigma Aldrich and were used as received. Solvents were dried following standard procedures. Phosphoramidite for automated solid-phase DNA synthesis was purchased from Glen Research. Other reagents needed for DNA synthesis were purchased from Sigma Aldrich. TLC analyses were done on aluminum plates coated with silica gel 60 F_{254}. Column chromatography was performed on 200- 400 mesh silica gel. ^{1}H, ^{13}C, and ^{19}F-NMR spectra were recorded on a 500 MHz Bruker spectrometer using 1,1,1,1-tetramethylsilane (TMS) as the internal standard. The water used for all studies was Milli Q deionized water. ESI-HRMS measurements were performed on a Q Exactive TM- Bench top LC-HRMS mass spectrometer. Measurements were performed in multi-mode, i.e. ESI and APCI simultaneously in negative ion mode. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analyses were performed on Bruker UltrafleXtreme MALDI-TOF mass spectrometer. Oligonucleotides were synthesized on H-8 K&A DNA synthesizer. The miRNA-21 was purchased from Sigma Aldrich. All the DNA was purified using a reverse phase column on a Shimadzu HPLC system equipped with a diode array detector. AFM analyses were carried out on Brucker Multimode 8. Samples were prepared by drop casting 2 μL solution of the sample on a freshly cleaved mica surface and dried under air. Imaging was done under ambient conditions in tapping mode. The probe used for imaging was an antimony doped silicon cantilever with a resonant frequency of 300 kHz and spring constant of 40 Nm^{-1}. TEM analyses were carried out on FEI Tecnai G2 Spirit Bio Twin F20 (120 kV). Samples were prepared by depositing 2 μL of the sample on a 400-mesh carbon-coated copper grid (Ted Pella, Inc.), which was negatively glow discharged prior to use. Samples were allowed to adsorb on the grid for 2 min, and then excess sample was wicked with a piece of filter paper. Then the grid was dried under air. Absorption spectra were recorded using quartz cuvette of 10 mm path length on a Shimadzu UV-3600 Vis-NIR Spectrophotometer. Dynamic Light Scattering (DLS) was performed on Malvern Zetasizer Nano ZS equipped with 655 nm laser. Experiments were done at 20℃ at a back-scattering angle of 173˚. CD analysis was carried out on Jasco J-815 Circular Dichroism (CD) Spectropolarimeter using a quartz cuvette of 10 mm path length.

Extraction of telomerase from HeLa cells

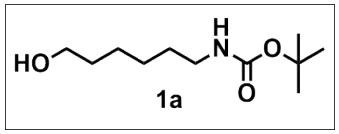
Cells were collected in the exponential phase of growth, and 5×10^7 cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS (0.1 M, pH 7.4), and resuspended in 200 μL of ice-cold CHAPS lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5 % CHAPS and 10 % glycerol. The mixture was incubated for 30 min on ice and centrifuged at 16000 rpm and 4 °C for 20 min. The supernatant was collected and diluted to 200 μL as cell extract for detection or storage at -80 °C. After incubating the mixtures of 10 μL cell extract (corresponding to 2.5×10^6 cells), 10 μL dNTPs (10 mM each) and 0.5 mL probe) at 37 °C for 10 min to 4 h, the ^{19}F NMR analysis was done.

Synthesis and characterization

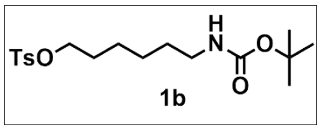


Scheme S1: Synthesis scheme for **1**.

Synthesis of 1a: A 100 ml two-necked RB flask was charged with 6-aminohexanol (4.0 g, 34.0 mmol) in 30 ml of dry DCM, and the contents were kept in an ice bath. Di-tert-butyl dicarbonate (8.5 g, 37.0 mmol) was added to the solution, and the reaction mixture was stirred at room temperature overnight. Reaction completion was confirmed by TLC analysis. Then the reaction mixture was extracted with ethyl acetate from aqueous citric acid, saturated $NaHCO_3$, and washed with brine. The organic phases were collected, dried over anhydrous sodisulfateate, and concentrated. The crude mixture was purified using silica column chromatography to afford the desired product as yellow oily liquid (83%). TLC (PE/EA): R_f = 0.62; ^{1}H NMR (500 MHz, DMSO), δ (ppm) =1.33-1.261 (m, 4H) 1.41(s, 9H) 1.43-1.45 (m, 2H), 2.9258 (q, J= 7.5Hz, 2H), 3.42 (t, J= 5.Hz, 2H) and 4.35(t, J=4.9Hz, 1H), 6.762(s, 1H); ^{13}C NMR (125 MHz, DMSO) δ(ppm) = 156.04, 77.69, 61.14, 4, 32.98, 28.72, 27.31, 26.67, 25.71; HR-MS (m/z): $[M+Na]^+$ for $C_{11}H_{24}NO_3$: 240.17 (cal.); 240.14 (expt.).



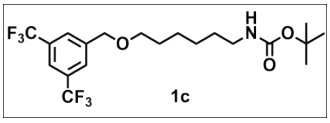
Synthesis of 1b: A 100 ml double necked RB flask was charged with compound **1a** (1.0 g, 4.6 mmol) in 20 ml of DCM and the contents were kept in an ice bath.Triethyl amine (1.29 ml, 9.2 mmol) was added to the solution. Then p-TsCl (0.963 g, 5.06 mmol) in 10ml DCM was added slowly at 0° C. After addition, contents were allowed to warm up to ambient temperature and stirred for 8 h. Reaction completion was confirmed by TLC analysis. Then the reaction mixture was extracted with DCM and water. The



organic layers were collected and dried over anhydrous sodium sulphate, and the solvent was removed under reduced pressure. The

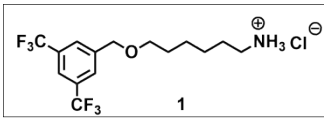
crude mixture was purified using silica column chromatography to afford the desired product as a pale-yellow liquid (90%). TLC (PE/EA): R_f = 0.51; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 1.18-1.22 (m, 2H), 1.23-1.27(m, 2H), 1.366 (s, 9H), 1.5-1.58 (m, 4H), 2.38 (s, 3H), 3.0 (q, J=7.75, 2H), 3.945 (t, J=6.3Hz, 2H), 4.5(s,1H), 7.278 (d, J=8.1Hz, 2H) and 7.717(d, J=8.25Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) = 21.64, 25.07, 26.11, 28.42, 28.75, 29.82, 40.36 70.47, 79.09, 127.88, 129.84, 133.15, 144.71, 155.98; HR-MS (m/z): $[M+Na]^+$ for $C_{18}H_{30}NO_5$: 394.18 (cal.); 394.06 (expt.).

Synthesis of 1c: A 100 ml two-necked RB flask was charged with 3,5- bistrifluromethyl benzyl alcohol (0.7 g, 2.86 mmol) and evacuated for 15 minutes. To a suspension of 60% NaH (0.344 g, 8.604 mmol) in 15ml of freshly dried DMF, was added 10ml solution of dry DMF containing compound **1b** (4.96 g,14.2 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h and then quenched with ice. Reaction completion was confirmed by TLC analysis. Then the reaction mixture was extracted with DCM/water, washed with brine, and dried over anhydrous sodium sulfate. The organic layers were collected and dried over anhydrous sodium sulphate, and the solvent was removed under reduced pressure. The crude mixture was purified using silica column chromatography to afford the desired product as a colorless liquid (72%). TLC (PE/EA): R_f = 0.51; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) =1.35 (s, 9H), 1.3010-1.2643 (m, 2H), 1.41-1.39 (m, 2H), 1.5603 (t, J=7.25Hz, 2H), 3.03 (s, 2H), 3.4658-3.433 (m, 2H), 4.51 (d, J= 3.4Hz, 2H) and 7.71 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) = 24.86, 25.59, 27.37, 28.55, 29.04, 39.54, 70.12, 77.95, 120.31, 123.45, 126.22, 130.48, 130.75, 140.53, 155.06; HR-MS (m/z): $[M+Na]^+$ for $C_{20}H_{28}F_6NO_3$: 466.19 (cal.); 466.08 (expt.).



