Hexadecameric Self-Assembled Dendrimers Built from 2'-Deoxyguanosine Derivatives

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A. General Experimental Procedures

DMF was purchased anhydrous from Aldrich and used without further treatment. MeOH (1L) was distilled from Magnesium (5g) and iodine (0.5g). THF was distilled under argon from sodium and benzophenone. All other reagents were from commercial sources and used without further purification. Unless otherwise noted, all compounds were purified by column chromatography on silica gel 60, 0.04-0.063 mm, and TLC and PTLC (from Sorbent Technologies) were performed using EMD silica gel 60 F_{254} glass backed plates from Sorbent Technologies. Visualization of spots was effected with UV light, iodine, 3,5-dinitrophenylhydrazine and phosphomolybdic acid in ethanol stains. Reactions requiring anhydrous conditions were carried out using flame-dried glassware under Argon.

B. Characterization of the target compounds

^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-500 spectrometer, with nominal frequencies of 500.13 MHz for proton or 125.77 MHz for carbon respectively. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in parts per million relative to the undeuterated solvent as an internal reference. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; b, broad. FT-IR analysis were performed on a Bruker Tensor 27 Infrared Spectrometer equipped with a Helios Attenuated Total Reflectance (ATR) accessory with a diamond crystal. Melting temperatures were determined using Fisher brand electro-thermal digital melting point apparatus from Fisher Scientific. High-resolution mass spectral data were obtained from Emory University Mass Spectrometry Center on a Micromass VG AutoSpec magnetic sector mass spectrometer (70 eV)

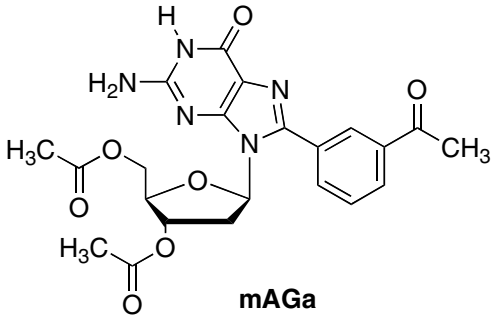
C. Nomenclature

The dendronized subunits **mAGDX** result from linking 8-(*meta*-acetylphenyl)-(3',5'-bis-O-(6-azidohexanoyl))-2'-deoxyguanosine (**mAGhaz**) with the corresponding alkynyl dendrons (**Dg_n**) and haz is the liker 6-azidohexanoic ester. **X** indicates the generation of

the dendronized **mAG** scaffold and equals **n** + 1 where **n** is the generation of the alkynyl dendrons (**Dg_n**).

*D. General method for the esterification of **mAG***

Procedure 1. Crystalline **mAG** (0.260 mmol), pre-dried by suspension in acetonitrile and solvent evaporation (3x), was suspended in anhydrous acetonitrile. To this suspension, TEA (0.571 mmol), isobutyric anhydride (0.571 mmol) (or the corresponding anhydride) and DMAP (0.026 mmol) were added. The reaction mixture was stirred until TLC ($CHCl_3$/MeOH; 80:20, Rf = 0.75) showed complete conversion of starting material. The reaction mixture was quenched by adding excess of MeOH followed by solvent evaporation. The resulting solid material was dissolved in EtOAc and washed with 10% $NaHCO_3$ (2 x 15 ml) and brine (1 x 15 ml). The organic phase was separated, dried over $MgSO_4$ and evaporated into silica gel. Dry loading of the silica column followed by flash chromatography ($CHCl_3$/MeOH; 95:5) afforded the target compounds **mAGa**, **mAGi**, **mAGb**, **mAGh**, and **mAGDp** as solids.



mAGa

8-(3-Acetylphenyl)-(3', 5'-bis-*O*-acetyl)-2'-deoxyguanosine (mAGa)

White powder, mp 166.5 – 167.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.85 (s, 1H), 8.19 (s, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 6.53 (s, 2H), 6.08 (t, J = 6.9 Hz, 1H), 5.40 (m, 1H), 4.41 (dd, J = 4.6, 11.5 Hz, 1H), 4.23 (dd, J = 4.6, 11.4 Hz, 1H), 4.16 (m, 1H), 3.51 (m, 1H), 2.63 (s, 3H), 2.36 (m, 2H), 2.00 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.9, 170.6, 170.4, 157.1, 153.6, 152.5, 146.5, 137.6, 133.7, 130.9, 129.6, 129.3, 129.3, 117.6, 85.1, 82.1, 75.1, 64.1, 34.3, 27.2, 21.2, 21.0. IR (ν_{max}): 2969, 1736, 1675, 1592, 1151, 1067 cm^{-1}. Anal Calcd for $C_{22}H_{23}N_5O_7$: C, 56.29; H, 4.94; N, 14.92; Found: C, 56.26; H, 5.03, N, 14.47.

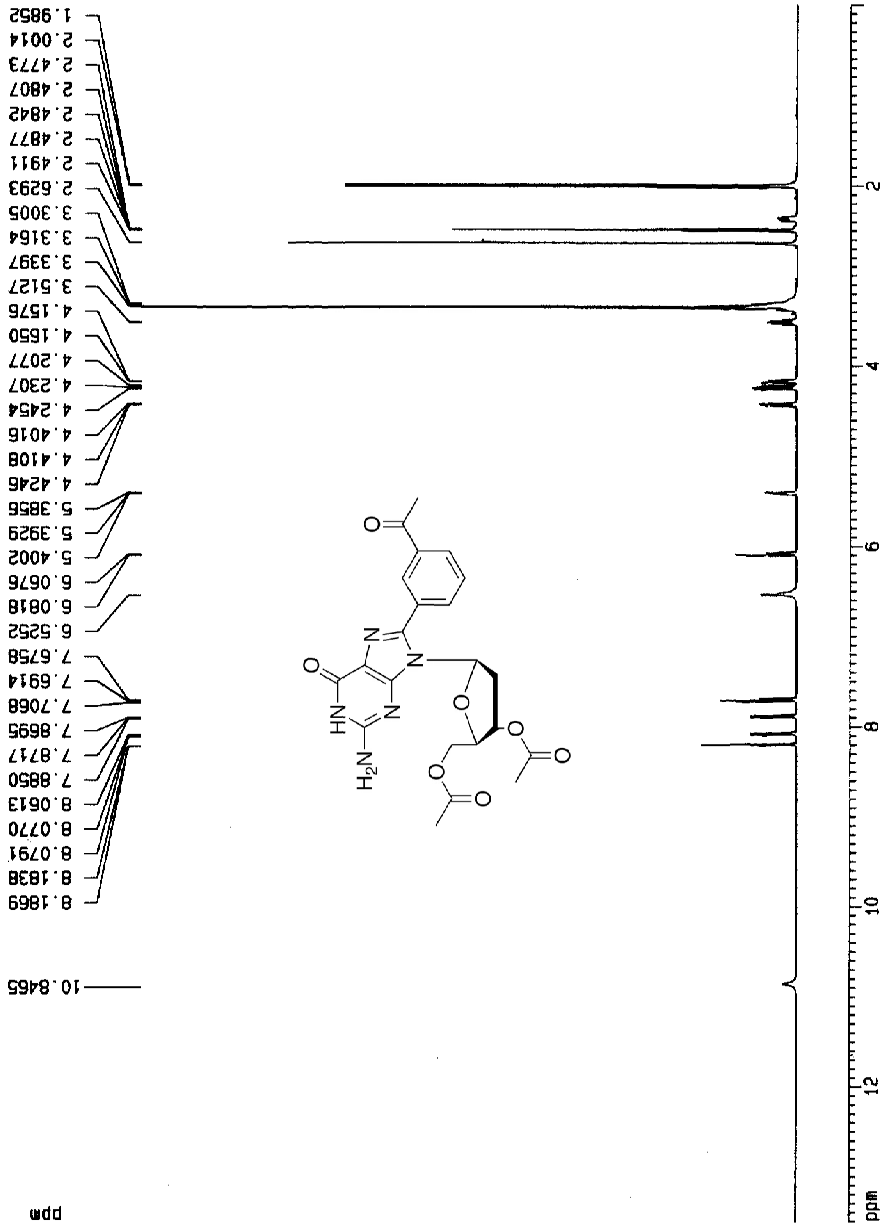


Figure D1 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGa**

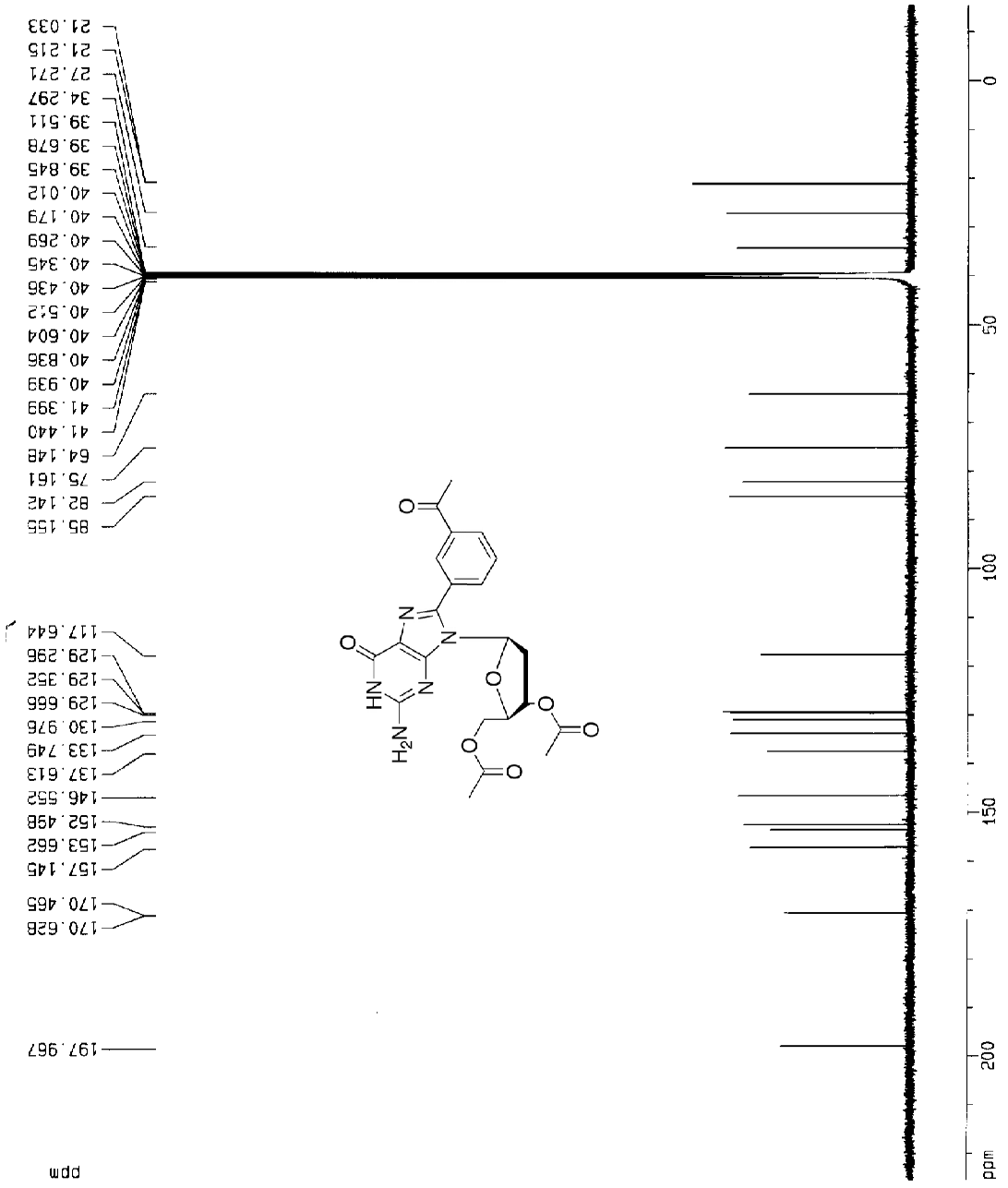
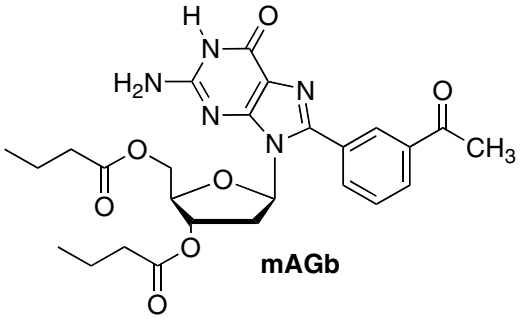


Figure D2 ^{1}H NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGa**



mAGb

8-(3-Acetylphenyl)-(3', 5'-bis-*O*-butiryl)-2'-deoxyguanosine (mAGb)

White powder, mp 167.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.85 (s, 1H), 8.20 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.70 (t, *J* = 7.7 Hz, 1H), 6.52 (s, 2H), 6.10 (t, *J* = 6.9 Hz, 1H), 5.45 (m, 1H), 4.44 (dd, *J* = 4.7, 11.6 Hz, 1H), 4.33 – 4.22 (m, 1H), 4.16 (s, 1H), 3.54 (dt, *J* = 7.0, 13.8 Hz, 1H), 2.64 (s, 3H), 2.37 (m, 1H), 2.27 (q, *J* = 7.1 Hz, 4H), 1.51 (dt, *J* = 7.4, 14.9 Hz, 4H), 0.85 (dd, *J* = 7.5, 16.8 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.4, 172.6, 172.3, 156.7, 153.2, 152.0, 146.1, 137.1, 133.3, 130.4, 129.1, 128.8, 117.2, 84.7, 81.7, 74.6, 63.5, 35.2, 35.1, 33.9, 26.8, 17.8, 13.3. IR (ν_{max}): 2964, 1733, 1681, 1592, 1568, 1253, 1173, 1091 cm^{-1}.

