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Polycationic adamantane-based dendrons form nanorods in complex with plasmid DNA

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General Material and Characterization Details

All starting materials, chemicals, and anhydrous solvents were obtained from commercial suppliers and used without purification. ^{1}H and ^{13}C NMR spectra were recorded using a Brucker 400 or 500 MHz spectrometer; the protons of the residual solvent were used to reference the chemical shift in ppm. Coupling constants *J* are reported in Hertz (Hz), the splitting patterns are designated as *s* (singlet), *d* (doublet), *dd* (doublet of doublet), *t* (triplet), *m* (multiplet), br (broad), and the protons named “ada”, “chol” or “biot” refer to the protons of the structure of adamantane, cholesterol or biotin, respectively. IR spectra were measured on a Perkin–Elmer Spectrum One ATR-FTIR spectrometer. MS experiments were performed on a Bruker Daltonics microTOF spectrometer (Bruker Daltonik GmgH, Bremen, Germany) equipped with an orthogonal electrospray (ESI) interface. Calibration was performed using Tunning mix (Agilent Technologies). Sample solutions were introduced into the spectrometer source with a syringe pump (Harvard type 55 1111: Harvard Apparatus Inc., South Natick, MA, USA) with a flow rate of 5 µL/min. Reversed phase high-performance liquid chromatography analyses ($RP\text{-}HPLC_{anal}$) were performed on a Beckman Coulter instrument, equipped with a System Gold 508 autosampler and a System Gold 166 NMP detector, using a Macherey–Nagel Nucleodur 100–3 C18 column (gradient: 0-100% B in 20 min at 1.2 mL/min flow rate; λ = 214 nm). The purifications by preparative RP-HPLC were performed on a Beckman Coulter instrument equipped with a PerkinElmer series 200 detector with a Macherey–Nagel Nucleodur 100–16 C18 column (gradient: 5–65% B in 20 min at 6 mL/min flow rate; λ = 220 nm). Eluent for both analytical and preparative RP-HPLC: A = H_2O+0.1% TFA (trifluoroacetic acid), B = MeCN+0.08% TFA. The following compounds were synthesized according to reported procedures: polycationic *HYDRAmers*[1] (Figure S1) and Biotin-N_3[2]. The CuAAC reaction corresponds to copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction. The plasmid DNA (pDNA) investigated in this study is the pcDNA3-EGFP plasmid obtained from the non-profit plasmid repository Addgene (www.addgene.org) (plasmid #13031) with the following specifications: vector type = mammalian expression (pcDNA3 backbone); gene name: Enhanced Green Fluorescent Protein; size = 6160 pair bases; bacterial resistance: ampicillin. N/P means: *HYDRAmer* cationic charge per pDNA negative charge.