1c

Synthesis of 1: A 50 ml double-necked RB flask was charged with compound **1c** (0.54 g, 2.8 mmol) in DCM. TFA (1.5 ml, 2.0 mmol) was added and kept stirring for 1 h at room temperature. After completion of the reaction monitored by TLC, the reaction mixture was neutralized with aqueous $NaHCO_3$ and extracted with chloroform/water, washed with brine, and dried over anhydrous sodium sulfate. The organic layers were collected, and dried over anhydrous sodium sulphate, and the solvent was removed under reduced pressure. The crude mixture was purified using silica column chromatography to afford the desired product as a viscous liquid. The purified product was further washed with 1N NaOH followed by 1N HCl to replace the trifluoroacetate counter ion with a chloride ion. The washed product was then vacuum dried and then used for the experiments (10%). TLC ($CHCl_3$/MeOH): R_f = 0.2; ^{1}H NMR (500 MHz, D_2O), δ (ppm) = 1.26 (s, 4H), 1.506 (t, J=5.5Hz, 2H) 1.561 (t, J=6.5Hz, 2H), 2.896 (t, J= 7.5Hz, 2H), 4.714 (s, 2H), 7.859 (s, 2H) and 7.937(s, H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) = 24.37, 25.08, 26.29,



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28.08, 38.79, 69.88, 121.42, 123.59, 126.49, 130.43, and 140.48; ^{19}F NMR (470 MHz, D_2O) δ(ppm) = -62.73; MALDI-TOF (m/z): $[M+H]^+$ for $C_{15}H_{20}F_6NO$: 344.19 (cal.); 344.27 (expt.).

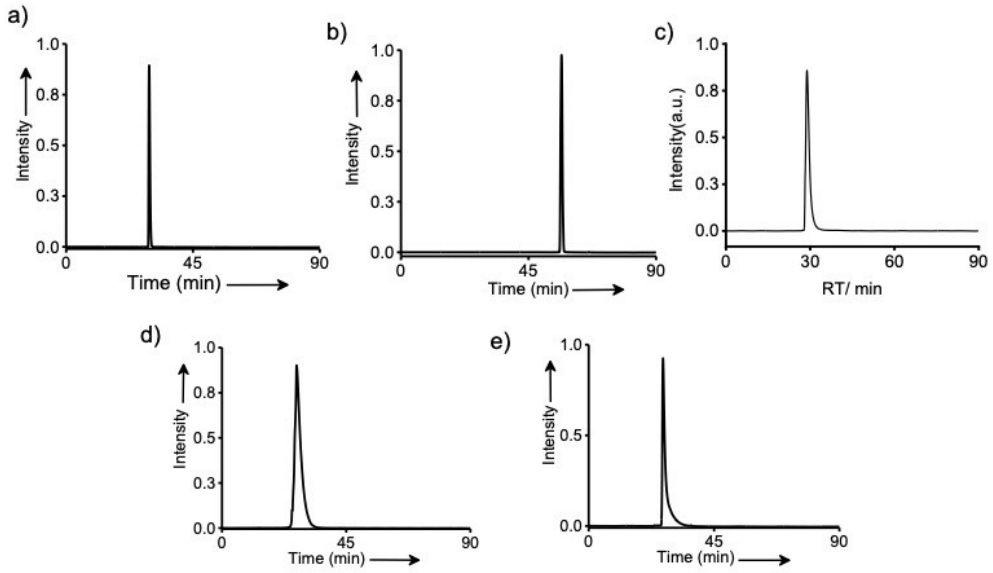

Figure S1. HPLC traces of (a) **DNA1**, (b) **DNA2**, (c) **DNA3**, (d) **DNA4** and (e) **DNA5**.

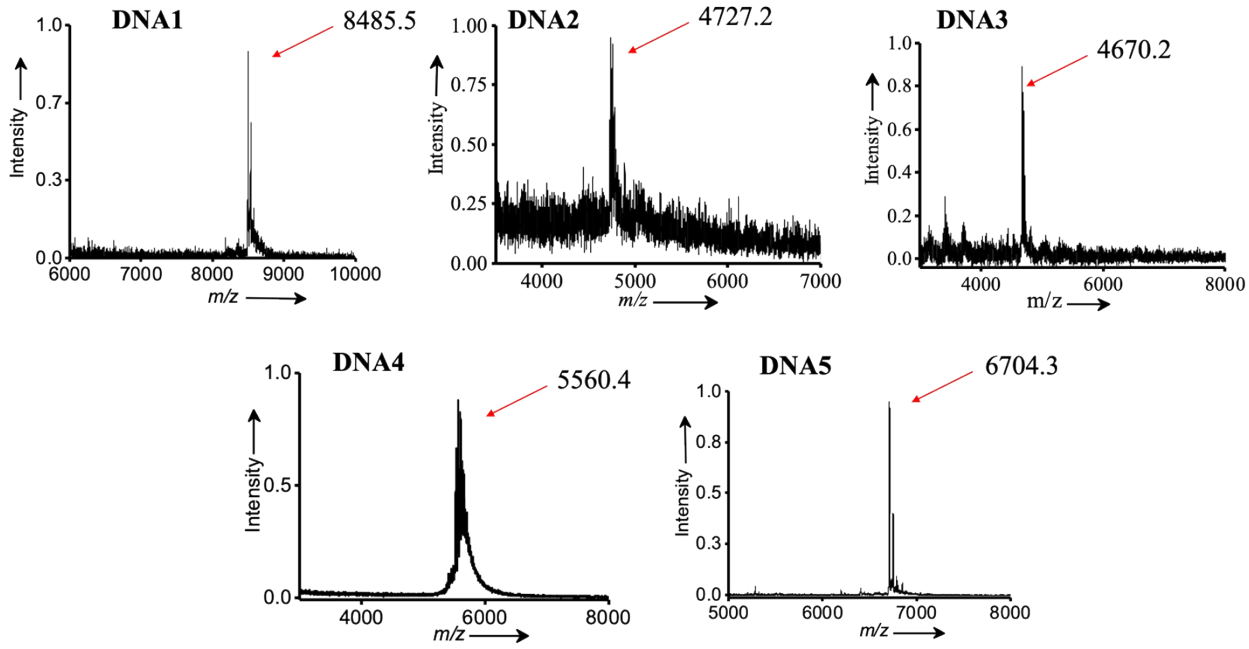

Figure S2. MALDI-TOF spectra of (a) **DNA1**, (b) **DNA2**, (c) **DNA3**, (d) **DNA4** and (e) **DNA5**.

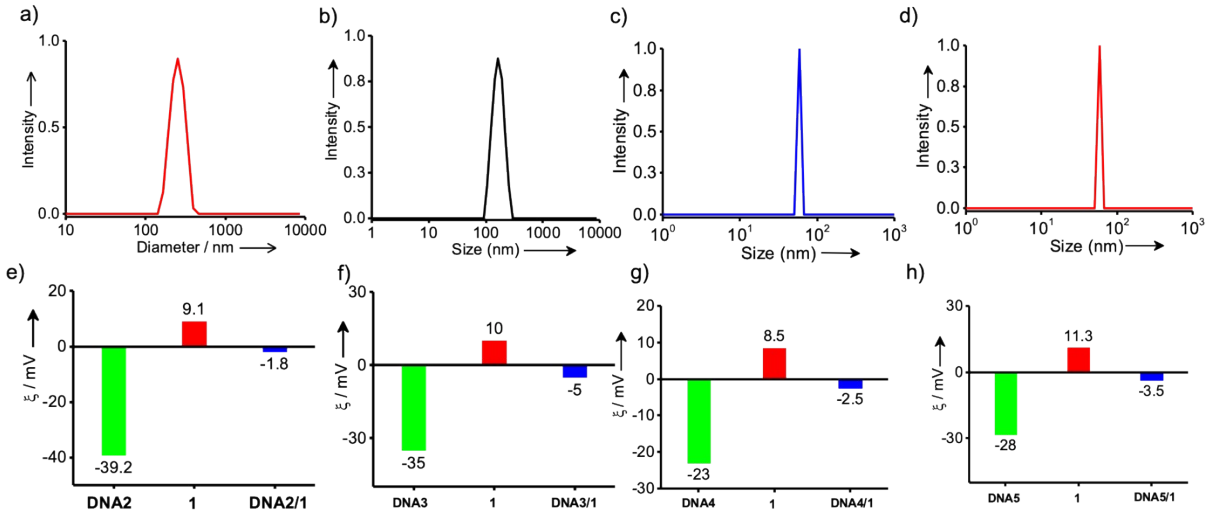

Figure S3. (a-d) DLS analyses of 1:10 molar ratio solutions of (a) **DNA2/1**, (b) **DNA3/1**, (c) **DNA4/1**, (d) **DNA5/1** NPs and (e-h) their respective zeta potential measurements.

