Supporting Information

Size Controllable DNA Nanogels from the Self-assembly of DNA Nanostructures through Multivalent Host-Guest Interactions

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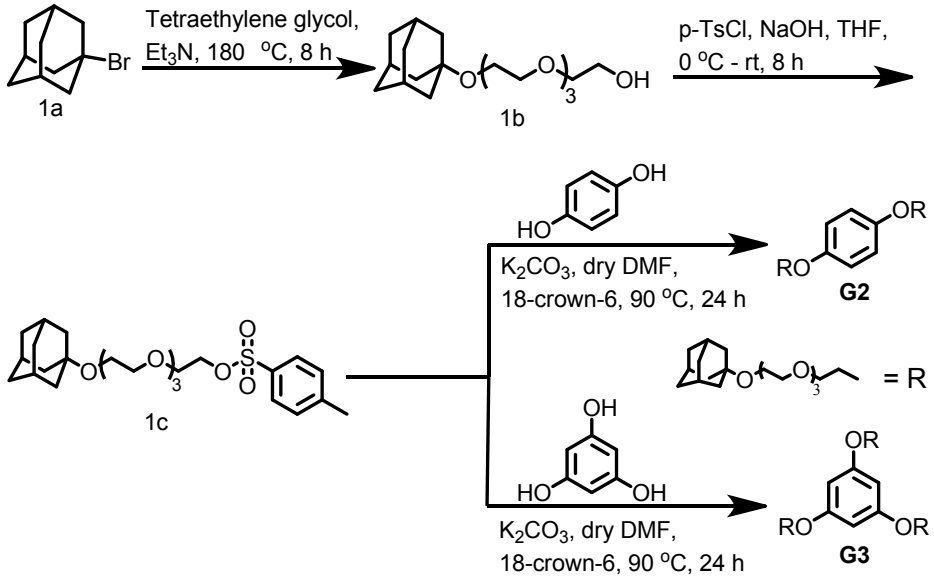
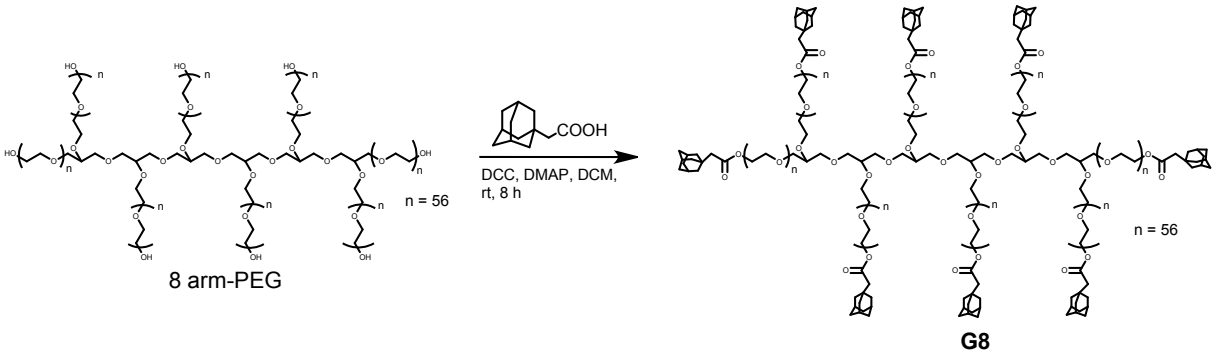
Synthesis of G2 and G3



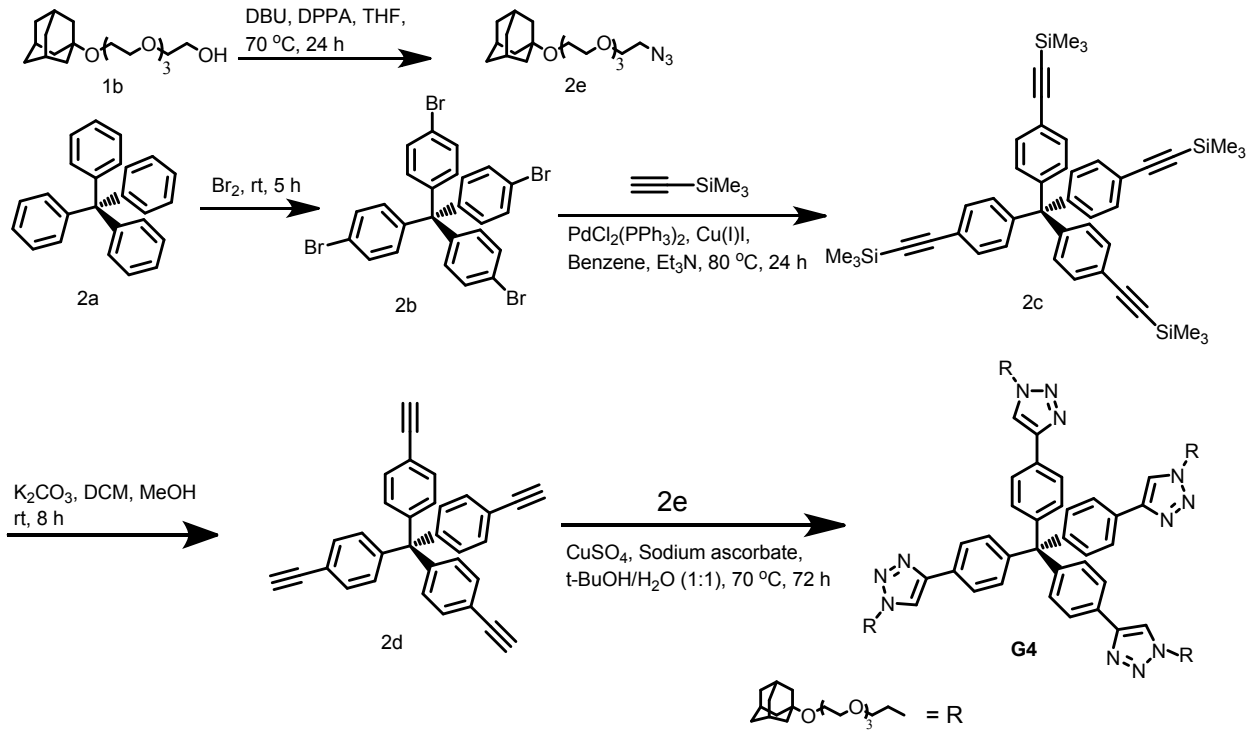
Scheme S1. Synthetic scheme for **G2** and **G3**.

Synthesis of G8



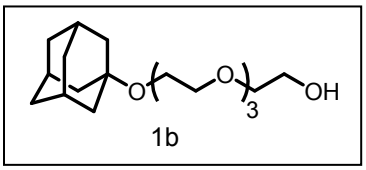
Scheme S2. Synthetic scheme for **G8**.

Synthesis of G4



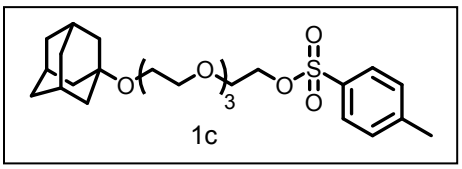
Scheme S3. Synthetic scheme for **G4**.

Synthesis of 1b: In a 100 mL round bottom flask, **1a** (2 g, 9.29 mmol) was suspended in triethylamine (4 mL) and tetraethyleneglycol (TEG) (37.45 g, 192.86 mmol). The reaction mixture was stirred 8h at 180 ℃. Subsequently it was extracted with DCM, washed with 2M HCl (20 mL), and once with brine (20 mL). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to yield the crude product as yellow oil (78%). TLC in 5% MeOH in DCM, R_f = 0.61; ^{1}H NMR (500 MHz, $CDCl_3$ δ ppm): 3.65 (t, *J* = 3.50 Hz, 2H), 3.590-3.490 (m, 14H), 2.89 (bs, 1H), 2.069 (bs, 3H), 1.681 (bs, 6H), 1.580-1.508 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$ δ ppm): 72.79, 72.60, 71.27, 70.61, 70.56, 70.46, 70.18, 61.70, 59.24, 41.35, 36.43, 30.51; HRMS-m/z of $C_{18}H_{32}O_5Na$: $[M+Na]^+$: 351.2150 (calc.), 351.2150 (exp.).



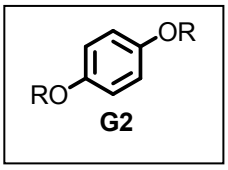
Synthesis of 1c: To an aqueous solution of NaOH (0.7311g, 18.279 mmol), **1b** (2.0 g, 6.093 mmol) in THF (20 mL) was added at 0 ℃. To this mixture, a solution of p-TsCl (1.742 g, 9.140

mmol) in dry THF (20 mL) was added drop wise. After the addition, reaction mixture was allowed towarm to room temperature and stirred for 8h. After completion of the reaction, solvent was removed under reduced pressure and product was extracted with EtOAc (50 mL), washed with water (50 mL) and brine. The organic layer was dried over the anhydrous sodium sulphate and the solvent was removed under reduced pressure. The crude product obtained was purified using column chromatography (silica gel, 40% EtOAc in pet-erther) to get the desired product as colorless oil (70%). TLC (40% EtOAc in pet-ether), R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 7.730 (d, *J*=8.00 Hz, 2H), 7.270 (d, *J* = 8.5 Hz, 2H), 4.092 (t, *J* = 5 Hz, 2H), 3.618 (t, *J* = 5.5 Hz, 2H), 3.568-3.501 (m, 12H), 2.378 (s, 3H), 2.066 (bs, 3H), 1.669 (d, *J* = 2.0 Hz, 6H),1.580-1.503 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm):143.74, 132.06, 128.79, 126.98, 71.25, 70.28, 69.75, 69.63, 69.57, 69.52, 68.22, 67.68, 58.25, 40.48, 35.45, 29.50, 20.62; HRMS-m/z of $C_{25}H_{38}O_7SNa$: $[M+Na^+]^+$: 505.2238 (calc.), 505.2248 (exp.).



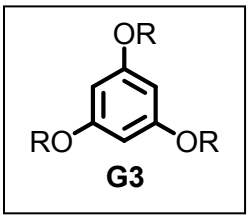
1c

Synthesis of G2: In a round bottom flask, 1,4-dihydroxybenzene (0.05 g, 0.4540 mmol) was dissolved in dry DMF (5 mL). **1c** (0.657 g, 1.3632 mmol), K_2CO_3 (0.3750g, 2.724 mmol) and 18-crown-[6] (catalytic amount) were added to the above solution. Reaction was heated to 90 ^{o}C for 24 h. After the reaction was completed, solvent was removed under reduced pressure. Reaction mixture was washed with water, extracted with EtOAc and dried over the sodium sulphate. The crude compound was purified by column chromatography (silica gel, 2% MeOH in DCM) and the desired product was obtained as yellow oil (60%). TLC (2% MeOH in DCM), R_f = 0.25; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 6.857 (s, 4H), 4.096 (t, *J* = 4.75 Hz, 4H), 3.852 (t, *J* = 5.1 Hz, 4H,), 3.758-3.684 (m, 16H), 3.628-3.588 (m, 8H), 2.157 (bs, 6H), 1.765 (d, *J* = 2.6 Hz, 12H), 1.67-1.595 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 153.12, 115.58, 72.29, 71.29, 70.81, 70.65, 70.61, 69.87, 68.09, 59.28, 41.49, 36.47, 30.52; HRMS-m/z of $C_{42}H_{66}O_{10}Na$: $[M+Na^+]^+$: 753.4556 (calc.), 753.4526 (exp.)