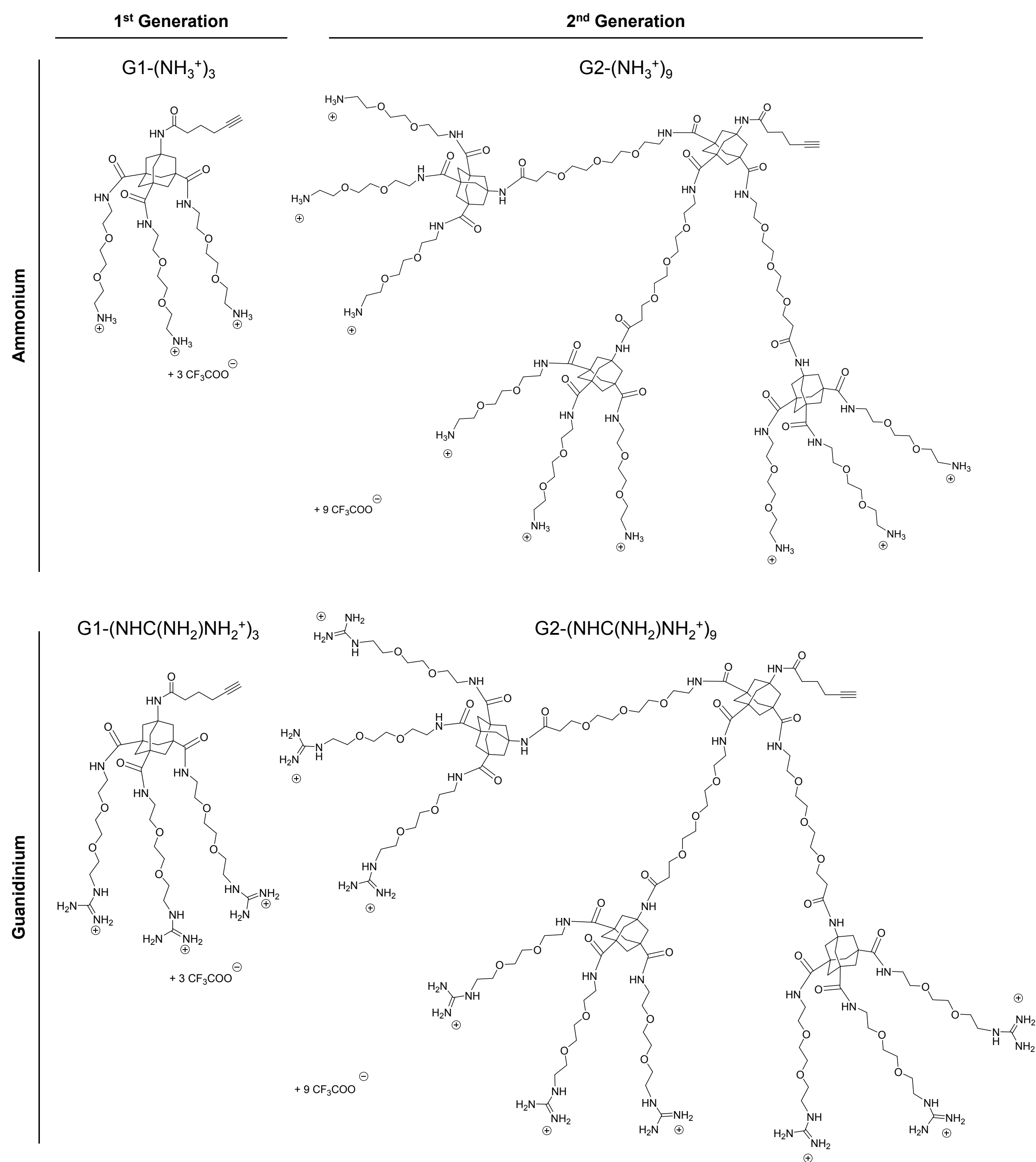
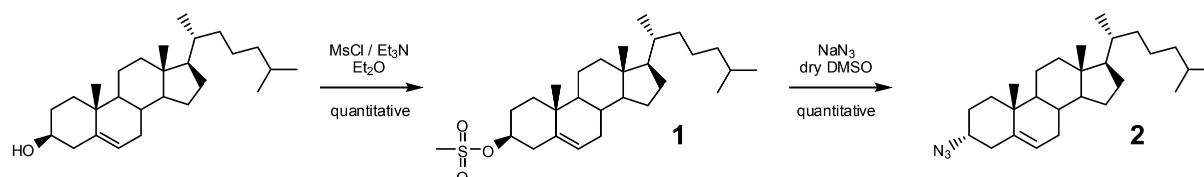


Figure S1. Molecular structures of ammonium first and second generation *HYDRAmers* (G1-$(NH_3^+)_3$ and G2-$(NH_3^+)_9$, respectively) and guanidinium first and second generation *HYDRAmers* (G1-$(NHC(NH_2)NH_2^+)_3$ and G2-$(NHC(NH_2)NH_2^+)_9$, respectively).

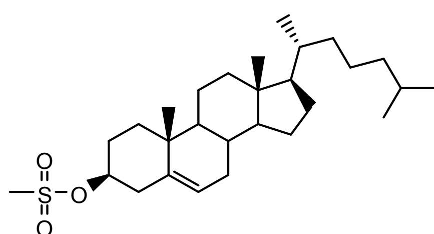
Synthesis and Characterization Data

Synthesis of cholesteryl derivative with an azide functional group

Scheme S1. Synthesis of cholesteryl derivative **(2)**.

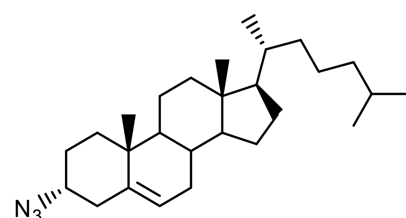


Cholest-5-en-3β-ol, methanesulfonate (1)