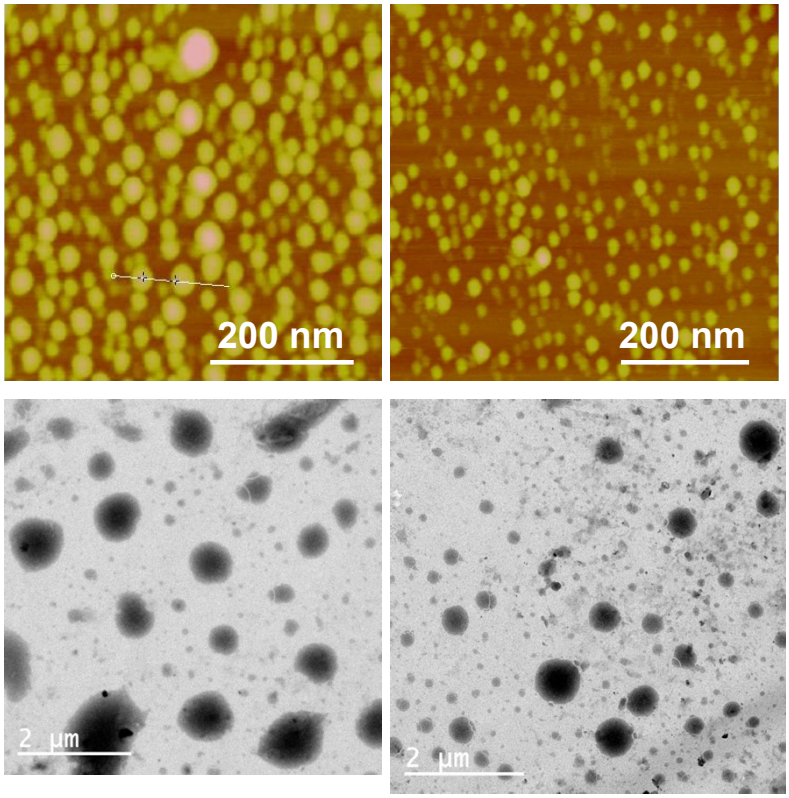

Figure S4. Additional AFM (top) and TEM (bottom) images of **DNA1/1** NPs (1:10 molar ratio) at different magnifications.

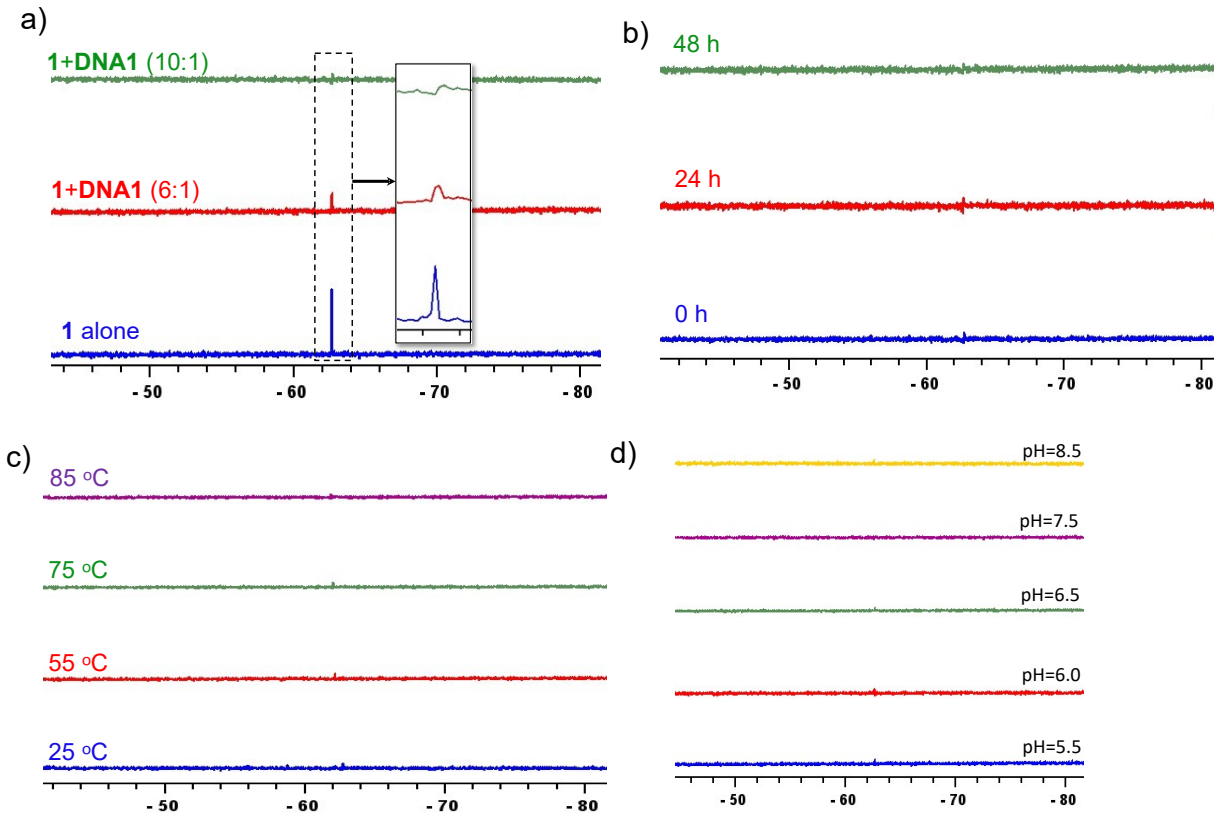

Figure S5. (a) ^{19}F NMR spectral changes of **1** with the gradual addition of **DNA1**. (b-d) Time, temperature and pH-dependent ^{19}F NMR studies of **DNA1/1** NPs, respectively.

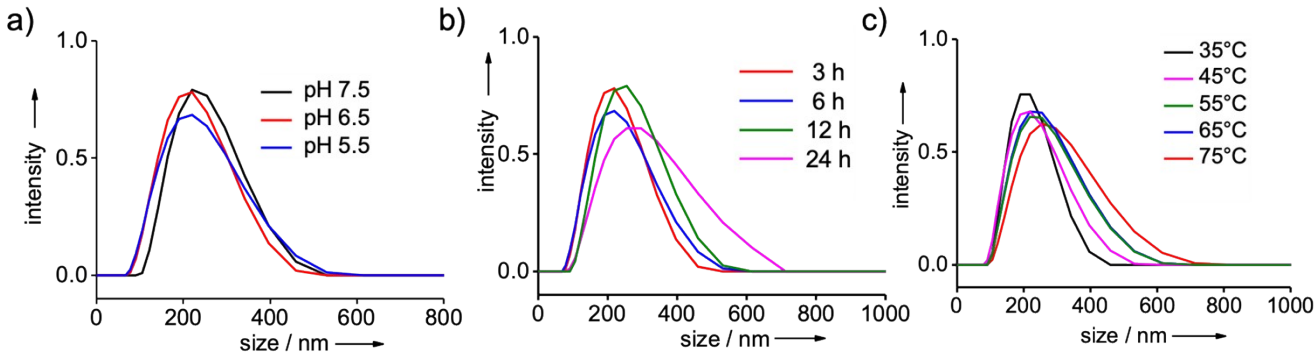

Figure S6. Stability of **DNA1/1** NPs with respect to (a) pH, (b) time and (c) temperature.

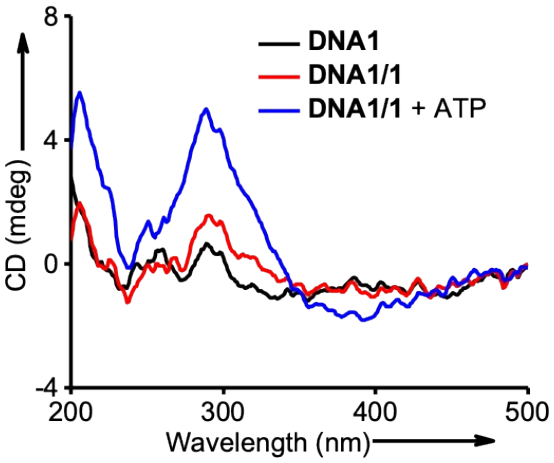

Figure S7. CD spectra of **DNA1**, **DNA1/1** NPs and **DNA1/1** NPs after the addition of ATP.

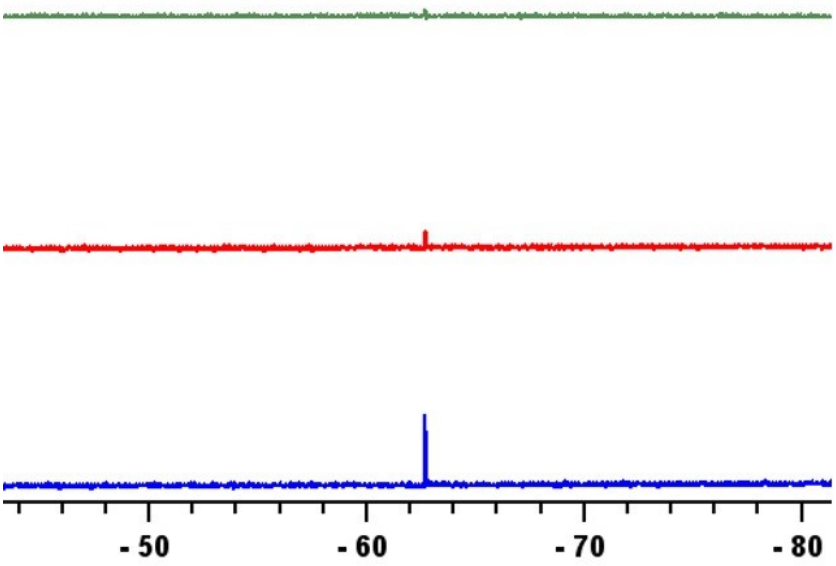

Figure S8. ^{19}F-NMR spectral changes of **1** with the gradual addition of **DNA2**. Probe **1** alone (blue trace), **DNA2/1** (1:6) (red trace) and **DNA2/1** (1:10) (green trace).