G2

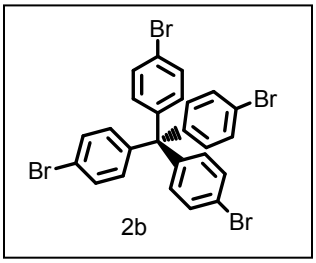
Synthesis of G3: In dry 50 mL round bottom flask, 1,3,5-trihydroxybenzene (0.05 g, 0.3964 mmol) was dissolved in dry DMF (5 mL). **1c** (0.765 g, 1.585 mmol), K_2CO_3 (0.4366g, 3.1712 mmol) and 18-crown-[6] (catalytic amount) were added to the above solution. Reaction was



G3

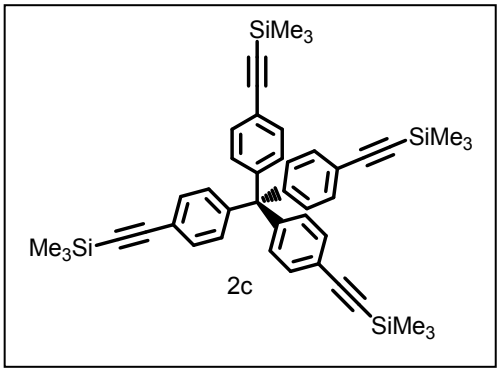
heated to 90 °C for 24 h. After the reaction was completed, solvent was removed under reduced pressure. Reaction mixture was washed with water, extracted with EtOAc and dried on anhydrous sodium sulphate. The crude compound was purified by column chromatography (silica gel, 2% MeOH in DCM) and the desired product was obtained as yellow oil (54%). TLC (4% MeOH in DCM), R_f = 0.62; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 6.024 (s, 3H), 3.985 (t, *J* = 4.5 Hz, 6H), 3.75 (t, *J* = 4.5 Hz, 6H), 3.642-3.584 (m, 24H), 3.528-3.498 (m, 12H), 2.058 (bs, 9H), 1.665 (d, *J* = 2.5 Hz, 18H), 1.545-1.52 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 160.53, 94.41, 72.27, 71.31, 70.82, 70.68, 70.65, 70.62, 69.68, 67.46, 59.28, 41.50, 36.49, 30.52; HRMS-m/z of $C_{60}H_{96}O_{15}Na$: $[M+Na^+]^+$: 1079.6649 (calc.), 1079.6634 (exp.)

Synthesis of 2b: In dry 50 mL round bottom flask, bromine (4.79 mL, 93.62 mmol) was added to tetraphenylmethane (**2a**) (3.0 g, 9.362 mmol) with continuous stirring under nitrogen atmosphere. Then the reaction mixture was stirred for additional 6 h. After completion, the solid reaction mixture was poured into ethanol (65 mL) and cooled to -78 ℃. Then the mixture was allowed to warm to room temperature and stirred for another 1 h. Subsequently the precipitated solid was filtered and washed with saturated $NaHSO_3$ solution (80 mL) and dried at 60 ℃ under vacuum. The crude product was purified by column chromatography (silica gel, pet-ether) and the desired product was obtained as white solid (99%). TLC (pet-ether), R_f = 0.64; ^{1}H NMR (500, MHz, d_6-DMSO, δ ppm): 7.459 (d, *J* = 8.65 Hz, 8H), 6.994 (d, *J* = 8.7 Hz, 8H); ^{13}C NMR (125 MHz, d_6- DMSO, δ ppm): 144.51, 132.32, 130.92, 119.91, 63.21; GC-MS (EI) -m/z of $C_{25}H_{16}Br_4$: 631.79 (calc.), 632 (exp.).



2b

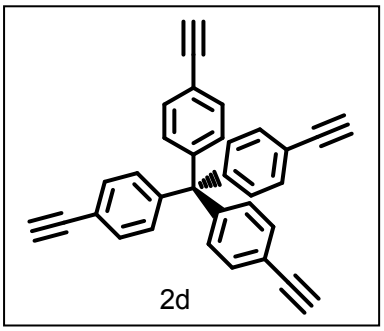
Synthesis of 2c: Triethylamine (10 mL) was added to a solution of **2b** (0.5 g, 0.801 mmol) in dry benzene (3.5 mL) under nitrogen atmosphere and degassed for 10 minutes. Subsequently, $PdCl_2(PPh_3)_2$ (0.0285 g, 0.04 mmol), Cu(I)Br (0.00572 g, 0.004) and trimethylsilyl-acetylene (0.912 mL, 0.629 g, 6.408 mmol) were added to the degassed solution. The resulting mixture was heated to 80 °C for 24 h. After



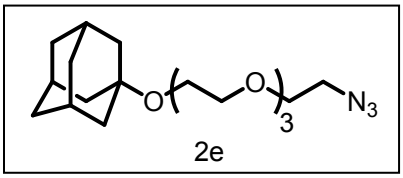
2c

removal of solvent under reduced pressure, the residue was extracted with DCM and HCl (1 M, 20 mL). Organic layer was washed with water, dried on anhydrous sodium sulphate and purified by column chromatography, (silica gel, 3% toluene in cyclohexane as eluent). The desired product was obtained as a yellow solid (37%). TLC (3% toluene in cyclohexane), R_f = 0.47; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 7.362 (d, *J* = 8.4 Hz, 8H), 7.075 (d, *J* = 8.4 Hz, 8H), 0.266 (s, 36H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 0.0033, 146.06, 131.43, 130.78, 121.28, 104.67, 94.83, 64.83; GC-MS (EI)-m/z of $C_{45}H_{52}Si_4$: 704.31 (calc.), 705 (exp.).

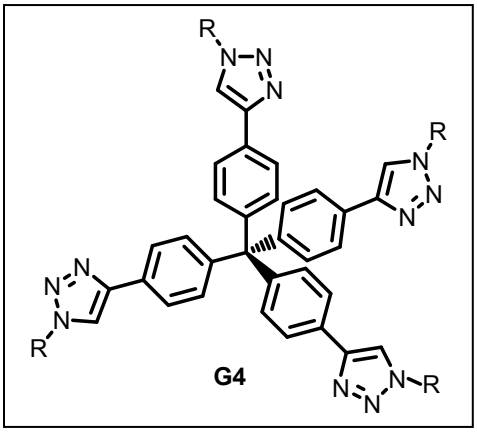
Synthesis of 2d: To a solution of **2c** (80 mg, 0,1134 mmol) in DCM (10 mL) and MeOH (10 mL), K_2CO_3 (625 mg, 4.539 mmol) was added and the reaction mixture was stirred for 8h at room temperature. After the completion of the reaction, the mixture was washed with water (10 mL, 2 times), extracted with DCM and dried on an anhydrous sodium sulphate. Solvent was removed under reduced pressure to give the desired product as a yellow solid (97%). TLC (20% toluene in cyclohexane), R_f = 0.48; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 7.387 (d, *J* = 7.95 Hz, 8H), 7.118 (d, *J* = 7.9 Hz, 8H), 3.061 (s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 146.21, 131.66, 130.75, 120.31, 83.18, 77.61, 64.81; GC-MS (EI)-m/z of $C_{33}H_{20}$: 416.16 (calc.), 416.0 (exp.).



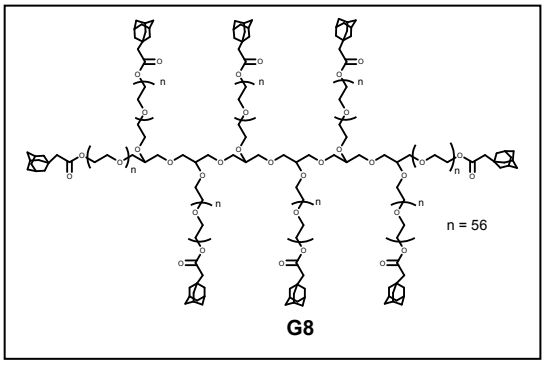
Synthesis of 2e: To a solution of **1b** (1.0 g, 3.446 mmol) in dry THF (15 mL), DBU (0.6 mL, 4.262 mmol) and DPPA (0.727 mL, 3.958 mmol) were added and stirred at 70℃ for 24 h. After the completion of the reaction, the mixture was quenched with aqueous ammonium chloride, extracted with ethylacetate and dried on anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel, 2% MeOH in DCM) to get the desired product as colorless oil (87%). TLC (2% MeOH in DCM), R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.621-3.500 (m, 14H), 3.321 (t, *J* = 5 Hz, 2H), 2.07 (bs, 3H), 1.677 (d, *J* = 1.5 Hz, 6H), 1.583-1.508 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 72.24, 71.31, 70.72, 70.68, 70.64, 70.04, 59.28, 50.73, 41.51, 36.49, 30.53; HRMS-m/z of $C_{18}H_{31}NO_4$: $[M-N_2]^+$ 325.2253 (calc.), 326.2337 (exp.)



Synthesis of G4: To a solution of **2d** (100 mg, 0.24 mmol) in t-BuOH/H_2O (10 mL, 1/1), $CuSO_4.5H_2O$ (12 mg, 0.048 mmol), sodium ascorbate (38.08 mg, 0.1922 mmol) and 1-adamantyl-tetraethylene glycol azide (**2e**) (511 mg, 1.44 mmol) were added. The reaction mixture was stirred at 70 ℃ for 72 h. After completion, the reaction mixture was allowed to cool to room temperature and the solvent was removed by reduced pressure. It was extracted with DCM and washed with water and brine solution (10 mL). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed by reduced pressure. The crude compound was purified by column chromatography (silica gel, 3% MeOH in DCM) to yield the desired product as a yellow sticky solid (31%). TLC (4% MeOH in DCM), R_f = 0.25; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 7.921 (s, 4H), 7.689 (d, *J* = 8.45 Hz, 8H), 7.287 (d, *J* = 8.5 Hz, 8H), 4.52 (t, *J* = 4.8 Hz, 8H), 3.836 (t, *J* = 4.8 Hz, 8H), 3.556-3.455 (m, 54H), 2.028 (bs, 12H), 1.626-1.618 (m, 24H), 1.546-1.464 (m, 24H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 147.32, 146.27, 131.46, 128.70, 125.09, 121.04, 72.27, 71.31, 70.65, 70.59, 70.52, 69.61, 59.26, 50.39, 41.46, 36.44, 30.48; HRMS-m/z of $C_{105}H_{144}N_{12}Na$: $[M+2H^+]^+$: 1831.0823 (calc.), 1831.0947 (exp.).



Synthesis of G8: Synthesis of **G8** was achieved following a reported procedure.[S1] In a dry 50 mL round bottom flask a mixture of 8-arm-PEG (purchased from Creative PEGWorks, USA; 1.0 g, 0.05 mmol) and DMAP (0.0488 g, 0.4 mmol) were dried under vacuum at 50 ℃ overnight. The mixture was cooled room temperature and subsequently a solution of adamantine acetic acid (0.155 g, 0.8 mmol) in dry DCM (10 mL) and DCC (0.2063 g, 0.99 mmol) were added. The reaction mixture was stirred 8h at room temperature. After the completion of the reaction, insoluble DCU was filtered and filtrate was concentrated to 3 mL by reduced pressure and precipitated by adding 200



mL of diethylether. The precipitate thus obtained was dissolved in methanol, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to yield the desired product as a white solid. ^{1}H NMR (500 MHz, D_2O, δ ppm): 4.242 (t, J = 4.5 Hz, 16H), 3.825-3.522 (m, 1995H), 2.137 (s, 16H), 1.939 (bs, 24H), 1.708-1.584 (m, 96H); ^{13}C NMR (175 MHz, d_6-DMSO, δ ppm): 171.16,70.26, 68.87, 63.13, 48.61, 42.17, 36.71, 32.70, 28.45.