HR-MS (M+H) 526.22920.

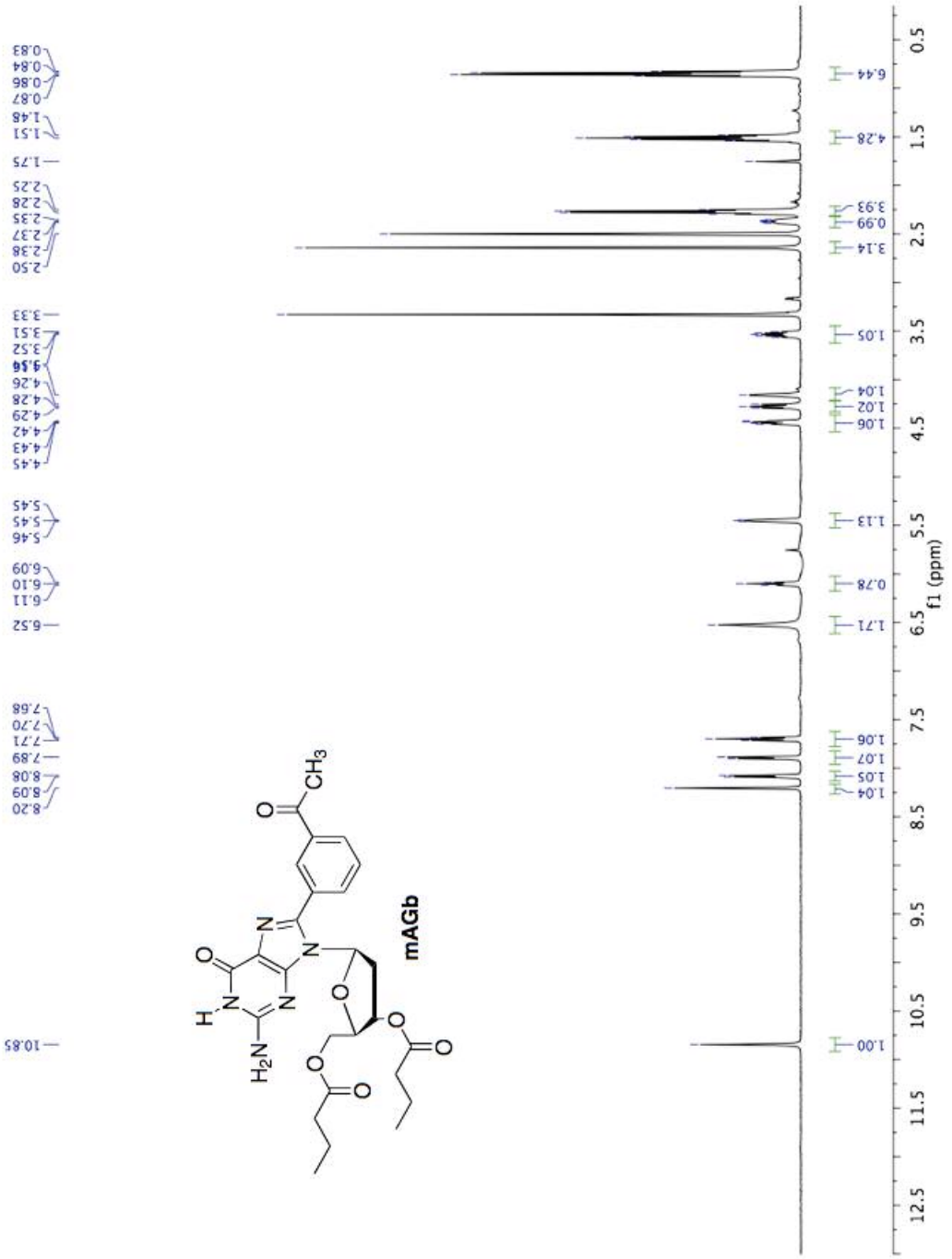


Figure D3 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGb**

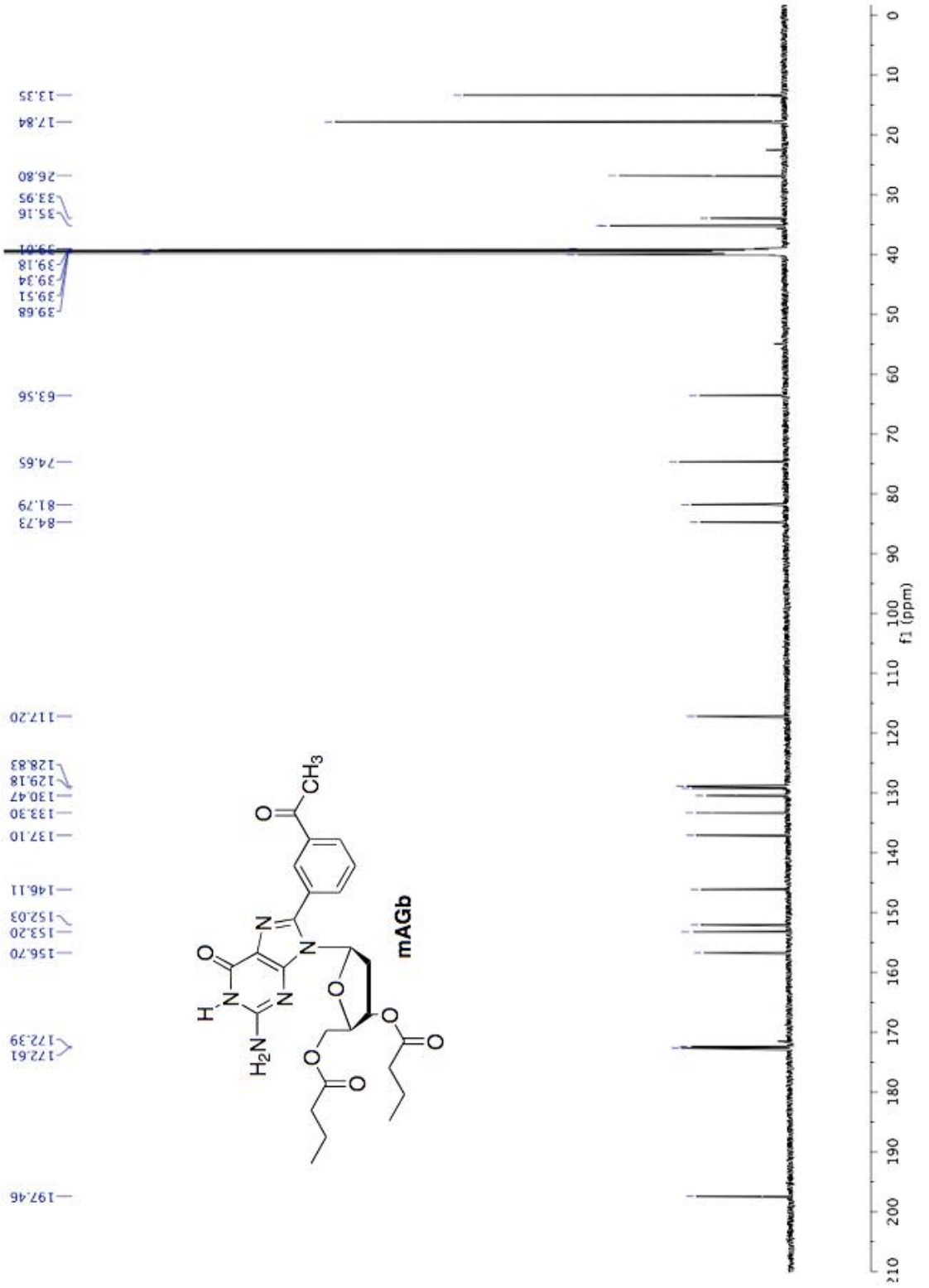
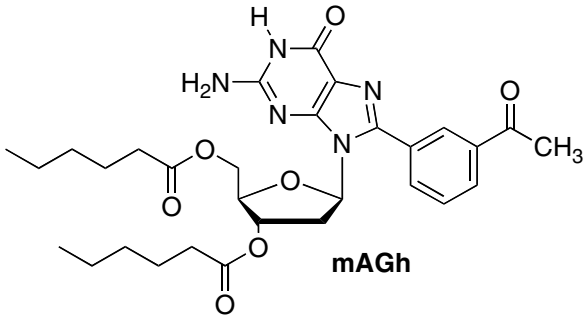


Figure E4 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGb**



mAGh

8-(3-Acetylphenyl)-(3', 5'-bis-*O*-hexanoyl)-2'-deoxyguanosine (mAGh)

White-bone powder, mp 167.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.84 (s, 1H), 8.21 (s, 1H), 8.08 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 7.4 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 6.51 (s, 2H), 6.10 (t, J = 6.9 Hz, 1H), 5.44 (d, J = 3.5 Hz, 1H), 4.43 (dd, J = 4.8, 11.6 Hz, 1H), 4.27 (dd, J = 7.3, 11.4 Hz, 1H), 4.15 (s, 1H), 3.54 (dt, J = 6.9, 13.9 Hz, 1H), 2.64 (s, 3H), 2.38 (m, 1H), 2.28 (q, J = 7.1 Hz, 4H), 1.49 (dd, J = 7.2, 14.3 Hz, 4H), 1.22 (m, 8H), 0.82 (q, J = 6.3 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.4, 172.7, 172.4, 156.7, 153.1, 152.0, 146.1, 137.0, 133.2, 130.4, 129.1, 128.8, 117.2, 84.7, 81.7, 74.6, 63.5, 33.8, 33.3, 33.2, 30.5, 30.5, 26.7, 24.0, 23.9, 21.7, 13.7. IR (ν_{max}): 2969, 1736, 1681, 1631, 1592, 1358, 1246, 1166, 1066 cm^{-1}. HR-MS (M+H) 582.29156