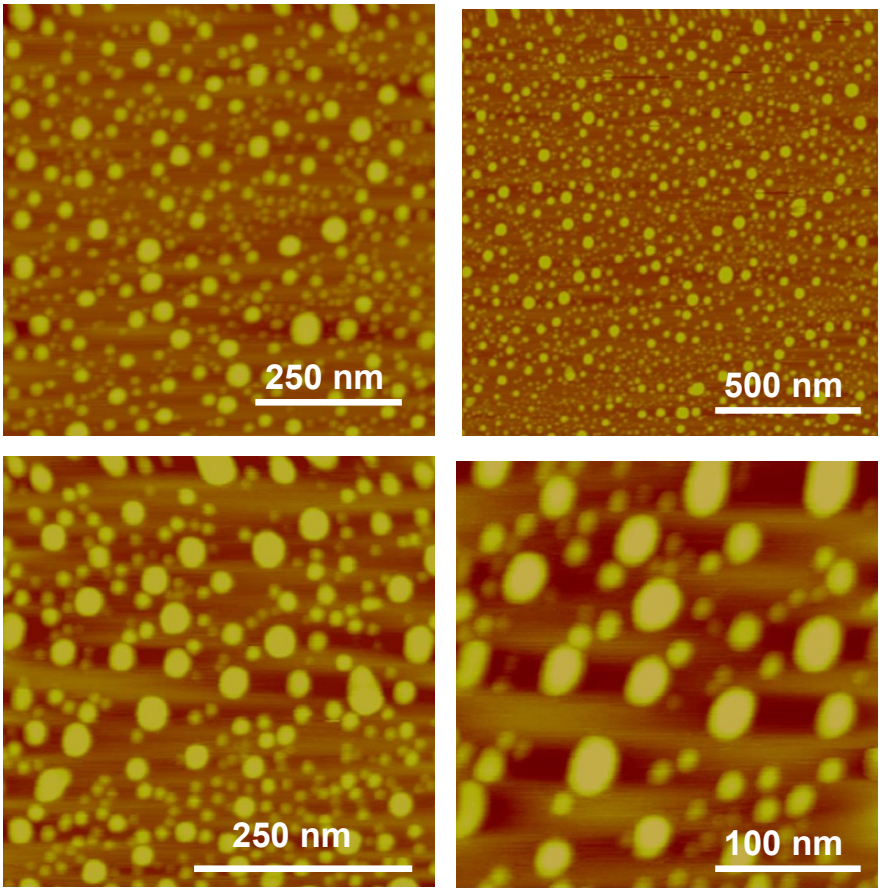

Figure S9. Additional AFM images of **DNA2/1** NPs (1:10 molar ratio) at different magnifications.

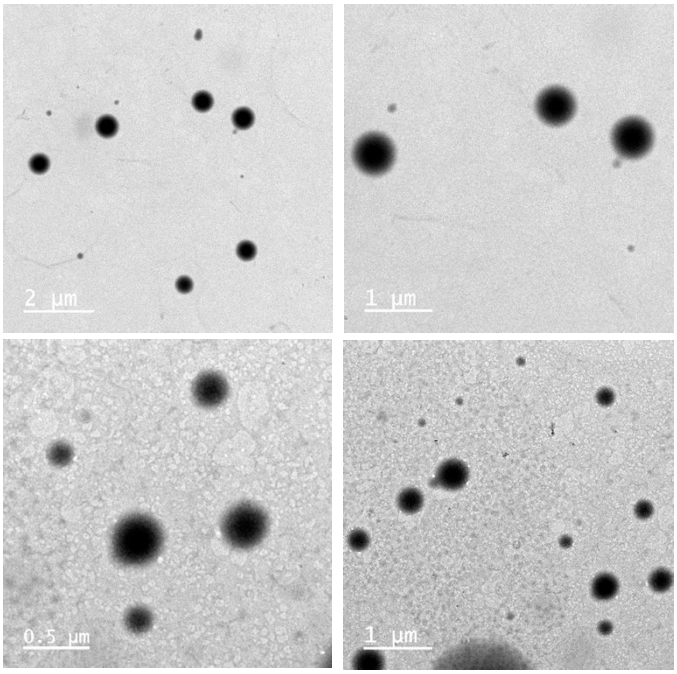

Figure S10. Additional TEM images of **DNA2/1** NPs (1:10 molar ratio) at different magnifications.

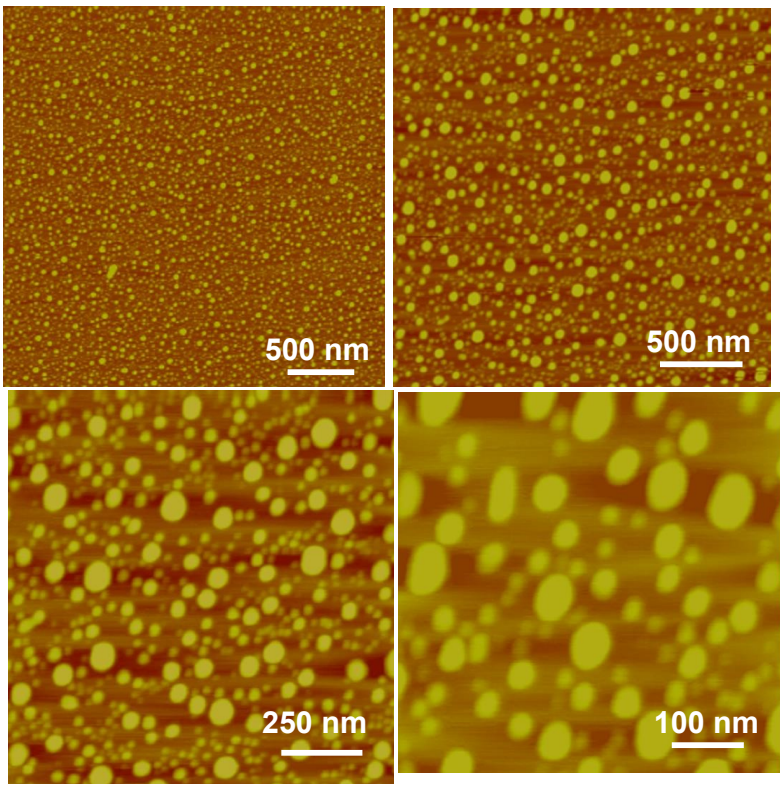

Figure S11. Additional AFM images of **DNA3/1** NPs (1:10 molar ratio) at different magnifications.

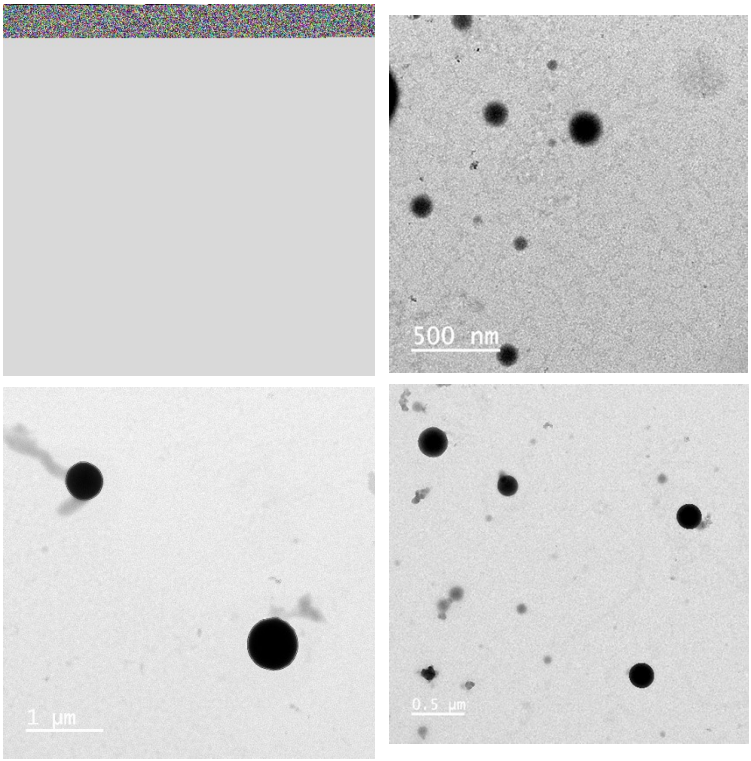

Figure S12. TEM images of **DNA3/1** NPs (1:10 molar ratio) at different magnifications.

