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Supplementary Data

Dendrimer-based Micelles as Cyto-compatible Nanocarriers

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1. Experimental Section

1.1. Materials

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich Chemicals and solvents were obtained from Spectrochem Pvt. Ltd., India. Curcumin and Nile red used in the encapsulation studies were procured from Fluka Chemie GmbH. The cells were purchased from the DSMZ (The Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany). The progress of the reactions was monitored using pre-coated TLC plates (Merck silica gel 60 F254) and the spots on TLC were visualized by staining in ceric solution. Silica gel (100-200 mesh) was used for the purification of the compounds through column chromatography. The encapsulation study and physico-chemical characterization were carried out in fresh Milli-Q water. The higher generation dendrimers were purified by dialysis using benzoylated dialysis membrane (MW cut off size 1200-2000 Da) procured from Sigma-Aldrich Chemicals.

1.2. Cytotoxicity Studies

The cytotoxicity study of the dendrimers was carried out at a concentration of 1, 0.5, and 0.1 mg/mL after 72 h using CellTiter 96® AQ_{ueous} one solution cell proliferation assay from Promega (Mannheim, Germany) according to the manufacturer's instructions. In short, A549 cells were seeded in a 96-well plate (4,000 cells/well in 90 µL cell culture medium) and cultured overnight at 37 °C before adding the sample substances (10 µL) dissolved in PBS in serial dilutions (1, 0.5, 0.1 mg/mL). For background subtraction, wells containing no cells but only samples were used. Cells were incubated for 72 h at 37 °C before the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) solution (20 µL) was added. After an incubation period of 2 h and 30 min, absorbance was measured at a wavelength of 490 nm and a reference wavelength of 630 nm with a Tecan plate reader (Infinite pro200, TECAN-reader Tecan Group Ltd., Männedorf, Switzerland). The measurements were done in triplicates and repeated three times. The cell viability was calculated by setting the non-treated control to 100% and the non-cell control to 0% by subtracting the background using Microsoft Excel 2013. GraphPad Prism (5.01) served for data visualization.

1.3. Synthetic Procedure

*General procedure A: synthesis of acetyl-protected G1/G2 dendrimers (**3a-4a**/**6a-7a**) by click reaction*

To a solution of compound **2**/**5** (150 mg, 1 equivalent, 0.62/0.18 mmol) and 2-azidopropane-1,3-diyl diacetate (3/6 equivalent, 1.87/1.1 mmol) or 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate (3/6 equivalent, 1.87/1.1 mmol) in a THF/H_2O mixture (3:1, 8/12 mL), sodium ascorbate (0.8/1.6 equivalent, 0.5/0.3 mmol) and $CuSO_4 \cdot 5H_2O$ (0.3/0.6 equivalent, 0.19/0.11 mmol) were added. The resulting mixture was stirred at 45 °C for 18-24 h. On completion of the reaction, THF was removed under reduced pressure with rotary evaporator, and the mixture was extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with an aqueous ammonia solution (2 x 50 mL), saturated aq. NH_4Cl (2 x 50 mL), and brine solution (2 x 50 mL). The resulting solution was then filtered through celite, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product so obtained was purified by column chromatography ($CHCl_3$/MeOH) affording the desired *O*-acetyl derivative of G1/G2 dendrimer (**3a**-**4a**/**6a**-**7a**).

Acetyl-protected G1 dendrimer **3a**

Obtained as viscous liquid in 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73 (s, 3H, H-5''), 6.26 (s, 3H, H-2), 5.12 (s, 6H, H-1'), 5.07-5.05 (m, 3H, H-1'''), 4.54-4.45 (m, 12H, H-2'''), 1.99 ppm (s, 18H, 6 x -OAc); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.1, 159.9, 143.8, 122.5, 95.0, 62.4, 61.8, 58.5, 20.5 ppm; IR (film): ν_{max} = 2952, 1740, 1222, 1154, 1044 cm^{-1}.

Acetyl-protected G1 dendrimer **4a**

Obtained as a white solid in 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97 (s, 3H, H-5''), 6.30 (s, 3H, H-2), 5.94 (d, 3H, *J* = 9.16 Hz, H-a), 5.51-5.40 (m, 6H, H-b & H-c), 5.27 (t, 3H, *J* = 9.16 Hz, H-d), 5.16 (s, 6H, H-1'), 4.33-4.26 (m, 3H, H-f), 4.16-4.13 (m, 3H, H-f), 4.04-4.01 (m, 3H, H-e), 2.08-2.03 (m, 27H, 9 x -OAc), 1.86-1.85 (m, 9H, 3 x -OAc); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.4, 169.8, 169.3, 168.8, 159.8, 144.4, 121.4, 95.1, 85.5, 74.9, 72.5, 70.2, 67.6, 61.6, 61.4, 20.6, 20.5, 20.4, 20.0. IR (film): ν_{max} = 1741, 1596, 1456, 1367, 1213, 1163, 1036 cm^{-1}.

Acetyl-protected G2 dendrimer **6a**

Obtained as an off-white solid in 75% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.82 (s, 3H, H-5''), 7.60 (s, 6H, H-c), 6.28 (s, 3H, H-2), 5.08 (s, 6H, H-1'), 5.05-5.02 (m, 6H, H-d), 4.93-4.88 (m, 3H, H-1'''), 4.59 (s, 12H, H-a), 4.53-4.44 (m, 24H, H-e), 3.96-3.89 (m, 12H, H-2'''), 1.99 (s, 36H, 12 x -

OAc); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.1, 160.1, 144.2, 143.2, 123.3, 122.4, 95.1, 68.8, 64.5, 62.4, 62.0, 60.6, 58.5, 20.5; IR (film): ν_{max} = 2923, 1743, 1369, 1226, 1045 cm^{-1}.

Acetyl-protected G2 dendrimer **7a**

Obtained as a white solid in 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97-7.83 (m, 9H, H-5" & H-c), 6.31 (s, 3H, H-2), 5.98 (t, 6H, *J* = 9.16 Hz, H-d), 5.51-5.30 (m, 18H, H-e – H-g), 5.12 (s, 6H, H-1'), 4.99-4.96 (m, 3H, H-1'"), 4.65 (s, 12H, H-2'"), 4.33-4.29 (m, 6H), 4.17-4.06 (m, 12H), 3.93-3.92 (m, 12H) (H-a, H-h & H-i), 2.07-2.03 (m, 54H, 18 x -OAc), 1.82 (s, 18H, 6 x -OAc); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.3, 169.7, 169.3, 168.7, 159.9, 144.7, 143.0, 123.2, 121.4, 94.8, 85.3, 74.7, 72.3, 70.1, 68.2, 67.5, 64.2, 64.1, 61.6, 61.3, 60.0, 20.4, 20.4, 20.3, 19.8; IR (film): ν_{max} = 1746, 1596, 1456, 1367, 1213, 1149, 1036 cm^{-1}.

General procedure B: Synthesis of G1/G2 dendrimers (**3-4**/**6-7**)

In a 50 mL round-bottom, an aqueous solution of sodium hydroxide (1N aq. NaOH, 200/400 μL) was added to a solution of acetylated precursor of G1/G2 dendrimer (**3a-4a**/**6a-7a**) (100 mg) in MeOH (2.5 mL). The resulting mixture was stirred at room temperature for 4 to 6 h. On completion of the reaction, the pH of the solution was adjusted to 6-7 with an ion-exchange resin (Dowex 50WX8). After filtration, the solvent was removed under reduced pressure with a rotary evaporator and the residue was lyophilized to furnish the desired G1/G2 dendrimer (**3-4**/**6-7**).

G1 dendrimer **3**

Obtained as a white solid in 89% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (s, 3H, H-5"), 6.33 (s, 3H, H-2), 5.03 (s, 6H, H-1'), 4.58-4.52 (m, 3H, H-1'"), 3.74-3.73 (m, 12H, H-2'"); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 160.0, 141.8, 124.1, 94.2, 64.9, 61.2, 60.5; IR (neat): ν_{max} = 3298, 1597, 1564, 1459, 1155, 1053; HRMS: Calculated for $C_{24}H_{33}N_9O_9$ $[M+H]^+$ 592.2479, found 592.2478.

G1 dendrimer **4**

Obtained as a white solid in 86% yield; ^{1}H NMR (400 MHz, D_2O): δ = 8.02 (s, 3H, H-5"), 6.02 (s, 3H, H-2), 5.51 (d, 3H, *J* = 9.16 Hz, H-a), 4.81 (s, 6H, H-1'), 3.78 (t, 3H, *J* = 9.16 Hz, H-c), 3.68-3.65 (m, 3H, H-b), 3.56-3.48 (m, 9H, H-d – H-f), 3.42-3.38 (m, 3H, H-f), ; ^{13}C NMR (100.5 MHz, D_2O): δ = 159.4, 143.3, 124.3, 95.3, 87.5, 78.8, 75.9, 72.3, 68.9, 61.0, 60.4; IR (neat): ν_{max} = 3294, 1597, 1457, 1370, 1154, 1042 cm^{-1}; HRMS: calculated for $C_{33}H_{45}N_9O_{18}$ $[M+H]^+$ 856.2955, found 856.3000.

G2 dendrimer **6**

Obtained as an off-white solid in 94% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.23 (s, 3H, H-5''), 7.96 (s, 6H, H-c), 6.34 (s, 3H, H-2), 5.02-4.95 (m, 9H, H-1''' & H-1'), 4.52-4.49 (m, 6H, H-d), 4.46 (s, 12H, H-a), 3.89-3.79 (m, 12H, H-2'''), 3.71-3.70 (m, 24H, H-e); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 159.9, 142.7, 142.0, 124.3, 123.4, 94.2, 68.8, 64.7, 63.7, 61.2, 60.4, 59.9; IR (neat): ν_{max} = 3273, 1595, 1559, 1411, 1151, 1048 cm^{-1}; HRMS: calculated for $C_{60}H_{87}N_{27}O_{21}$ $[M+H]^+$ 1522.6648, found 1522.6616.

G2 dendrimer **7**

Obtained as a white solid in 90% yield; ^{1}H NMR (400 MHz, D_2O): δ = 7.81-7.80 (m, 9H, H-5'' & H-c), 6.06 (s, 3H, H-2), 5.46 (d, 6H, *J* = 9.16 Hz, H-d), 4.85 (s, 6H, H-1'), 4.79-4.76 (m, 3H, H-1'''), 4.34-4.24 (m, 12H), 3.77-3.64 (m, 24H), 3.55-3.36 (m, 24H) (H-2''', H-a, H-e – H-i); ^{13}C NMR (100.5 MHz, D_2O): δ = 159.3, 143.7, 142.8, 124.7, 124.1, 95.4, 87.4, 78.8, 75.8, 72.2, 68.8, 68.2, 63.1, 60.9, 60.3; IR (neat): ν_{max} = 3279, 1597, 1455, 1362, 1232, 1043 cm^{-1}; HRMS: calculated for $C_{78}H_{111}N_{27}O_{39}$ $[M+Na]^+$ 2072.7425, found 2072.7488.

Synthesis of 1,3,5-tris((1-(1,3-bis(prop-2-yn-1-yloxy)propan-2-yl)-1*H*-1,2,3-triazol-4-yl)methoxy) benzene (**5**)

To a stirred solution of G1 dendrimer **3** (100 mg, 0.17 mmol) and NaOH (162 mg, 4.05 mmol) in water (2 ml) and TBAI (12.5 mg, 20 mol% of G1), propargyl bromide (482 mg, 4.08 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 48 h. Progress of the reaction was monitored by TLC. On completion of the reaction, ethyl acetate (10 mL) was added and the resulting solution was transferred into a separation funnel containing ethyl acetate (20 mL) and a saturated aqueous solution of NH_4Cl (30 mL). The organic layer was washed with saturated aq. NH_4Cl (2 × 20 mL), water (2 × 20 mL), and brine (10 mL). The organic layer was then dried over Na_2SO_4 and concentrated under reduced pressure. The purification of the crude product so obtained using column chromatography ($CHCl_3$/MeOH) afforded the desired compound **5** in 40% yield as a viscous brown liquid.

Obtained as viscous brown liquid in 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 (s, 3H, H-5''), 6.27 (s, 3H, H-2), 5.11 (s, 6H, H-1'), 4.97-4.92 (m, 3H, H-1'''), 4.12 (d, 12H, *J* = 2.44 Hz, H-a), 3.97-3.96 (m, 12H, H-2'''), 2.42 (t, 6H, *J* = 2.44 Hz, H-c); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ =

160.3, 143.5, 123.3, 95.2, 78.8, 75.5, 68.5, 62.2, 60.5, 58.7; IR (film): ν_{max} = 1742, 1218, 1044, 743, 665 cm^{-1}; HRMS: calculated for $C_{42}H_{45}N_9O_9$ $[M+H]^+$ 820.3413, found 820.3414.

Synthesis of acetyl-protected alkylated G1/G2 dendrimers (**13a**-**14a**/**16a**-**17a**)

The compounds **13a-14a/16a-17a** were obtained by click reaction of compounds **12**/**15** with 2-azidopropane-1,3-diyl diacetate and 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate respectively by following the general procedure A.

Acetyl-protected alkylated G1 dendrimer **13a**

Obtained as a semi-solid in 88% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (s, 3H, H-5'''), 6.01 (s, 3H, H-2), 5.07-5.01 (m, 3H, H-a), 4.61 (s, 6H, H-1''), 4.54-4.45 (m, 12H, H-b), 3.86 (t, 6H, *J* = 6.7 Hz, H-1'), 3.51 (t, 6H, *J* = 6.7 Hz, H-6'), 2.02 (s, 18H, 6 x -OAc), 1.76-1.69 (m, 6H, H-2'), 1.65-1.59 (m, 6H, H-5'), 1.45-1.38 (m, 12H, H-3' & H-4'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.3, 161.3, 145.8, 122.0, 94.0, 71.0, 68.0, 64.5, 62.7, 58.6, 29.7, 29.3, 26.1, 20.7. IR (film): ν_{max} = 2954, 1744, 1600, 1565, 1228, 1150, 1040 cm^{-1}.

Acetyl-protected alkylated G1 dendrimer **14a**

Obtained as a white solid in 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (s, 3H, H-5'''), 6.04 (s, 3H, H-2), 5.87 (d, 3H, *J* = 9.16 Hz, H-a), 5.47-5.39 (m, 6H, H-b & H-c), 5.24 (t, 3H, *J* = 9.16 Hz, H-d), 4.62 (s, 6H, H-1''), 4.32-4.27 (m, 3H), 4.16-4.12 (m, 3H), 4.02-3.97 (m, 3H, H-e), 3.89 (t, 6H, *J* = 6.10 Hz, H-1'), 3.51 (t, 6H, *J* = 6.10 Hz, H-6'), 2.08, 2.07, 2.03, 1.87 (4 s, 9H each, 12 x -OAc), 1.77-1.74 (m, 6H, H-2'), 1.65-1.61 (m, 6H, H-5'), 1.46-1.40 (m, 12H, H-3' & H-4'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 172.4, 171.9, 171.3, 170.8, 162.8, 148.1, 122.7, 95.6, 87.6, 77.0, 74.6, 72.7, 72.2, 69.8, 69.6, 66.0, 63.4, 31.5, 31.1, 27.8, 27.8, 22.6, 22.5, 22.5, 22.1. IR (neat): ν_{max} = 1746, 1594, 1459, 1367, 1211, 1159, 1035 cm^{-1}.