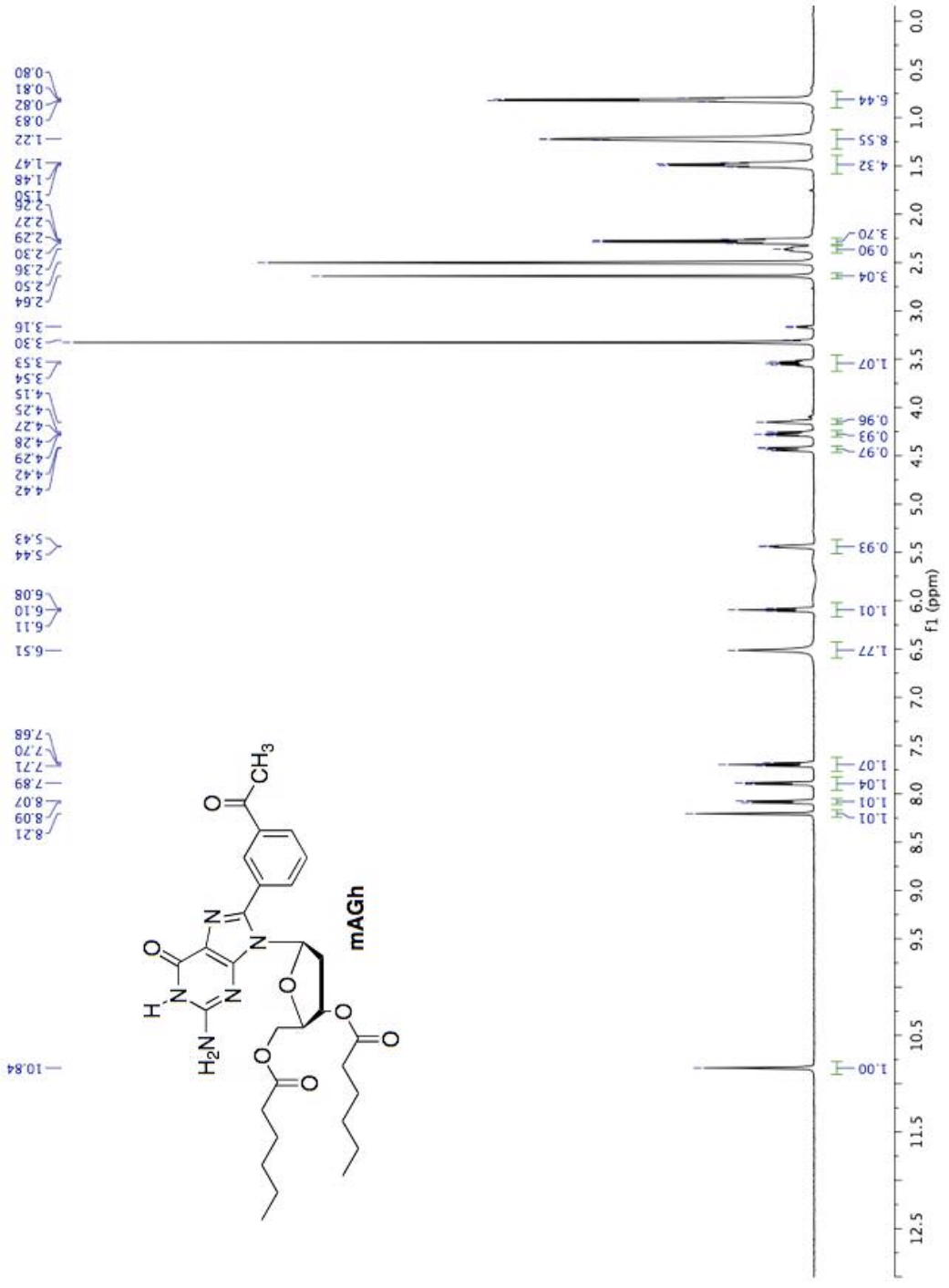


Figure D5 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGh**

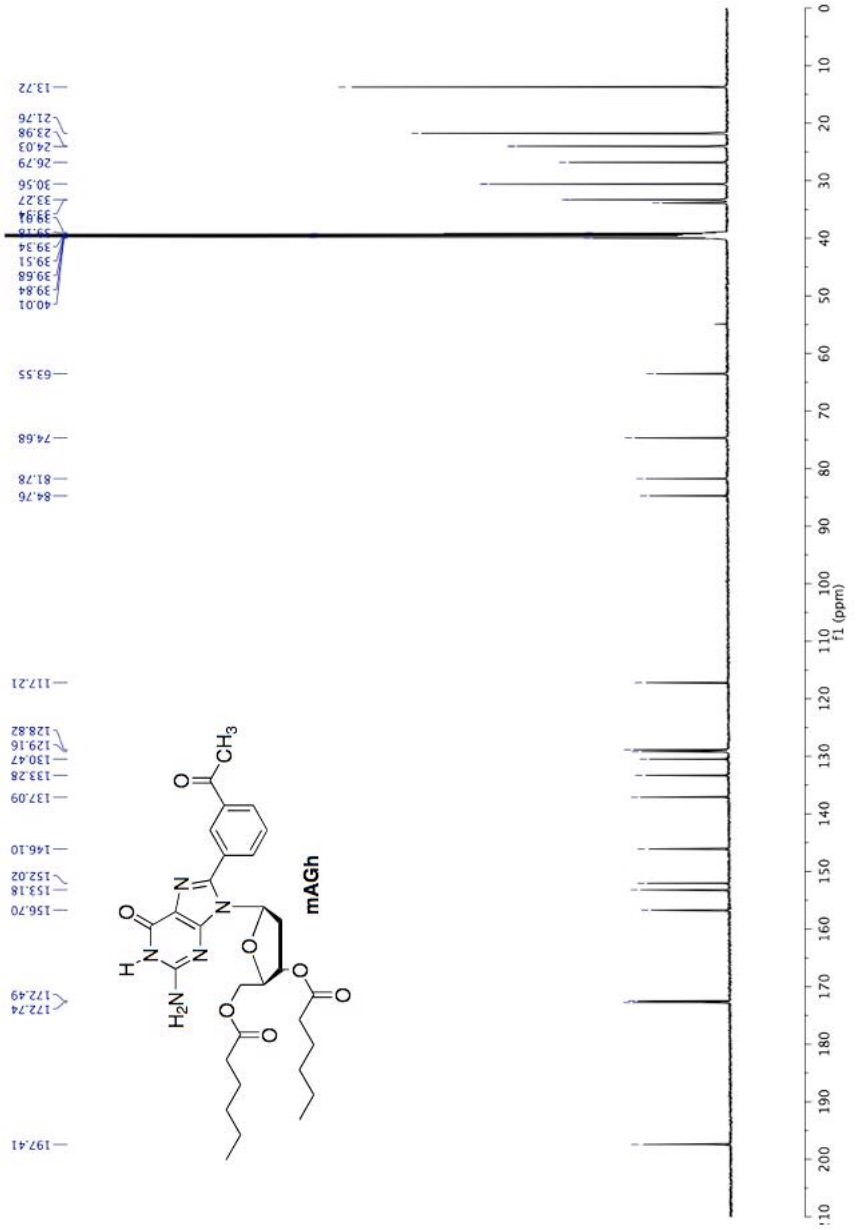
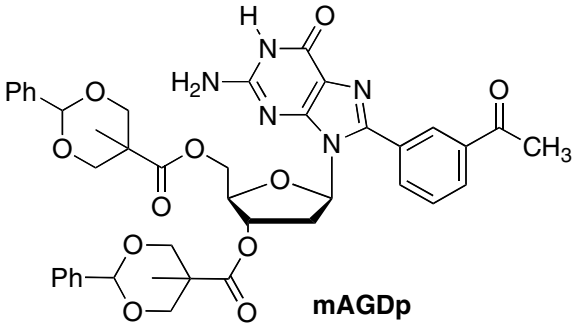


Figure D6 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGh**



mAGDp

8-(3-Acetylphenyl)-(3',5'-bis-O-(5-methyl-2-phenyl-1,3-dioxane-5-carbonyloxy)-2'-deoxyguanosine (mAGDp)

White-bone powder, mp 177.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.86 (s, 1H), 8.21 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.40 – 7.21 (m, 10H), 6.52 (s, 2H), 6.18 (t, J = 6.8 Hz, 1H), 5.62 (s, 1H), 5.49 (d, J = 4.1 Hz, 2H), 4.45 (ddd, J = 7.7, 20.8, 37.1Hz, 6H), 4.25 (s, 1H), 3.81 – 3.50 (m, 6H), 2.59 (s, 3H), 0.94 (dd, J = 18.2, 31.1 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.4, 173.5, 173.3, 156.7, 155.5, 153.5, 153.2, 152.0, 151.9, 146.1, 138.1, 137.0, 133.2, 130.3, 129.1, 128.8, 128.7, 128.7, 128.6, 128.0, 128.0, 127.9, 127.9, 126.1, 126.1, 126.1, 120.6, 117.4, 117.2, 100.5, 100.5, 85.2, 84.7, 81.9, 81.8, 75.2, 74.8, 72.4, 72.3, 64.1, 64.0, 54.9, 42.1, 42.0, 33.9, 26.7, 17.3, 17.0. IR (ν_{max}): 2887, 2852 1731, 1682, 1632, 1594, 1392, 1235, 1169, 1092 cm^{-1}. HR-MS (M+H) 794.30269

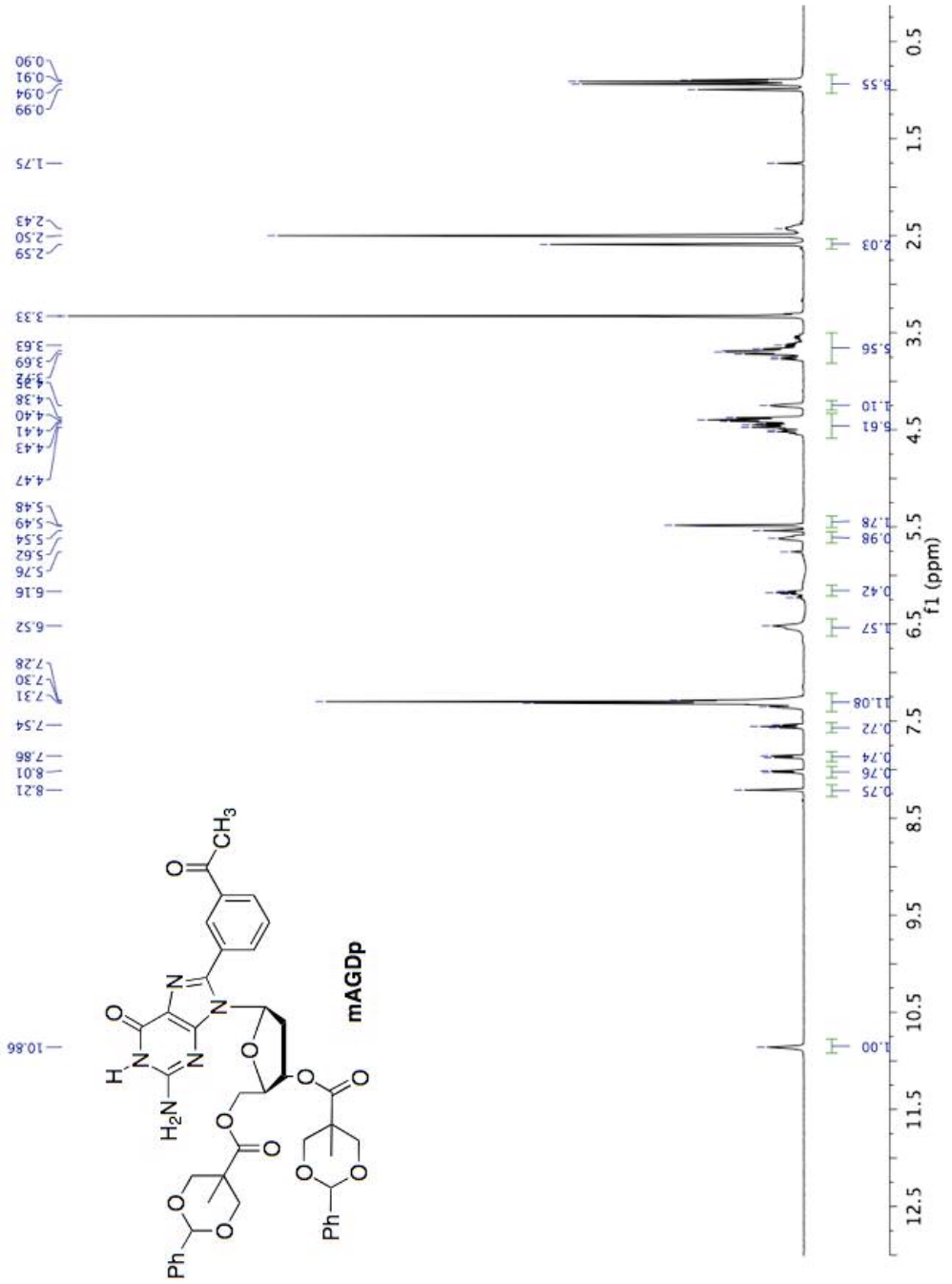


Figure D7 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGDp**

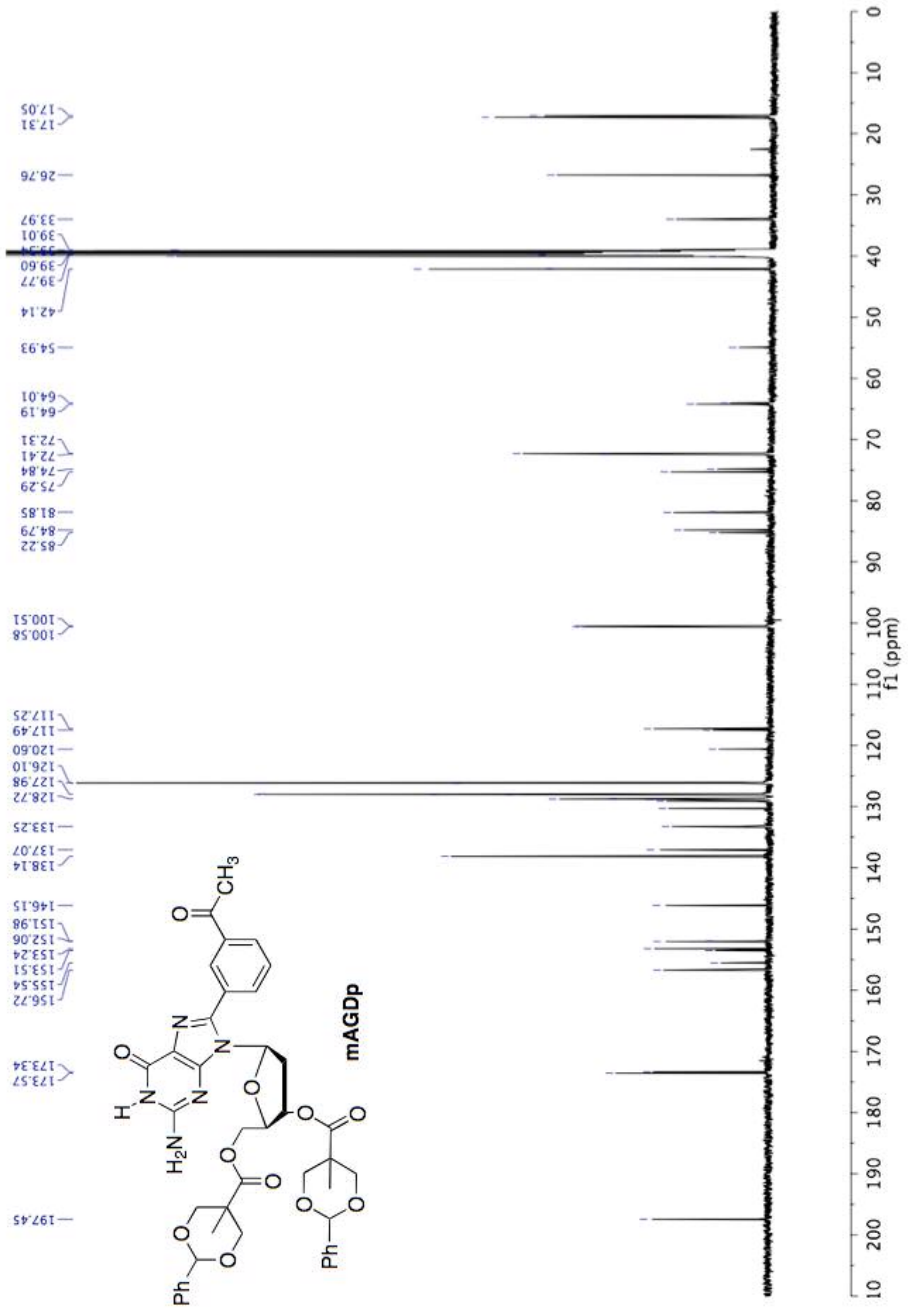
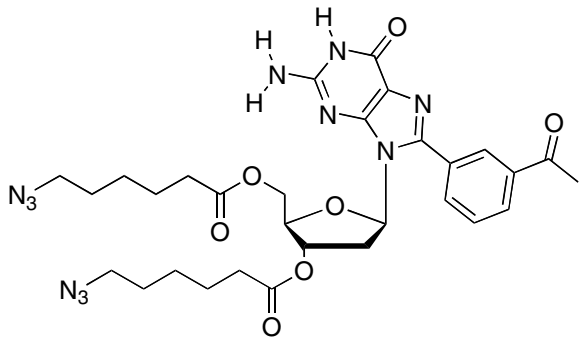