Table S1. HR-MS for DNAs

DNA	Molecular Formula	Calculated	Observed mass
DNAX1	$C_{418}H_{540}N_{147}O_{258}P_{37}$	12891.39	1288.1366 (z = 10), 1073.1833 (z =12), 990.5659 (z = 13), 858.6509 (z = 15), 715.1141 (z = 18), 357.0 (z = 36)
DNAX2	$C_{421}H_{541}N_{150}O_{260}P_{37}$	13002.42	1299.203 (z =10), 999.1849 (z = 13), 590.0968 (z =22), 499.0477 (z = 26), 393.0118 (z = 33)
DNAX3	$C_{420}H_{543}N_{142}O_{264}P_{37}$	12944.37	1848.066 (z = 7), 808.0038 (z = 16), 760.4208 (z = 17), 587.3937 (z = 22), 561.7936 (z = 23), 516.7278 (z = 25)
DNAX4	$C_{417}H_{540}N_{145}O_{258}P_{37}$	12851.39	802.1221 (z = 16), 675.114 (z = 19), 513.056 (z = 25), 355.990 (z = 36), 346.0428 (z = 37)
DNAY1	$C_{322}H_{419}N_{108}O_{200}P_{27}$	9833.89	645.5861 (z = 15). 613.6149 (z = 16), 577.4574 (z = 17), 545.3116 (z = 18), 363.2152 (z = 27)
DNAY2	$C_{321}H_{418}N_{109}O_{198}P_{27}$	9802.92	2449.7312 (z = 4), 1632.8163 (z = 6), 979.290 (z = 10), 753.0728 (z = 13), 699.9656 (z = 14), 489.1852 (20)
DNAY3	$C_{323}H_{420}N_{107}O_{202}P_{27}$	9864.88	1095.0902 (z = 9), 703.6896 (z = 14), 579.280 (z = 17), 492. 2270 (z = 20), 410.047 (z = 24)

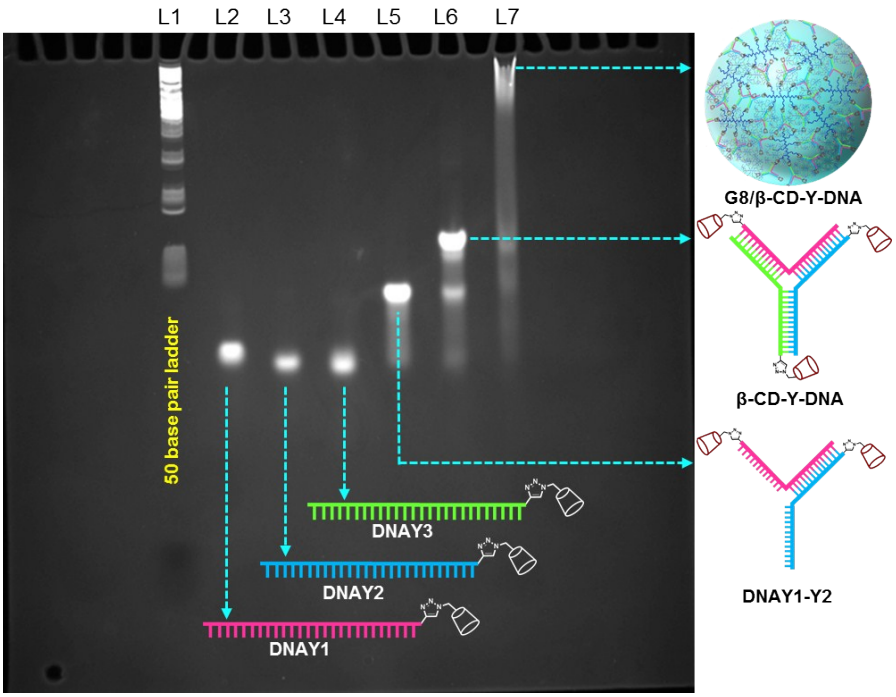


Fig. S1 Native PAGE analysis of **G8/β-CD-Y-DNA** (L1:50 base pair ladder, L2–L4: **DNAY1**, **DNAY2** and **DNAY3**, respectively. L5 = assembly of **DNAY1** and **DNAY2**, L6 = **β-CD-Y-DNA** and L7: **G8/β-CD-Y-DNA**.

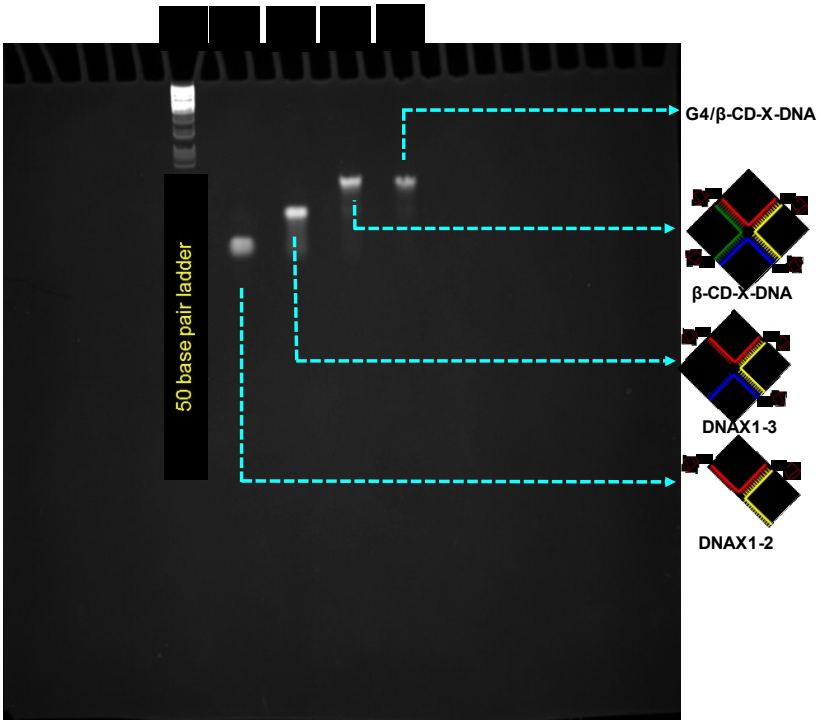


Fig. S2. Native PAGE analyses for the self-assembly of **β-CD-X-DNA** with **G4**. (L1:50 base pair ladder, L2 = assembly of **DNAX1** and **DNAX2**, L3 = assembly of **DNAX1-3**; L4 = **β-CD-X-DNA** and L5: **G4/β-CD-X-DNA**.

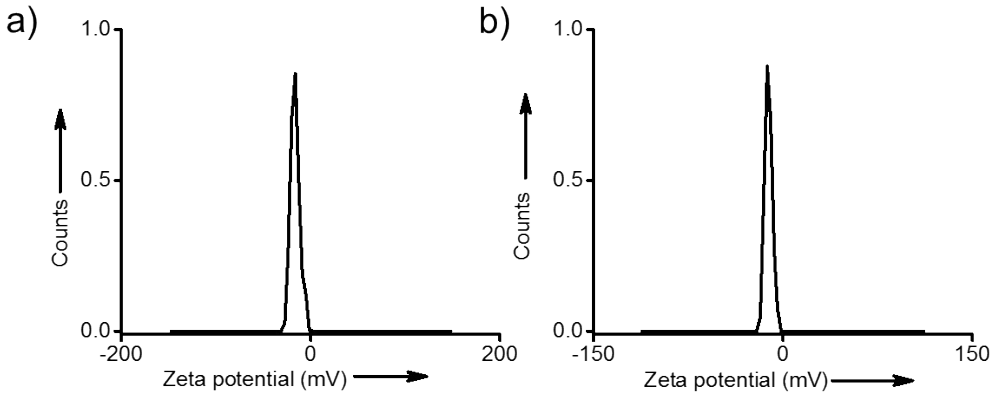


Fig. S3 Zeta potential plot for (a) **G8/β-CD-X-DNA** and (b) **G8/β-CD-Y-DNA** nanoparticles.

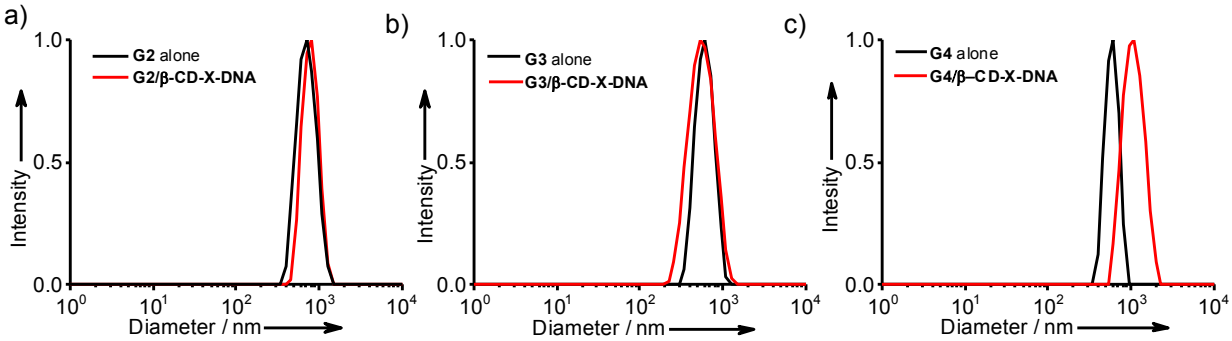


Fig. S4 DLS analyses of (a) **G2/β-CD-X-DNA**, (b) **G3/β-CD-X-DNA** and (c) **G4/β-CD-X-DNA**. No significant shifts are observed in the DLS size distribution curves with the addition of **β-CD-X-DNA** to **G2** and **G3**, clearly ruled out any oligomer formation for **G2/β-CD-X-DNA** and **G3/β-CD-X-DNA**. On the other hand, a noticeable shift is observed with the addition of **β-CD-X-DNA** to **G4**, suggesting the formation of oligomers in solution for **G4/β-CD-X-DNA**.

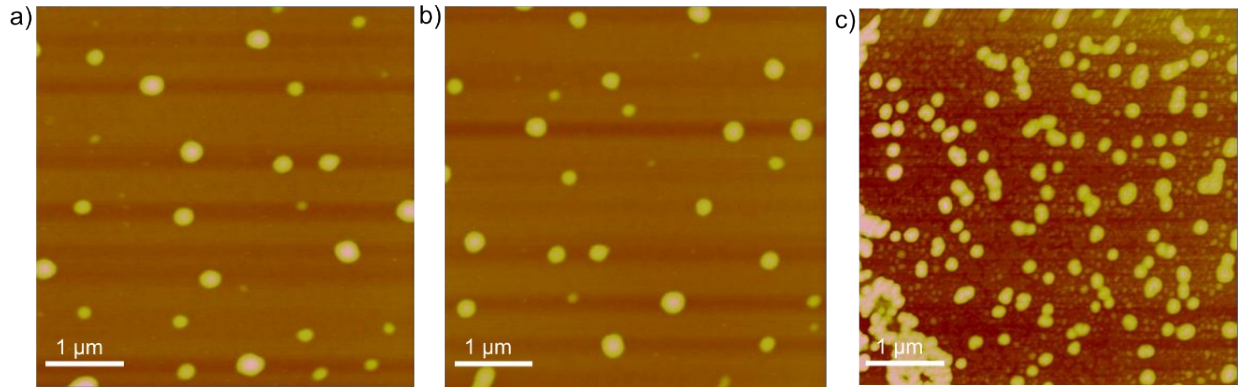


Fig. S5 Additional AFM images of **G8/β-CD-X-DNA** (z-scale = 25 nm for a & b and 45 nm for c).

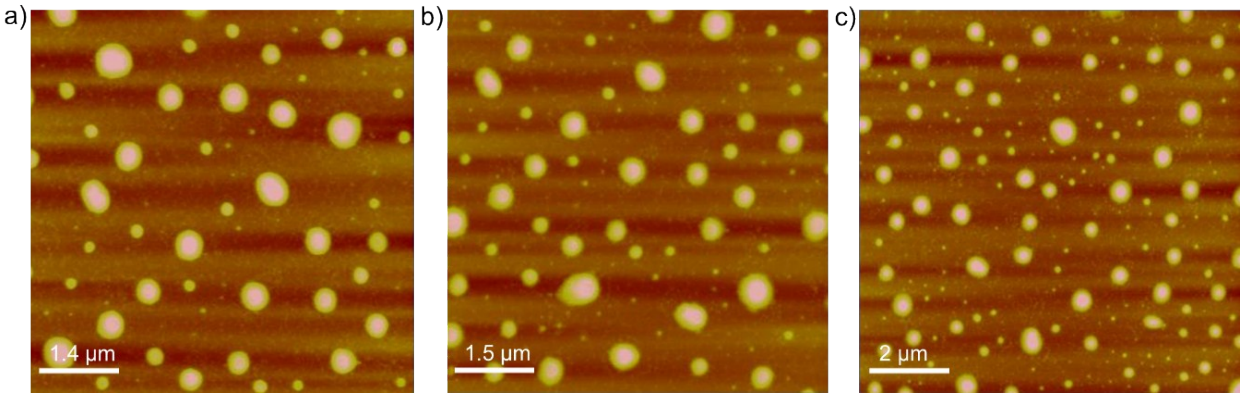


Fig. S6 Additional AFM images of **G8/β-CD-Y-DNA** (z-scale = 28 nm, 33 nm and 26 nm for a, b, c, respectively).