Acetyl-protected alkylated G2 dendrimer **16a**

Obtained as a semi-solid in 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.70-7.65 (m, 9H, H-5''', H-k), 6.03 (s, 3H, H-2), 5.11-5.07 (m, 6H, H-l), 4.88-4.84 (m, 3H, H-a), 4.62-4.61 (m, 18H), 4.56-4.49 (m, 24H), 3.89-3.81 (m, 18H), 3.53-3.38 (m, 30H), 2.05 (s, 36H, 12 x -OAc), 1.60-1.31 (m, 72H, H-2' – H-5' & H-d – H-g); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 169.9, 160.5, 145.0, 139.7, 123.2, 122.1, 93.5, 71.6, 71.3, 70.3, 70.0, 69.3, 68.0, 63.8, 63.3, 62.2, 61.9, 58.1, 52.2, 49.9, 29.2, 29.0, 28.9, 25.5, 25.4, 25.2, 20.1; IR (film): ν_{max} = 2944, 1738, 1598, 1561, 1198, 1148 cm^{-1}.

Acetyl-protected alkylated G2 dendrimer **17a**

Obtained as a white solid in 76% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.87-7.80 (m, 9H, H-5''' & H-k), 5.99 (s, 3H, H-2), 5.84 (d, 6H, *J* = 9.16 Hz, H-l), 5.44-5.37 (m, 12H), 5.22-5.17 (m, 6H), 4.87-4.84 (m, 3H, H-a), 4.56 (s, 18H, H-1'' & H-i), 4.26-4.23(m, 6H), 3.84 -3.78 (m, 10H), 3.46-3.35 (m, 39H), 2.03-1.98 (m, 54H, 18 x -OAc), 1.82 (s, 18H, 6 x -OAc), 1.70-1.20 (m, 72H, H-2' – H-5' & H-d – H-g); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.4, 169.8, 169.3, 168.8, 160.6, 146.0, 145.7, 121.0, 120.7, 93.5, 85.5, 79.9, 74.9, 74.1, 72.5, 71.4, 70.6, 70.5, 70.1, 69.9, 69.2, 67.7, 67.5, 64.1, 63.9, 61.4, 60.5, 57.9, 29.6, 29.4, 29.3, 29.2, 29.1, 25.8, 25.7, 20.6, 20.4, 20.1; IR (neat): ν_{max} = 1746, 1594, 1457, 1366, 1213, 1157, 1035 cm^{-1}.

Synthesis of alkylated G1 dendrimers (**13-14**/**16-17**)

The compounds **13-14** and **16-17** were obtained by deacetylation followed by the neutralization reaction of compounds **13a-14a** and **16a-17a** respectively, by following the general procedure B.

Alkylated G1 dendrimer **13**

Obtained as an off-white solid in 92% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.00 (s, 3H, H-5'''), 6.02 (s, 3H, H-2), 4.56-4.53 (m, 3H, H-a), 4.45 (s, 6H, H-1''), 3.86 (t, 6H, *J* = 6.7 Hz, H-1'), 3.76-3.75 (m, 12H, H-b), 3.43 (t, 6H, *J* = 6.7 Hz, H-6'), 1.66-1.61 (m, 6H, H-2'), 1.52-1.48 (m, 6H, H-5'), 1.40-1.32 (m, 12H, H-3' & H-4'); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 160.4, 143.4, 123.0, 93.3, 69.5, 67.3, 64.6, 63.4, 60.4, 29.0, 28.6, 25.4, 25.3; IR (neat): ν_{max} = 3352, 1596, 1561, 1452, 1150 cm^{-1}; HRMS: calculated for $C_{42}H_{69}N_9O_{12}$ $[M+H]^+$ 892.5144, found 892.5163.

Alkylated G1 dendrimer **14**

Obtained as a white solid in 93% yield; ^{1}H NMR (400 MHz, D_2O): δ = 7.93 (s, 3H, H-5'''), 5.81 (s, 3H, H-2), 5.46 (d, 3H, *J* = 9.16 Hz, H-a), 4.28 (s, 6H, H-1''), 3.76 (t, 3H, *J* = 9.16 Hz, H-c), 3.63-3.40 (m, 21H, H-b, H-d, H-e, H-1', H-6'), 3.21-3.16 (m, 6H, H-f), 1.36-1.02 (m, 24H, H-2' – H-5'); ^{13}C NMR (100.5 MHz, D_2O): δ = 160.6, 144.4, 123.8, 93.6, 87.5, 78.8, 76.0, 72.3, 70.6, 68.8, 67.7, 63.0, 60.4, 29.0, 25.6, 25.5; IR (neat): ν_{max} = 3325, 1593, 1459, 1383, 1229, 1159, 1041 cm^{-1}; HRMS: calculated for $C_{51}H_{81}N_9O_{21}$ $[M+H]^+$ 1156.5620, found 1156.5683.

Alkylated G2 dendrimer **16**

Obtained as an off-white solid in 90% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.04-7.94 (m, 9H, H-5''' & H-k), 6.00 (s, 3H, H-2), 4.79-4.76 (m, 3H, H-a), 4.59-4.54 (m, 6H, H-l), 4.48-4.43

(m, 18H), 3.90-3.71 (m, 42H), 3.40-3.27 (m, 30H), 1.74-1.22 (m, 72H, H-2' – H-5' & H-d – H-g); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ = 160.5, 143.5, 139.9, 124.8, 123.2, 93.5, 70.5, 69.5, 67.3, 64.7, 63.4, 60.6, 57.3, 52.5, 51.4, 29.0, 28.8, 25.4, 25.2; IR (neat): ν_{max} = 3298, 1600, 1560, 1426, 1150, 1038, 816 cm^{-1}; HRMS: calculated for $C_{114}H_{195}N_{27}O_{30}$ (exact mass: 2422.4563), $(M+3H)^{3+}$ 808.4933, found 808.7375.

Alkylated G2 dendrimer **17**

Obtained as a white solid in 85% yield; ^{1}H NMR (400 MHz, D_2O): δ = 8.02 (s, 9H, H-5''' & H-k), 5.82 (s, 3H, H-2), 5.53 (d, 6H, *J* = 9.16 Hz, H-l), 4.71-4.65 (m, 3H, H-1'''), 4.36 (s, 18H), 3.81-3.09 (m, 84H), 1.72-0.97 (m, 72H, H-2' – H-5' & H-d – H-g); ^{13}C NMR (100.5 MHz, D_2O): δ = 160.7, 144.3, 123.9, 87.5, 78.9, 76.0, 72.3, 71.2, 70.4, 70.1, 68.9, 62.9, 61.6, 60.4, 31.1, 28.9, 28.8, 28.4, 25.6, 25.3, 24.7; IR (neat): ν_{max} = 3336, 1595, 1457, 1365, 1219, 1161, 1034 cm^{-1}; HRMS: calculated for $C_{132}H_{219}N_{27}O_{48}$ (exact mass: 2950.5526), $[M+3Na]^{3+}$ 1006.5067, found 1006.5647.

Synthesis of 1,3,5-tris((6-((1-(4,11,15,22-tetraoxapentacosa-1,24-diyn-13-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)hexyl)oxy)benzene (**15**)

The title compound **15** was obtained by alkylation reaction of compound **13** with 1-bromo-6-(prop-2-yn-1-yloxy)hexane (**10**) by following the similar reaction procedure as given for synthesis of compound **5**.

Obtained as viscous brown liquid in 32% yield; ^{1}H NMR (100.5 MHz, $CDCl_3$): δ = 7.70 (s, 3H, H-5'''), 6.03 (s, 3H, H-2), 4.90-4.85 (m, 3H, H-a), 4.61 (s, 6H, H-1''), 4.12 (d, 12H, *J* = 2.2 Hz, H-i), 3.90-3.81 (m, 18H), 3.54-3.37 (m, 30H), 2.42 (t, 6H, *J* = 2.2 Hz, H-k), 1.58-1.31 (m, 72H, H-2' – H-5' & H-d – H-g); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 160.7, 144.5, 122.3, 93.8, 79.9, 74.1, 71.6, 70.6, 70.0, 69.3, 67.8, 64.3, 60.5, 57.9, 29.3, 29.3, 25.9, 25.8; IR (film): ν_{max} = 3292, 2189, 1589, 1460, 1140, 1028 cm^{-1}; HRMS: calculated for $C_{96}H_{153}N_9O_{18}$ $[M+H]^+$ 1743.1226, found 1743.1262.

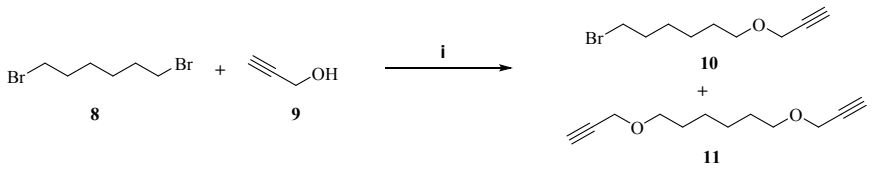
Synthesis of 1-bromo-6-(prop-2-yn-1-yloxy)hexane (**10**)

1-Bromo-6-(prop-2-yn-1-yloxy)hexane (**10**) was synthesized from propargyl alcohol (**9**) and 1,6-dibromohexane (**8**). *O*-Alkylation of propargyl alcohol (**9**) with 1,6-dibromohexane (**8**) using NaOH and TBAI as a phase transfer catalyst yielded the dialkyne **11** and the desired monoalkyne **10** derivatives in 1:3 ratio, which were separated by subjecting the mixture to column chromatography and using hexane as an eluent.

2. Cytotoxic Study Results

Cytotoxicity is one of the major areas of concern that needs to be addressed in order to develop efficient drug delivery nanocarriers for biomedical applications. To study the *in vitro* cytotoxicity of the synthesized dendritic architectures, A549 cells were treated with dendrimers using different concentrations (0.1, 0.5, and 1.0 mg/mL) for 72 h and analyzed using MTS assay (**Figure 6**). The first- and second-generation C_6 alkylated dendrimers **13**, **14**, and **17** were investigated for their cytotoxicity. The dendrimers exhibited a concentration dependence but low toxicity. However, at concentrations below 0.5 mg/mL the screened dendrimers were found to be non-toxic for A549 cells.

3. Schemes, Figures and Tables



Scheme S1. Synthesis of 1-bromo-6-(prop-2-yn-1-yloxy)hexane (**10**): (i) NaOH, TBAI, H_2O, 50 ^oC.

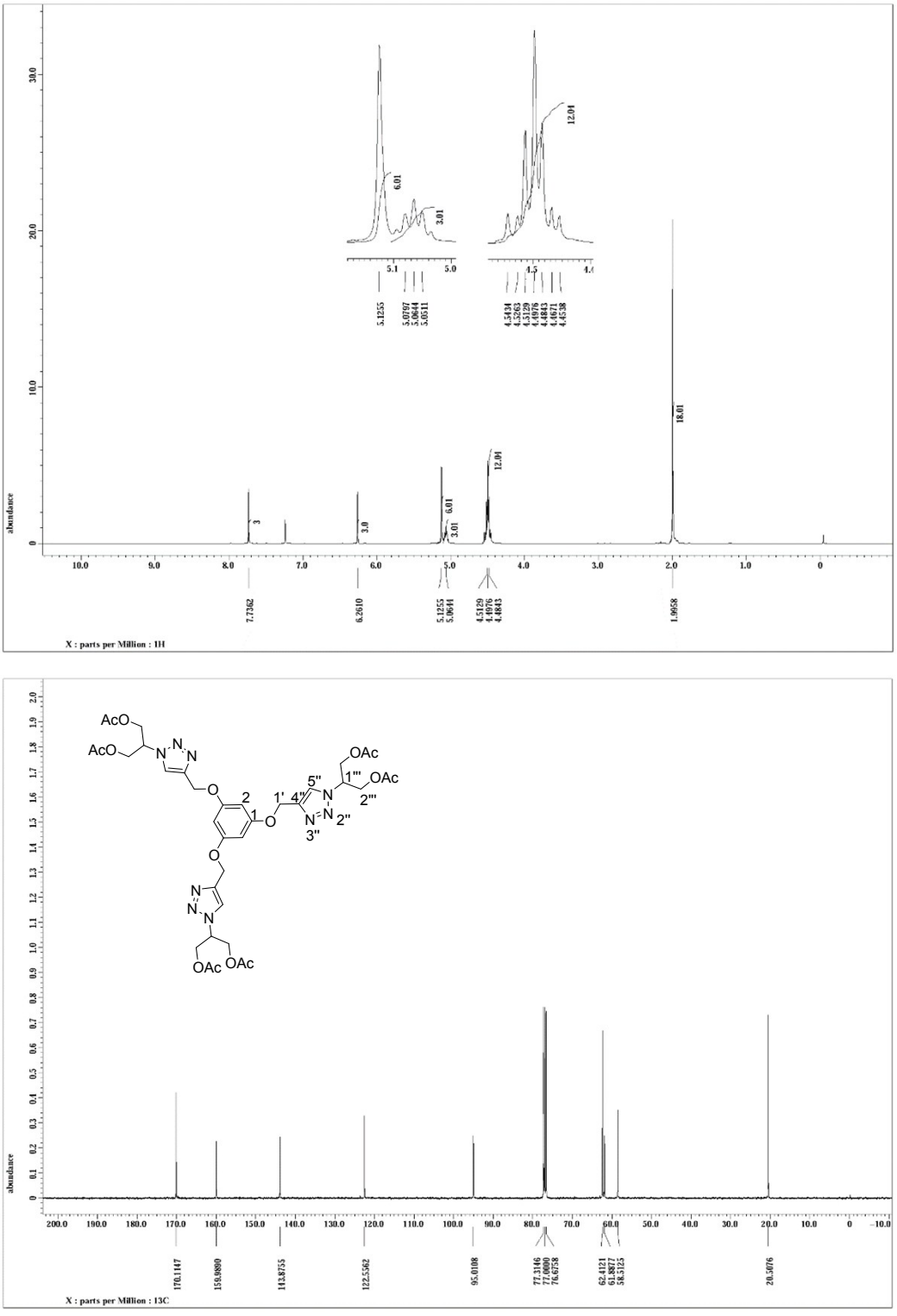


Figure S1. ^{1}H and ^{13}C NMR spectra of acetyl-protected G1 dendrimer **3a** in $CDCl_3$.