Figure D8 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGDp**

Procedure 2. Powder **mAG**, pre-dried by suspension in acetonitrile and solvent evaporation (3x), was suspended in anhydrous DMF. To this suspension, DPTS (0.5 equivalents), 6-Azidohexanoic acid (2.5 equivalents) and DCC (2.5 equivalents) were added. The reaction mixture was stirred until TLC ($CHCl_3$/MeOH, 80:20) showed complete conversion of starting material. The reaction mixture was quenched by adding excess of MeOH followed by solvent evaporation. The resulting solid material was dissolved in EtOAc and washed with 10% $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL). The organic phase was separated, dried over $MgSO_4$ and evaporated into silica gel. Dry loading of the silica column followed by flash chromatography ($CHCl_3$/MeOH, 95:5) afforded the target compound **mAGhaz** as solids.



Chemical Formula: $C_{30}H_{37}N_{11}O_7$
Molecular Weight: 663.68

8-(3-Acetylphenyl)-(3',5'-bis-O-(6-azidohexanoyl))-2'-deoxyguanosine. (mAGhaz)

Light yellow powder, mp 176.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.97 (s, 1H), 8.21 (s, 1H), 8.08 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.69 (t, J = 7.7Hz, 1H), 6.54 (s, 2H), 6.11 (t, J = 6.8 Hz, 1H,), 5.45 (s, 1H), 4.47 (dd, J = 24.7, 31.8 Hz, 1H), 4.21 (m, 2H), 3.55 (dt, J = 6.7, 13.6 Hz, 1H,), 3.27 (q, J = 6.8 Hz, 4H), 2.64 (s, 3H), 2.39 (d, J = 23.2 Hz, 1H,), 2.30 (dd, J = 6.4, 12.8 Hz, 4H), 1.51 (dd, J = 7.2, 14.5 Hz, 8H), 1.29 (dd, J = 7.3, 15.0 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.4, 172.6, 172.3, 156.6, 153.1, 152.0, 146.0, 137.0, 133.2, 130.4, 129.1, 128.8, 117.1, 84.7, 81.7, 74.6, 63.5, 50.4, 33.8, 33.2, 33.1, 27.9, 27.4, 26.8, 25.5, 23.8, 23.8. IR (ν_{max}): 3324, 2928, 2850, 2091, 1734, 1684, 1624, 1570, 1242, 1087, 640 cm^{-1}. HR-MS (M+H) 664.29470. Yield 75%.

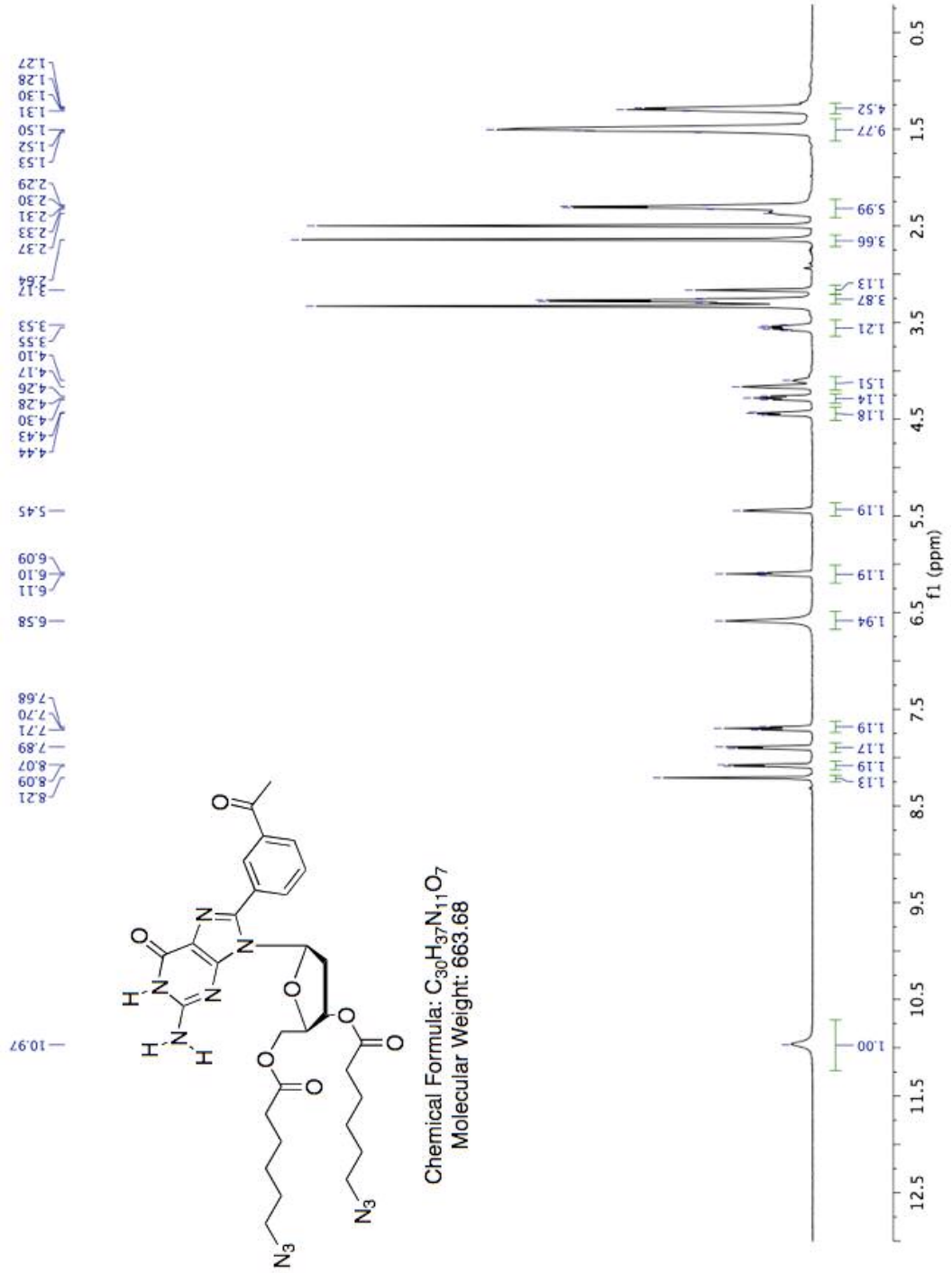


Figure D9 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGhaz**

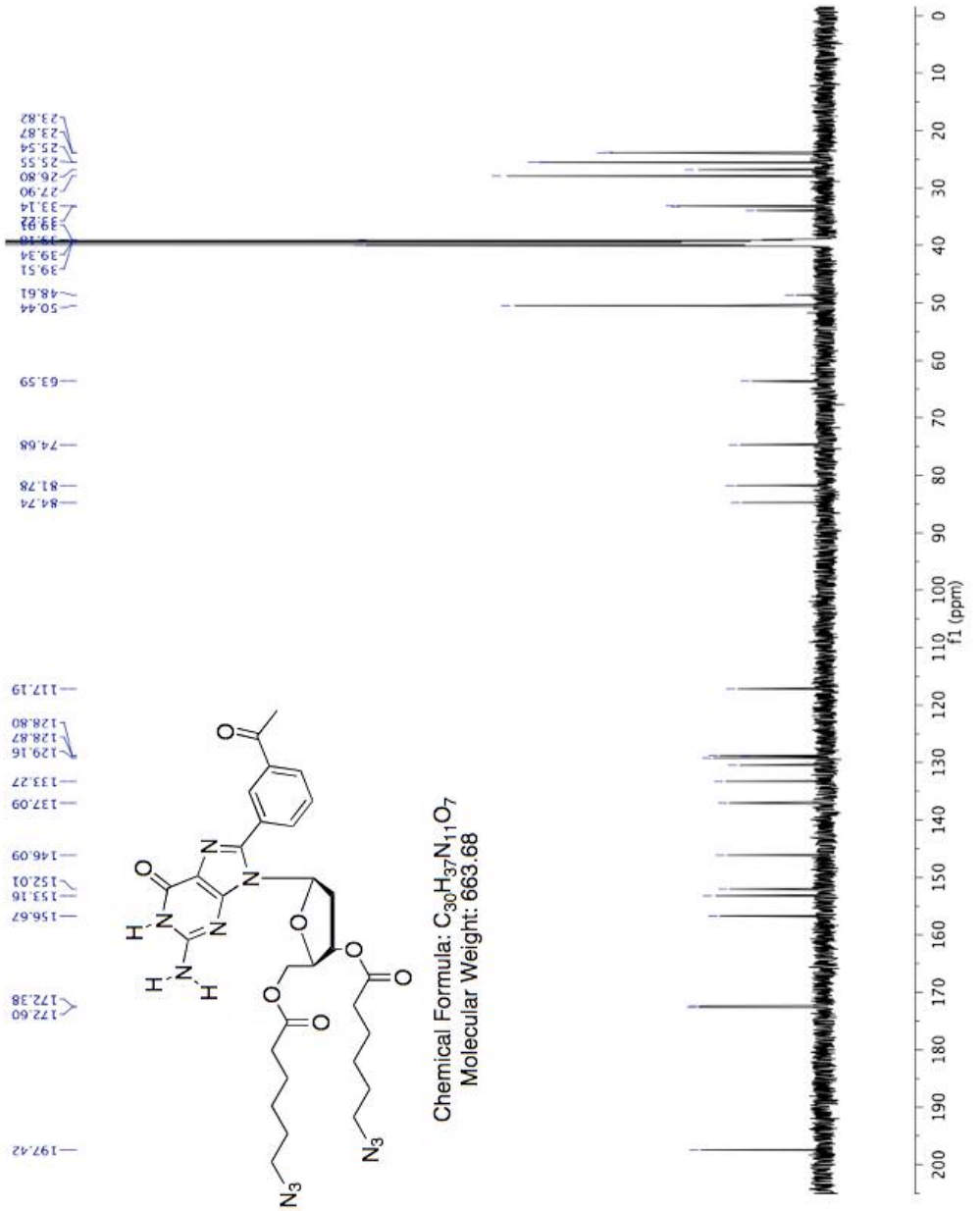
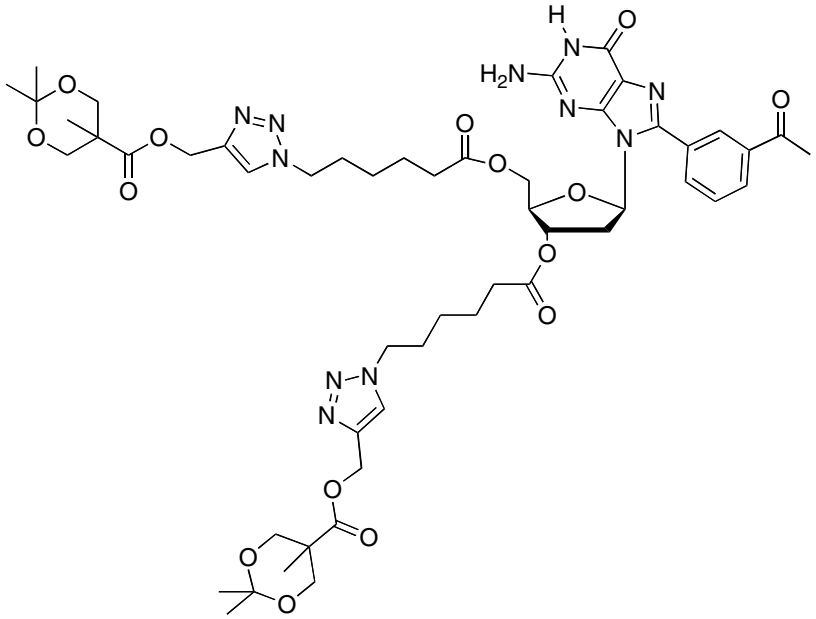