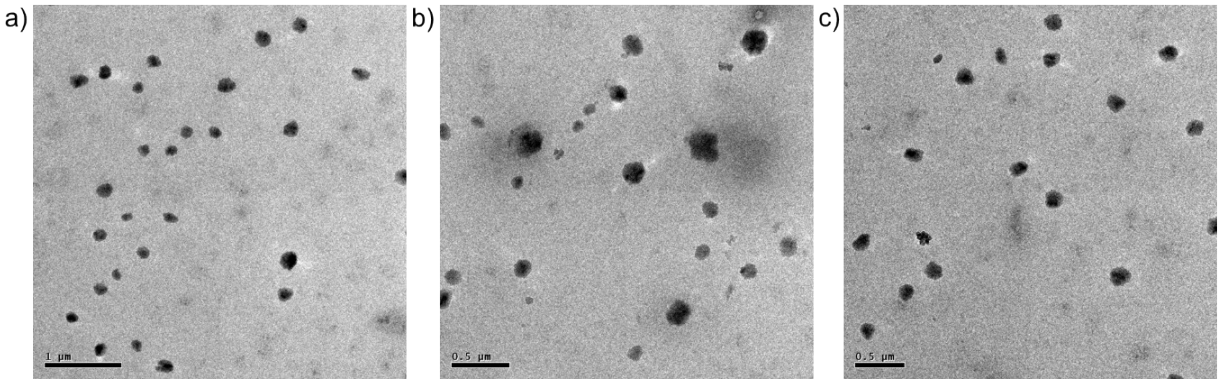


Fig. S7 Additional TEM images of **G8/β-CD-X-DNA** nanoparticles (1:2 µM).

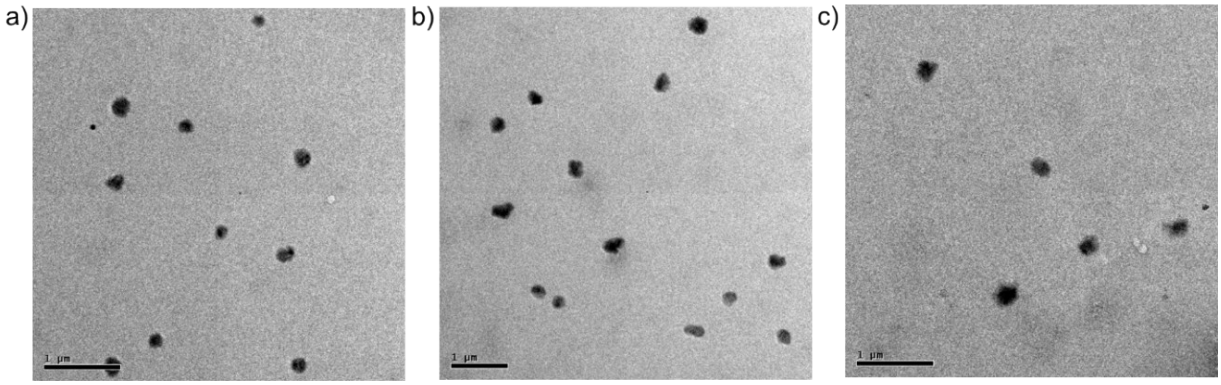


Fig. S8 Additional TEM images of **G8/β-CD-Y-DNA** nanoparticles (1:2 µM).

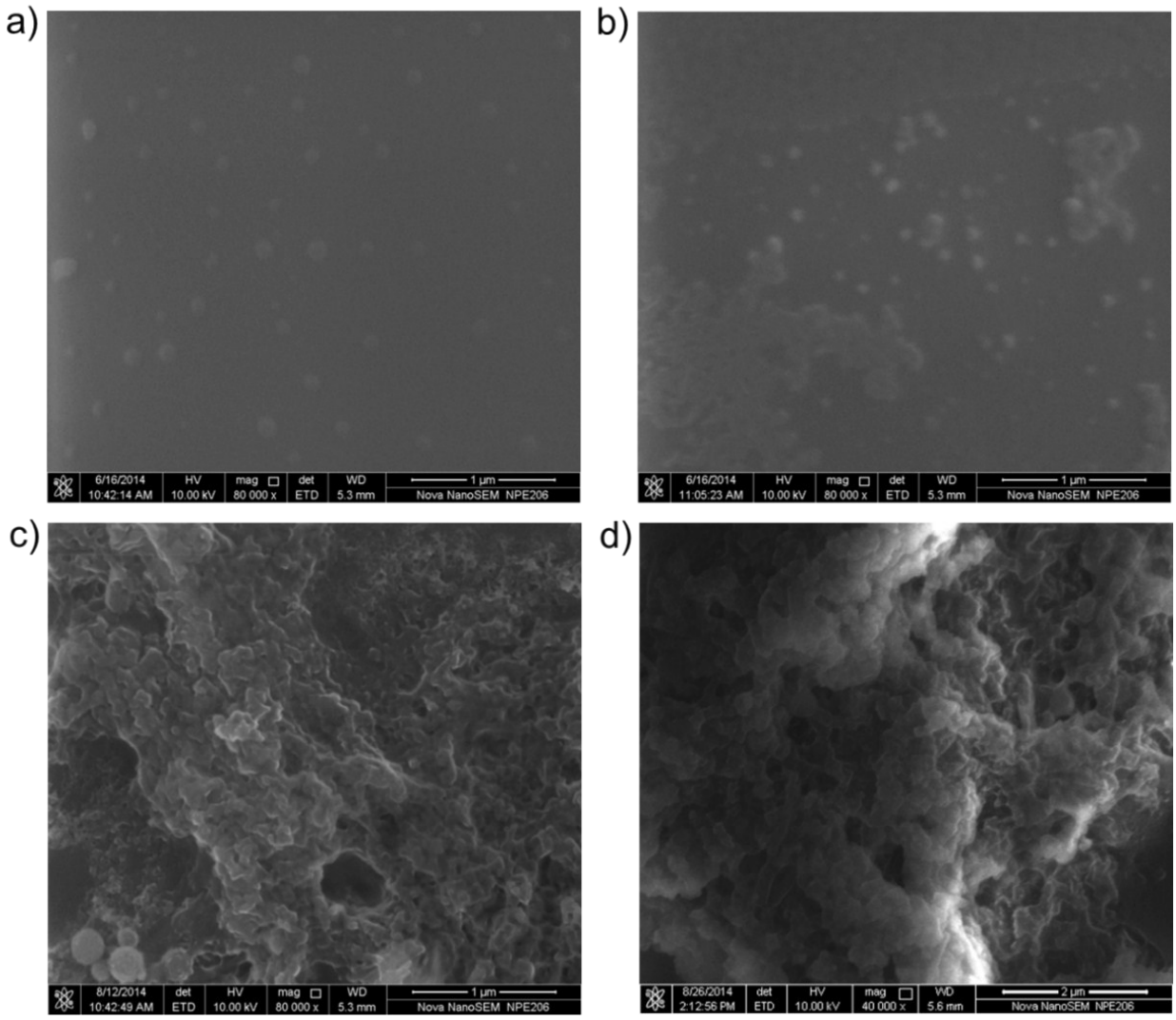


Fig. S9 SEM images of **G8/β-CD-X-DNA** nanoparticles (1:2 μM, a & b) and additional SEM images of **G8/β-CD-X-DNA** hydrogel at 1:2 mM (c & d).

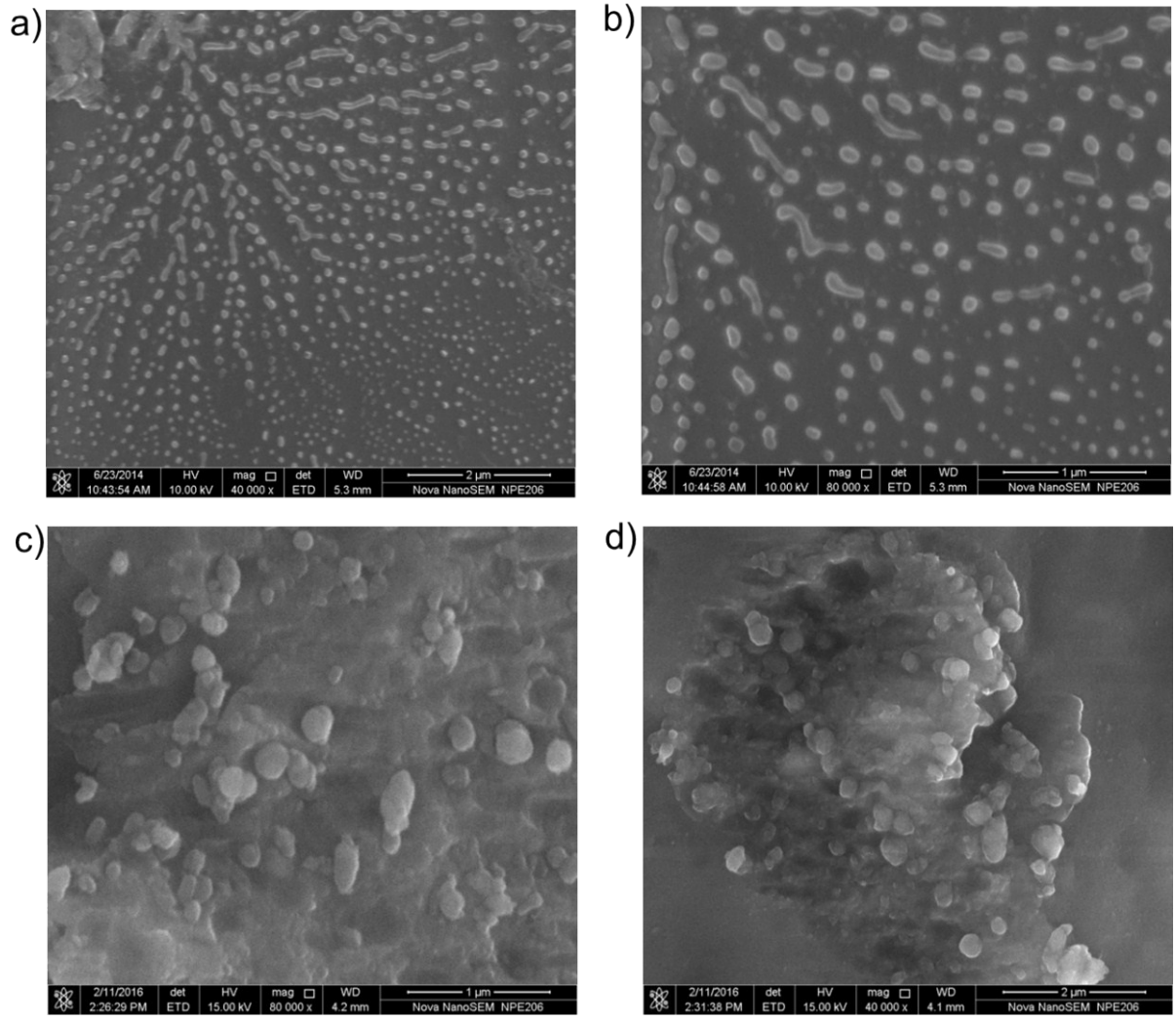


Fig. S10 SEM images of **G8/β-CD-Y-DNA** nanoparticles (1:2 μM, a & b) and SEM images of **G8/β-CD-Y-DNA** hydrogel at 1:2 mM (c & d).