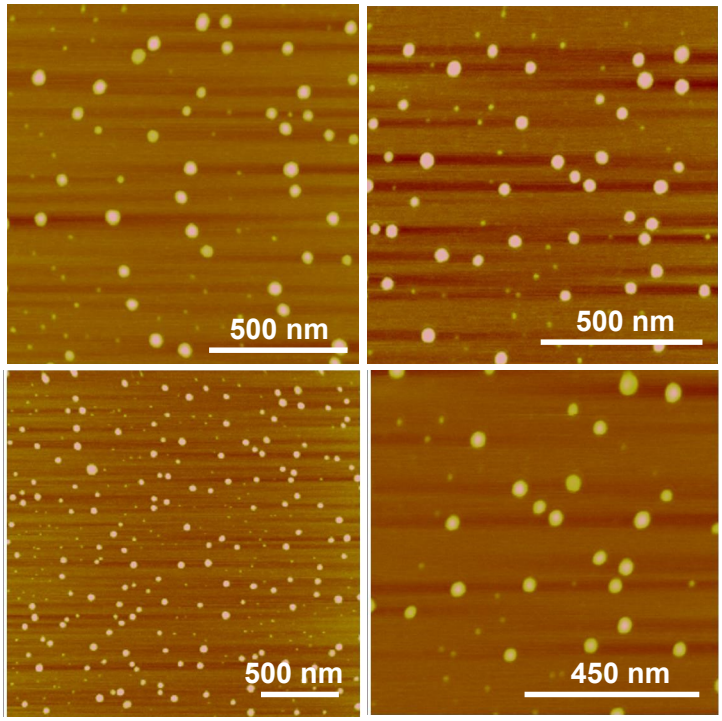

Figure S13. Additional AFM images of **DNA4/1** NPs (1:10 molar ratio) at different magnifications.

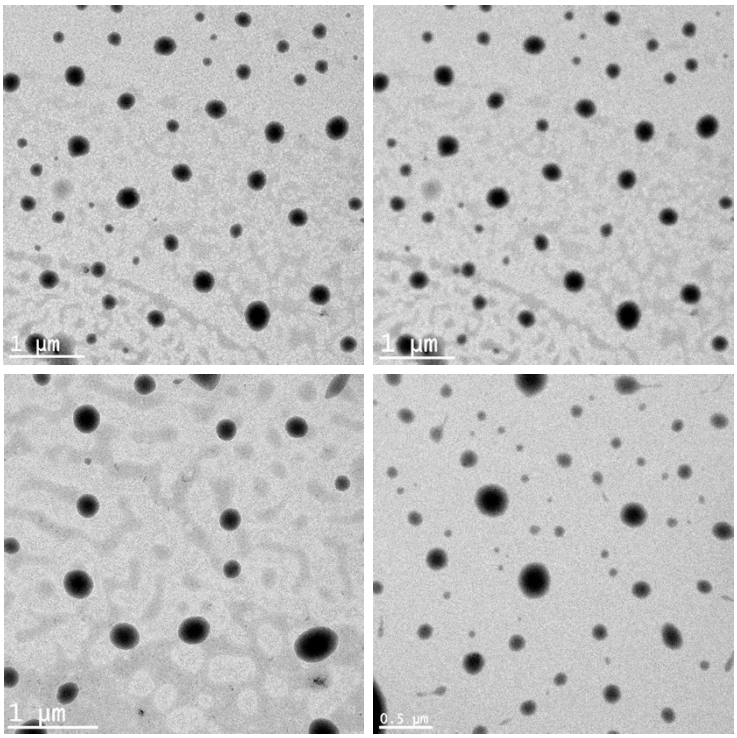

Figure S14. TEM images of **DNA4/1** NPs (1:10 molar ratio) at different magnifications.

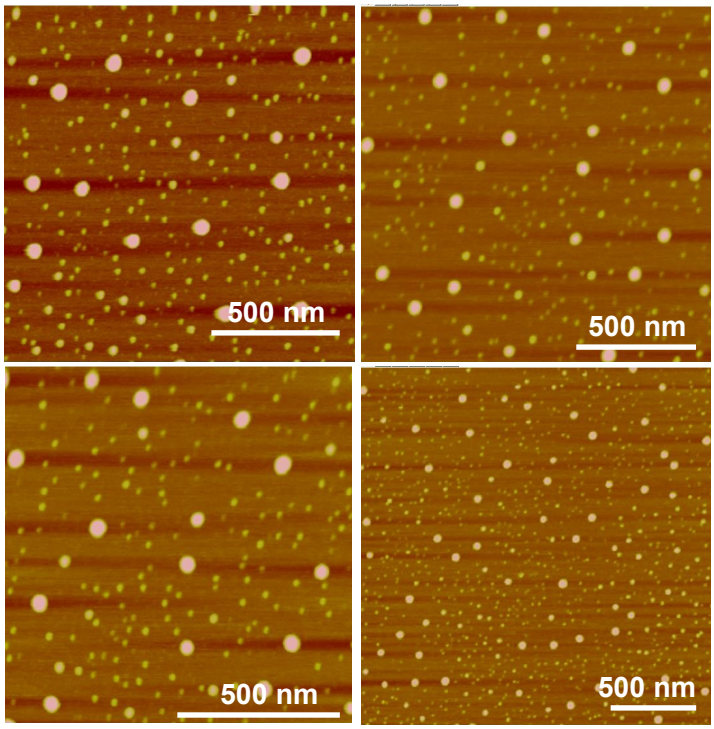

Figure S15: Additional AFM images of **DNA5/1** NPs (1:10 molar ratio) at different magnifications.

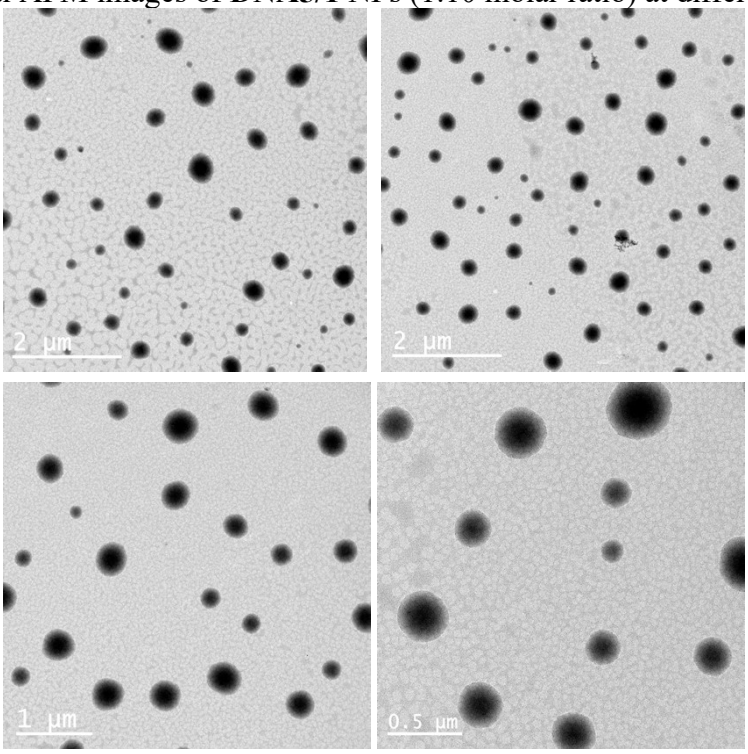

Figure S16. TEM images of **DNA5/1** NPs (1:10 molar ratio) at different magnifications.

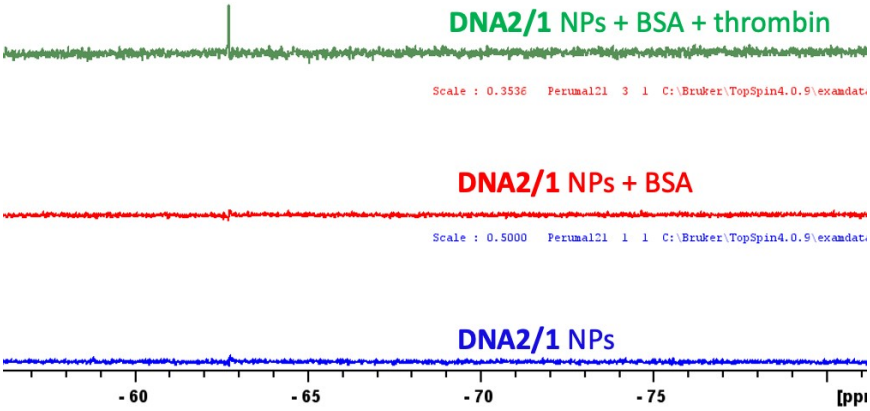

Figure S17. ^{19}F NMR response of **DNA2/1** NPs (6:60 μM) with the addition of thrombin (5 μM) in the presence of serum protein (BSA, 5 μM).