Triethylamine (1.6 mL, 11.4 mmol) and methanesulfonyl chloride (0.8 mL, 10.4 mmol) were sequentially added to a solution of cholesterol (200 mg, 0.52 mmol) in dry Et_2O. The reaction mixture was cooled to 0 °C for 10 min and was then stirred at room temperature for 3 h. The mixture was diluted with water and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the crude product was purified by column chromatography on silica gel (eluant: cyclohexane/AcOEt 8:2) to afford **1** as a white solid (235 mg, quantitative). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.41 (*m*, 1H, C=C*H*), 4.51 (*m*, 1H, CH_3SO_3CH), 3.00 (*s*, 3H, CH_3SO_3CH), 2.56-2.44 (*m*, 2H, CH_2C=CH), 2.06-0.83 (*m*, 38H), 0.67 (*s*, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 138.58, 123.77, 82.01, 56.56, 56.05, 49.86, 42.24, 39.59, 39.46, 39.11, 38.70, 36.84, 36.32, 36.12, 35.73, 31.83, 31.72, 28.92, 28.17, 27.97, 24.22, 23.77, 22.80, 22.53, 20.97, 19.16, 18.67, 11.81. FT-IR (neat, v/cm^{-1}): 2936, 2906, 2871, 1735, 1467, 1441, 1353, 1327, 1170, 1135, 1027. MS (ESI, *m/z*): 393.3 $[M-CH_3SO_3+Na]^+$, 763.6 $[2(M-CH_3SO_3)+Na]^+$.

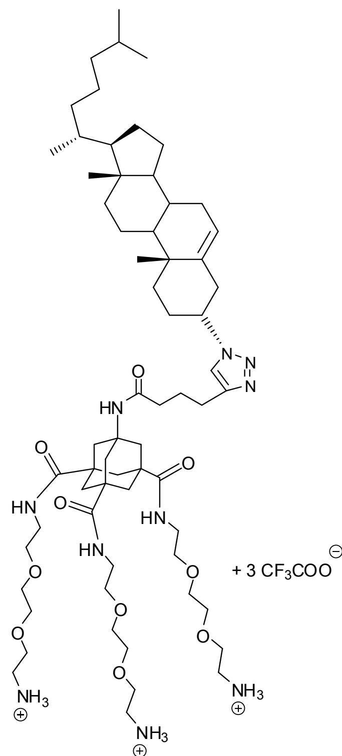
3α-Azido-5-cholestene (2)



A solution of **1** (100 mg, 0.22 mmol) in dry DMF (10 mL) along with sodium azide (143 mg, 2.20 mmol) was stirred at 60 °C overnight. The reaction mixture was washed with water and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo* to give **2** (85 mg, quantitative) as a white solid which was analyzed without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.40 (*m*, 1H, C=C*H*), 3.88 (*m*, 1H, N_3CH), 2.56-2.49 (*m*, 1H, CH_2C=CH), 2.21-2.16 (*m*, 1H, CH_2C=CH), 2.04-0.84 (*m*, 38H), 0.67 (*s*, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 138.04, 123.13, 58.25, 56.64, 56.04, 49.83, 42.25, 39.67, 39.49, 37.06, 36.15, 36.03, 35.79, 33.58, 31.79, 31.73, 28.22, 28.00, 26.05, 24.24, 23.81, 22.83, 22.55, 20.69, 18.98, 18.69, 11.83. FT-IR (neat, v/cm^{-1}): 2938, 2906, 2866, 2851, 2081, 1467, 1456, 1429, 1381, 1360, 1333, 1317, 1295, 1270, 1146, 1024. MS (ESI, *m/z*): 393.3 $[M-N_3+Na]^+$, 763.6 $[2(M-N_3)+Na]^+$. HR-MS (ESI): calcd for $C_{27}H_{45}N_3$ *m/z* = 434.351 $[M+Na]^+$. Found *m/z* = 434.351.

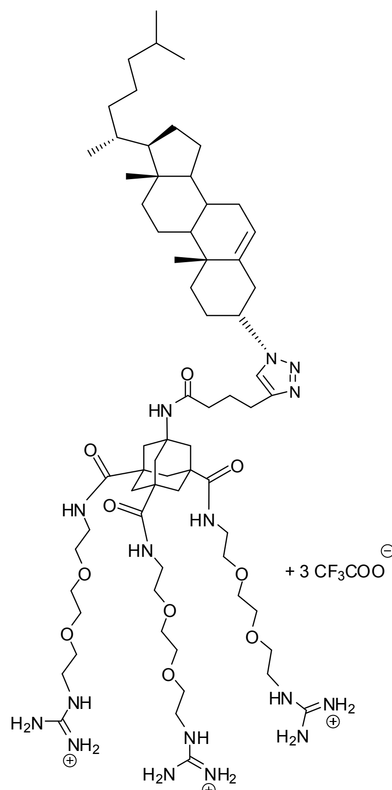
Synthesis of cholesterylated polycationic *HYDRAmers via* CuAAC reaction

Chol-G1-(NH_3^+)$_3$ (3)