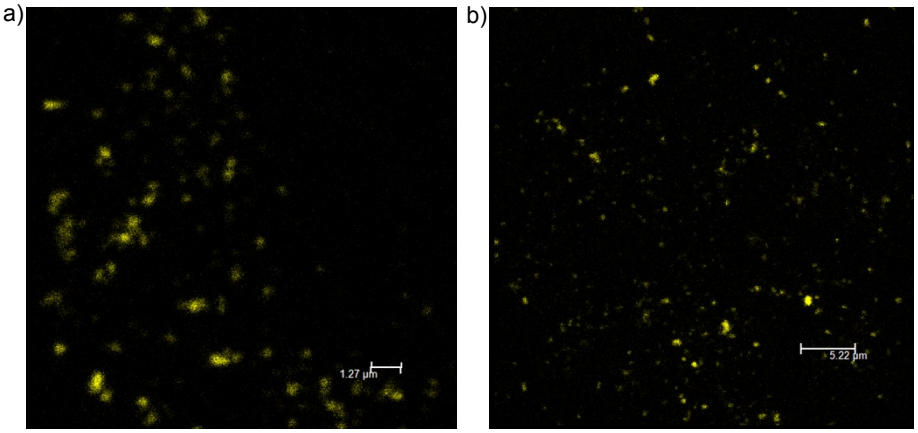


Fig. S11 Confocal microscopic images of (a) **G8/X-DNA-CY3** and **G8/Y-DNA-CY3** (b).

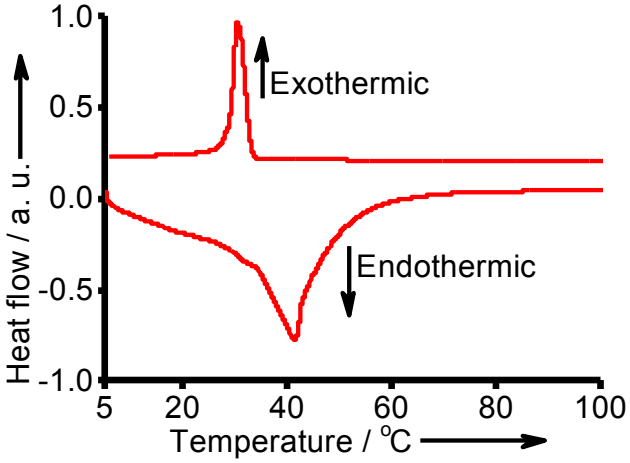


Fig. S12 DSC thermograms of **G8/β-CD-X-DNA** hydrogel (1:2 mM). The heating and cooling rates are 2 °C/min.

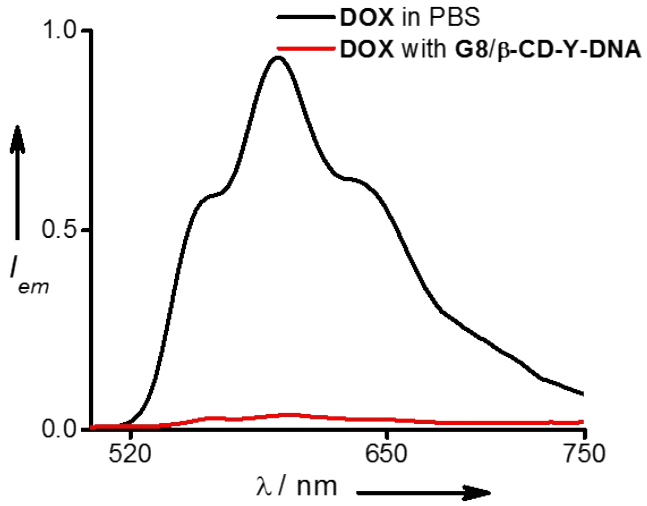


Fig. S13 Comparison of fluorescence spectra of **DOX** encapsulated in the **G8/β-CD-Y-DNA** nanogel network and free **DOX** (λ_{ex} = 470 nm).

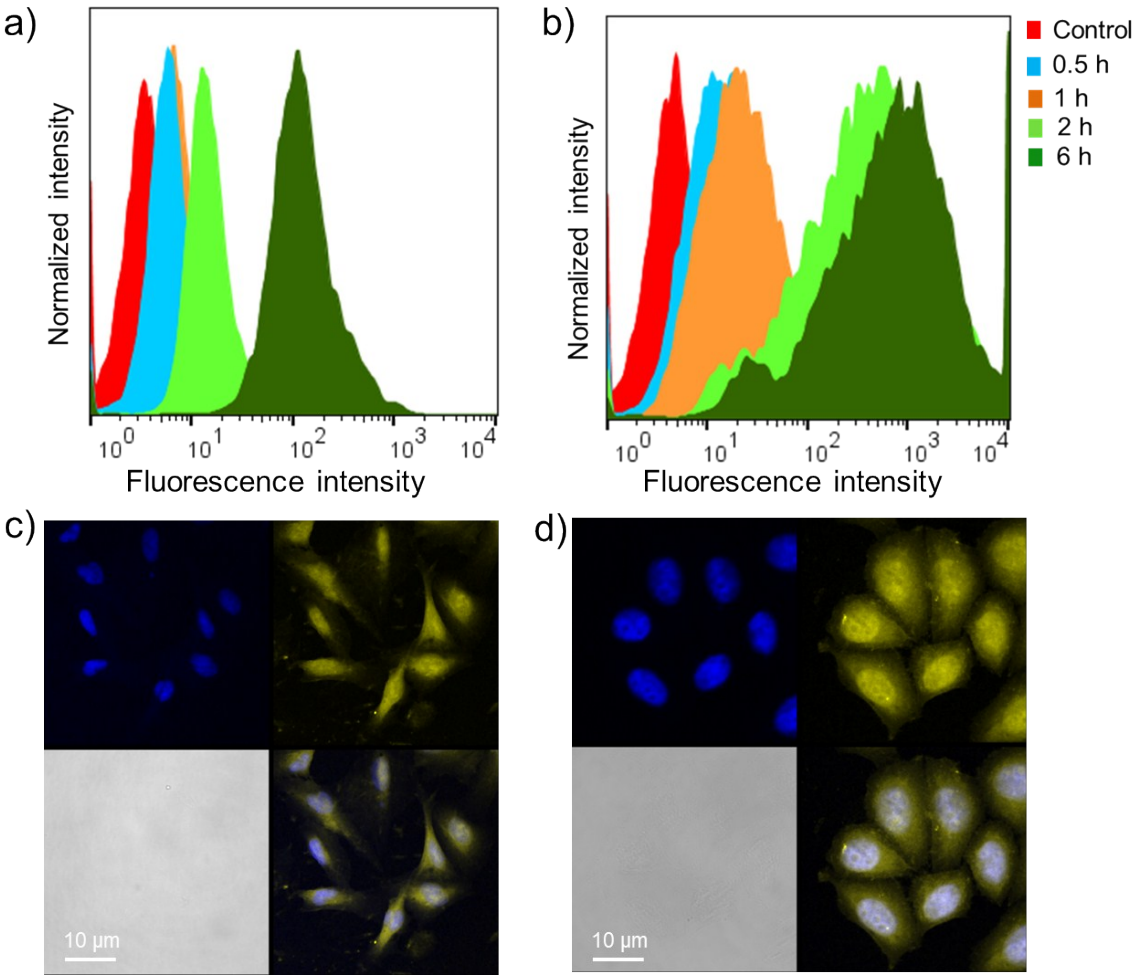


Fig. S14 Flow cytometry analyses to monitor the binding of **G8/β-CD-Y-DNA** nanogel with (a) A549 and (b) HeLa cell lines at different time intervals. CLSM analyses of (c) A549 and (d) HeLa cells after their incubation with **G8/β-CD-Y-DNA** nanogel for 24 h.

Calculation of **DOX** encapsulation efficiency with **G8/β-CD-X-DNA** nanogel

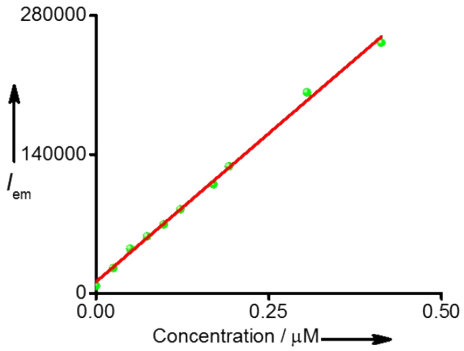


Fig. S15 Curve correlating the **DOX** concentration with its fluorescence intensity.

Amount of initial **G8/β-CD-X-DNA** nanogel concentration = 200 nM

Amount of initial **DOX** concentration = 5660 nM

Amount of **DOX** collected after centrifugation = 277 nM

Amount of encapsulated **DOX** with **G8/β-CD-X-DNA** =

$$\frac{[Initial\ DOX - Free\ DOX\ after\ centrifugation]}{Intial\ concentration} X\ 100$$

$$= \frac{[5660\ nM - 277\ nM]}{5660\ nM} X\ 100$$

= 95.1%

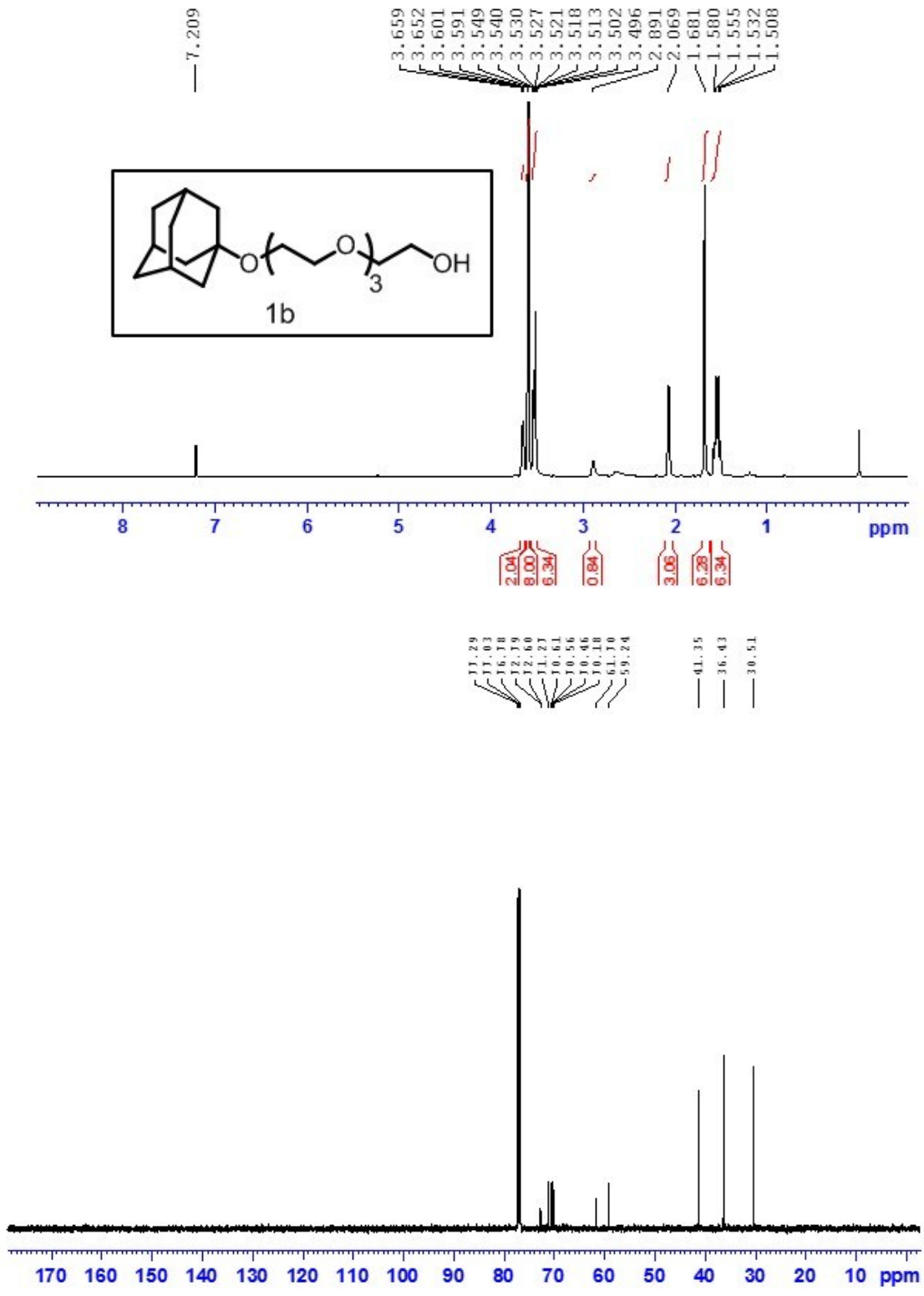


Fig. S16 ^{1}H (above) and ^{13}C (below) spectra of **1b**.