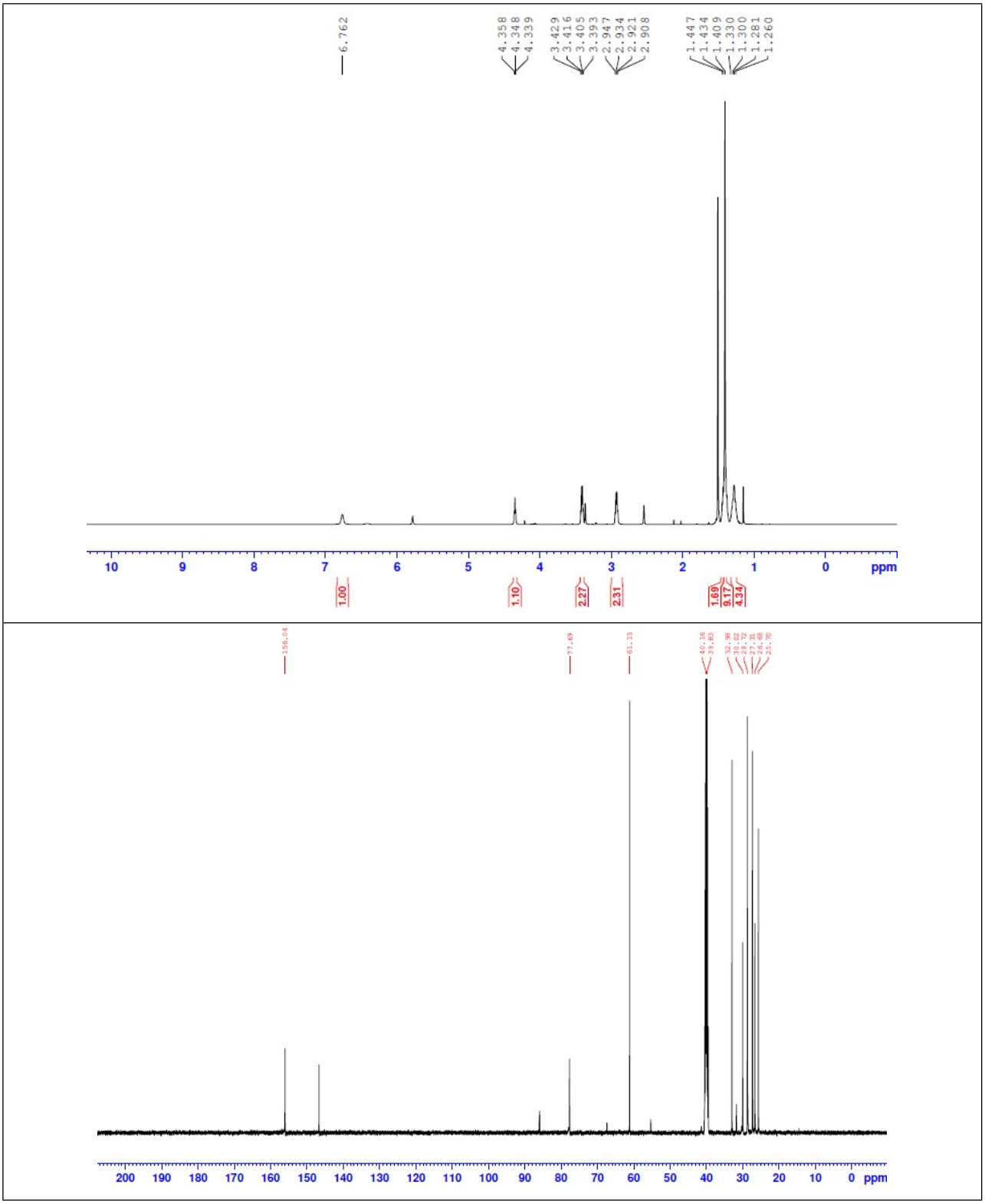

Figure S18. ^{1}H (above) and ^{13}C (below) NMR spectra of compound **1a**.

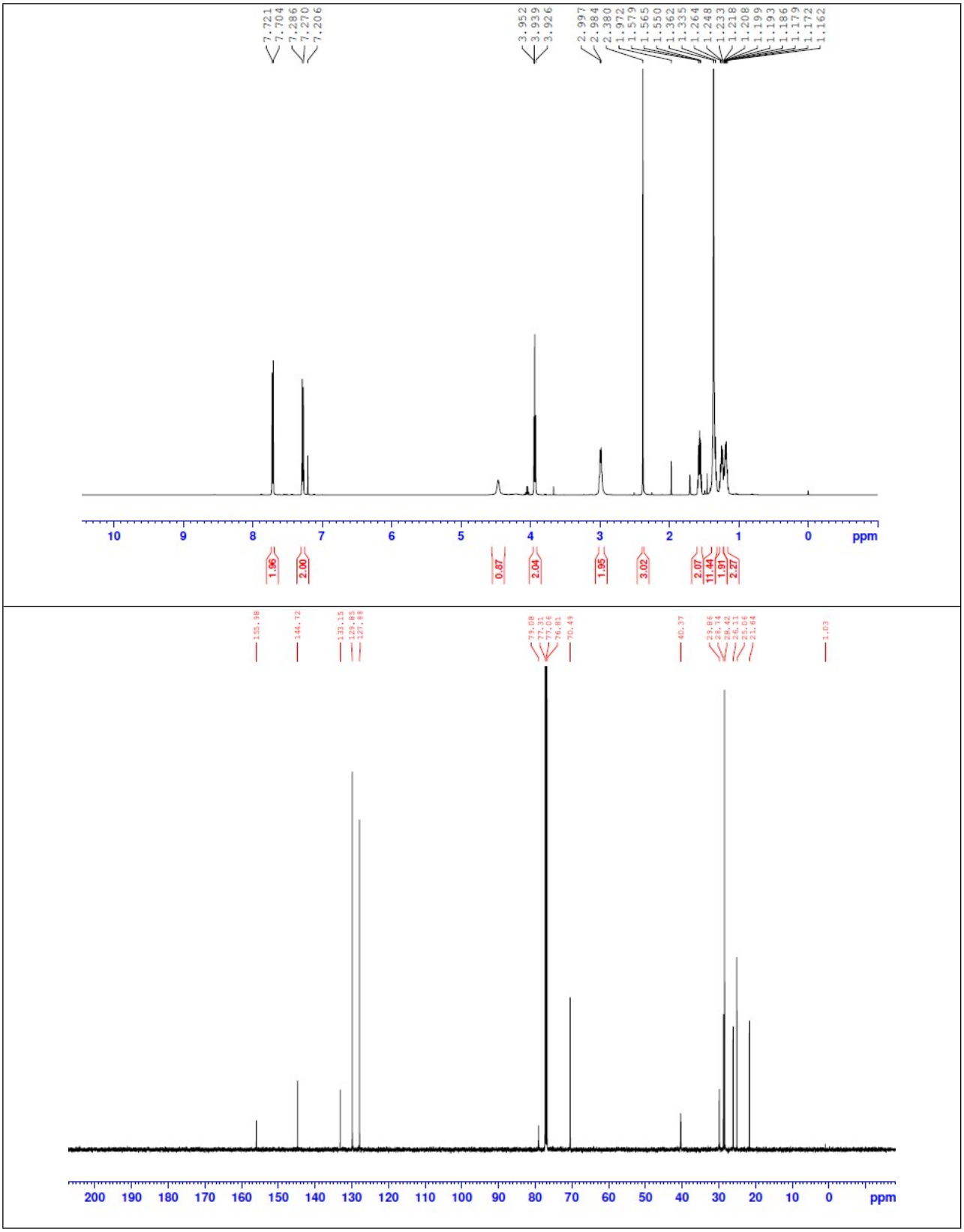

Figure S19. ^{1}H (above) and ^{13}C (below) NMR spectra of compound **1b**.