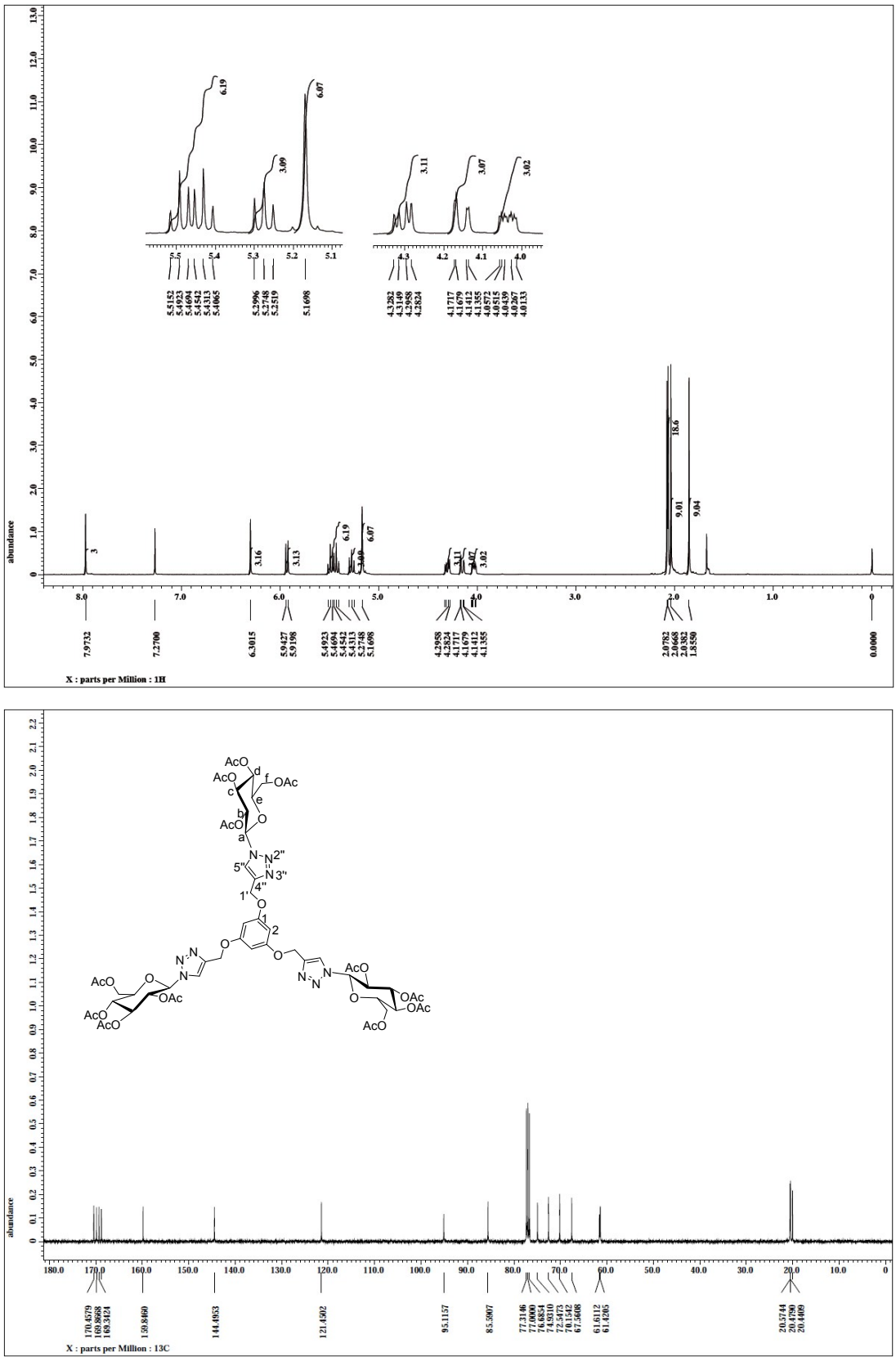
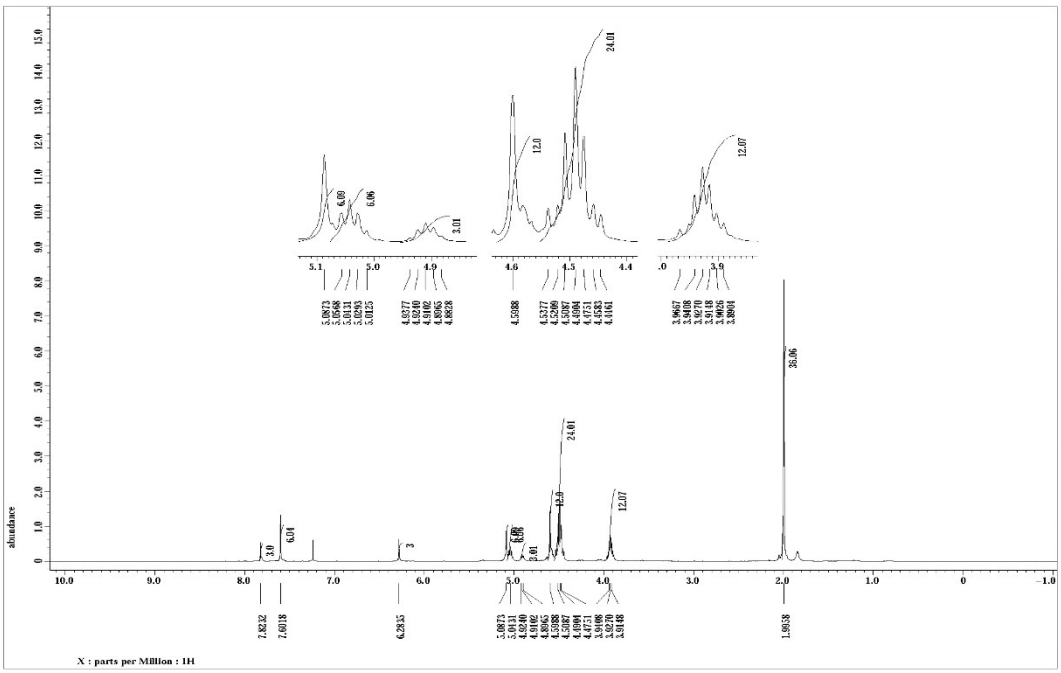


Figure S2. ^{1}H and ^{13}C NMR spectra of acetyl-protected G1 dendrimer **4a** in $CDCl_3$.



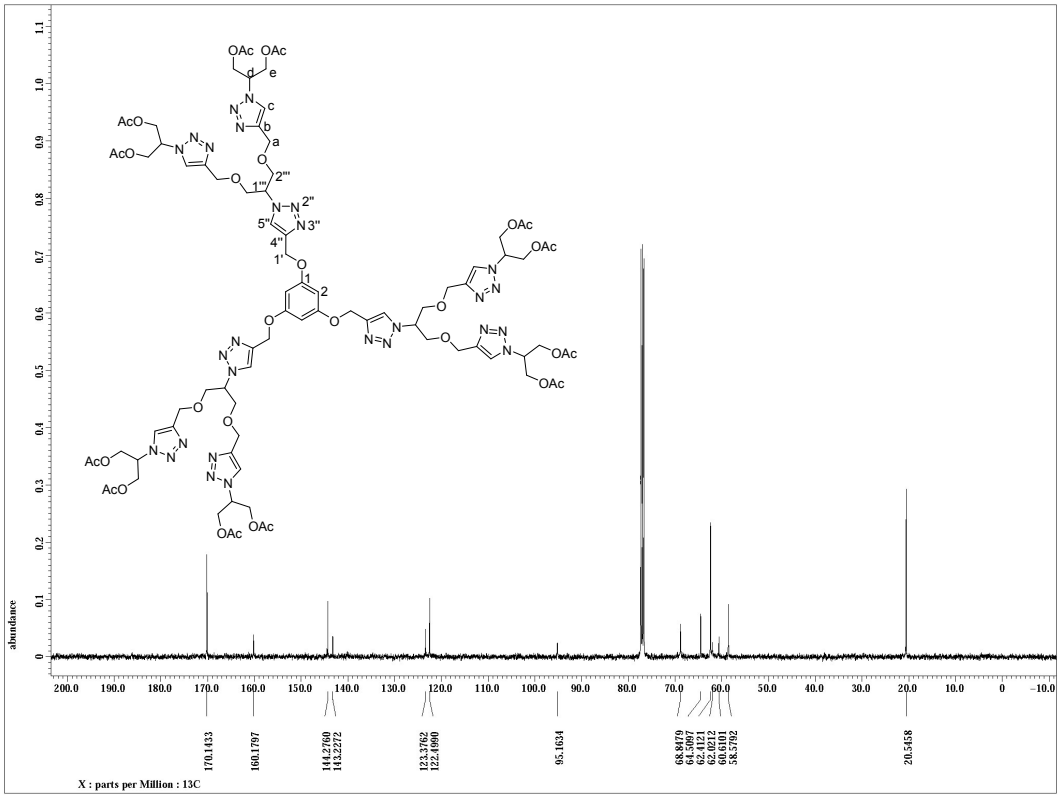


Figure S3. ^{1}H and ^{13}C NMR spectra of acetyl-protected G2 dendrimer **6a** in $CDCl_3$.

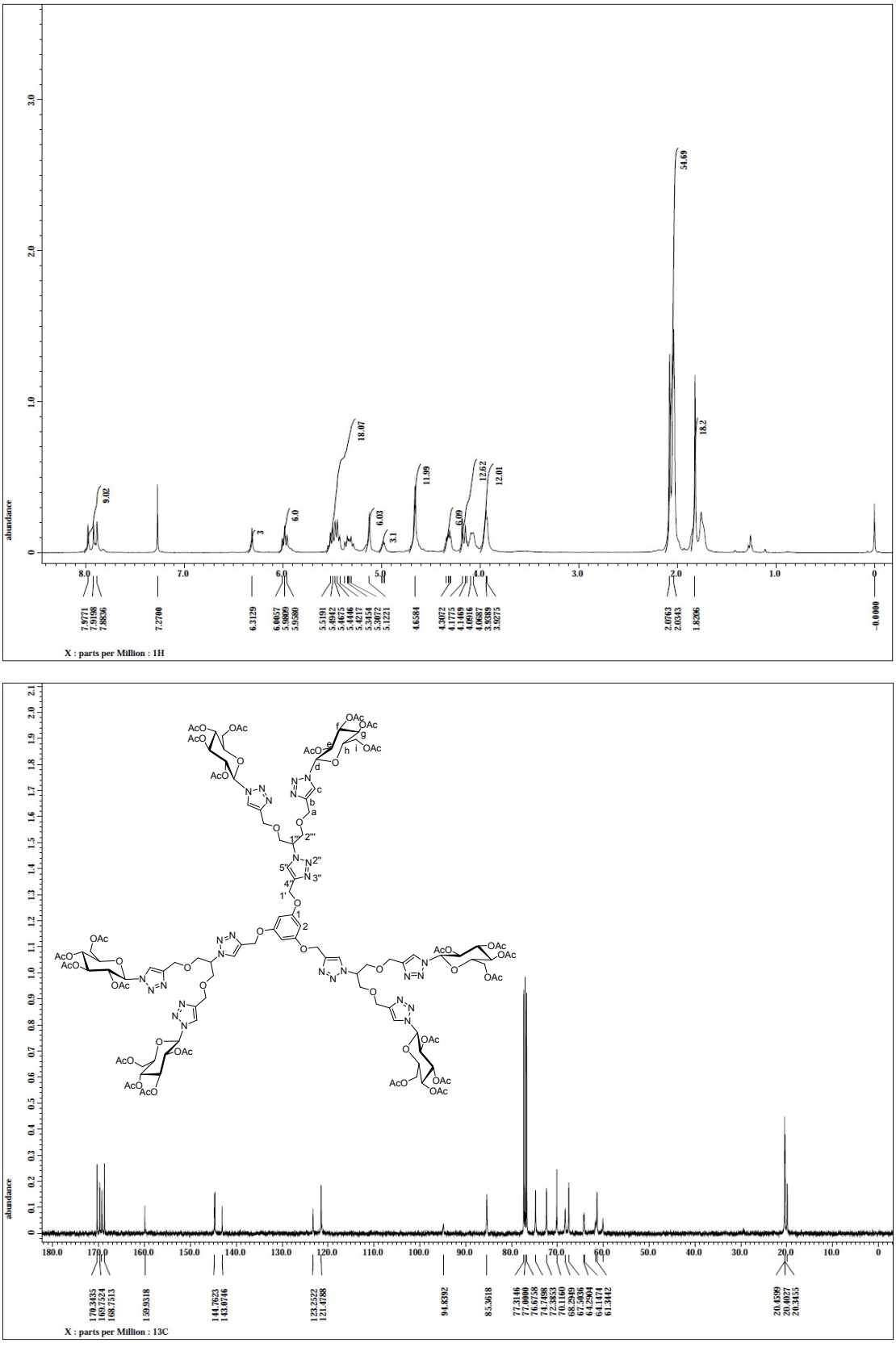


Figure S4. ^{1}H and ^{13}C NMR spectra of acetyl-protected G2 dendrimer **7a** in $CDCl_3$.

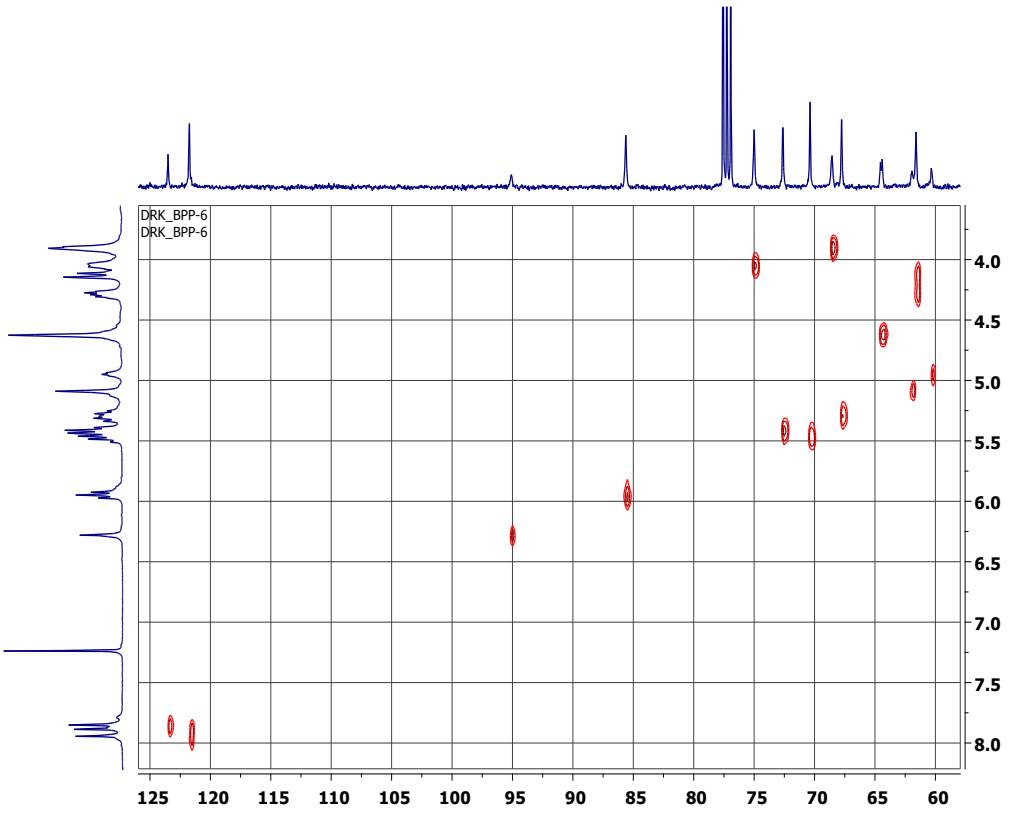


Figure S5. HETCOR spectrum of acetyl-protected G2 dendrimer **7a** in $CDCl_3$.