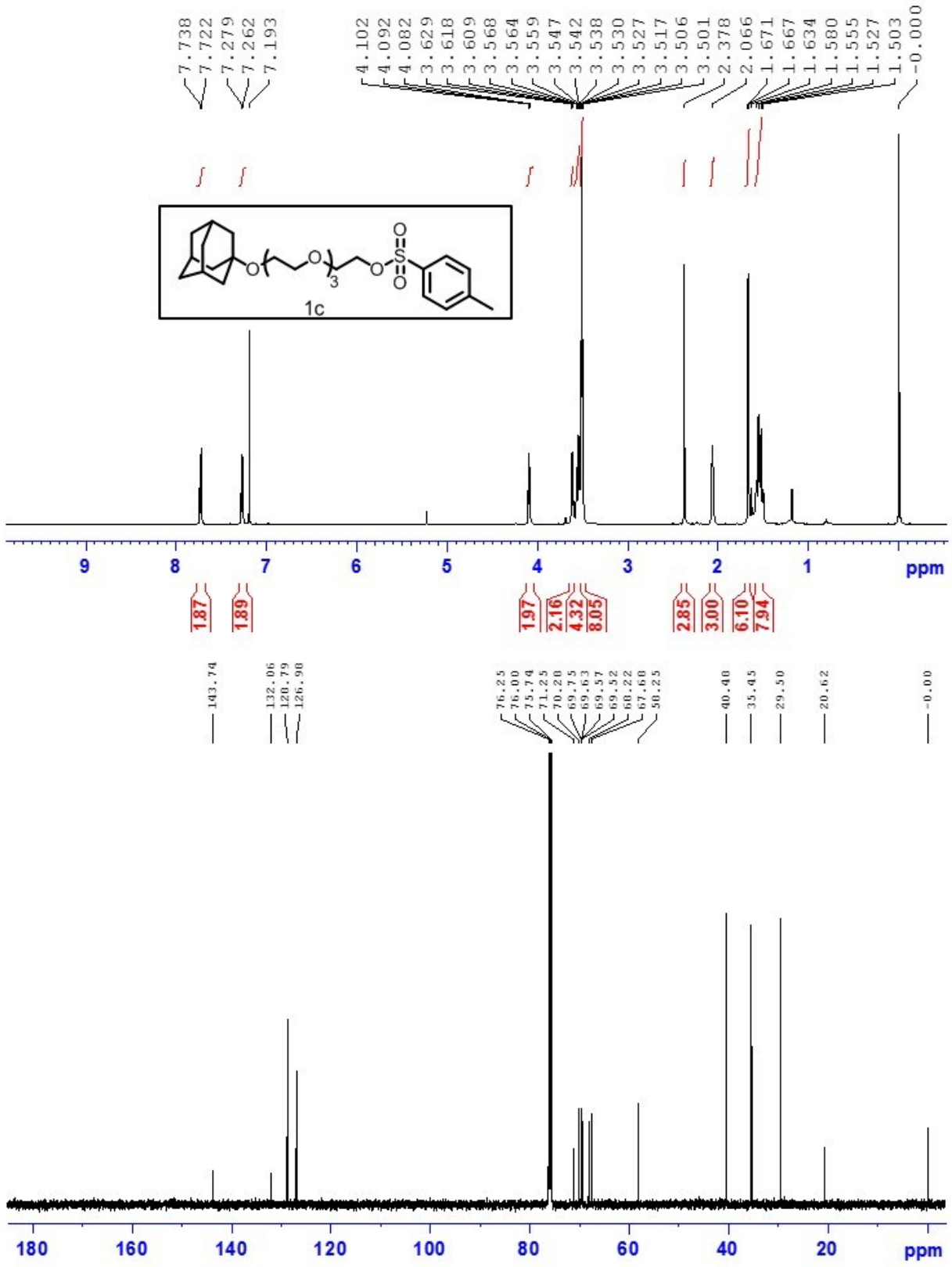


Fig. S17 ^{1}H (above) and ^{13}C (below) spectra of **1c**.

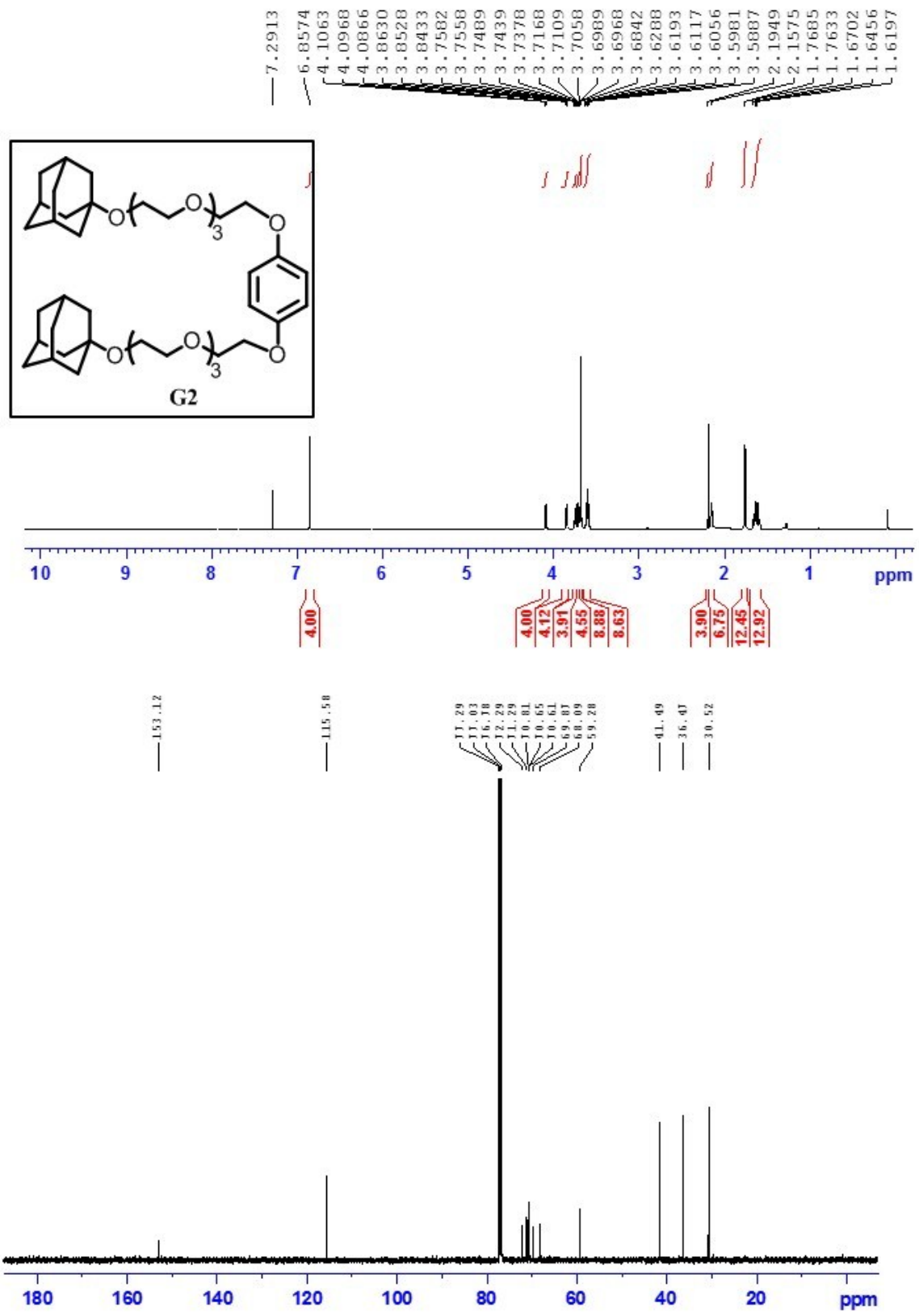


Fig. S18 ^{1}H (above) and ^{13}C (below) spectra of **G2**.

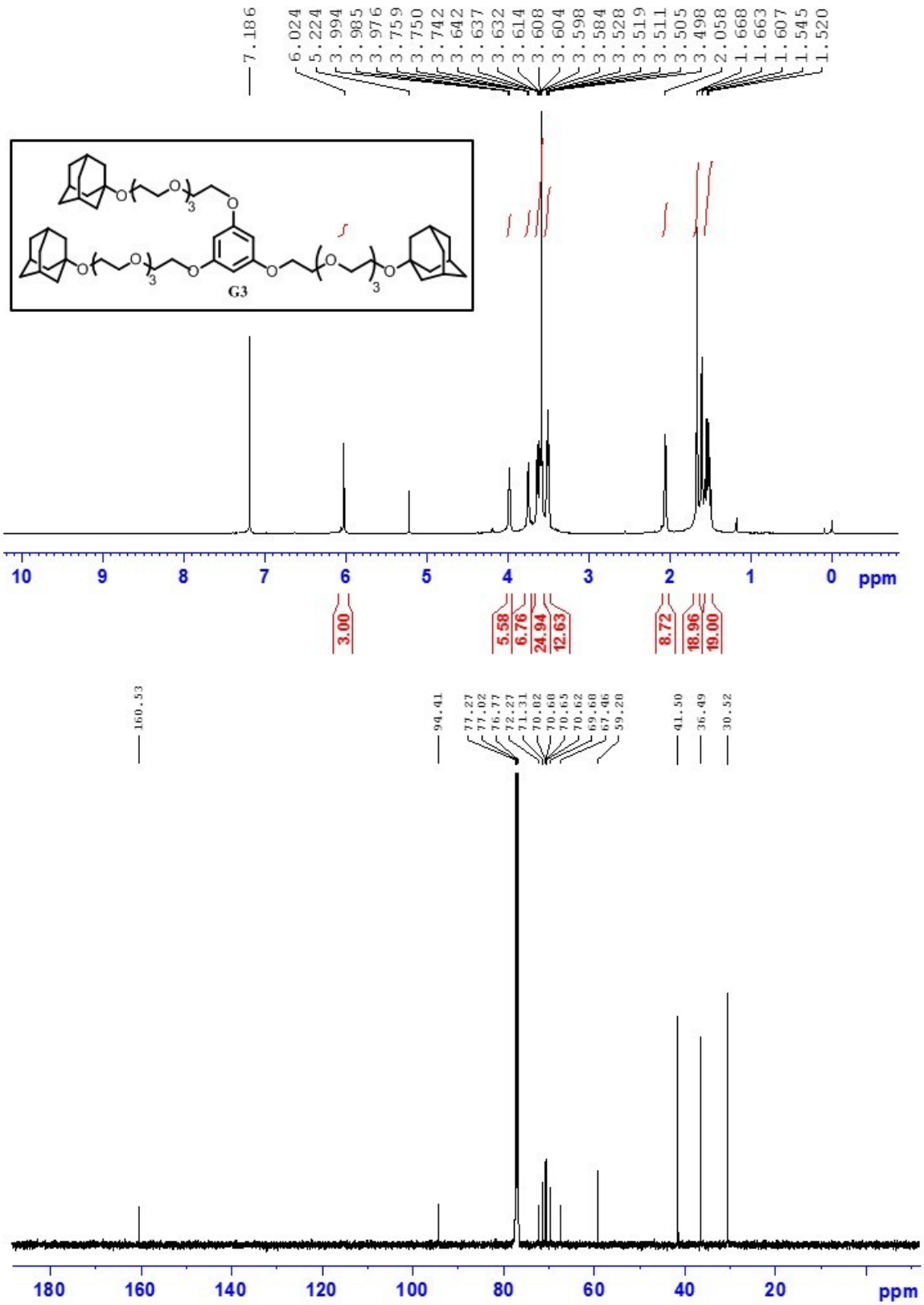


Fig. S19 ^{1}H (above) and ^{13}C (below) spectra of **G3**.