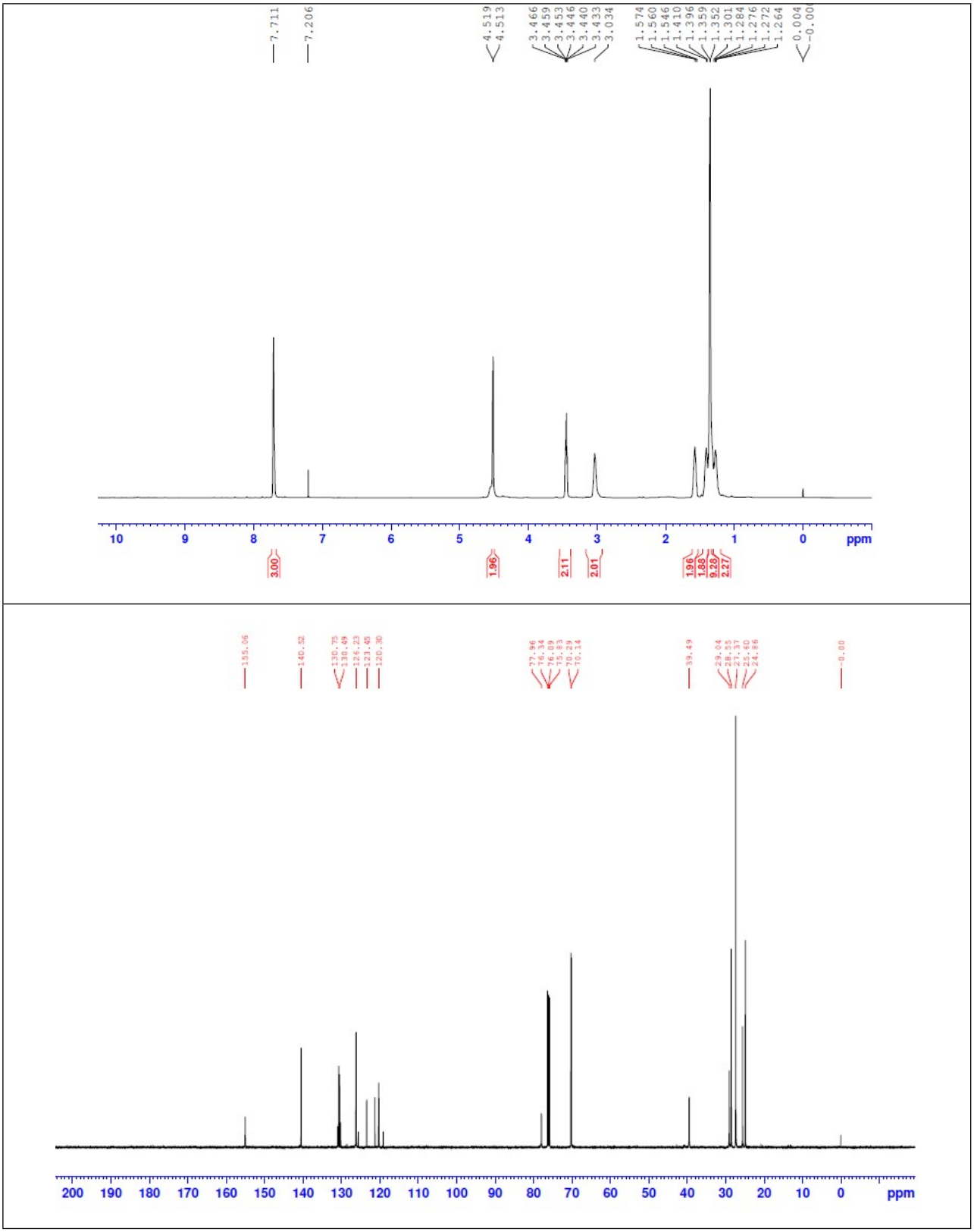

Figure S20. ^{1}H (above) and ^{13}C (below) NMR spectra of compound **1c**.

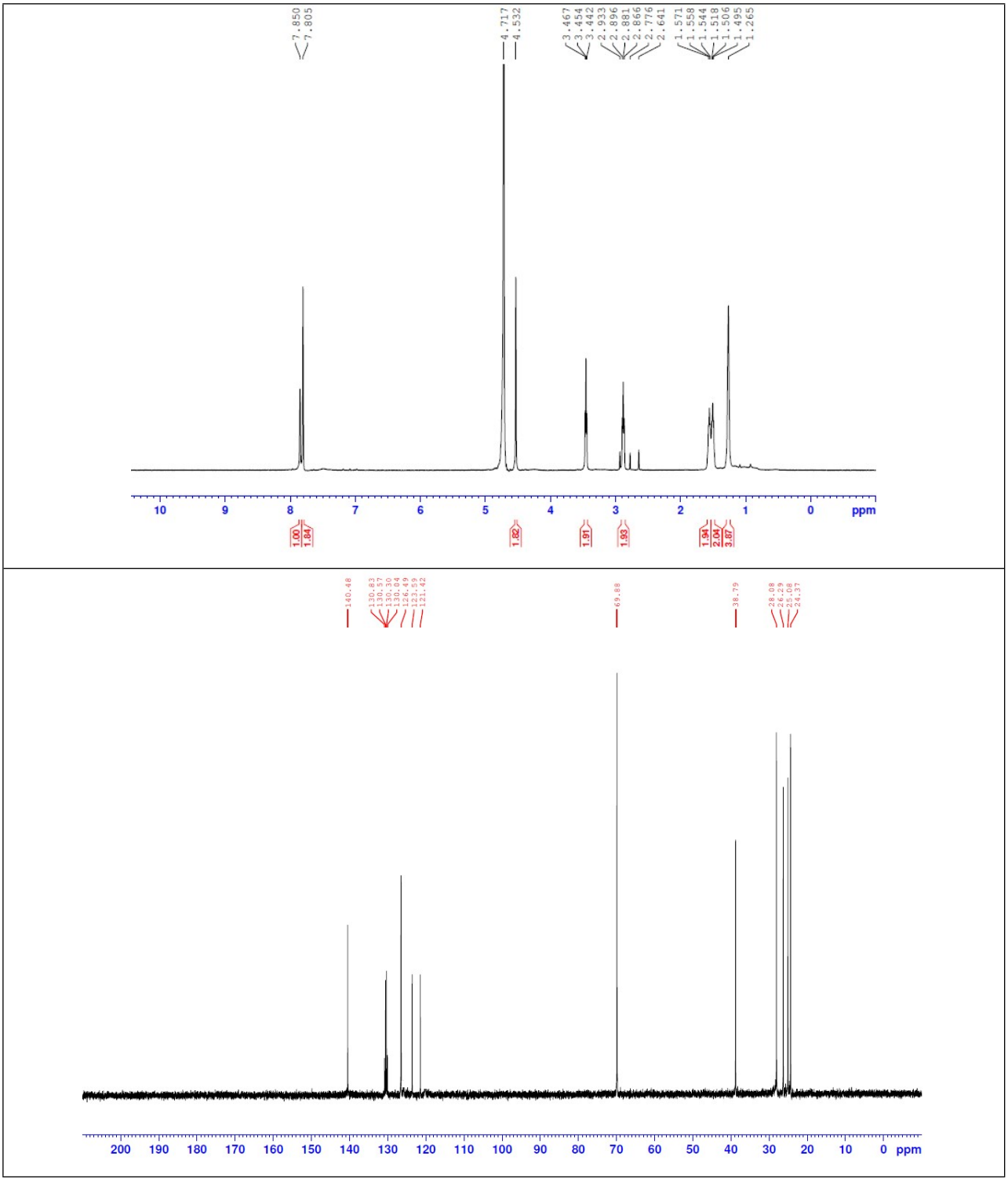

Figure S21. ^{1}H (above) and ^{13}C (below) NMR spectra of compound **1**.