Figure D10 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGhaz**

E. General method for the copper-catalyzed azide/alkyne cycloaddition

To a 10 mL THF:Phosphate buffer (pH 7.0; 3:1) solution of alkyne-**Dg_n** and **mAGhaz** were added sodium ascorbate (15% mol) and $CuSO_4 \cdot 5H_2O$ (5% mol). The reaction mixture was then allowed to stir at room temperature until completion, monitor by TLC ($CHCl_3$:MeOH, 90:10). The solvents were evaporated and the crude product was purified by dry-loading of the silica column followed by flash chromatography column eluting with chloroform and gradually increasing the polarity with methanol ($CHCl_3$:MeOH; 80:20), to give pure **mAGDX** as a solid. Yield >95 %



Chemical Formula: $C_{52}H_{69}N_{11}O_{15}$
Molecular Weight: 1088.17

mAGD2

Light yellow powder, mp 172.5°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.85 (s, 1H), 8.20 (s, 1H), 8.09 (s, 2H), 8.08 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 6.58 (bs, 2H), 6.09 (t, J = 7.1 Hz, 1H), 5.42 (m, 1H), 5.16 (s, 4H), 4.42 (dd, J = 4.5, 12.0 Hz, 1H), 4.28 (q, J = 6.5 Hz, 5H), 4.15 (b m, 1H), 3.99 (d, J = 11.5 Hz, 4H), 3.58 (d, J = 11.5 Hz, 4H) 3.51 (m, 1H), 2.62 (s, 3H), 2.33 (m, 1H), 2.27 (q, J = 6.5 Hz, 4H), 1.75 (q, J = 6.5 Hz, 4H), 1.50 (q, J = 6.5 Hz, 4H), 1.32 (s, 6H), 1.20 (s, 6H), 1.18 (m, 4H), 1.01 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 198.1, 174.1, 173.2, 173.0, 157.5,

154.0, 152.7, 146.6, 142.5, 137.7, 133.9, 131.1, 129.8, 129.5, 129.4, 124.9, 117.8, 98.0, 85.3, 82.4, 75.3, 65.6, 64.2, 58.3, 49.8, 41.9, 34.5, 33.8, 33.7, 28.4, 27.4, 26.1, 24.4, 22.4, 18.6. IR (ν_{max}): 3300, 2975, 2742, 1730, 1684, 1632, 1598, 1252, 1153, 1079 cm^{-1}. HR-MS (M+H) *1088.50293* . Yield 95 %.

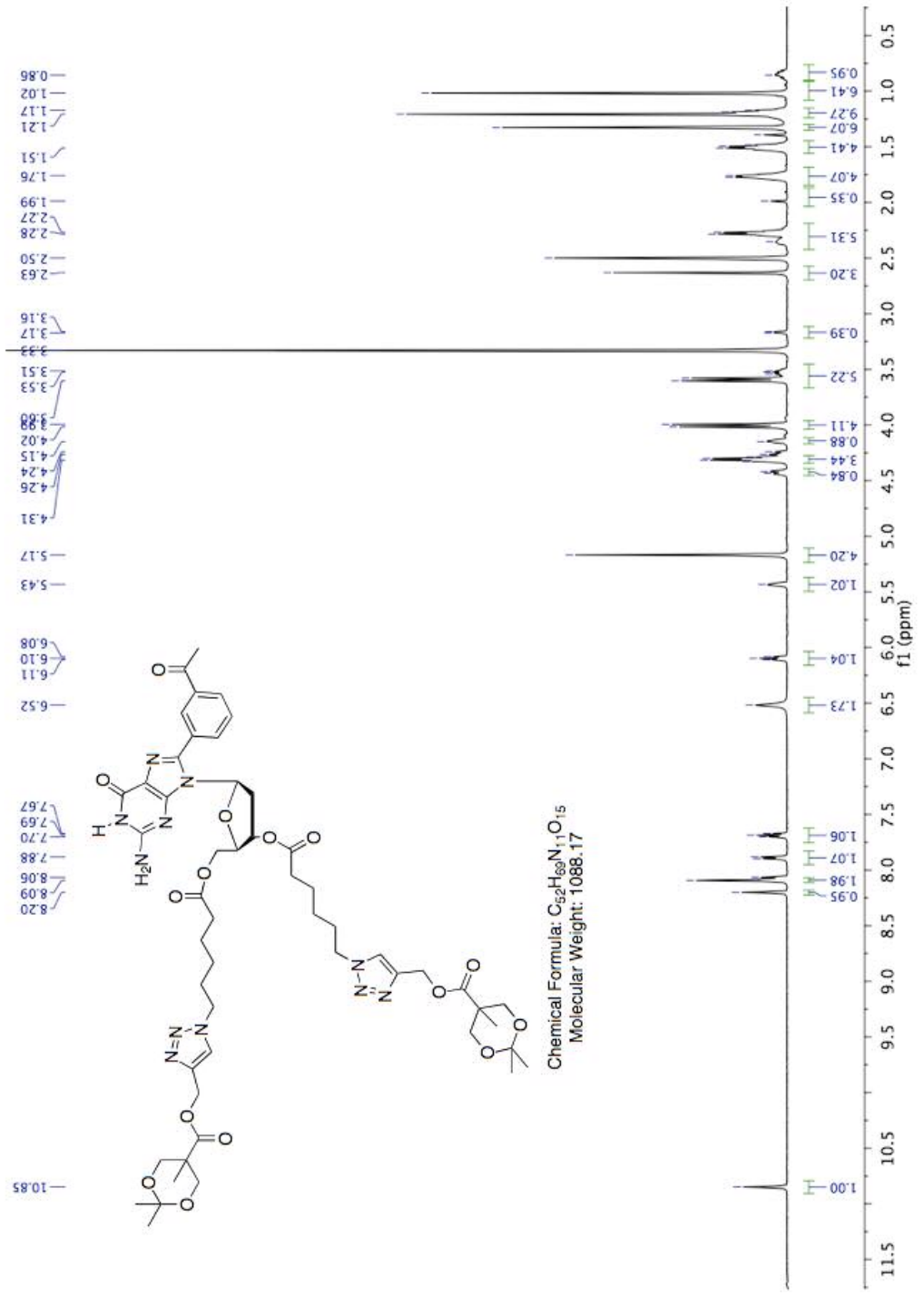


Figure 11 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGD2**

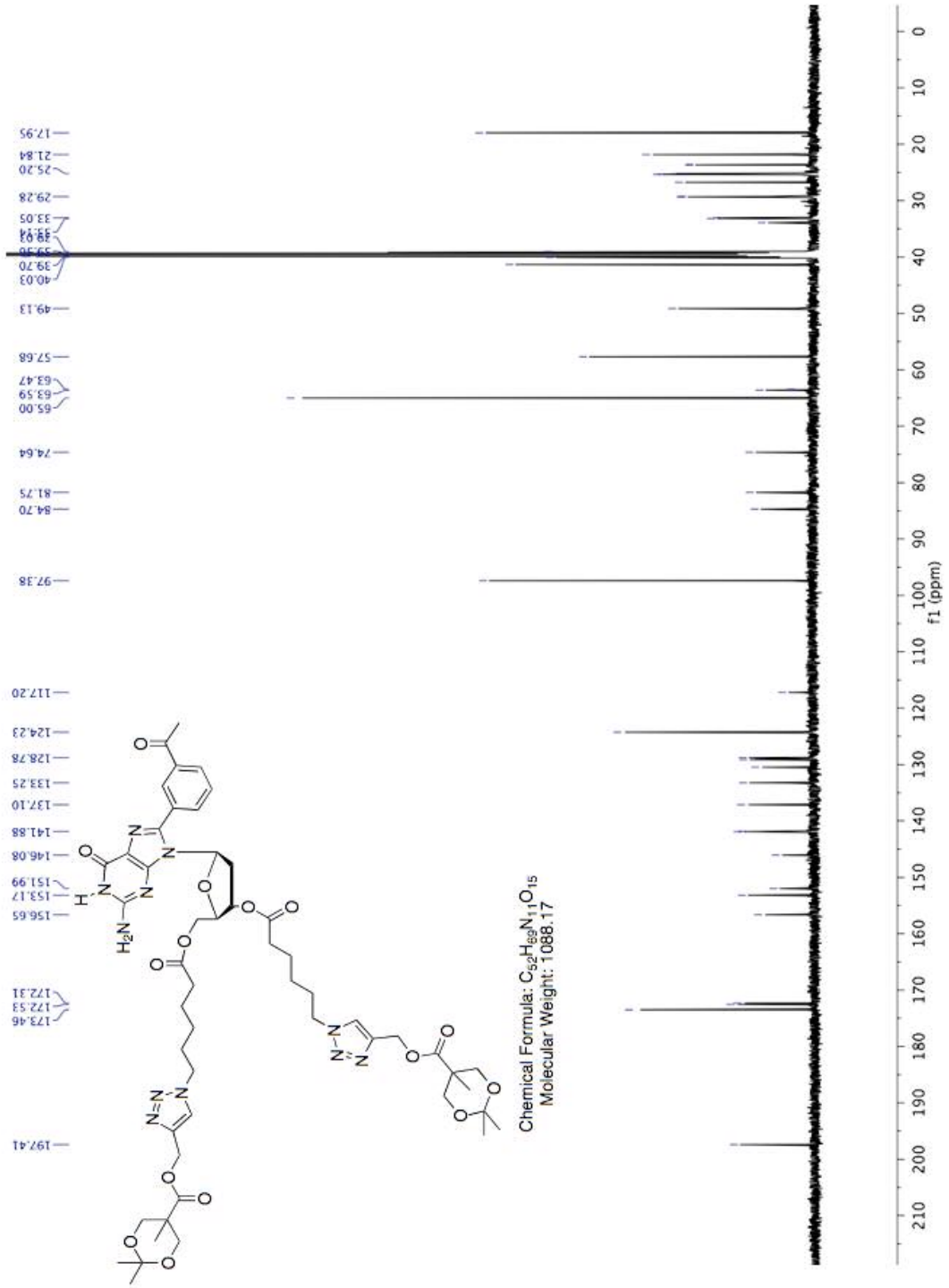
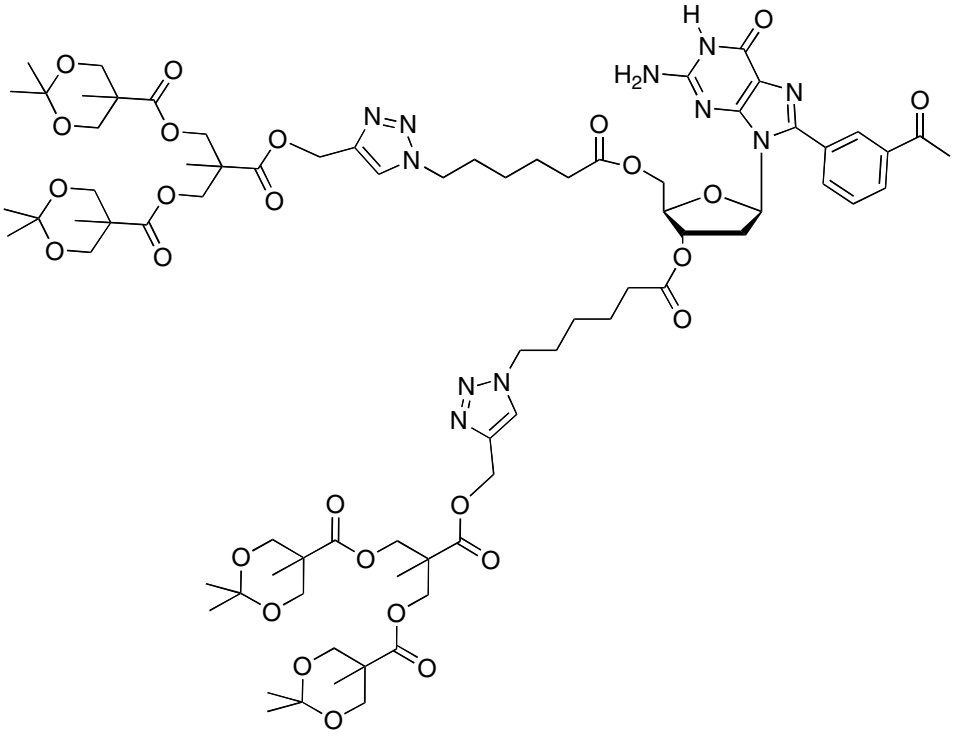


Figure 12 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGD2**



Chemical Formula: $C_{78}H_{109}N_{11}O_{27}$
Molecular Weight: 1632.76

mAGD3

Light yellow powder, mp 182.5°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.20 (s, 1H), 8.12 (d, J = 3.0 Hz, 2H), 8.07 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 6.53 (b s, 2H), 6.10 (t, J = 6.8 Hz, 1H), 5.44 (s, 1H), 5.17 (s, 4H), 4.44 (d, J = 7.4 Hz, 1H), 4.29 (dd, J = 6.9, 13.8 Hz, 5H), 4.19 (m, 8H), 3.92 (d, J = 11.6 Hz, 8H), 3.55 (d, J = 11.6 Hz, 9H), 2.63 (s, 3H), 2.31 (dd, J = 16.0, 23.4 Hz, 4H), 1.77 (d, J = 6.8 Hz, 4H), 1.51 (dd, J = 7.4, 14.9 Hz, 4H), 1.32 (s, 12H), 1.20 (s, 24H), 0.95 (s, 12H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.4, 173.1, 172.5, 172.3, 172.0, 156.6, 153.1, 137.0, 133.2, 130.4, 129.1, 128.7, 124.5, 117.1, 97.3, 74.6, 64.9, 64.9, 64.8, 57.9, 49.1, 46.2, 41.4, 33.1, 33.0, 29.3, 26.7, 25.6, 25.2, 23.6, 21.4, 17.7, 17.12. IR (ν_{max}): 3300, 2975, 2742, 1731, 1684, 1633, 1596, 1455, 1372, 1252, 1239, 1217, 1198, 1151, 1121, 1078, 1039, 1026 cm^{-1}. HR-MS (M+H) 1632.75685 Yield 96 %.