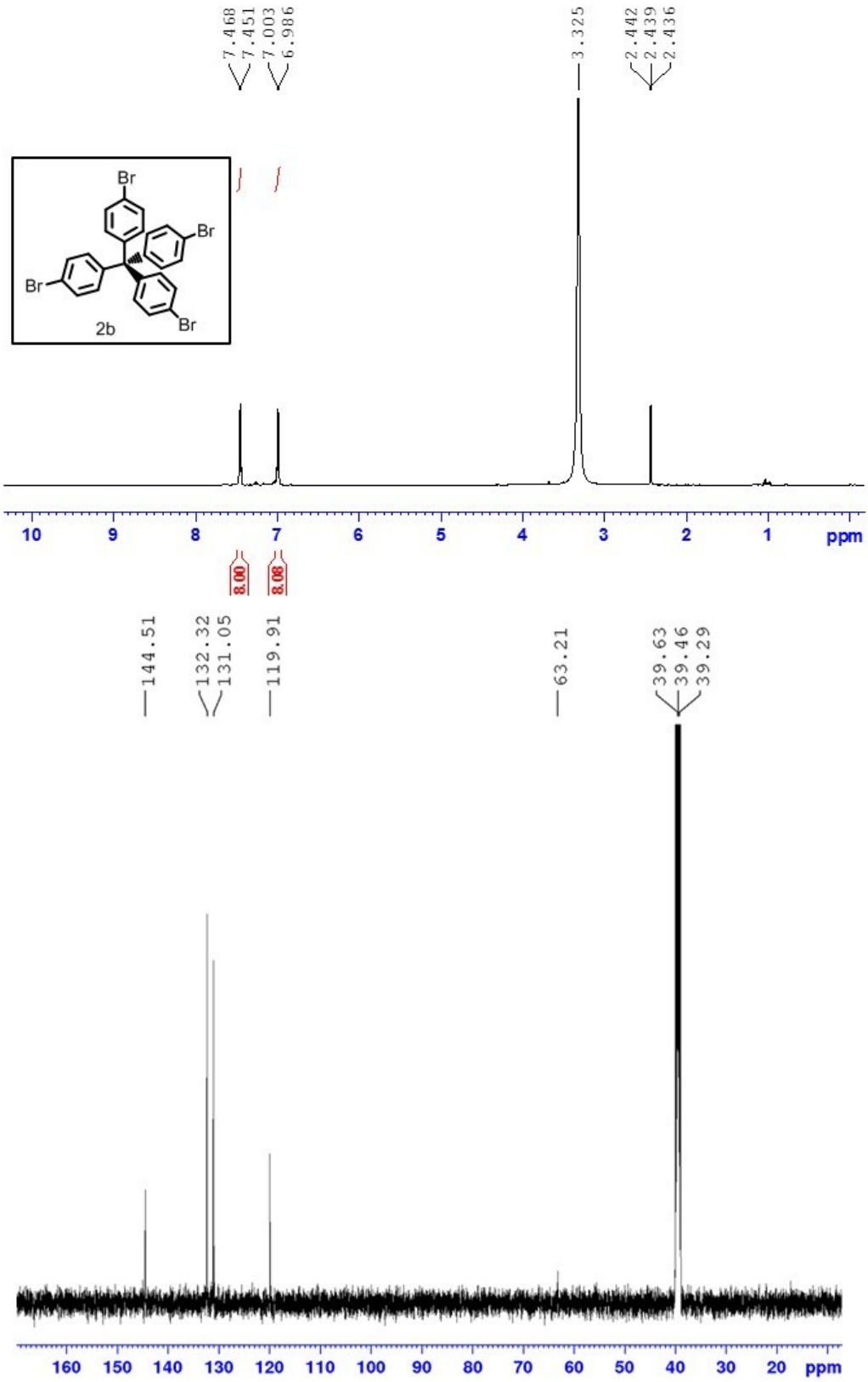


Fig. S20 ^{1}H (above) and ^{13}C (below) spectra of **2b**.

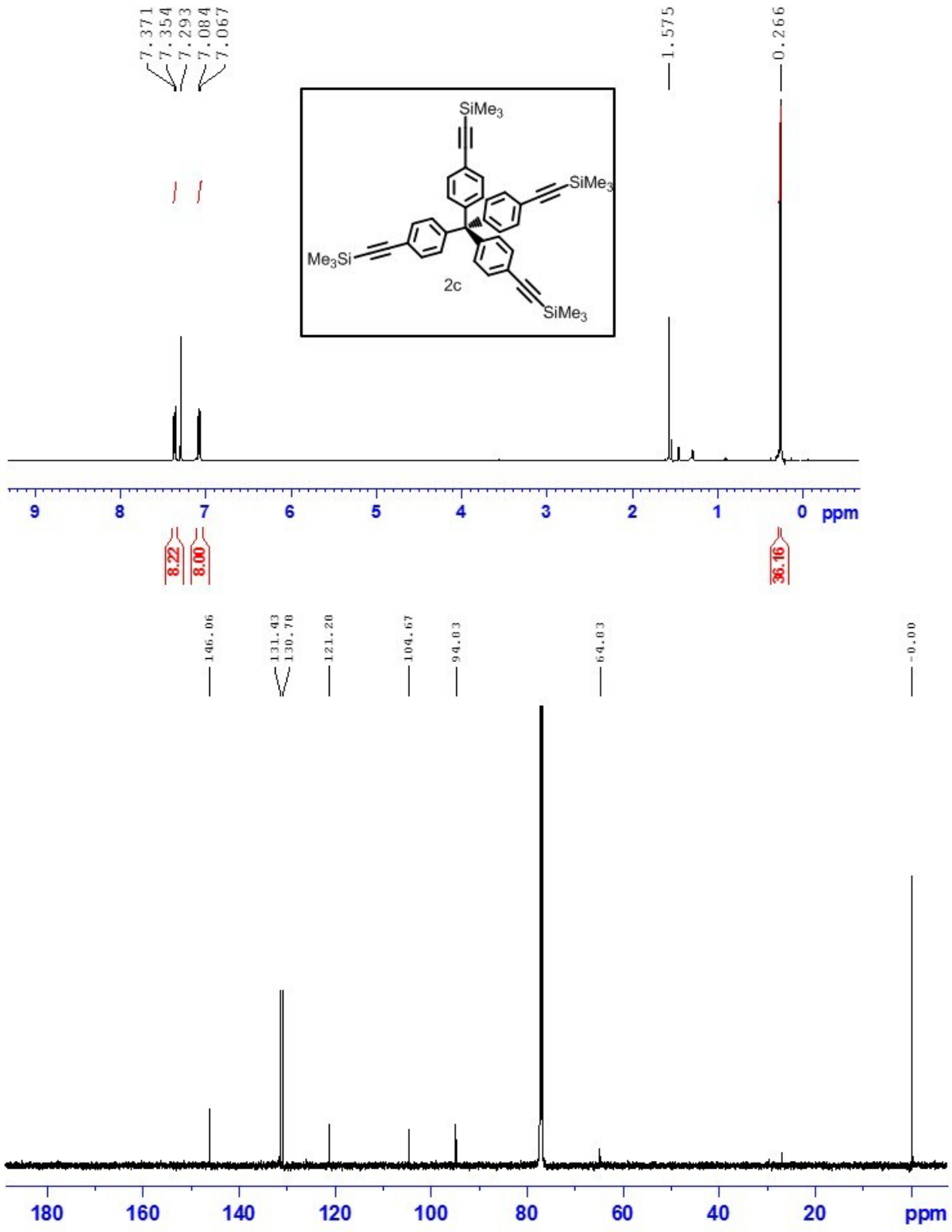


Fig. S21 ^{1}H (above) and ^{13}C (below) spectra of **2c**.

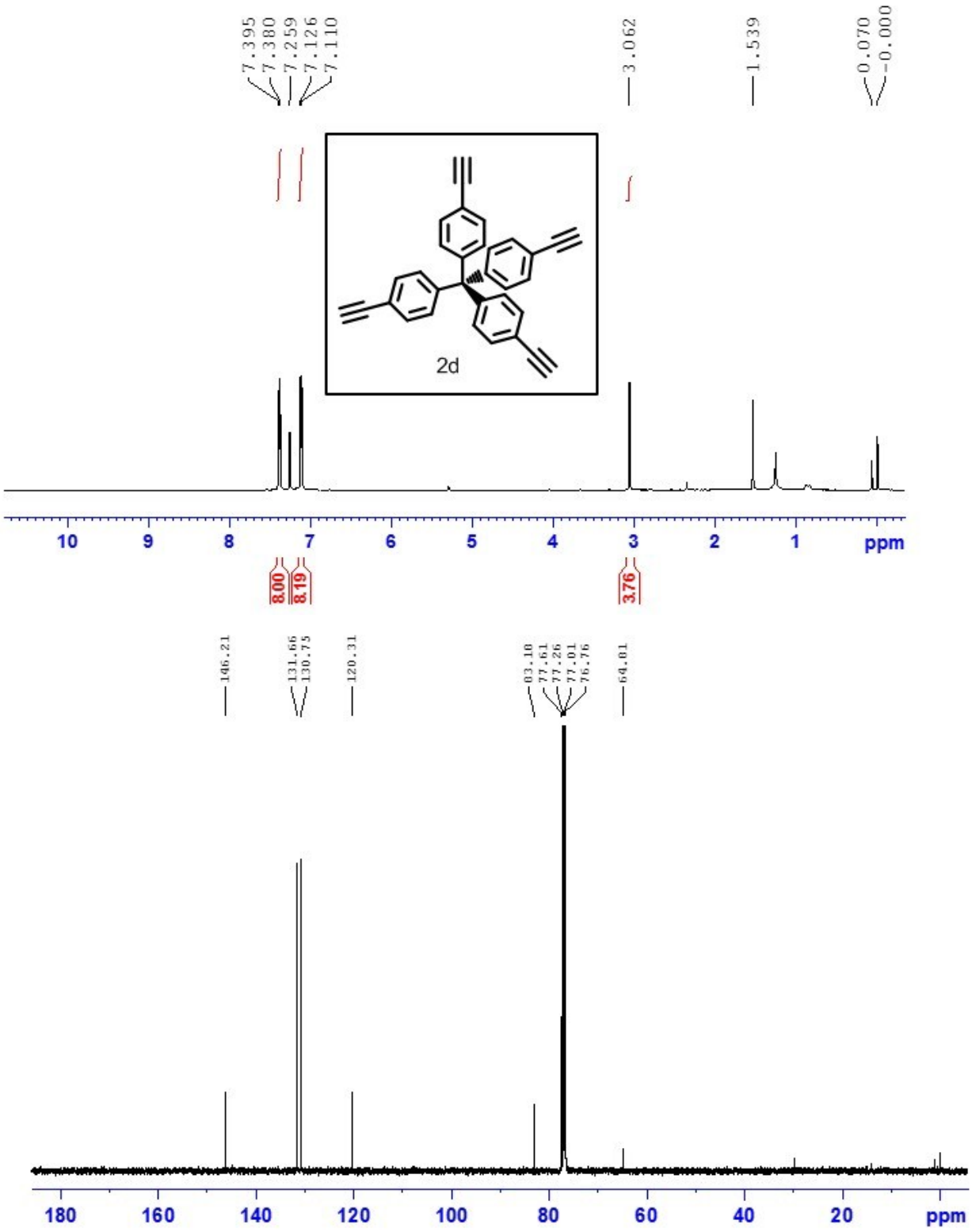


Fig. S22 ^{1}H (above) and ^{13}C (below) spectra of **2d**.