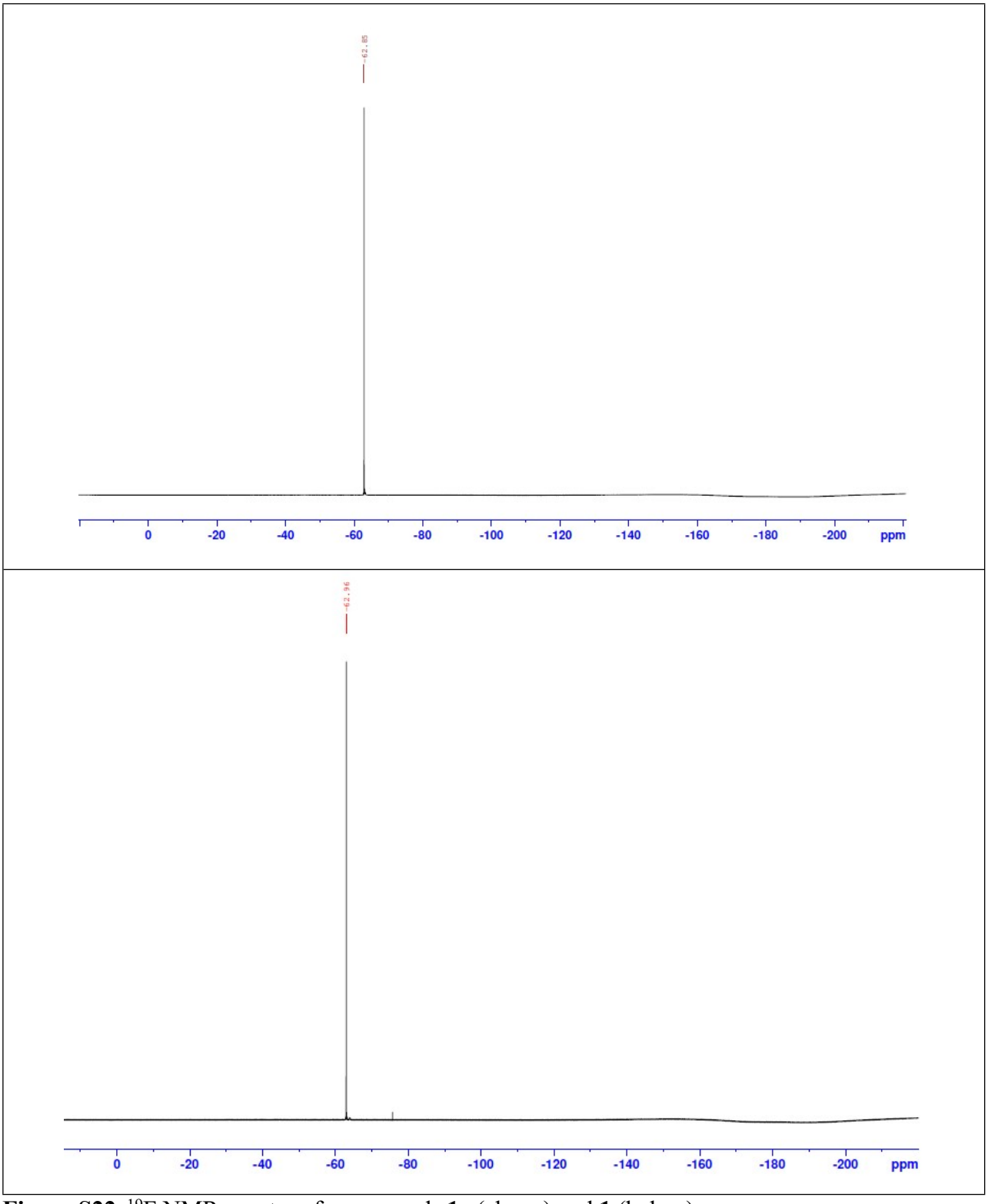

Figure S22. ^{19}F NMR spectra of compounds **1c** (above) and **1** (below).

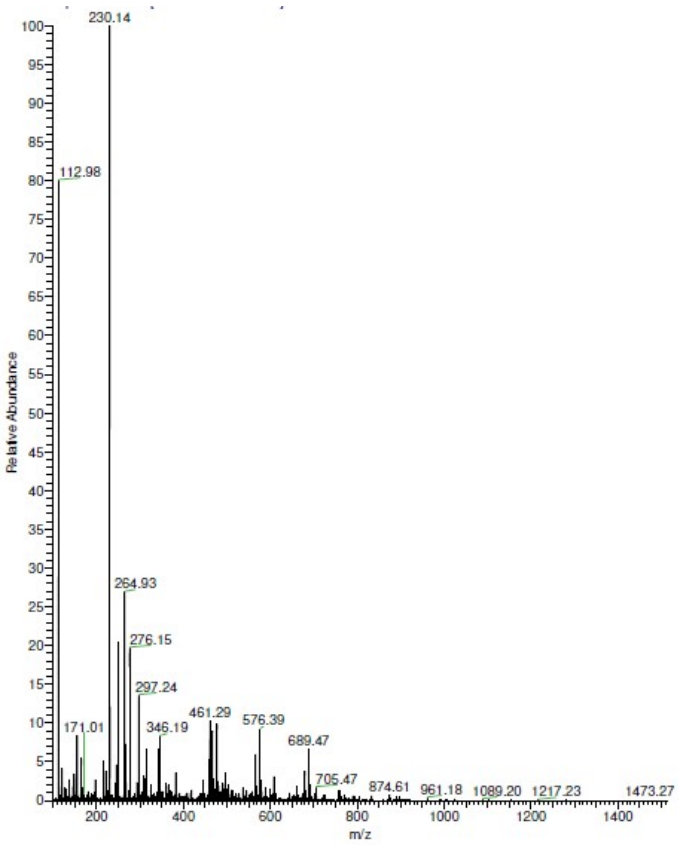

Figure S23. LCMS spectrum of compound **1a**.

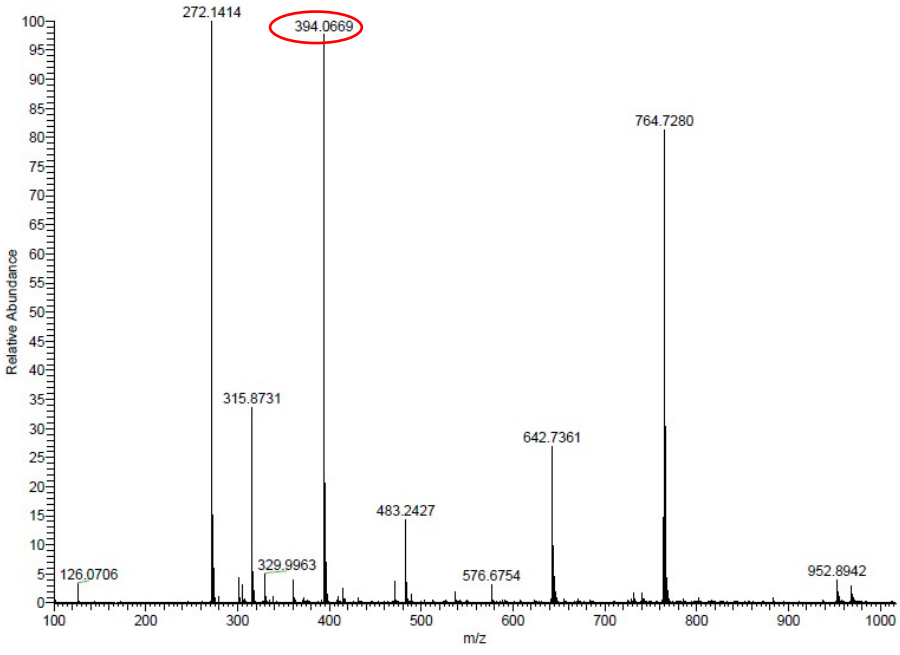

Figure S24. LCMS spectrum of compound **1b**.

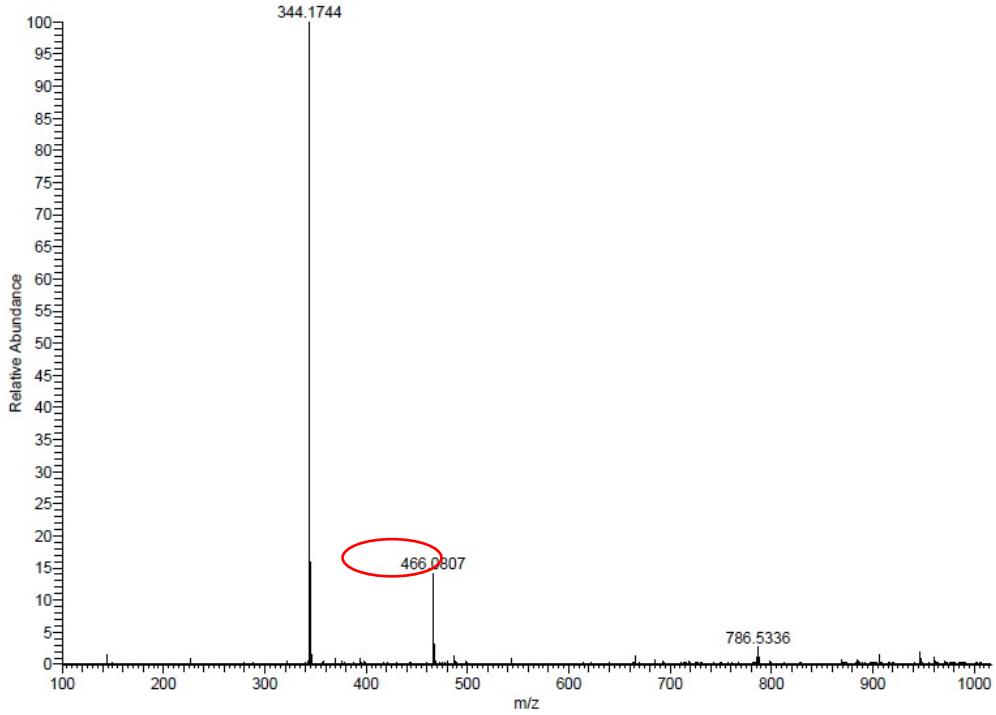

Figure S25. LCMS spectrum of compound **1c**.

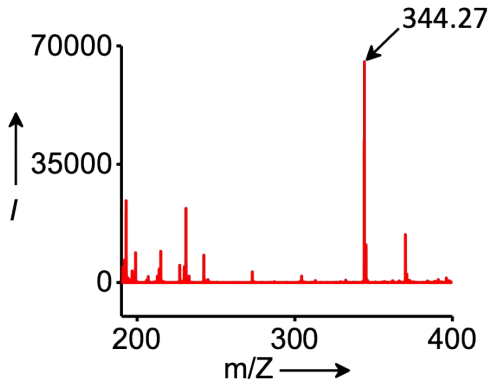

Figure S26. MALDI-TOF spectrum of compound **1**.