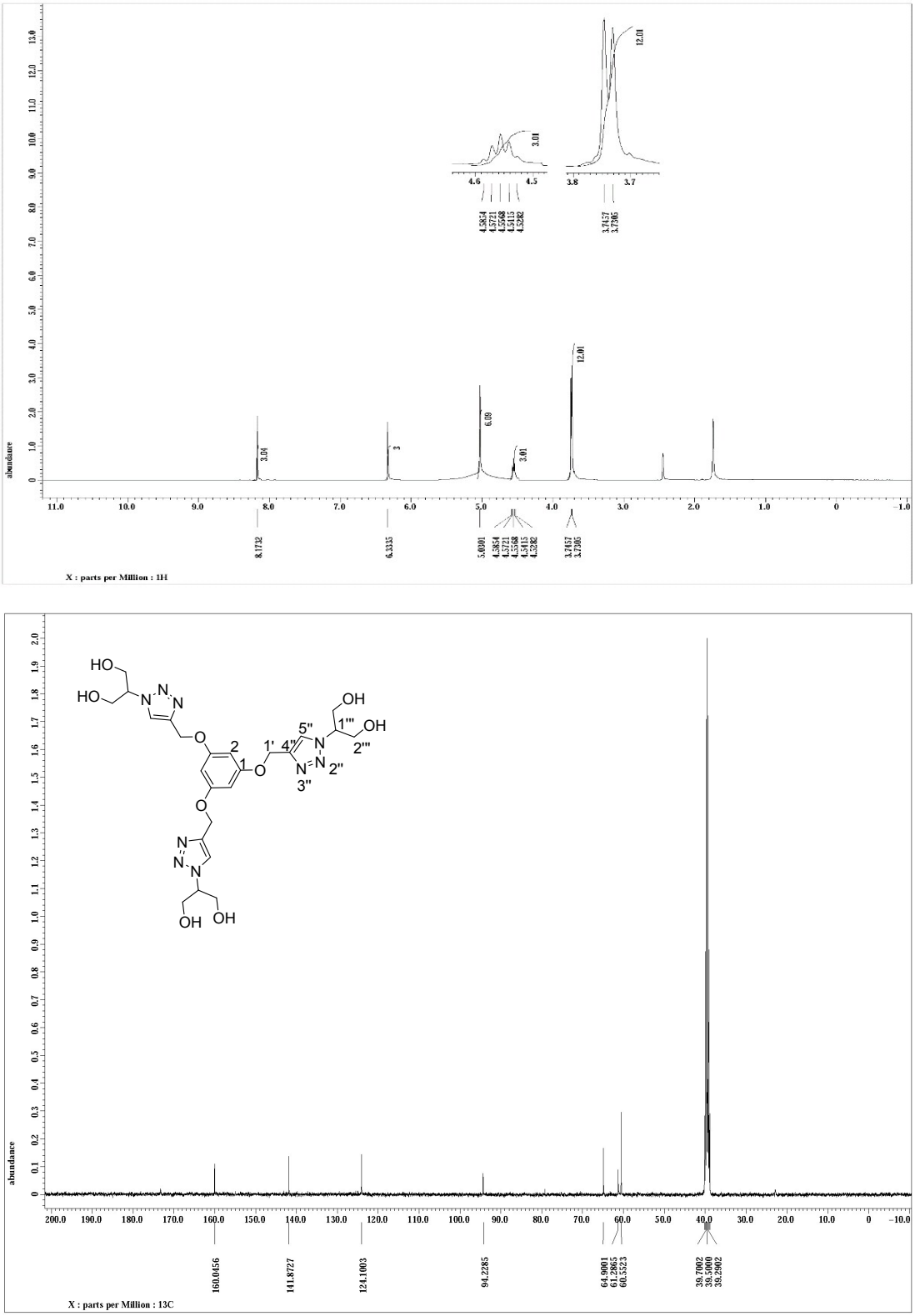


Figure S6. ^{1}H and ^{13}C NMR spectra of G1 dendrimer **3** in DMSO-d_6.

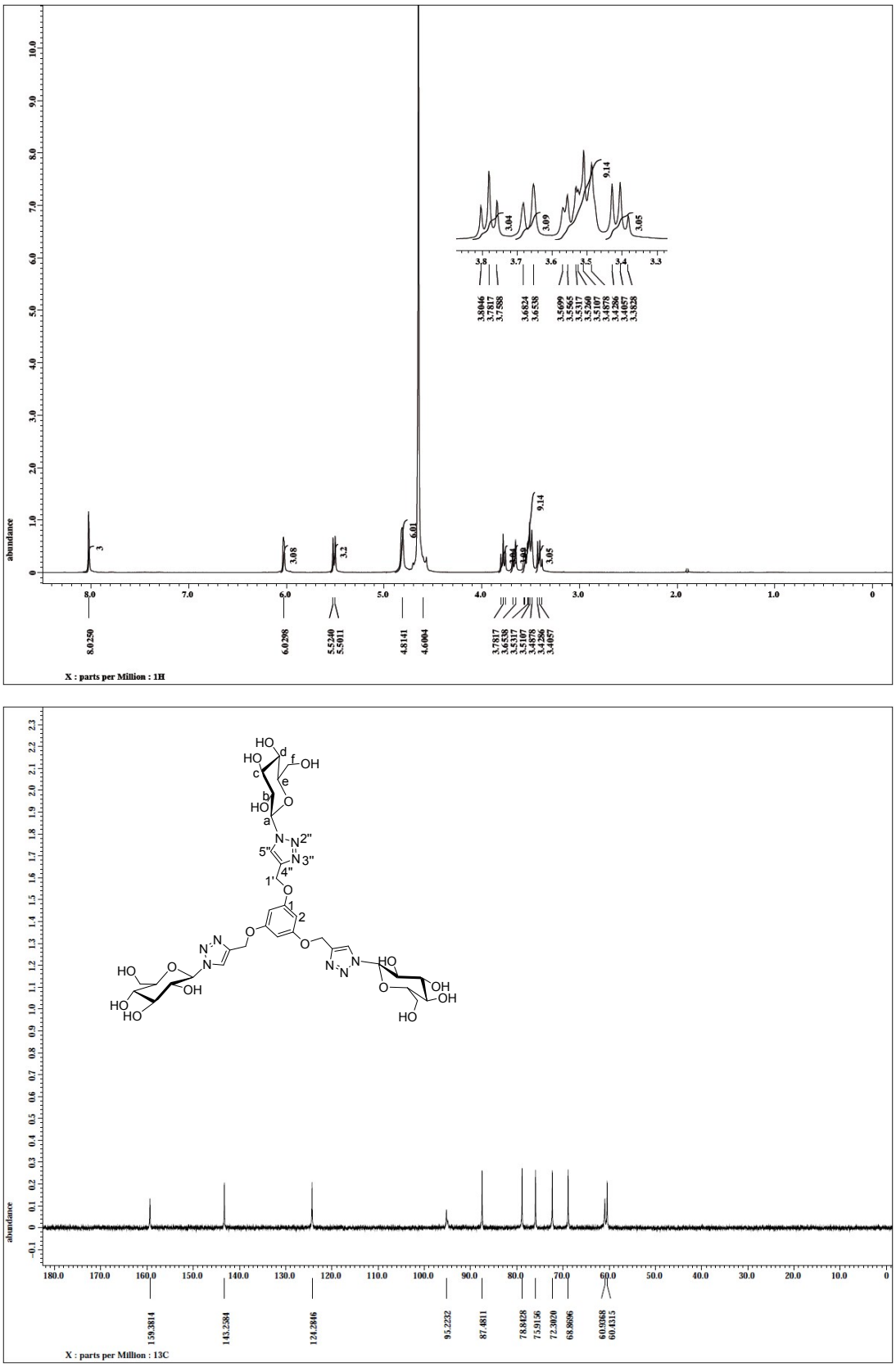


Figure S7. ^{1}H and ^{13}C NMR spectra of G1 dendrimer **4** in D_2O.

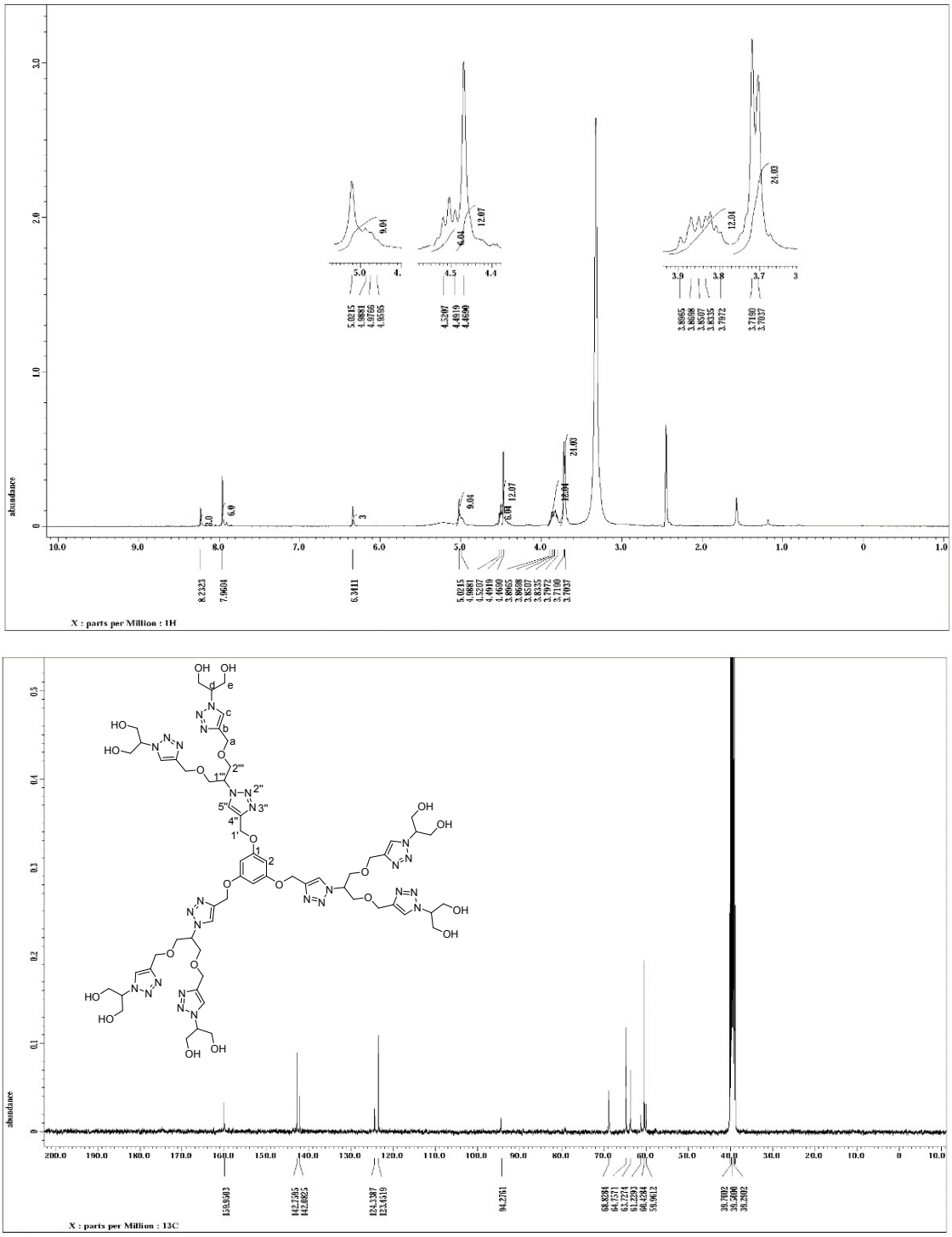


Figure S8. ^{1}H and ^{13}C NMR spectra of G2 dendrimer **6** in DMSO-d_6.

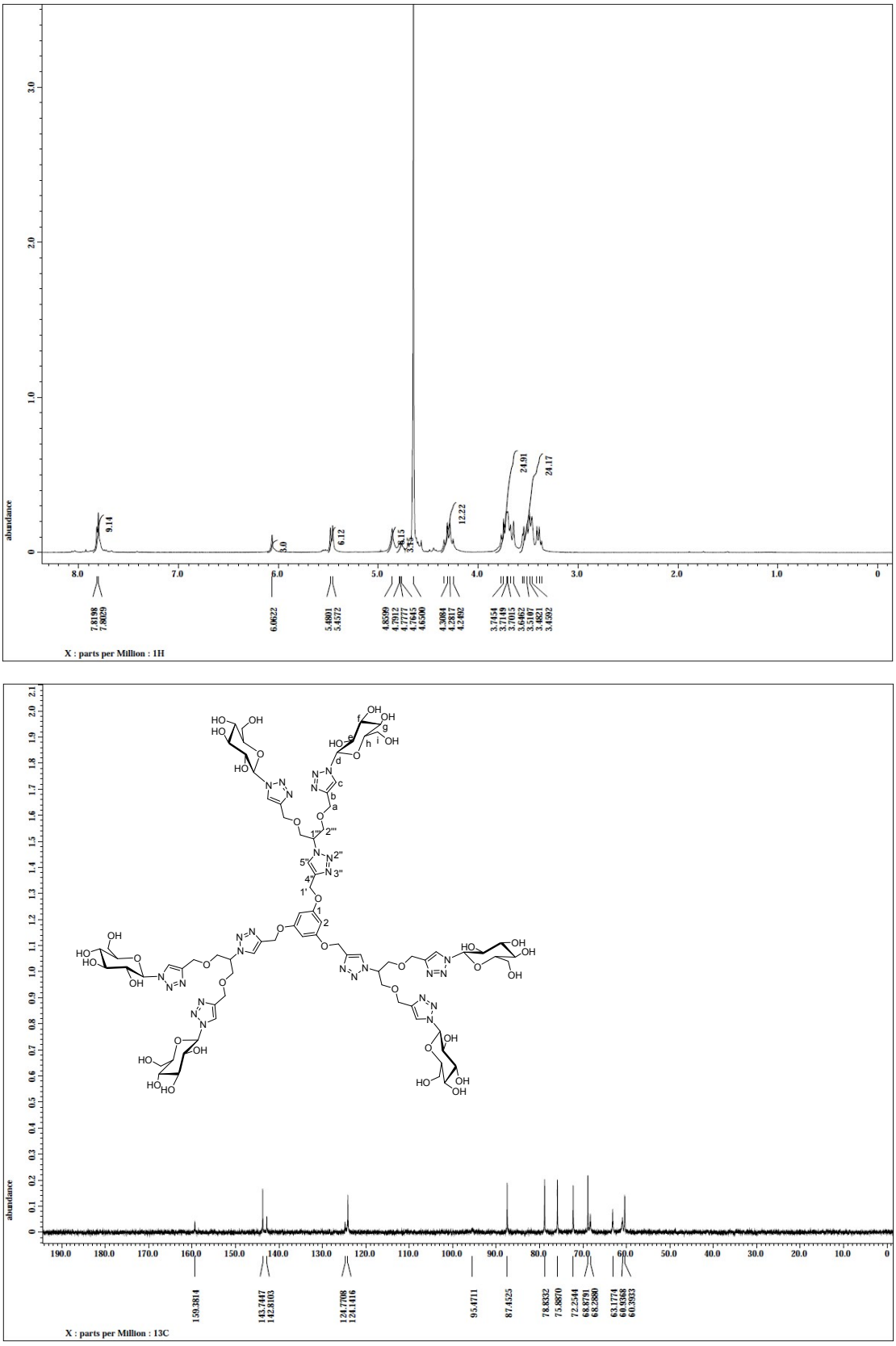


Figure S9. ^{1}H and ^{13}C NMR spectra of G2 dendrimer **7** in D_2O.