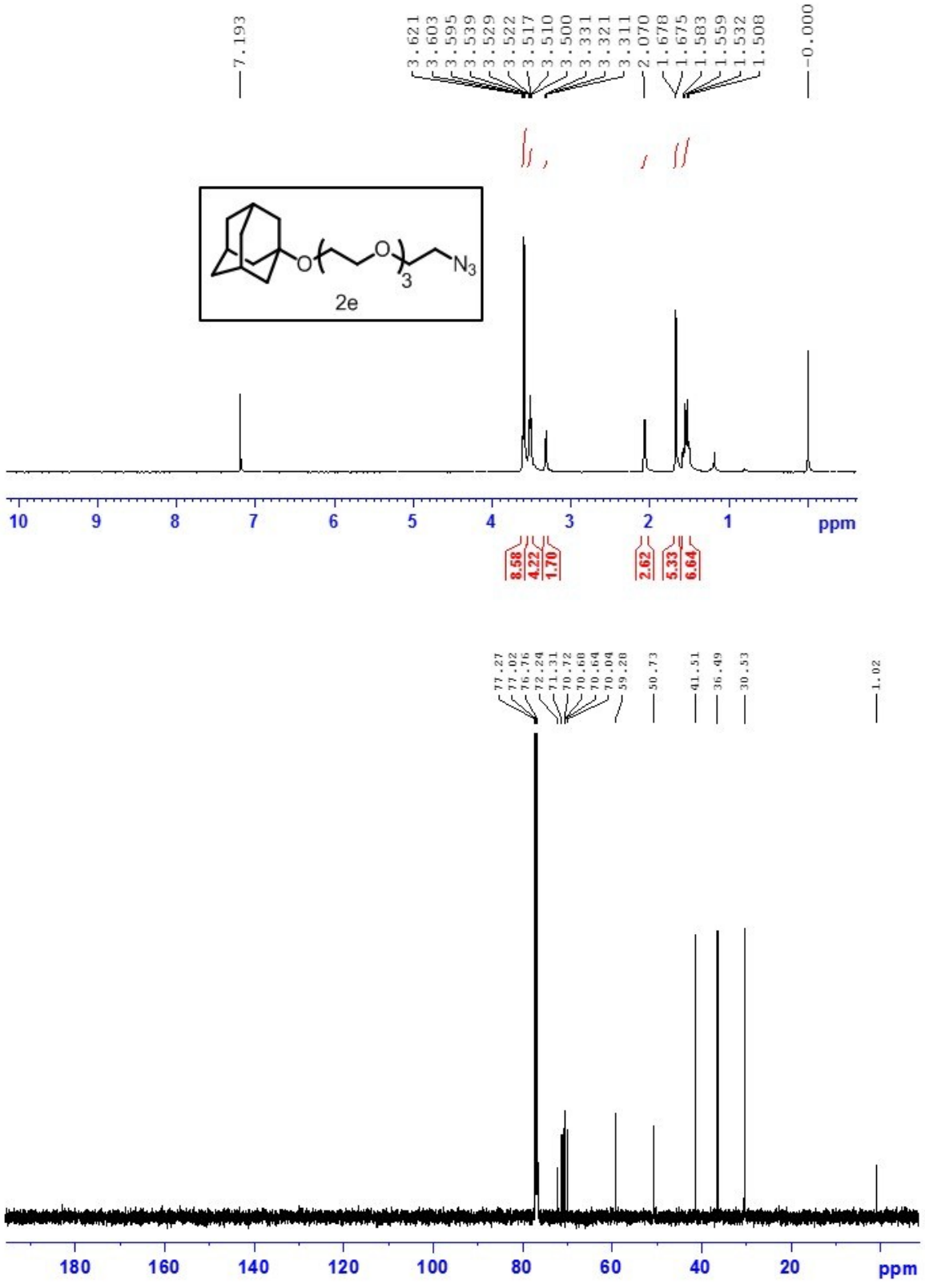


Fig. S23 ^{1}H (above) and ^{13}C (below) spectra of **2e**.

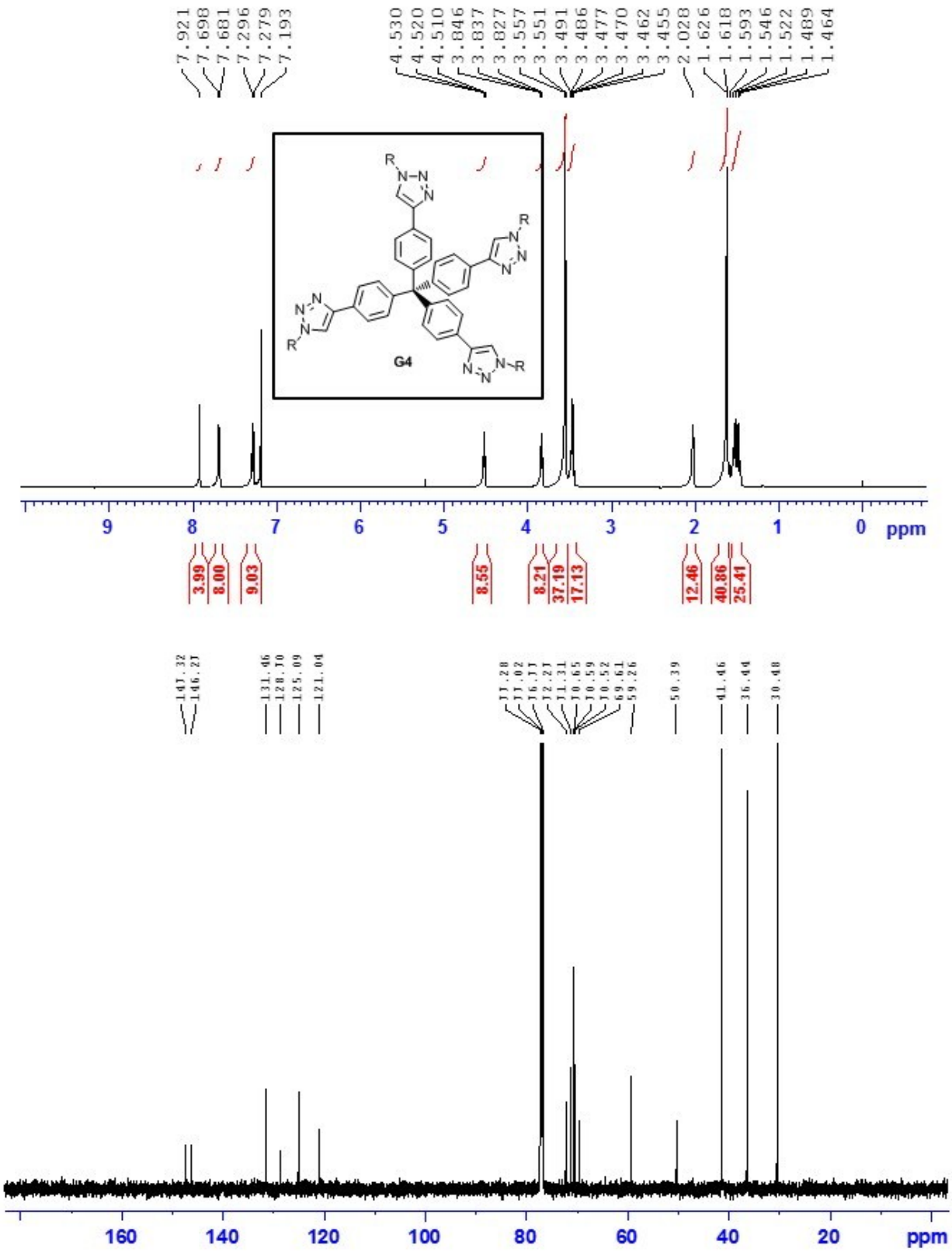


Fig. S24 ^{1}H (above) and ^{13}C (below) spectra of **G4**.

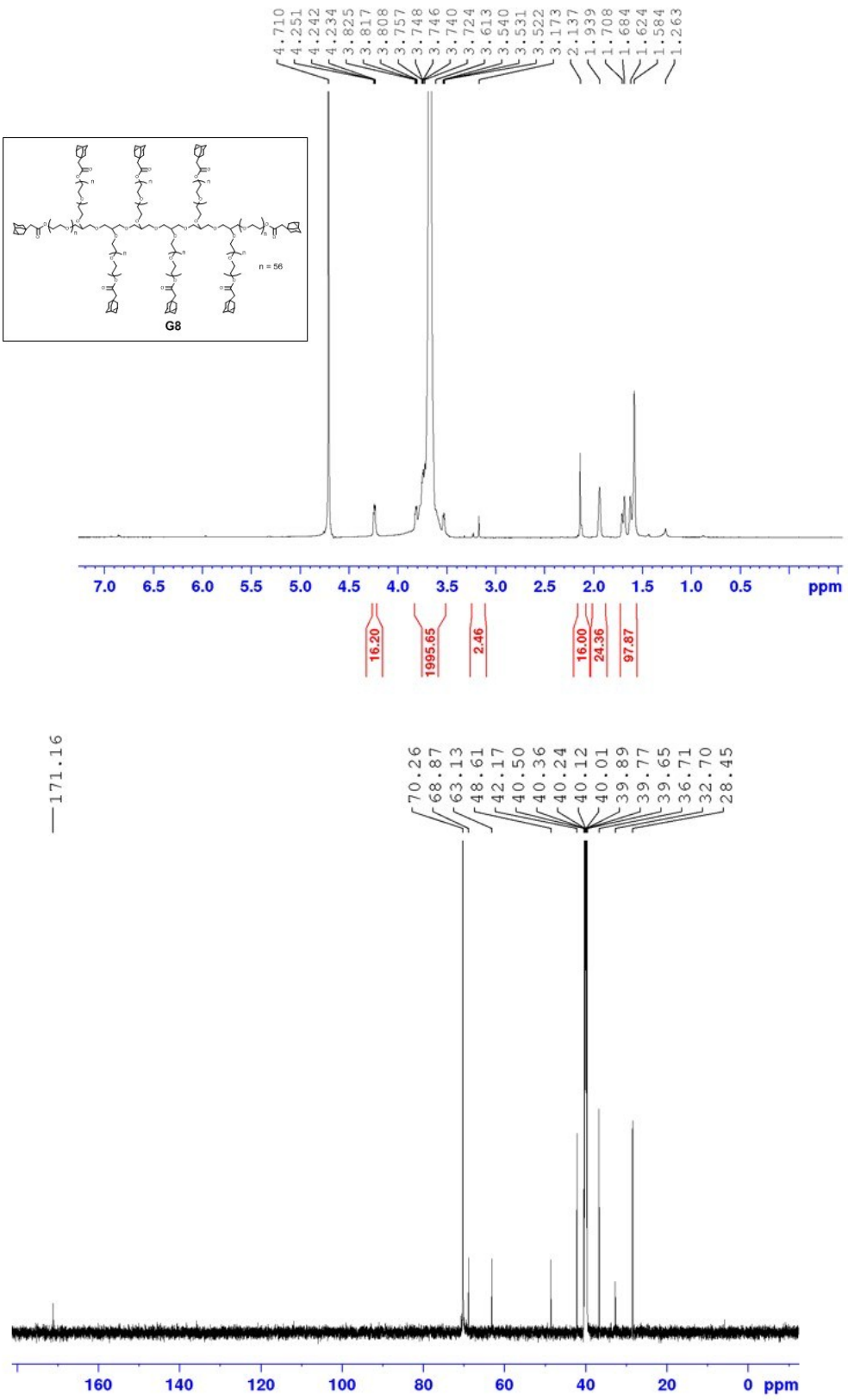


Fig. S25 ^{1}H (above) and ^{13}C (below) spectra of **G8**.

References:

S1. Z.-X. Zhang, K. L. Liu and J. Li, *Angew. Chem., Int. Ed.*, 2013, **52**, 6180–6184.