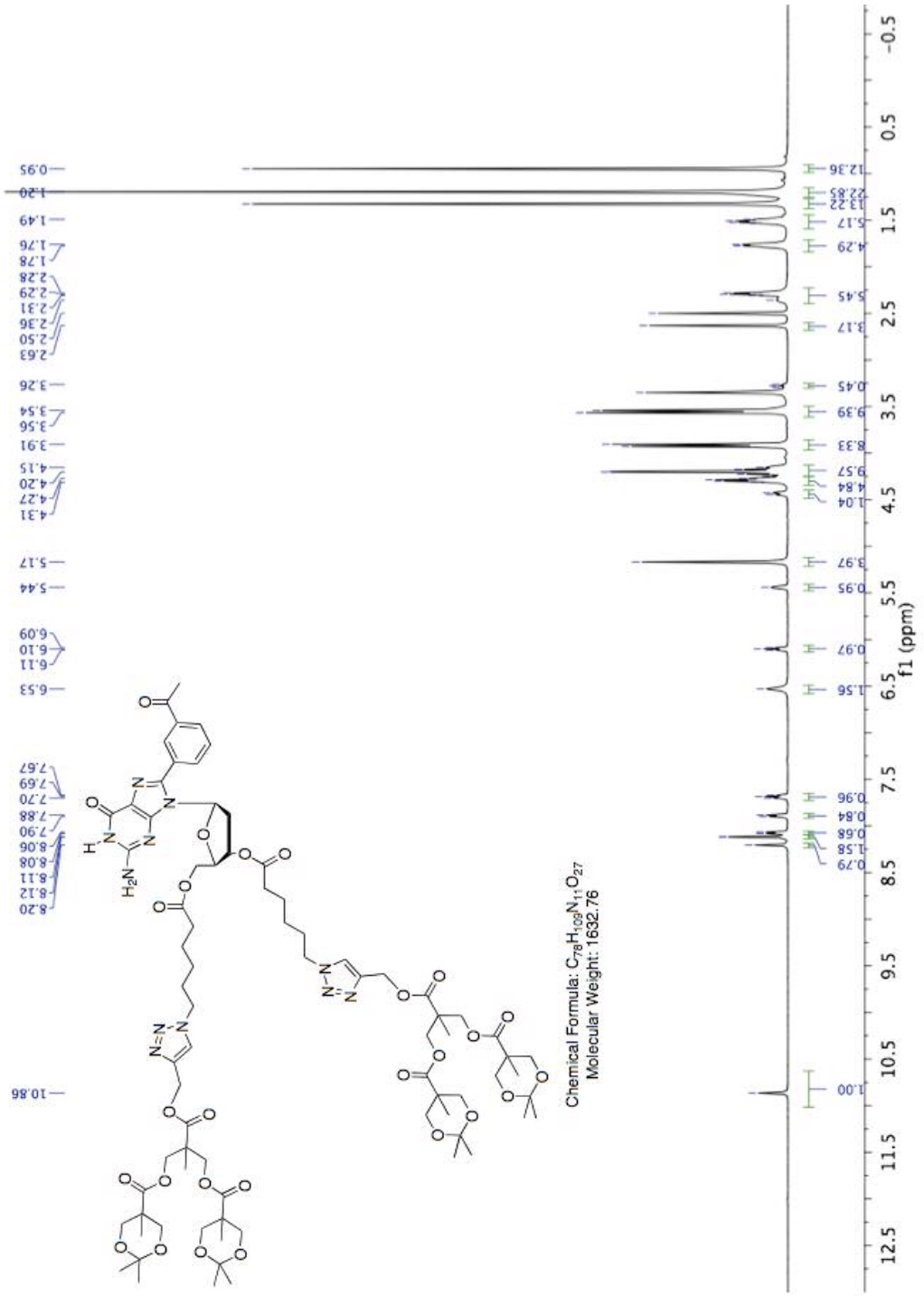


Figure E3 ^{1}H NMR (DMSO-d_6, 500 MHz, 298 K) spectrum of **mAGD3**

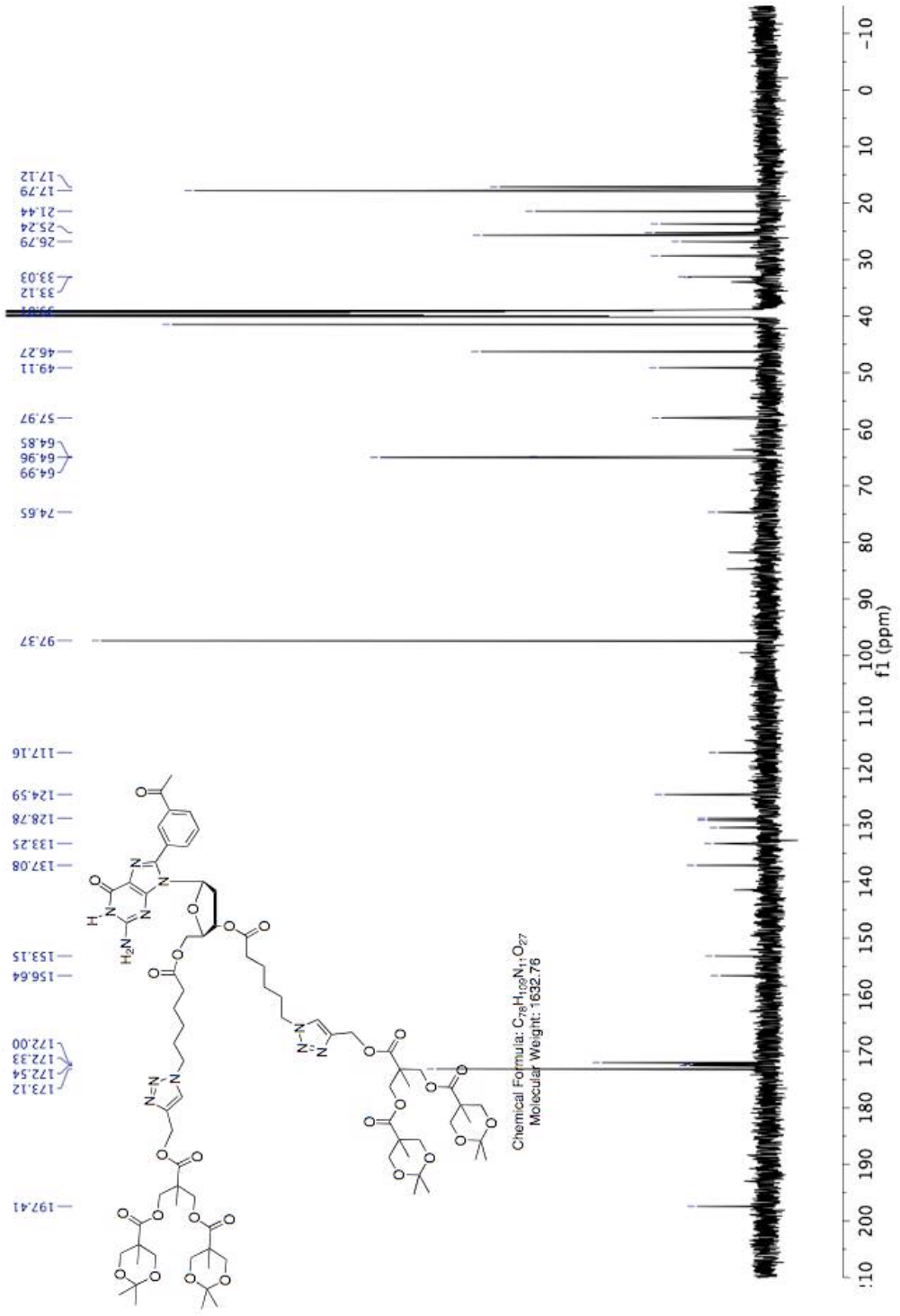


Figure 13 ^{13}C NMR (DMSO-d_6, 125 MHz, 298 K) spectrum of **mAGD3**

*F. Titration studies of **mAGDX** with potassium iodide monitored by ^{1}H NMR*

The main characterization method used to measure the formation of the self-assembled species was ^{1}H-NMR spectroscopy in deuterated solvents. The self-assembly studies was using K^+ (as KI) as a template for the self-assembly of **mAGDX**. Potassium is a bio-relevant cation and the low basicity of iodide as a counterion prevent its interference with the resulting supramolecule; in addition, KI is readily soluble in methanol and partially soluble in acetonitrile.

Upon controlled addition of potassium iodide into 30-50 mM solutions of **mAGDX** we observed the formation of G-quadruplexes with different levels of fidelity. Fidelity is the relative amounts of quadruplexes formed by various **mAGDX** derivatives determined by ^{1}H NMR (298 K) in $CDCl_3$ and CD_3CN using the potassium cation as a template.

Figure S1. Titration of a solution of **mAGD2** (30 mM) with potassium iodide as measured by ^{1}H-NMR (500 MHz, CD_3CN, 298.2 K)

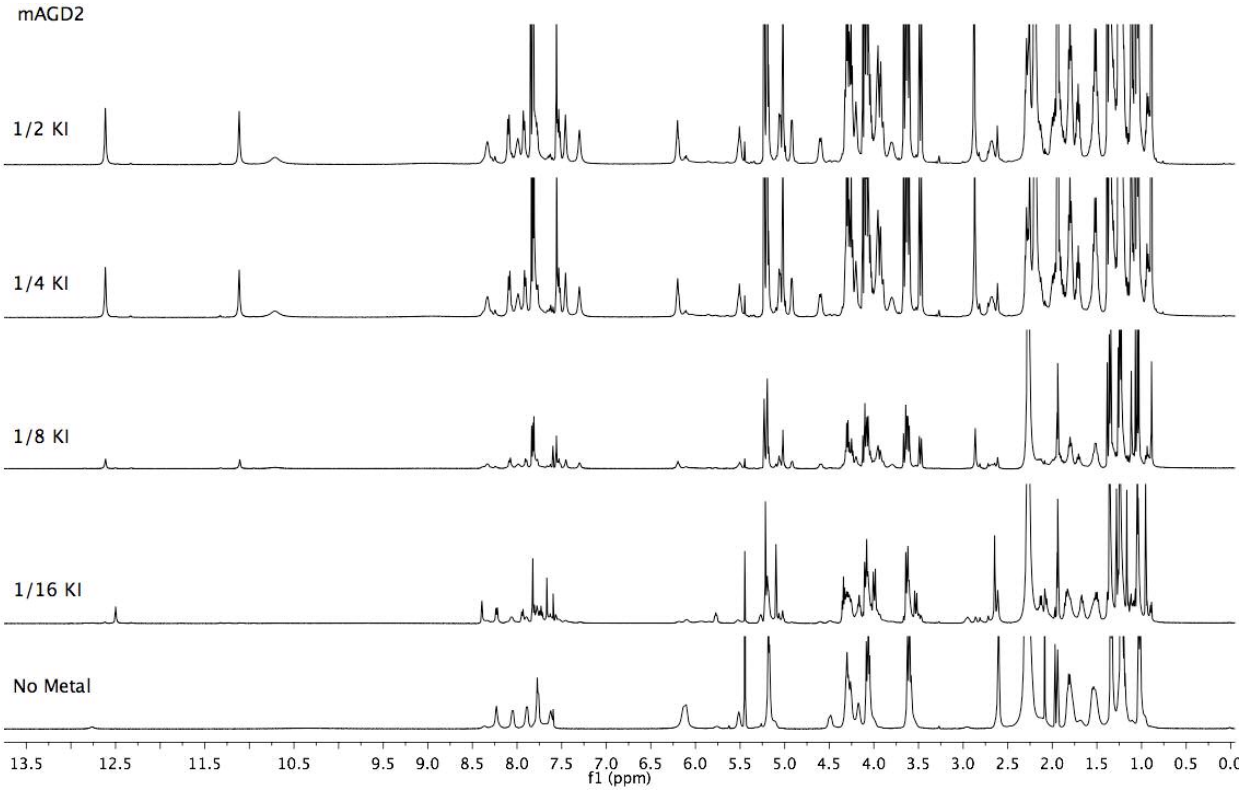
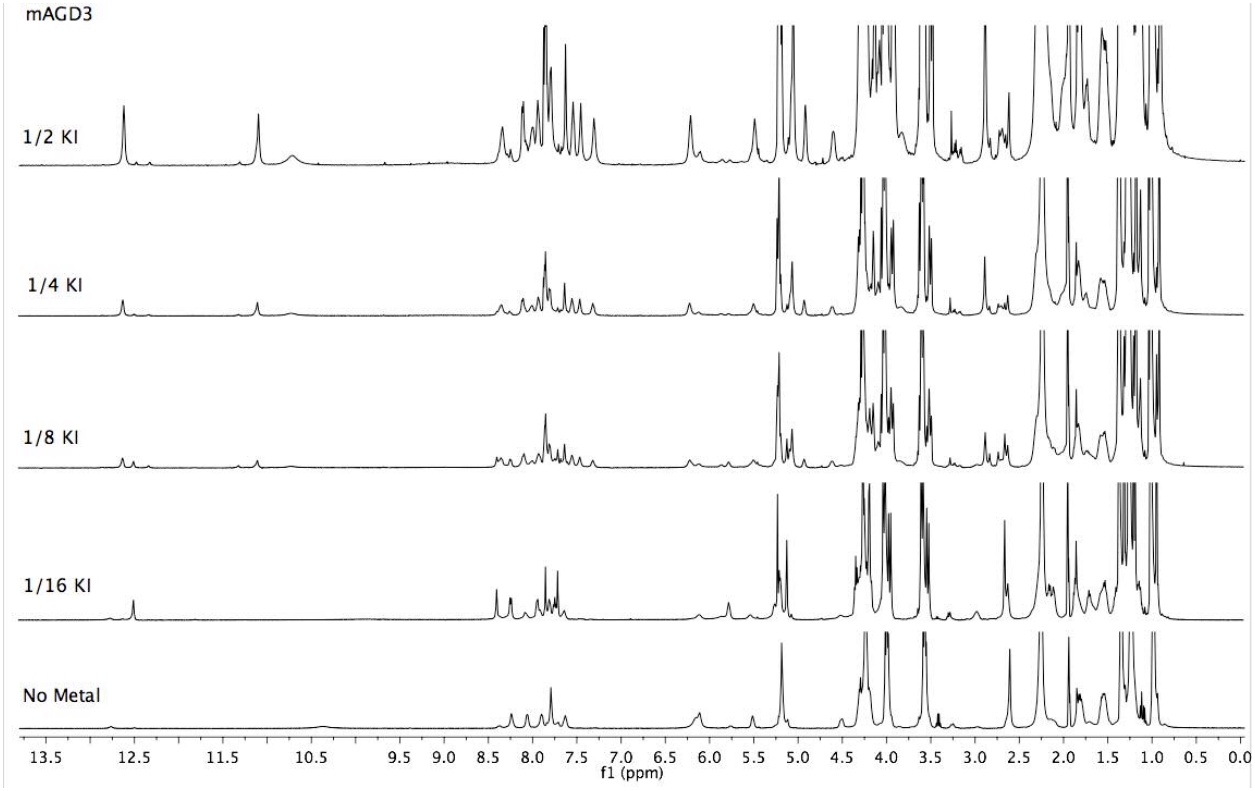