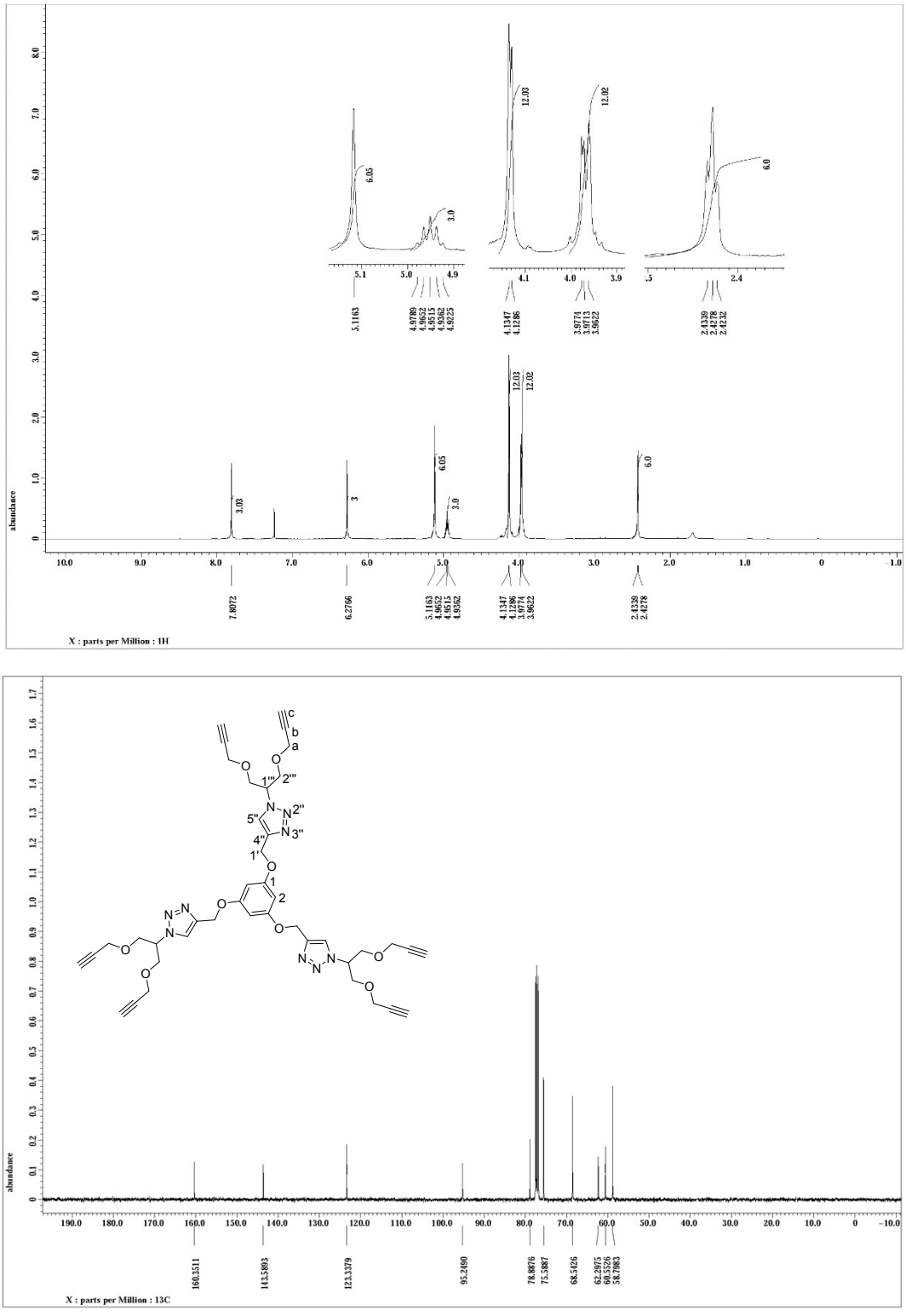


Figure S10. ^{1}H and ^{13}C NMR spectra of dendrimer **5** in $CDCl_3$.

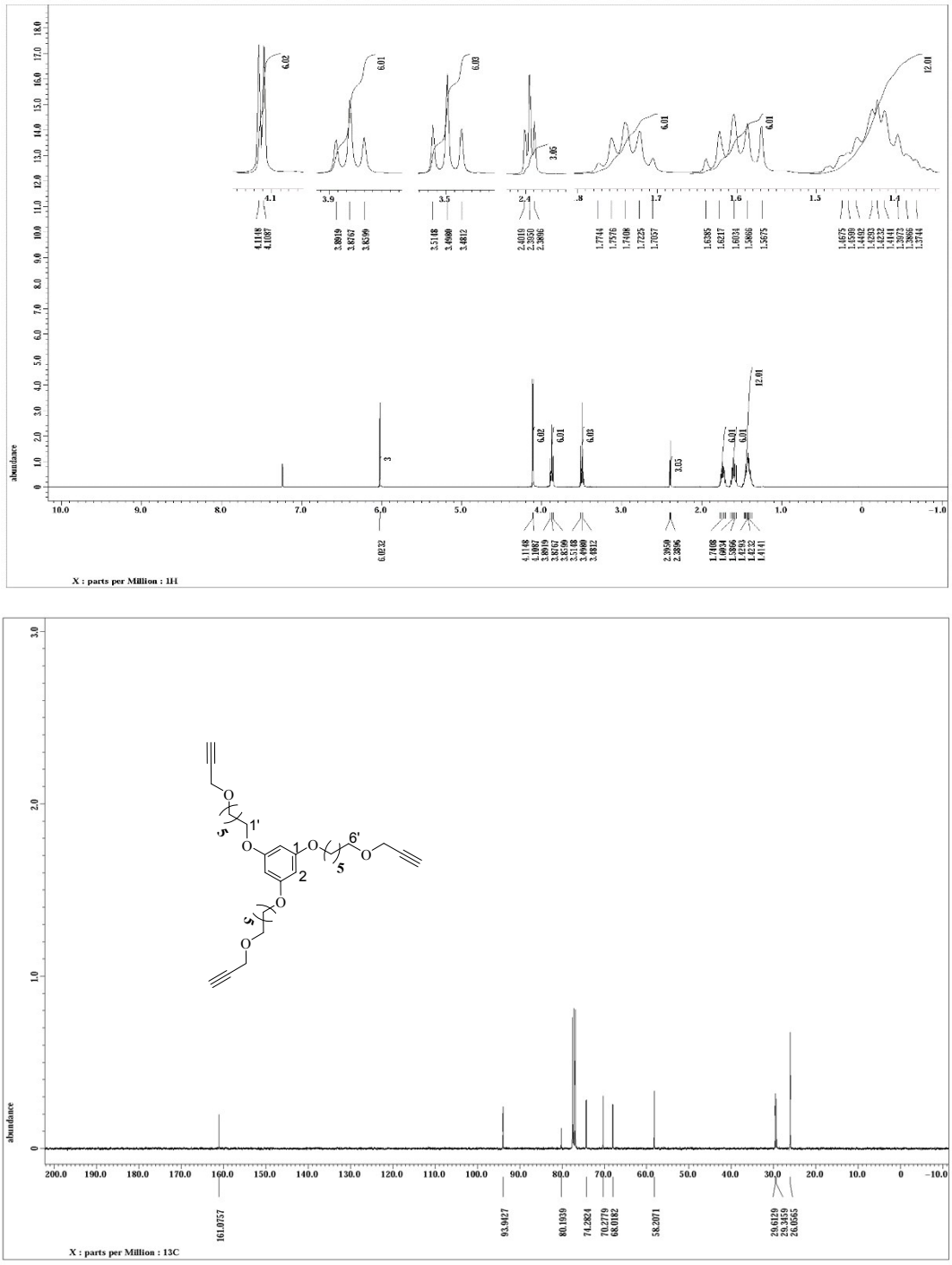


Figure S11. ^{1}H and ^{13}C NMR spectra of 1,3,5-tris((6-(prop-2-yn-1-yloxy)hexyl)oxy)benzene (**12**) in $CDCl_3$.

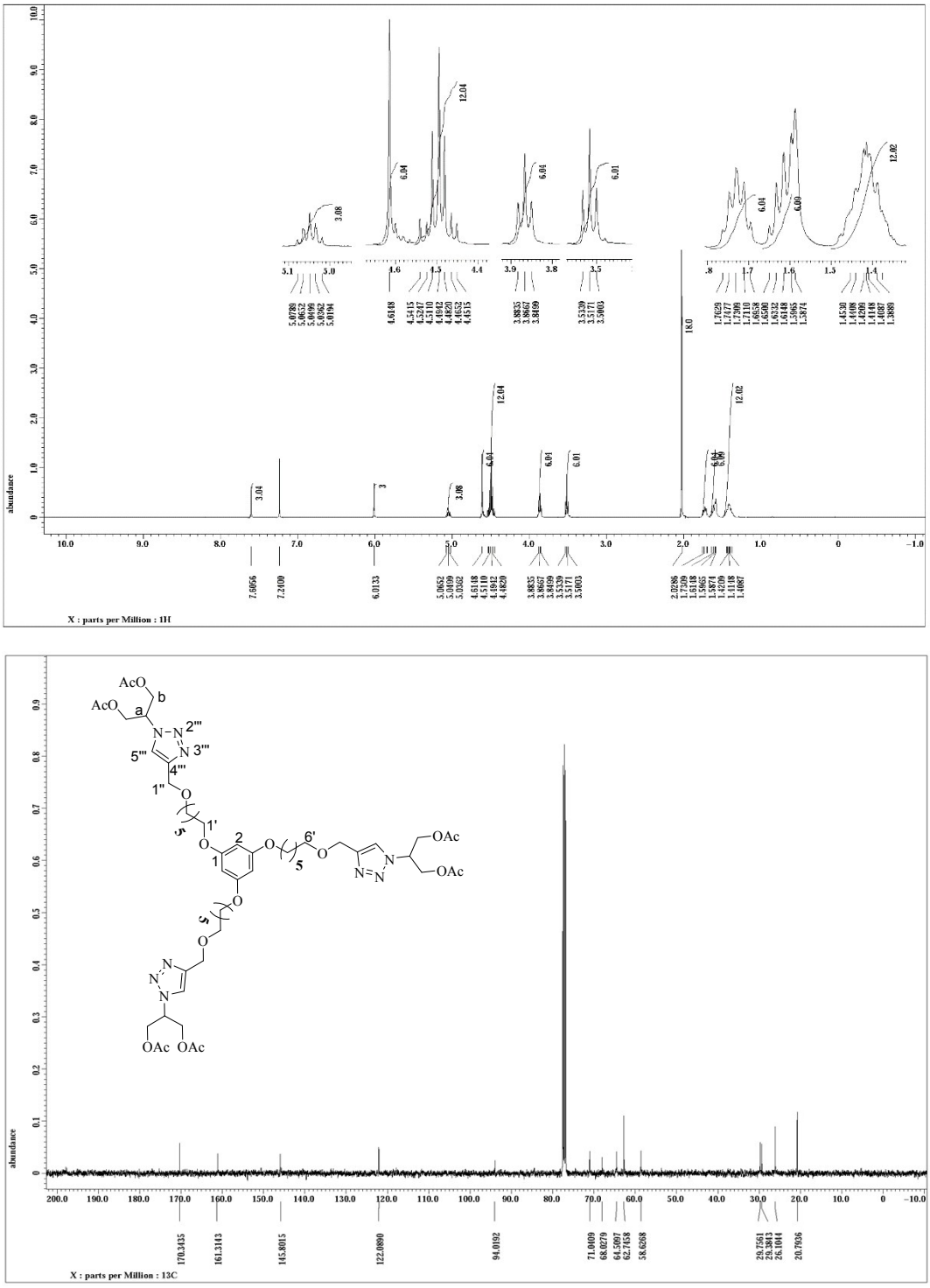


Figure S12. ^{1}H and ^{13}C NMR spectra of acetyl-protected alkylated G1 dendrimer **13a** in $CDCl_3$.

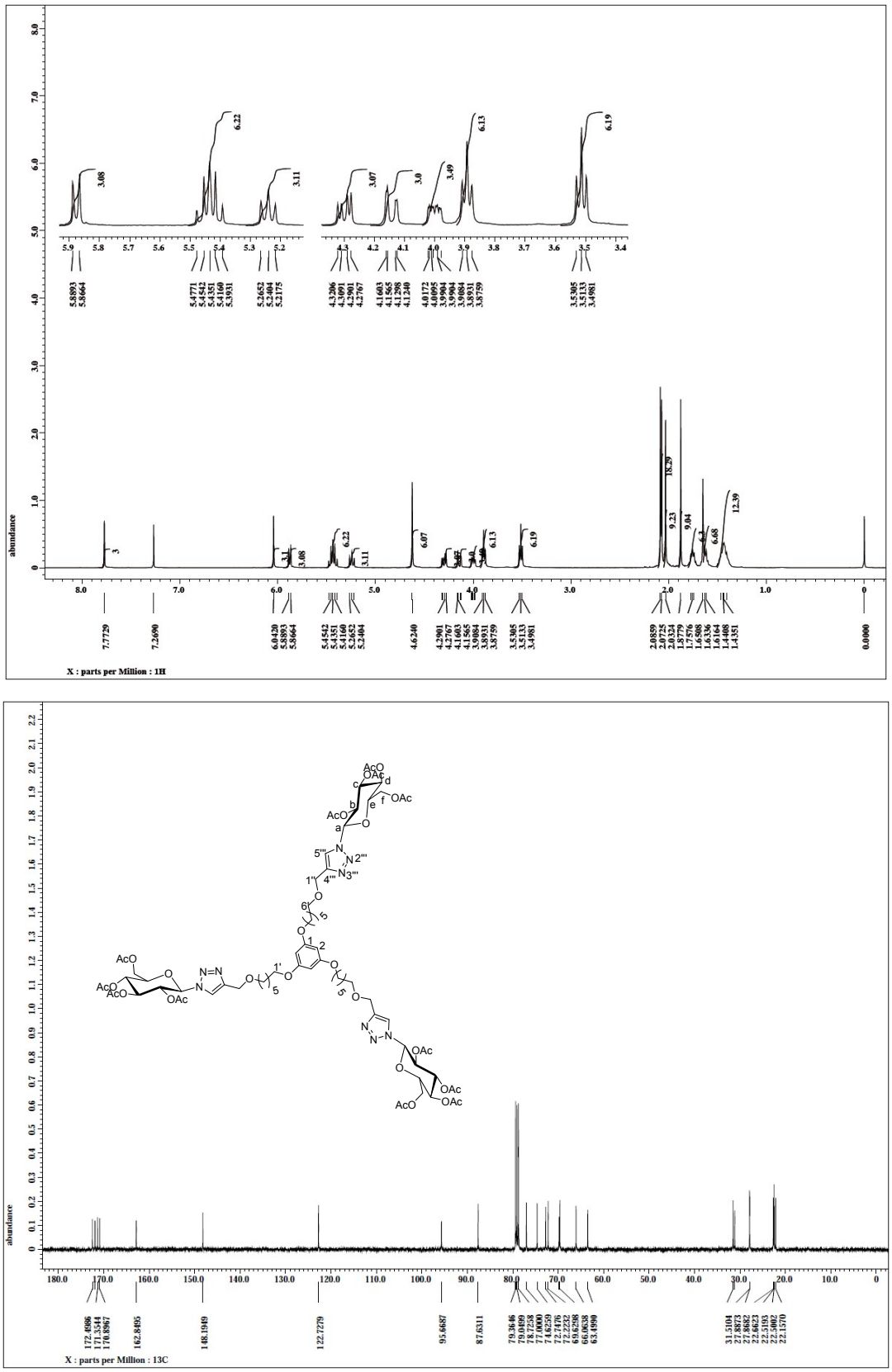


Figure S13. ^{1}H and ^{13}C NMR spectra of acetyl-protected alkylated G1 dendrimer **14a** in $CDCl_3$.