3α-Azido-5-cholestene (2) (3.0 mg, 7.2 µmol), sodium ascorbate (285 µg, 1.44 µmol), and copper(II) sulfate pentahydrate (180 µg, 0.72 µmol) were added to a solution of G1-(NH_3^+)$_3$ (8.0 mg, 7.2 µmol) in THF/H_2O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Chol-G1-(NH_3^+)$_3$ (3)** as a white solid (5.9 mg, 3.9 µmol, 54%). RP-HPLC$_{anal}$: t_r = 17.9 min, λ = 214 nm. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.78 (*s*, 1H, =C*H* triazole), 7.64 (br *t*, N*H*), 5.49 (*m*, 1H, C=C*H* chol), 3.71 (*t*, *J* = 5.2 Hz, 6H, 3x C*H₂*O), 3.68-3.63 (*m*, 13H, NC*H* chol, 3x 2 C*H₂*O), 3.55 (*t*, *J* = 6 Hz, 6H, 3x C*H₂*O), 3.43-3.37 (*m*, 6H, 3x C*H₂*NHC=O), 3.14 (*t*, *J* = 5.2 Hz, 6H, 3x C*H₂*NH_2), 3.06-2.98 (*m*, 1H, C*H₂*C=CH chol), 2.71 (*t*, *J* = 7.6 Hz, 2H, C*H₂*C=CH), 2.65-2.58 (*m*, 1H, C*H₂*C=CH chol), 2.27-0.86 (*m*, 54H, 6 C*H₂* ada, COC*H₂*CH_2, COCH_2C*H₂*, 38 chol), 0.73 (*s*, 3H chol). FT-IR (neat, *v*/cm^{-1}): 3357, 3078, 2935, 2866, 1675, 1633, 1537, 1456, 1432, 1363, 1355, 1297, 1201, 1178, 1125. MS (ESI, *m/z*): 1179.8 $[M+H]^+$, 590.4 $[M+2H]^{2+}$. HR-MS (ESI): calcd for $C_{64}H_{110}N_{10}O_{10}$ *m/z* = 1179.845 $[M+H]^+$. Found *m/z* = 1179.848.

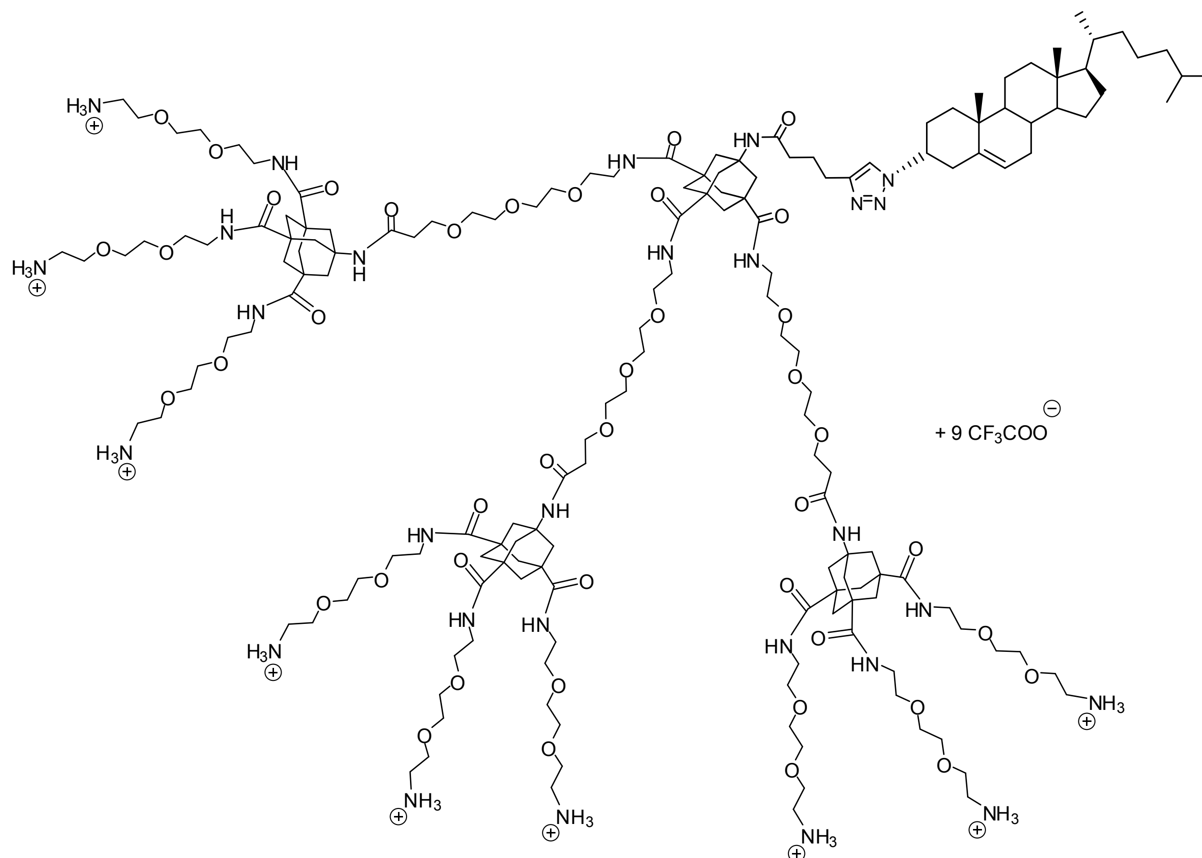
Chol-G1-($NHC(NH_2)NH_2^+$)$_3$ (4)