Figure S2. Titration of a solution of **mAGD3** (30 mM) with potassium iodide as measured by ^{1}H-NMR (500 MHz, CD_3CN, 298.2 K)



G. Vapor Pressure Osmometry studies.

The Vapor Pressure Osmometer (VPO) instrumet used was a Knauer K-7000 (Dampfdrukosometer) with software EuroOsmo® version 1.3. Calibrating solutions were prepared to construct a calibration curve and obtain a calibration constant (K_{calib}) or intercept in concentrations ranging from 0.02 to 0.05 molar solutions of a standard compound, an iron pyrazolato complex of formula $Fe_8O_4(C_3H_3N_2)_{12}Cl_4$ and a molecular weight of 1457 g/mol. Each trial solution, for the **mAGDX** derivatives and the calibration standard were tested 3-5 times per solution, using dry acetonitrile and in increasing order of concentration. A stock solution of **mAGDX** derivatives (45 – 50 mM) in CH_3CN containing excess of crystalline KI was decanted and divided into three 45 mM, 47.5 mM and 50 mM solutions. The start gain was set to 128 and the chamber temperature was set to 40°C (acetonitrile).

Validation of the calibration method showed a molecular weight for the standard of 1397±4 g/mol whereas the theoretical molar mass is 1457 g/mol. The percentage of difference is 4%. The chamber and instrument were kept in stabilization periods of 30 min to an hour. Auto-zeroing periods followed every measurement to obtain a stable baseline in the instrument's monitor. Input of the concentrations, volumes and molecular weight of the monomer used prior the injection of samples yielded into computer calculated average molecular weight for a given sample.

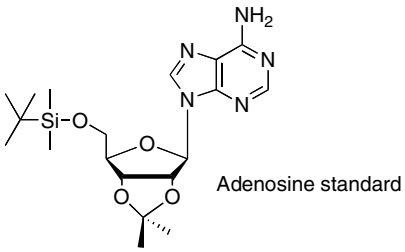
H. Diffusion NMR studies

Diffusion experiments were carried out with a Bruker DRX-500 spectrometer, using the Stimulated Echo Pulse Gradient sequence in FT mode. To improve homogeneity a "13 interval pulse sequence" was used with two pairs of bipolar gradients. All samples for the diffusion measurements were prepared in Shigemi tubes (Shigemi, Inc., Allison Park, PA) and the temperature was actively controlled at 25.0 ± 0.5°C. Diffusion coefficients were derived using integration of the desired peaks to a single exponential decay, using the T1/T2 Relaxation (Bruker TopSpin v 2.0). Calculation of the hydrodynamic radii in CD_3CN was done by using the viscosity value (η = 0.341 $Kg \bullet m^{-1} \bullet s^{-1}$, 298.15°K) provided by the solvent supplier. The hydrodynamic radii were calculated according to the

spherical approximation using the Einstein-Stokes equation: $D = \frac{k_B T}{6\pi\eta r}$ where T denotes the temperature, η is the viscosity of the solvent at the given temperature and k_B is the Boltzmann-Constant. All the measurements were performed in triplicate and the uncertainty is given by the standard deviation.

Figure S3. Stejskal-Tenner plot of SADs and the standard in CD_3CN.

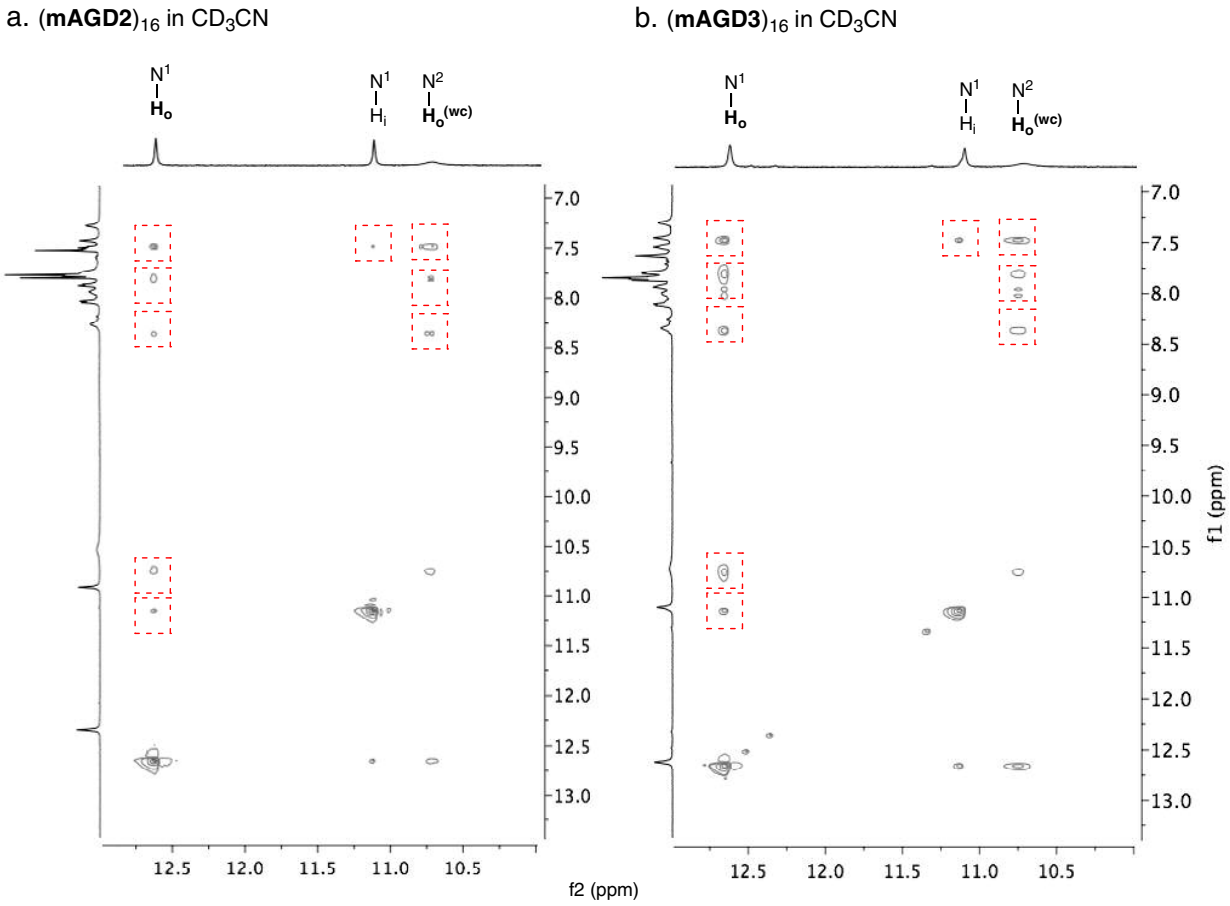
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



Adenosine standard

I. 2D-NOESY experiments

Figure S4. A portion of the NOESY spectra (500 MHz, CD_3CN, τ_m = 500 msec) for (a) (**mAGD2**)$_{16}$•3KI and (b) (**mAGD3**)$_{16}$•3KI. The red squares point to the signature cross peaks that are characteristic of the hexadecamer formed by the **mAG** scaffold.



J. Variable Concentration ^{1}H NMR Experiments

^{1}H NMR of **mAGDX** solution at variuos concentrations were recorded in CD_3CN while containing an excess of crystalline KI. The fraction of ordered species was determined by integration of the area under the peaks and reported as the ratio between the monomer and the G-quadruplex.

Figure S5. Variable concentration ^{1}H-NMR experiments (500 MHz, CD_3CN, 298.2 K) of **$(mAGD2)_{16}$•3KI**.

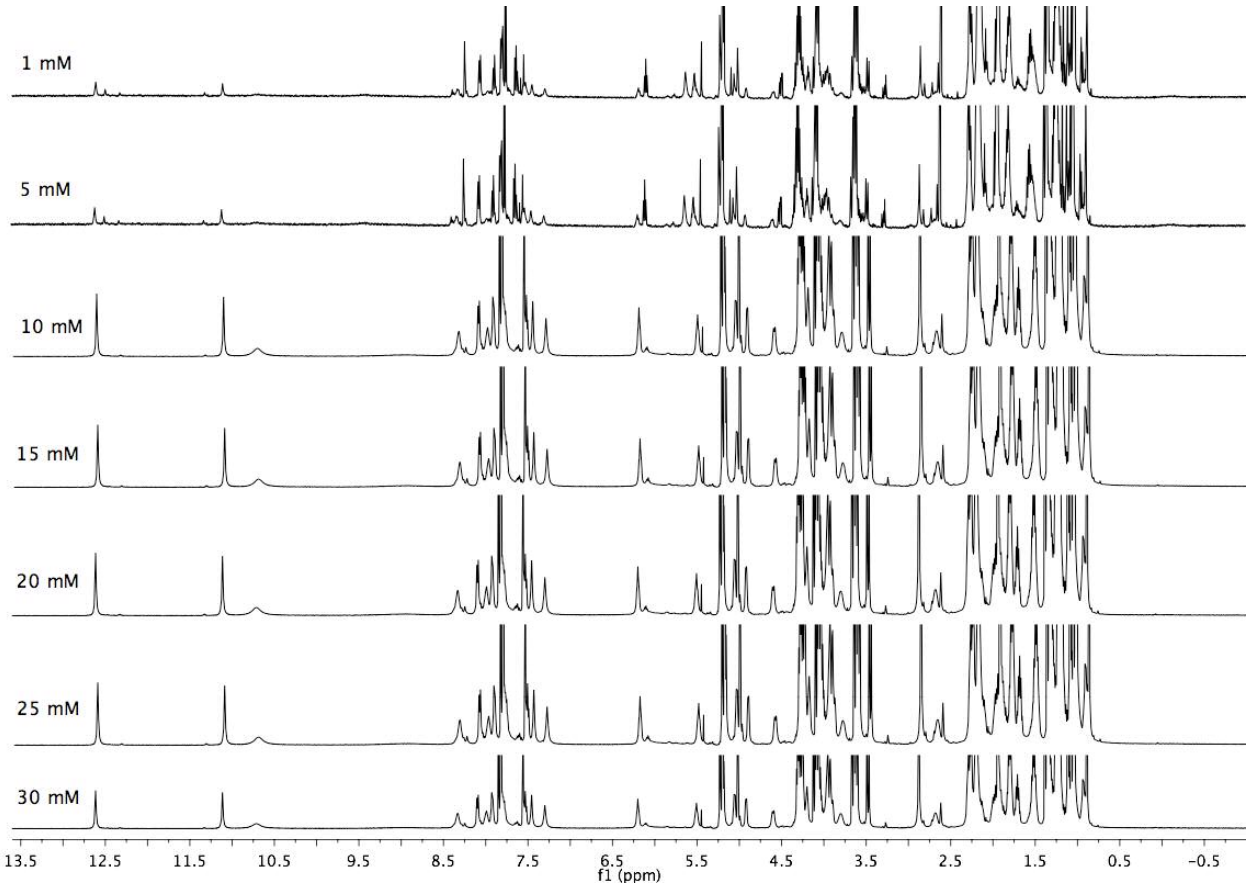


Figure S6. Variable concentration ^{1}H NMR experiments (500 MHz, CD_3CN, 298.2 K) of **$(mAGD3)_{16}$•3KI**.