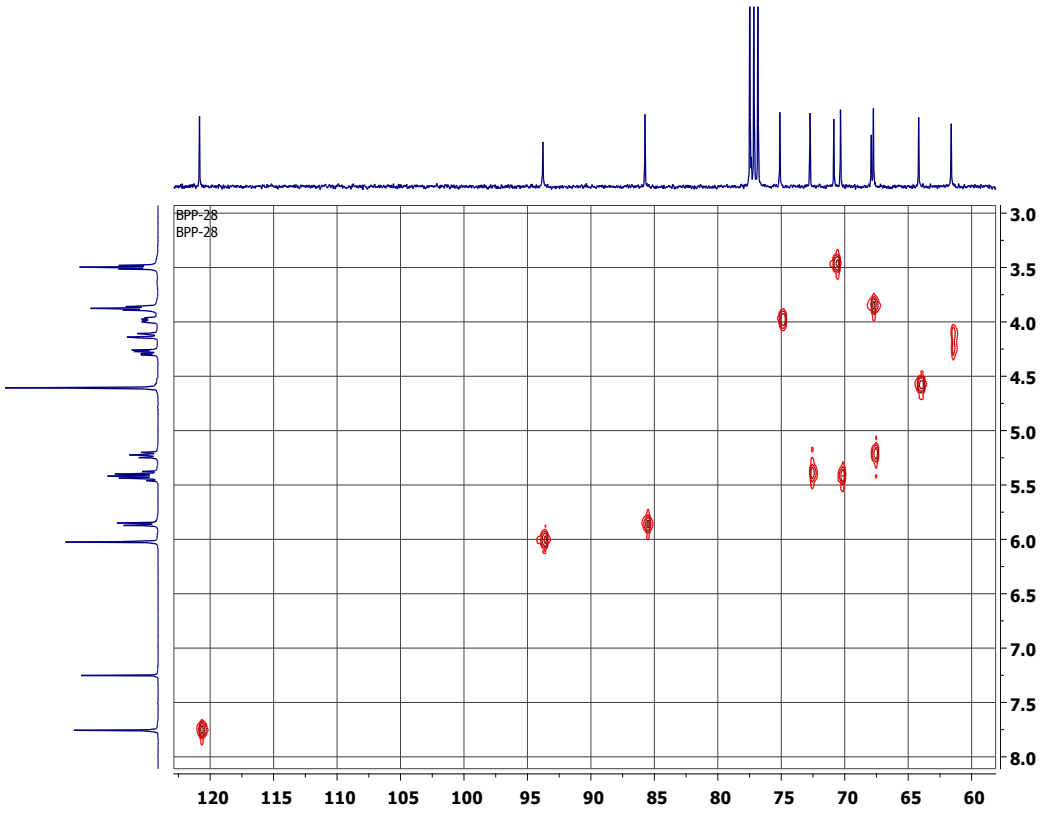


Figure S14. HETCOR spectrum of acetyl-protected alkylated G1 dendrimer **14a** in $CDCl_3$.

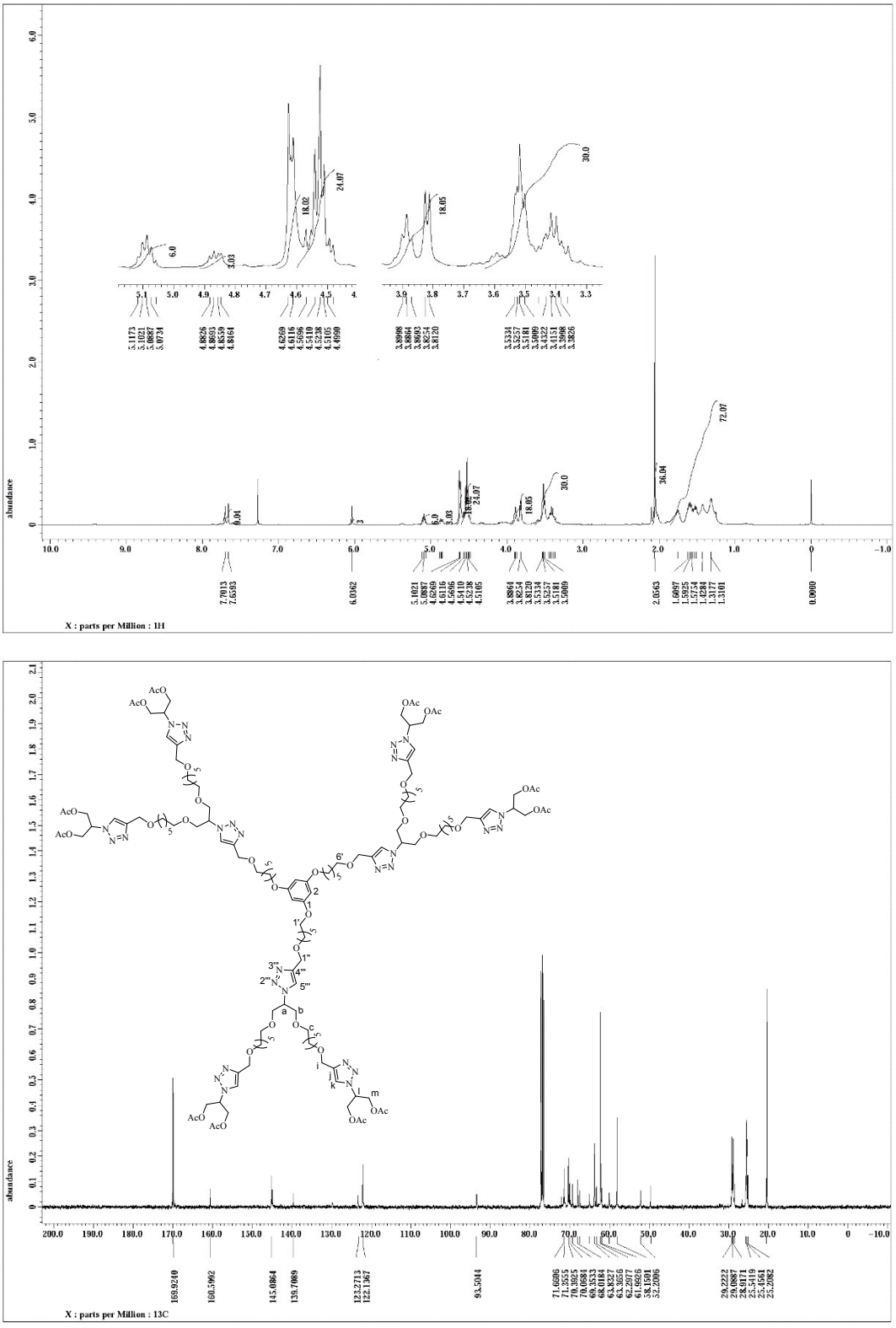


Figure S15. ^{1}H and ^{13}C NMR spectra of acetyl-protected alkylated G2 dendrimer **16a** in $CDCl_3$.

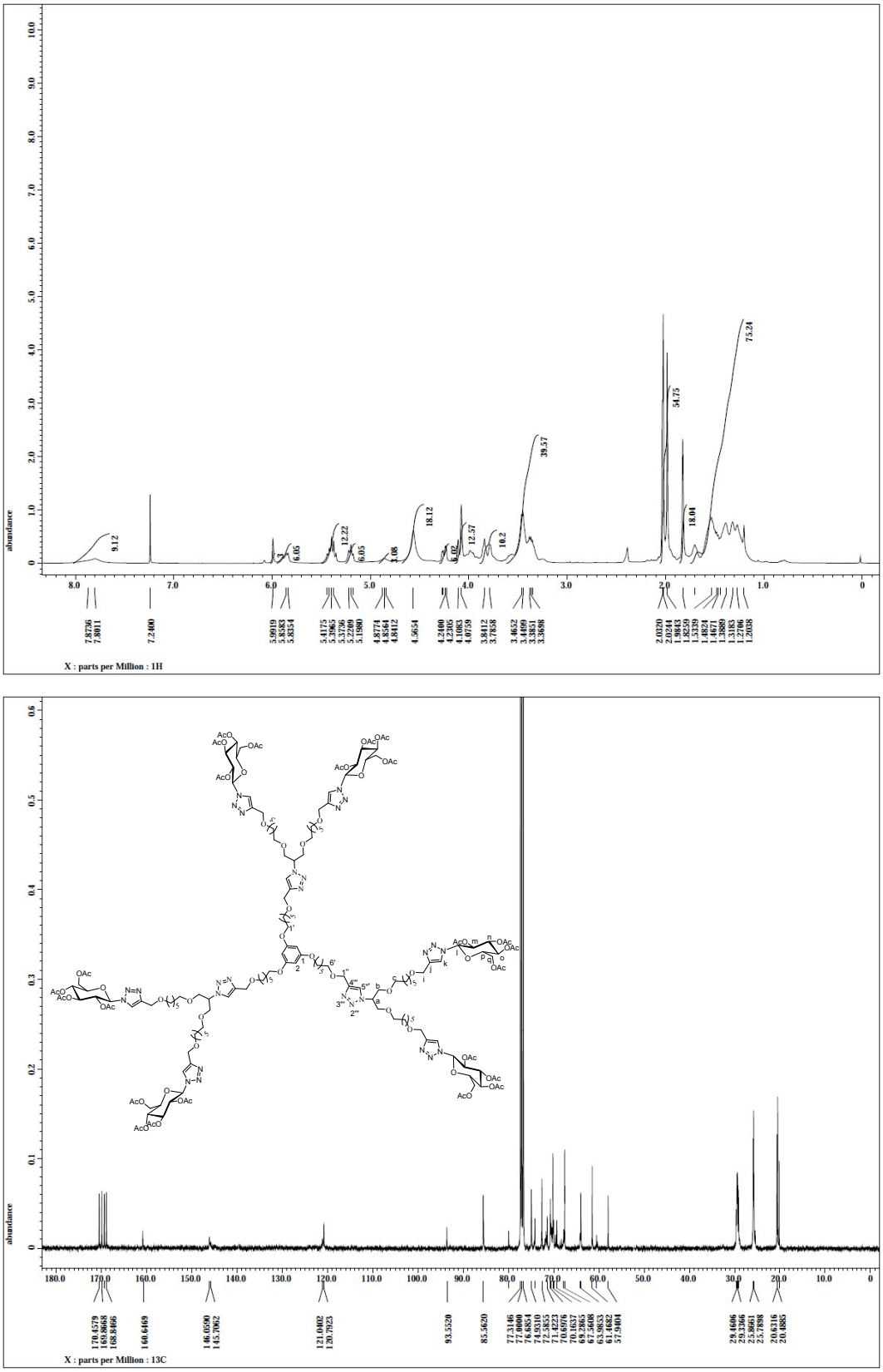


Figure S16. ^{1}H and ^{13}C NMR spectra of acetyl-protected alkylated G2 dendrimer **17a** in $CDCl_3$.

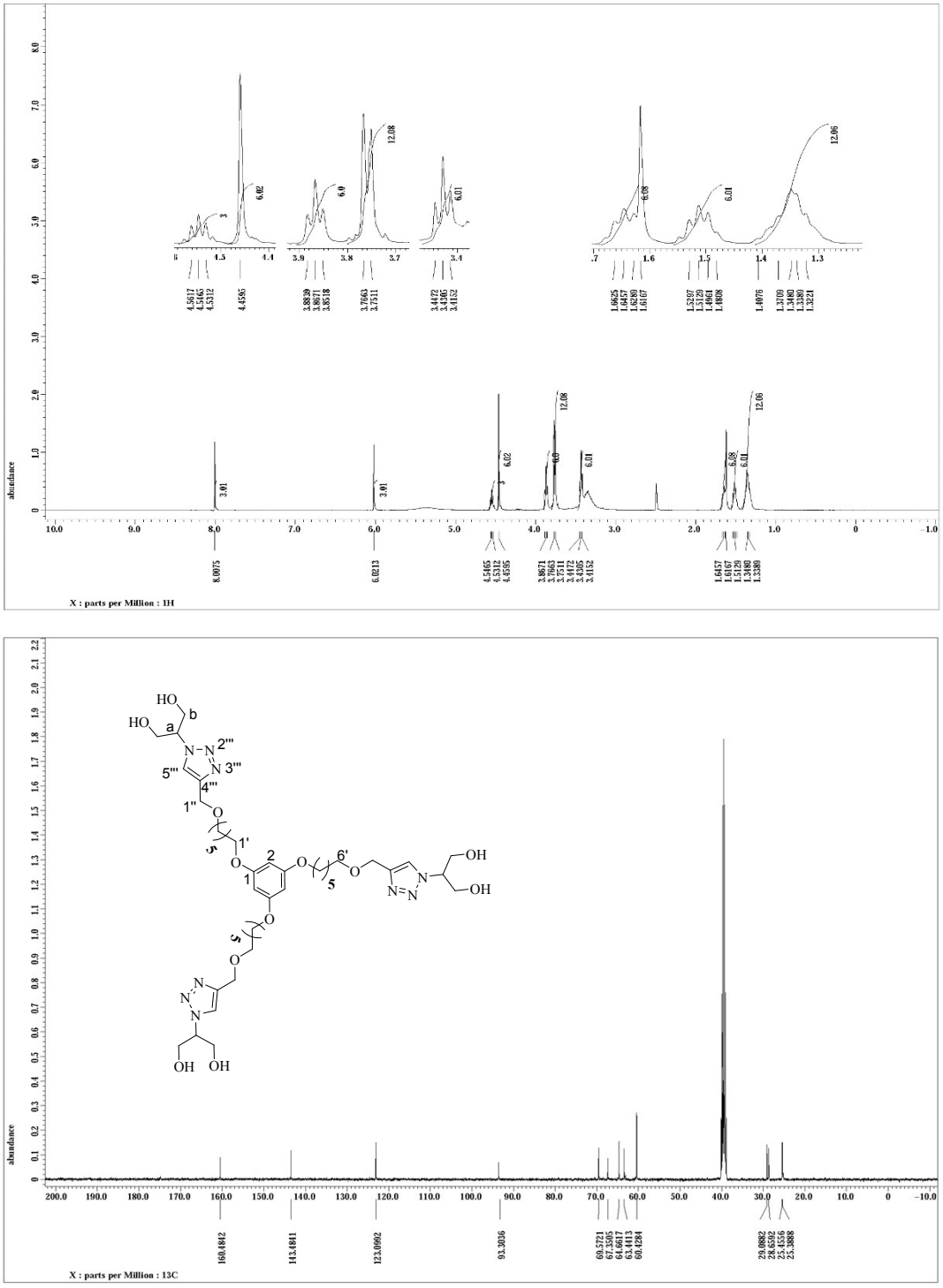


Figure S17. ^{1}H and ^{13}C NMR spectra of alkylated G1 dendrimer **13** in DMSO-d_6.