3α-Azido-5-cholestene (2) (3.3 mg, 8.1 µmol), sodium ascorbate (321 µg, 1.62 µmol), and copper(II) sulfate pentahydrate (202 µg, 0.81 µmol) were added to a solution of G1-($NHC(NH_2)NH_2^+$)$_3$ (10.0 mg, 8.1 µmol) in THF/H_2O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Chol-G1-($NHC(NH_2)NH_2^+$)$_3$ (4)** as a white solid (7.7 mg, 4.7 µmol, 58%). RP-HPLC$_{anal}$: t_r = 17.1 min, λ = 214 nm. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.79 (*s*, 1H, =C*H* triazole), 7.64 (br *t*, N*H*), 5.48 (*m*, 1H, C=C*H* chol), 3.66-3.60 (*m*, 19H, NC*H* chol, 3x 3 C*H₂*O), 3.54 (*t*, *J* = 5.6 Hz, 6H, 3x C*H₂*O), 3.38 (*t*, *J* = 5.2 Hz, 12H, 3x C*H₂*NHC=O, 3x C*H₂*NHC=NH_2), 3.06-2.98 (*m*, 1H, C*H₂*C=CH chol), 2.71 (*t*, *J* = 7.6 Hz, 2H, C*H₂*C=CH), 2.65-2.58 (*m*, 1H, C*H₂*C=CH chol), 2.26-0.86 (*m*, 54H, 6 C*H₂* ada, COC*H₂*CH_2, COCH_2C*H₂*, 38 chol), 0.73 (*s*, 3H chol). FT-IR (neat, *v*/cm^{-1}): 3340, 3178, 2937, 2868, 1679, 1633, 1537, 1456, 1429, 1362, 1352, 1292, 1200,

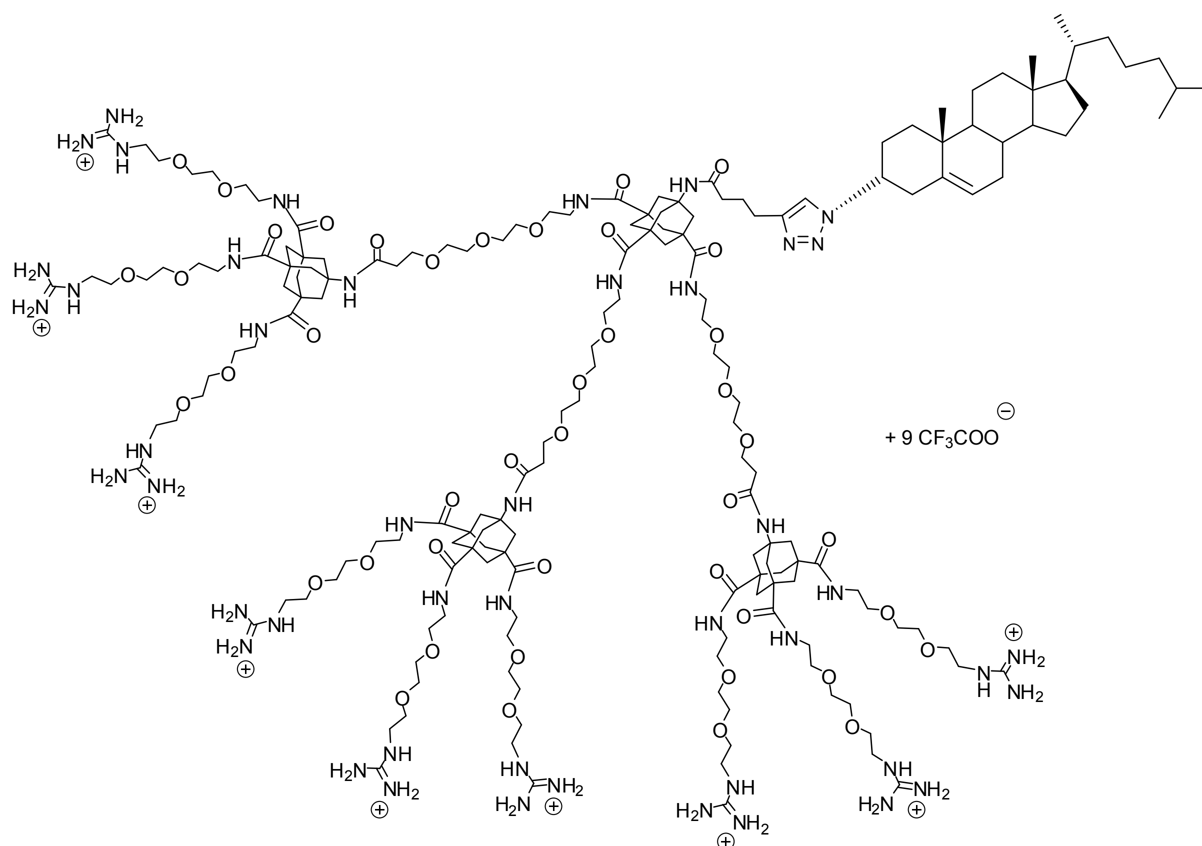
1178, 1127. MS (ESI, *m/z*): 1327.9 $[M+Na]^+$. HR-MS (ESI): calcd for $C_{67}H_{116}N_{16}O_{10}$ *m/z* = 1327.895 $[M+Na]^+$. Found *m/z* = 1327.884.