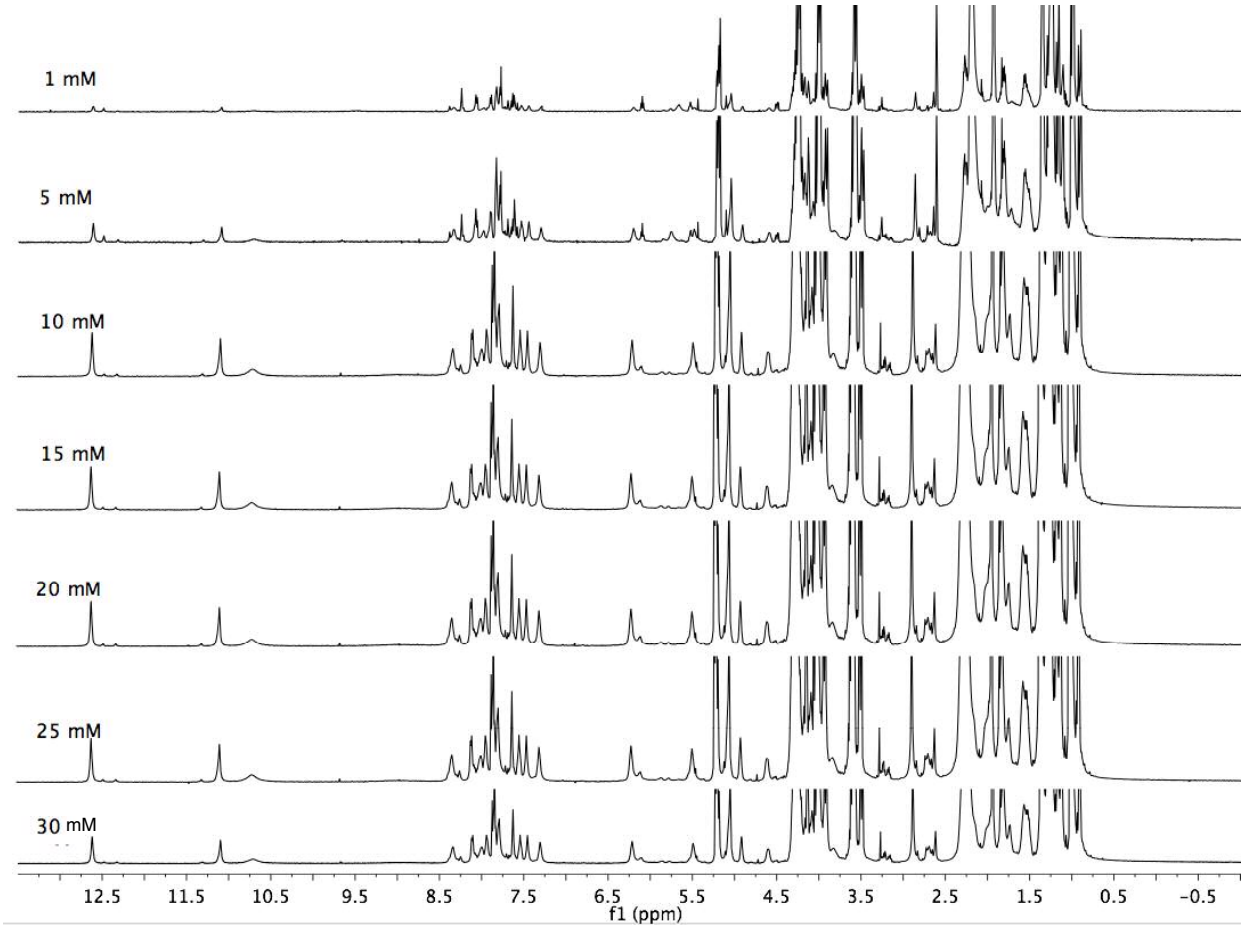


Table S1. Fraction of ordered species (f) at different concentrations (G_T) of SADs in CD_3CN[b] at 298.2 K

G_T	f/mAGD2[c]	f/mAGD3[c]
30	0.93 (100%H)	0.92 (100%H)
25	0.93 (100%H)	0.92 (87%H, 1%O, 4%D)
20	0.93 (100%H)	0.92 (87%H, 1%O, 4%D)
15	0.93 (100%H)	0.92 (87%H, 1%O, 4%D)
10	0.93 (100%H)	0.92 (87%H, 1%O, 4%D)
5	0.93 (83%H, 2%O, 8%D)	0.92 (71%H, 10%O, 11%D)
1	0.41(25%H, 6%O, 10%D)	0.41 (23%H, 10%O, 8%D)

[a] Ratio $f = [X_O]/[X_T]$, where $[X_O]$ and $[X_T]$ represent the concentration in ordered form (i.e. sum of all the SADs formed) and the total concentration of **mAGD2** and **mAGD3**, respectively.

[b] In CD_3CN, both monomeric and assembled species can be clearly distinguished in the ^1H NMR spectra.

[c] The numbers in parenthesis represent the percent composition of the fraction of ordered species (f).

K. Variable temperature ^{1}H NMR experiments

A solution of **mAGDX** (5 mM) containing an excess of KI was placed in a threaded cap sealed NMR tube and ^{1}H NMR (SW = 20, NS = 64, DS = 2) was recorded in CD_3CN at 298.15 K and subsequently at every additional 5 K up to 323.15 K. The fraction of ordered species was determined by integration of the area under the peaks and reported as the ratio between the monomer and the G-quadruplex.

Figure S7. Variable temperature ^{1}H-NMR experiments (500 MHz, CD_3CN) of **(mAGD2)$_{16}$•3KI**.

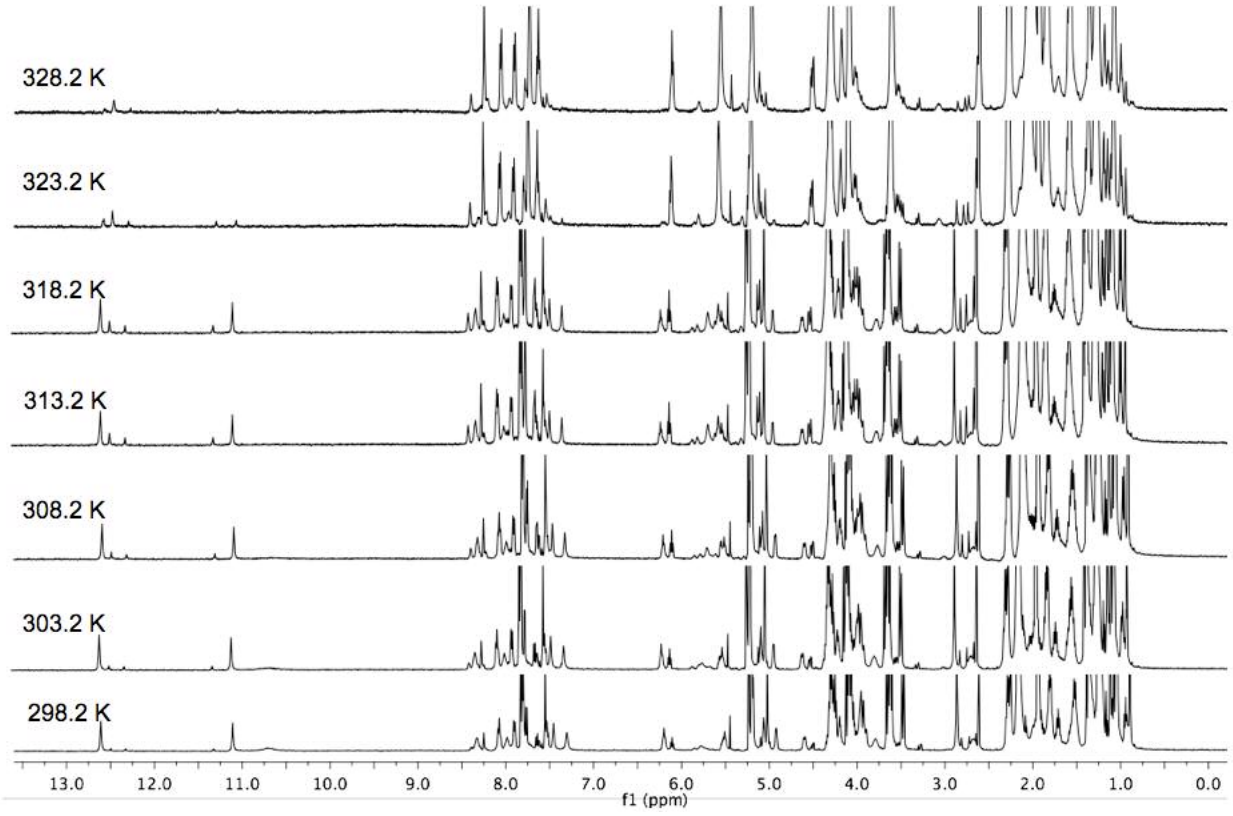


Figure S8. Variable temperature ^{1}H-NMR experiments (500 MHz, CD_3CN) of **(mAGD3)$_{16}$•3KI**.

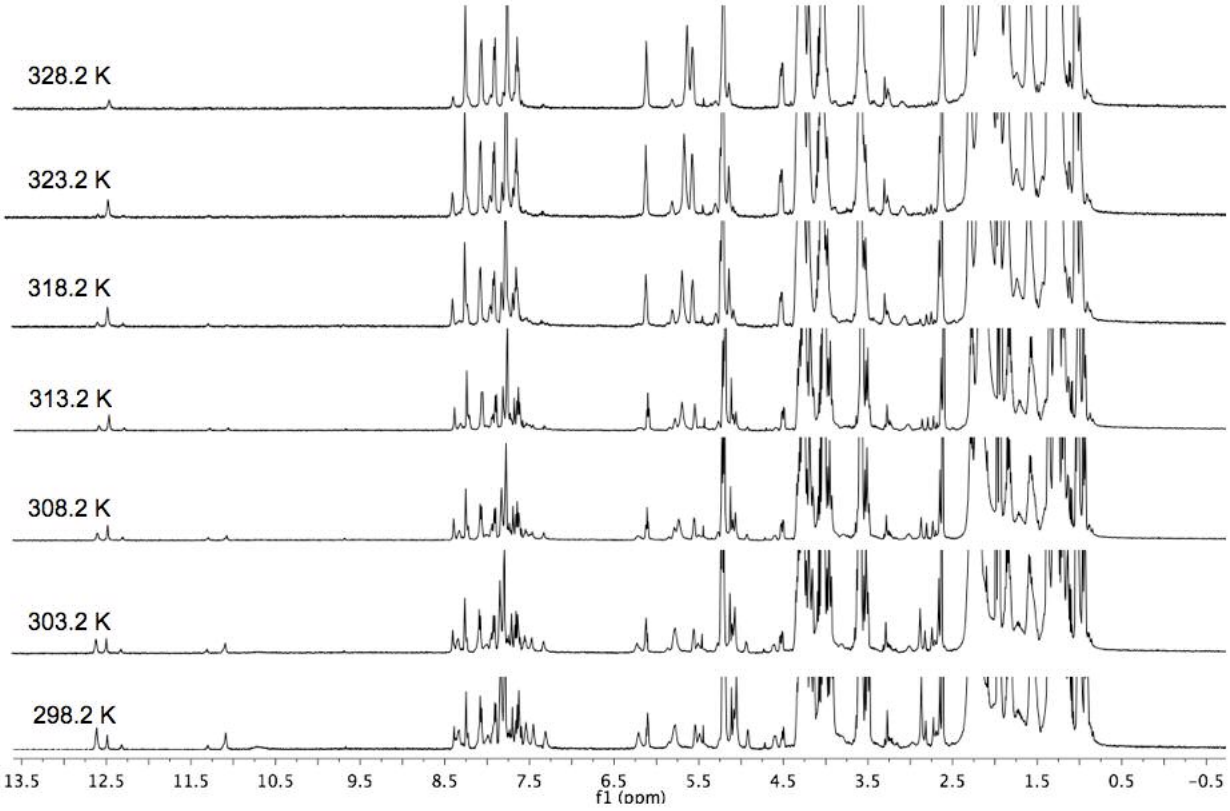


Table S2. Thermal stability of the SADs in CD_3CN with 0.5 eq of KI as determined by ^{1}H NMR (500 MHz, CD_3CN).

SAD	Molecularity	Tm* [K]	Composition
(mAGD2)$_{16}$•3KI	hexadecamer	316	25%H, 19%O, 6%D
(mAGD3)$_{16}$•3KI	hexadecamer	312	14%H, 26%O, 10%D

* We define Tm as f = 0.5; f = $[X_O]/[X_T]$, where $[X_O]$ and $[X_T]$ represent the concentration in ordered form (i.e. sum of all the SADs formed) and the total concentration of **mAGD2** and **mAGD3**, respectively.