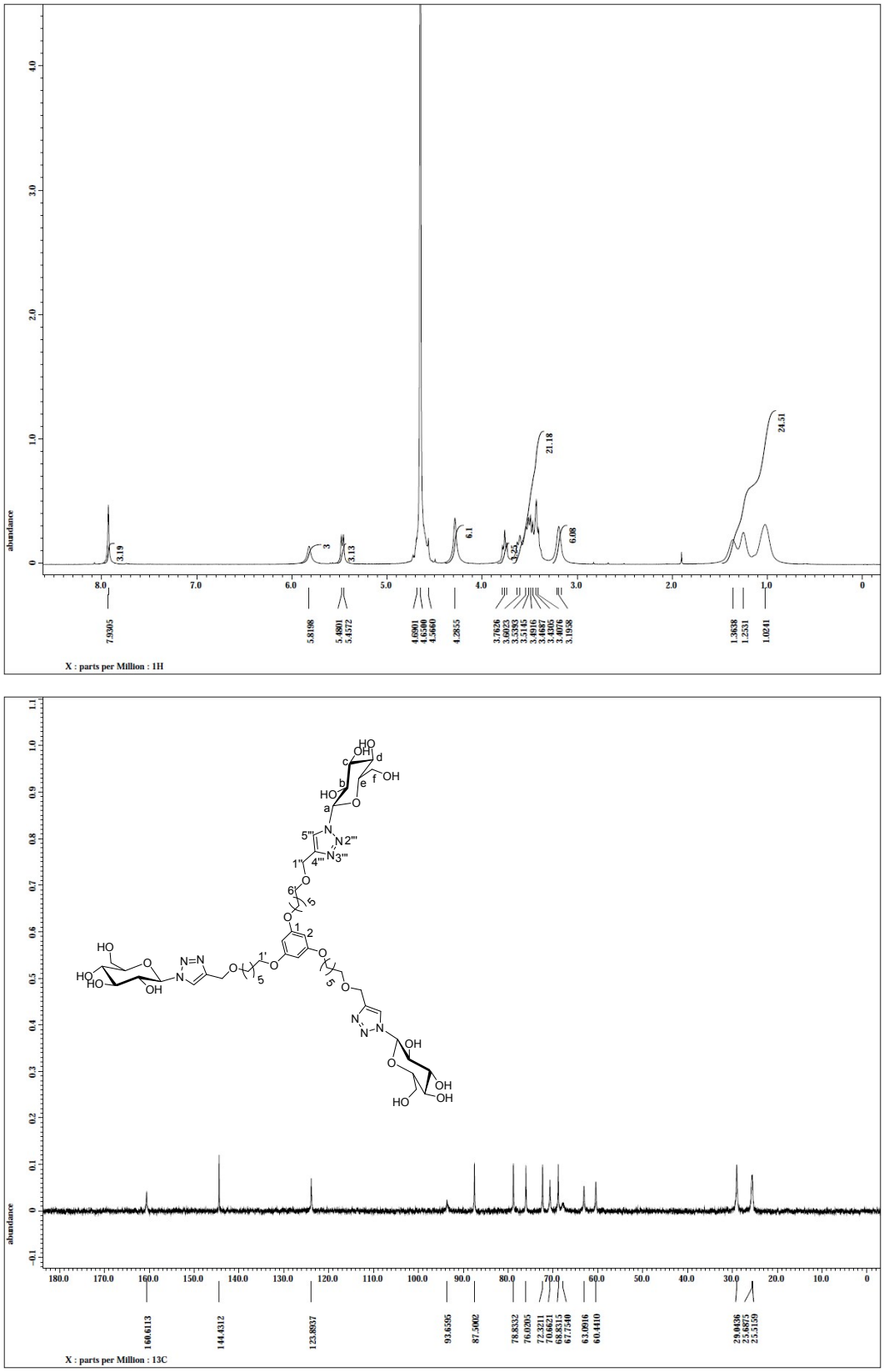


Figure S18. ^{1}H and ^{13}C NMR spectra of alkylated G1 dendrimer **14** in D_2O.

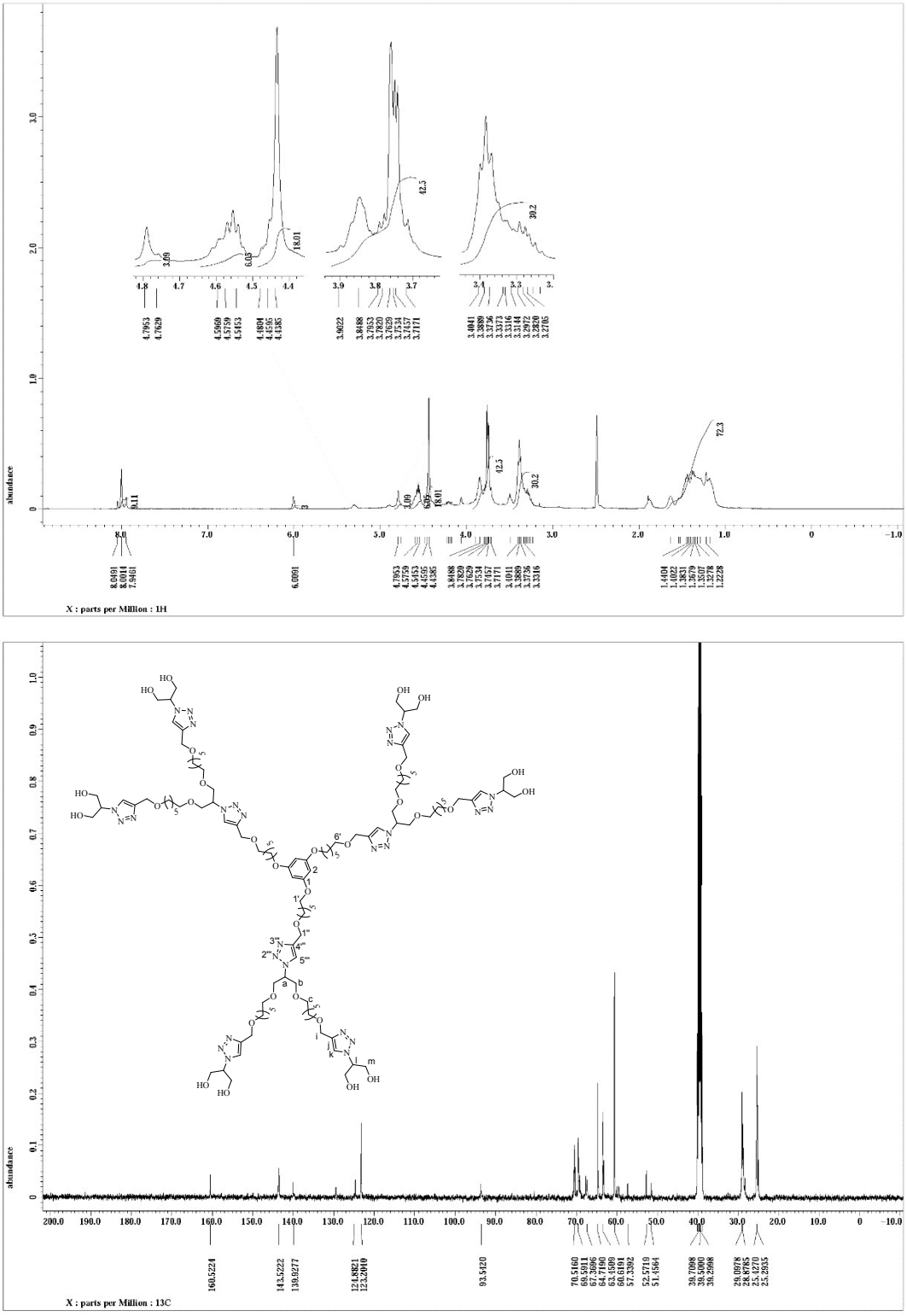


Figure S19. ^{1}H and ^{13}C NMR spectra of alkylated G2 dendrimer **16** in DMSO-d_6.

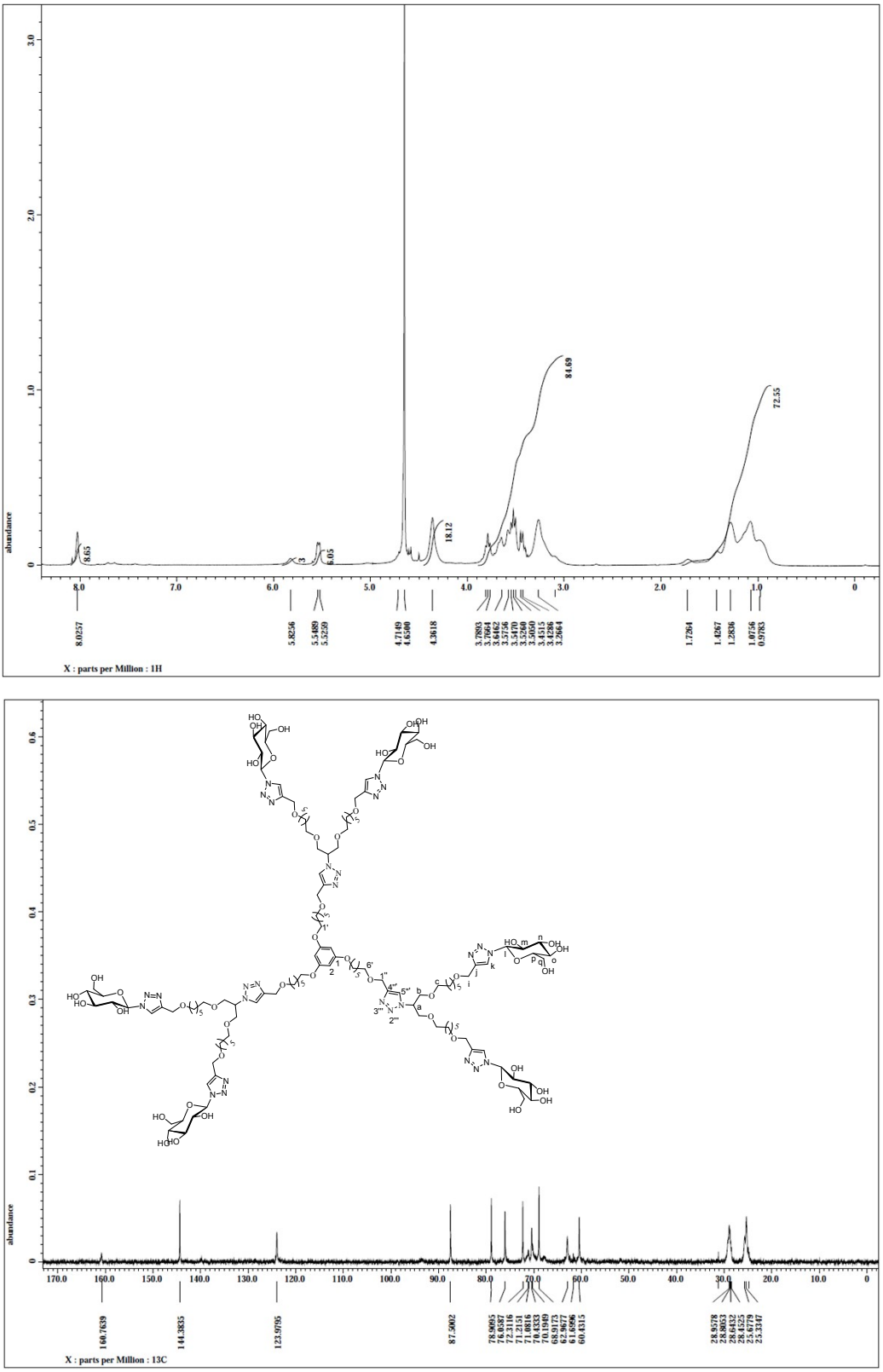


Figure S20. ^{1}H and ^{13}C NMR spectra of alkylated G2 dendrimer **17** in D_2O.

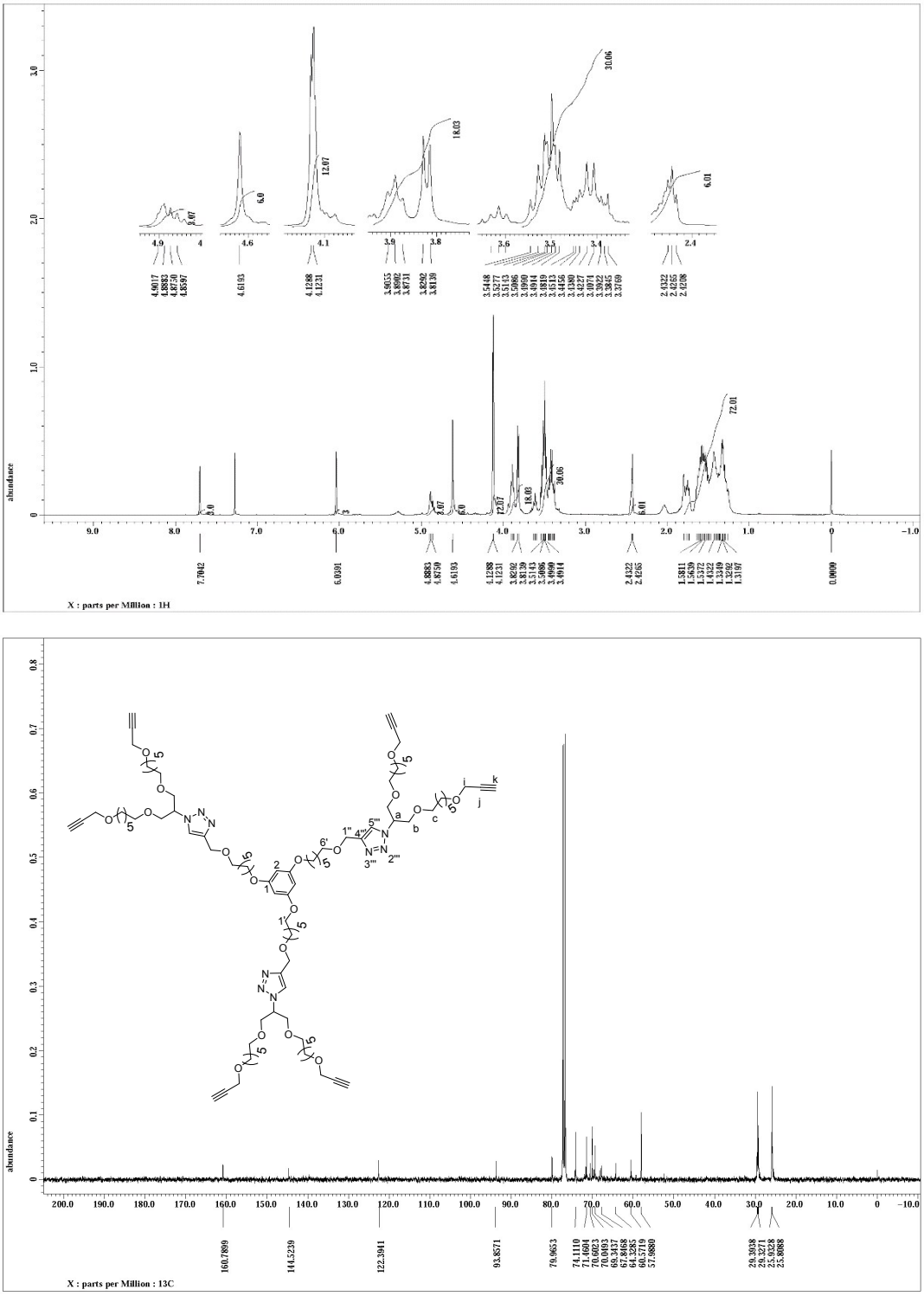


Figure S21. ^{1}H and ^{13}C NMR spectra of 1,3,5-tris((6-((1-(4,11,15,22-tetraoxapentacosa-1,24-diyn-13-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)hexyl)oxy)benzene (**15**) in $CDCl_3$.