Chol-G2-(NH_3^+)$_9$ (5)



3α-Azido-5-cholestene (2) (519 µg, 1.26 µmol), sodium ascorbate (50 µg, 0.25 µmol), and copper(II) sulfate pentahydrate (31 µg, 0.13 µmol) were added to a solution of G2-(NH_3^+)$_9$ (5.0 mg, 1.26 µmol) in THF/H_2O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Chol-G2-(NH_3^+)$_9$ (5)** as a white solid (2.8 mg, 0.64 µmol, 51%). RP-HPLC$_{anal}$: t_r = 12.6 min, λ = 214 nm. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.79 (*s*, 1H, =C*H* triazole), 7.75 (br *s*, N*H*), 7.64 (br *t*, N*H*), 5.49 (*m*, 1H, C=C*H* chol), 3.74-3.51 (*m*, 109H, NC*H* chol, 54 C*H$_2$*O), 3.43-3.37 (*m*, 24H, 12x C*H$_2$*NHC=O), 3.14 (*t*, *J* = 5.2 Hz, 18H, 9x C*H$_2$*NH$_2$), 3.06-2.98 (*m*, 1H, C*H$_2$*C=CH chol), 2.71 (*t*, *J* = 7.6 Hz, 2H, C*H$_2$*C=CH), 2.65-2.58 (*m*, 1H, C*H$_2$*C=CH chol), 2.43 (*t*, *J* = 6.4 Hz, 6H, 3x OCH$_2$C*H$_2$*C=O), 2.27-0.86 (*m*, 90H, 24 C*H$_2$* ada, COC*H$_2$*CH$_2$, COCH$_2$C*H$_2$*, 38 chol), 0.73 (*s*, 3H chol). FT-IR (neat, *v*/cm^{-1}): 3327, 3078, 2929, 2876, 1675, 1638, 1536, 1456, 1430, 1353, 1295, 1201, 1178, 1125. MS (ESI, *m/z*): 1706.1 $[M+2Na]^{2+}$. HR-MS (ESI): calcd for $C_{166}H_{290}N_{28}O_{43}$ *m/z* = 1705.058 $[M+2Na]^{2+}$. Found *m/z* = 1705.057.

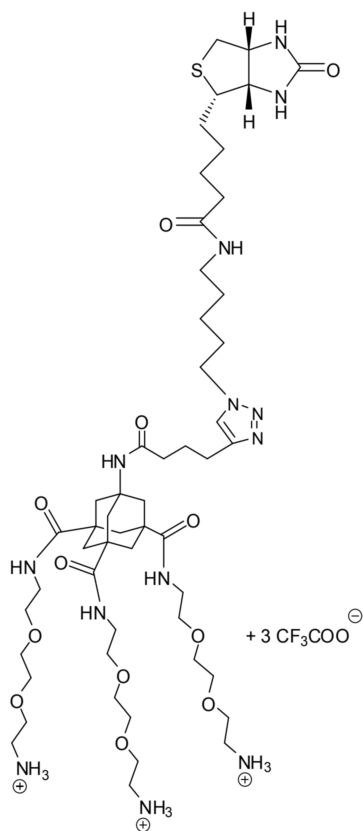
Chol-G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (6)



3α-Azido-5-cholestene (2) (473 µg, 1.15 µmol), sodium ascorbate (46 µg, 0.23 µmol), and copper(II) sulfate pentahydrate (29 µg, 0.12 µmol) were added to a solution of G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (5.0 mg, 1.15 µmol) in THF/H_2O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Chol-G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (6)** as a white solid (3.0 mg, 0.62 µmol, 54%). RP-HPLC$_{anal}$: t_r = 13.4 min, λ = 214 nm. ^{1}H NMR (CD$_3$OD, 400 MHz) δ: 7.85 (*s*, N*H*), 7.79 (*s*, 1H, =C*H* triazole), 7.74 (*s*, N*H*), 7.64 (br *t*, N*H*), 5.49 (*m*, 1H, C=C*H* chol), 3.72-3.51 (*m*, 109H, NC*H* chol, 54 C*H$_2$*O), 3.42-3.36 (*m*, 42H, 12x C*H$_2$*NHC=O, 9 C*H$_2$*NHC=NH$_2$), 3.06-2.98 (*m*, 1H, C*H$_2$*C=CH chol), 2.71 (*t*, *J* = 8 Hz, 2H, C*H$_2$*C=CH), 2.66-2.59 (*m*, 1H, C*H$_2$*C=CH chol), 2.42 (*t*, *J* = 6.4 Hz, 6H, 3x OCH$_2$C*H$_2$*C=O), 2.28-0.85 (*m*, 90H, 24 C*H$_2$* ada, COC*H$_2$*CH$_2$, COCH$_2$C*H$_2$*, 38 chol), 0.73 (*s*, 3H chol). FT-IR (neat, *v*/cm^{-1}): 3339, 3183, 2955, 2873, 1659, 1635, 1536, 1455, 1431, 1353, 1292, 1199, 1178, 1124. MS (ESI, *m/z*): 1249.1 [M+3H]$^{3+}$, 937.1 [M+4H]$^{4+}$, 749.9 [M+5H]$^{5+}$. HR-MS (ESI): calcd for $C_{175}H_{308}N_{46}O_{43}$ *m/z* = 936.591 [M+4H]$^{4+}$. Found *m/z* = 936.595.

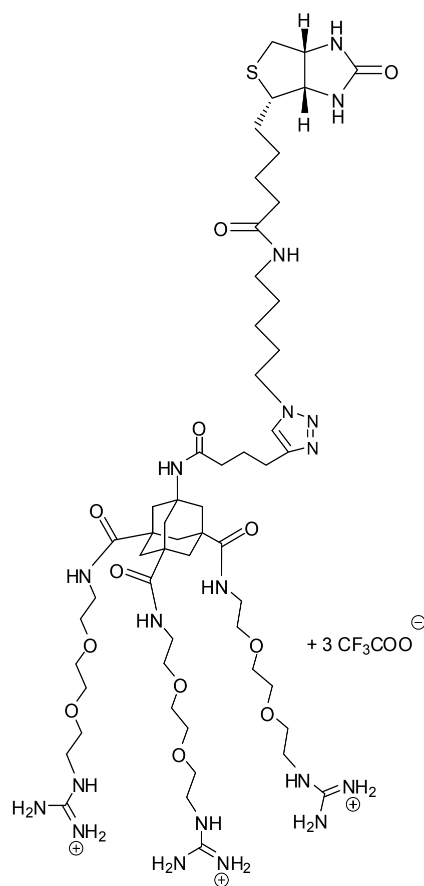
Synthesis of biotinylated polycationic *HYDRAmers via* CuAAC reaction

Biot-G1-(NH_3^+)$_3$ (7)