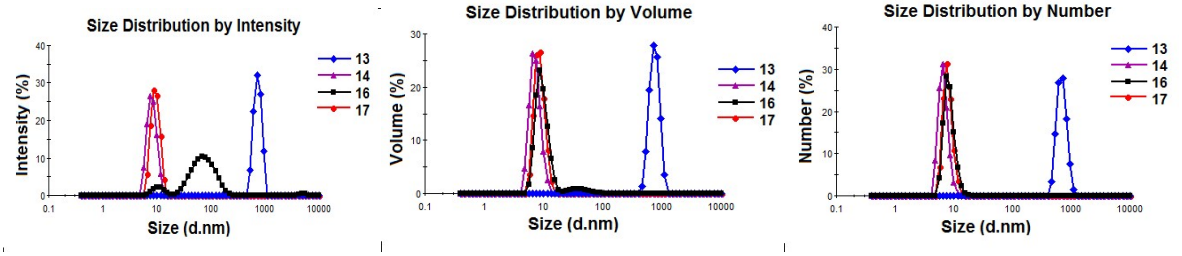


Figure S22. DLS size distribution graphs of dendrimers **13**, **14**, **16**, and **17**.

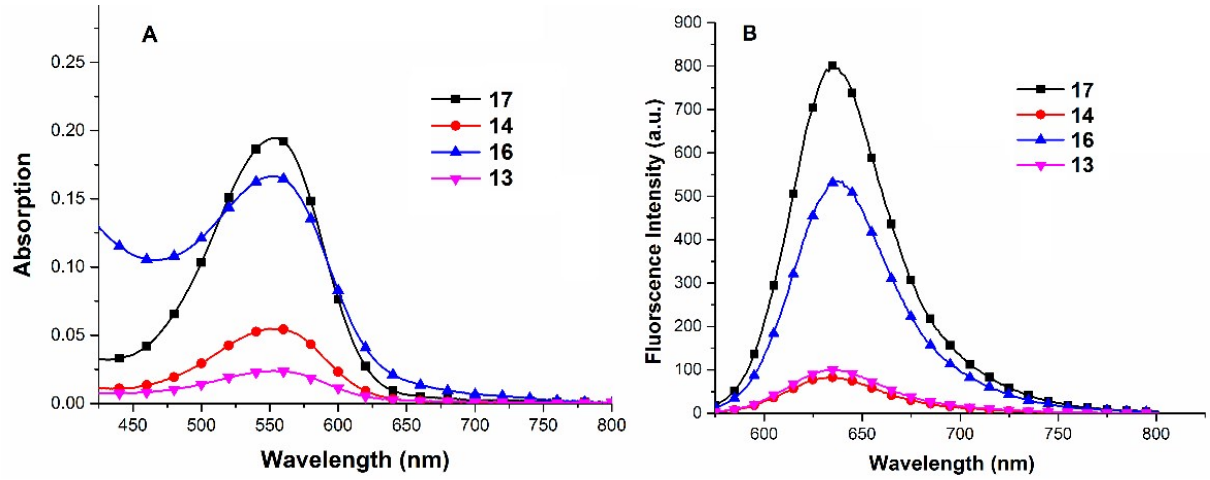


Figure S23. (A) UV absorbance and (B) fluorescence spectra of Nile red loaded samples in methanol.

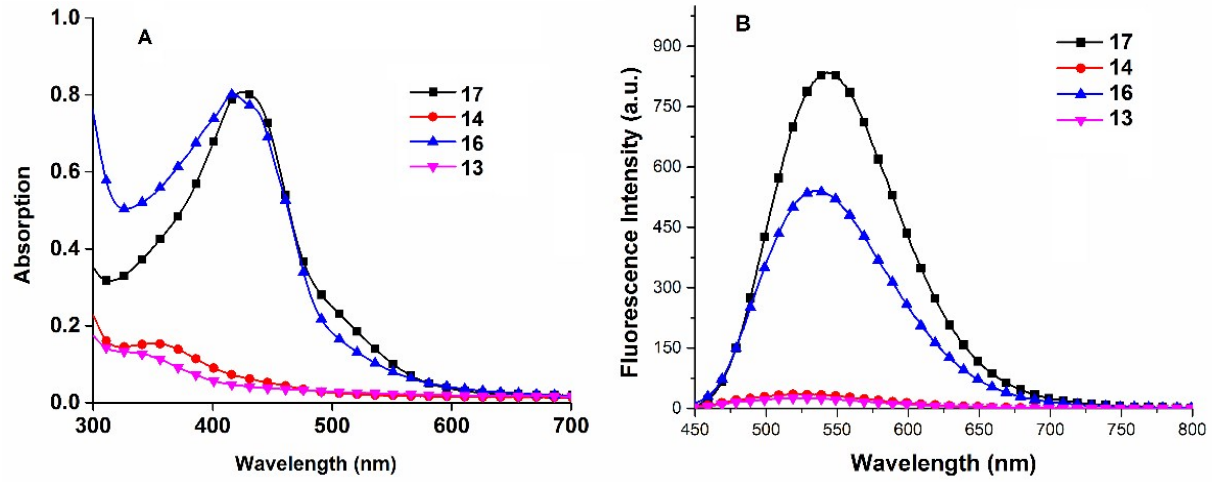


Figure S24. (A) UV absorbance and (B) fluorescence spectra of curcumin loaded samples in methanol.

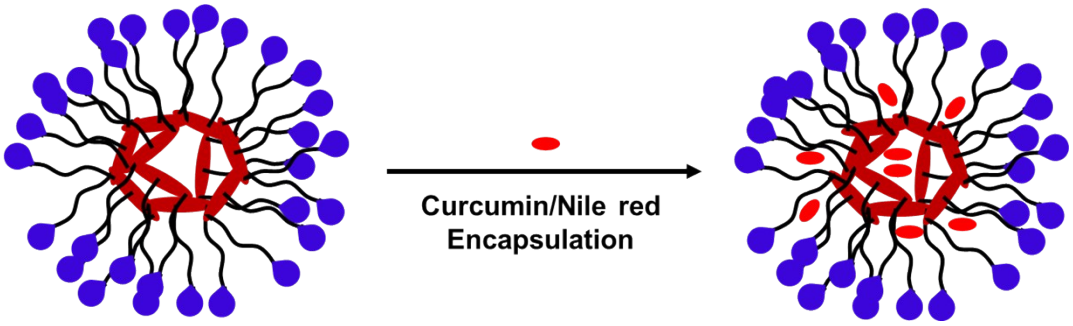


Figure S25. Schematic representation of dendritic micellar aggregates encapsulating the hydrophobic entity curcumin and Nile red in their core via hydrophobic interactions.

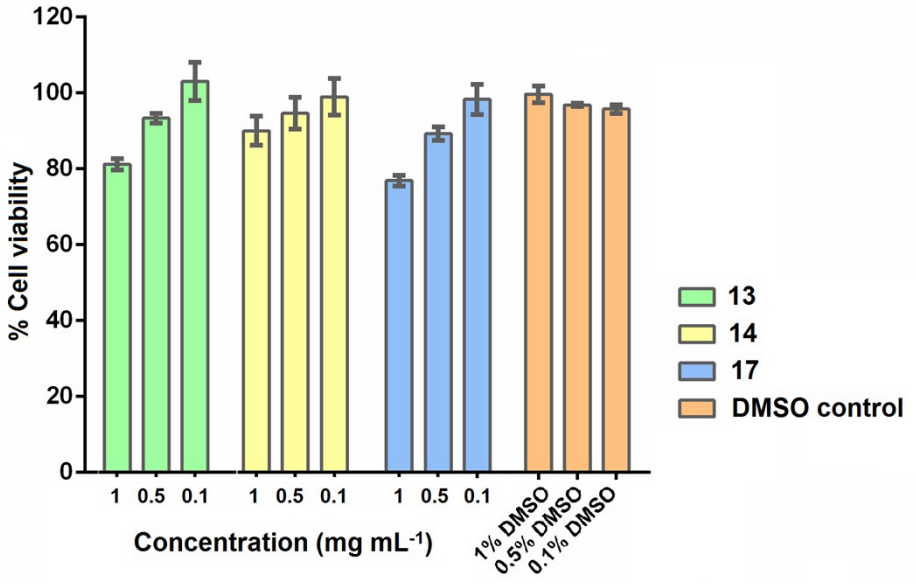
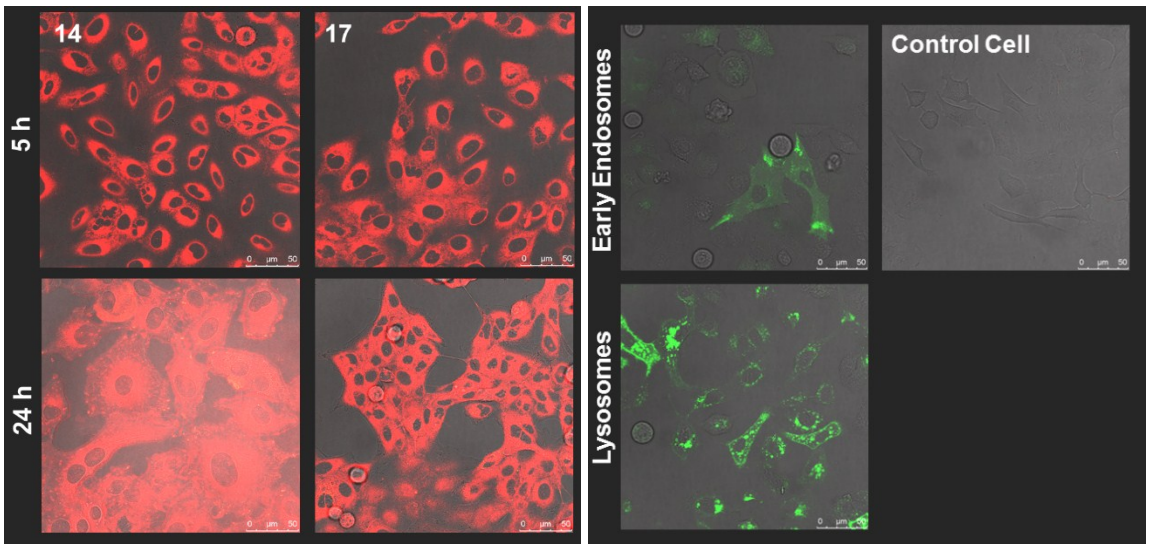


Figure S26. Cytotoxicity profile of dendrimers **13**, **14**, and **17** at three different concentrations for 72 h.

A



B

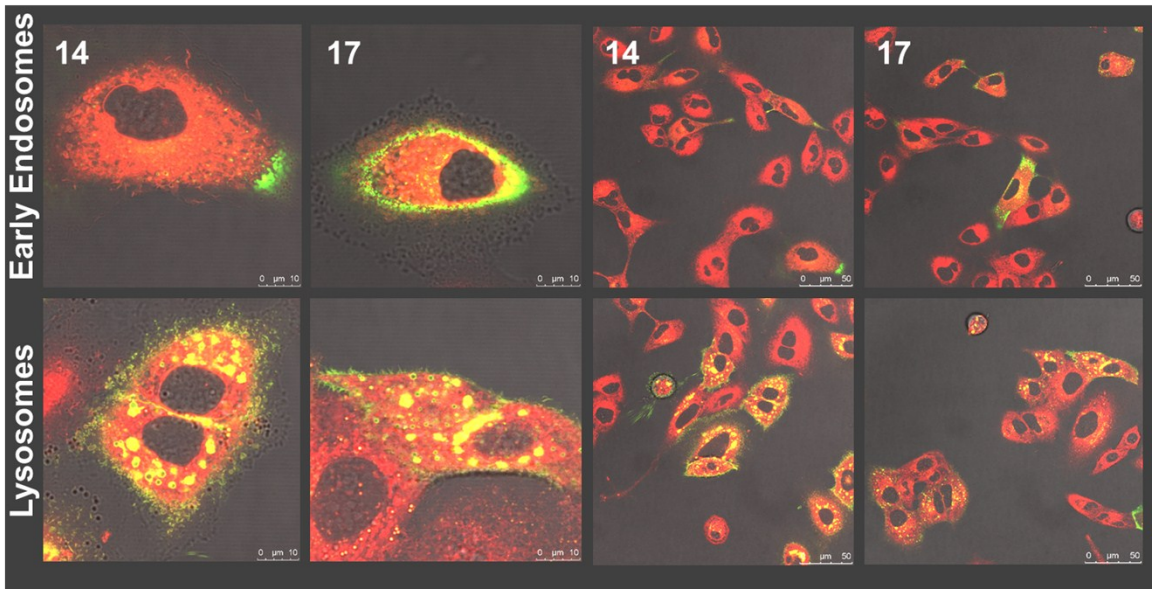


Figure S27. Confocal microscopy images. (A) Confocal laser scanning fluorescence microscopy images from A549 cells after 5 h and 24 h incubation with Nile red loaded dendrimers **14** and **17** and non-treated control cells at a final concentration of 0.5 mg/mL. Nile red is shown in red color and labeled early endosomes and lysosomes in green. The bright field channel is shown in grey scale. (B) Overlay images A549 cells after 5 hours incubation with Nile red loaded dendrimers **14** and **17** and non-treated control cells at a final concentration of 0.5 mg/mL. In the images, Nile red is shown in red color, labeled early endosomes and lysosomes in green. Yellow color indicates a merge of green and red color and therefore shows co-localization of Nile red and early endosomes or lysosomes. The bright field channel is shown in grey scale.

Table S1. CAC measurement and DLS size distribution of the dendrimer nanoparticles.

Dendrimer	CAC (M)	DLS Size (nm)			
		Intensity	**Volume**	**Number**	**PDI**
13	1.30×10^{-4}	737	752	696	0.321
14	6.62×10^{-4}	8.69	7.49	6.74	0.427
16	6.60×10^{-5}	8.48	7.54	6.83	0.214
17	6.65×10^{-5}	7.62	7.07	6.59	0.256

Table S2. Yields of synthesized compounds in mg and percentage value.

S.No.	Reactant (mg)	Product obtained (mg)	Yield (%)	S.No.	Reactant (mg)	Product obtained (mg)	Yield (%)
1.	**2** (150 mg)	**3a** (442 mg)	84%	11.	**3a** (100 mg)	**3** (66 mg)	89%
2.	**2** (150 mg)	**4a** (739 mg)	87%	12.	**4a** (100 mg)	**4** (54 mg)	86%
3.	**3** (100 mg)	**5** (55 mg)	40%	13.	**6a** (100 mg)	**6** (70 mg)	94%
4.	**5** (150 mg)	**6a** (278 mg)	75%	14.	**7a** (100 mg)	**7** (60 mg)	90%
5.	**5** (150 mg)	**7a** (448 mg)	80%	15.	**13a** (100 mg)	**13** (72 mg)	92%
6.	**12** (150 mg)	**13a** (279 mg)	88%	16.	**14a** (100 mg)	**14** (65 mg)	93%
7.	**12** (150 mg)	**14a** (368 mg)	80%	17.	**16a** (100 mg)	**16** (74 mg)	90%
8.	**13** (100 mg)	**15** (62 mg)	32%	18.	**17a** (100 mg)	**17** (63 mg)	85%
9.	**15** (150 mg)	**16a** (178 mg)	70%				
10.	**15** (150 mg)	**17a** (262 mg)	76%				