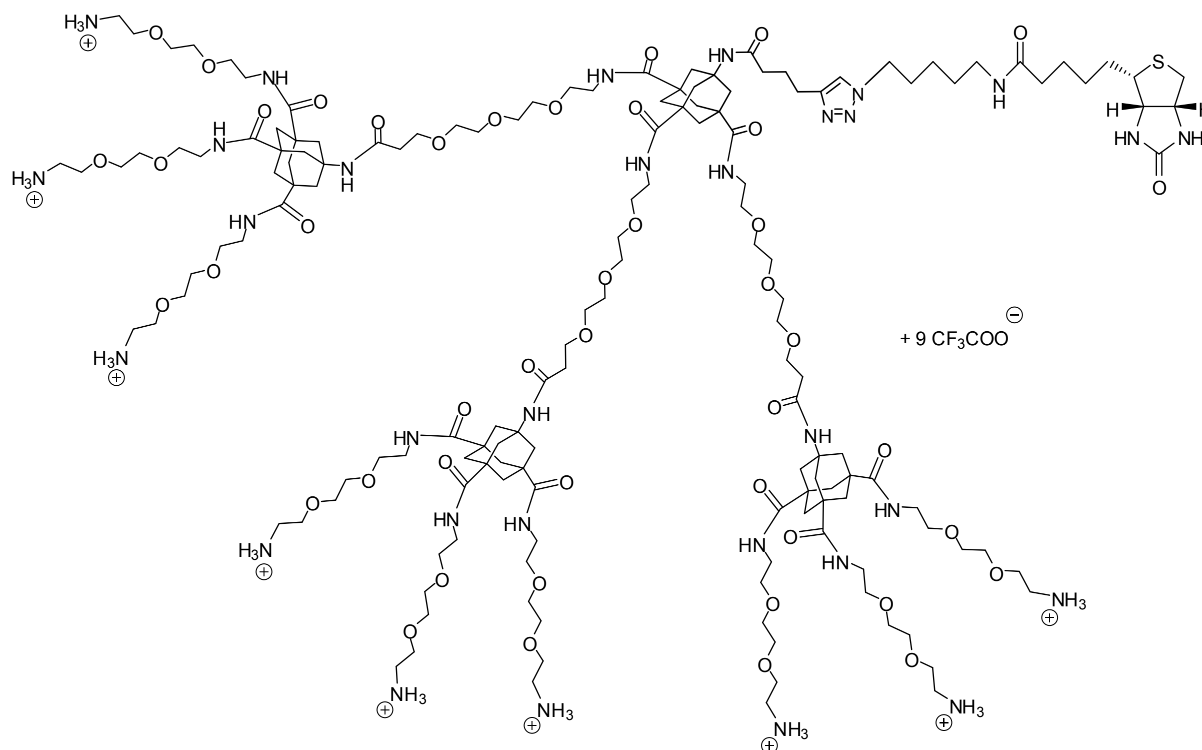
Biotin-N_3 (9.6 mg, 27.0 µmol), sodium ascorbate (1.1 mg, 5.4 µmol), and copper(II) sulfate pentahydrate (674 µg, 2.7 µmol) were added to a solution of G1-(NH_3^+)$_3$ (30.0 mg, 27.0 µmol) in THF/H_2O (2 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Biot-G1-(NH_3^+)$_3$ (7)** as a white solid (24.1 mg, 16.5 µmol, 61%). RP-$HPLC_{anal}$: t_r = 8.0 min, λ = 214 nm. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.77 (*s*, 1H, =C*H* triazole), 4.50 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CH_2S biot), 4.38 (*t*, *J* = 6.8 Hz, 2H, C*H$_2$*NN=N), 4.31 (*dd*, $^3J_1$ = 7.6 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CHS biot), 3.71 (*t*, *J* = 5.2 Hz, 6H, 3x C*H$_2$*O), 3.68-3.63 (*m*, 12H, 3x 2 C*H$_2$*O), 3.55 (*t*, *J* = 5.6 Hz, 6H, 3x C*H$_2$*O), 3.39 (*t*, *J* = 5.6 Hz, 6H, 3x C*H$_2$*NHC=O), 3.21 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHCHC*H*S biot), 3.19-3.12 (*m*, 8H, C*H$_2$*NHCOCH_2, 3x C*H$_2$*NH_2), 2.93 (*dd*, $^2J_1$ = 12.8 Hz, $^3J_2$ = 5.2 Hz, 1H, NHCH(*H*CH)S biot), 2.75-2.68 (*m*, 3H, C*H$_2$*C=CH, NHCH(HC*H*)S biot), 2.24-2.16 (*m*, 4H, C*H$_2$*C=O, C*H$_2$*C=O biot), 2.09 (*s*, 6H, 3 C*H$_2$* ada), 1.98-1.83 (*m*, 10H, 3 C*H$_2$* ada, COCH_2C*H$_2$*, SCHC*H$_2$* biot), 1.79-1.38 (*m*, 10H, 5 CH_2C*H$_2$*CH_2 biot). FT-IR (neat, *v*/cm^{-1}): 3296, 3073, 2929, 2871, 1671, 1632, 1535, 1457, 1429, 1353, 1287, 1199, 1175, 1123. MS (ESI, *m/z*): 1122.7 $[M+H]^+$, 561.8 $[M+2H]^{2+}$, 374.9 $[M+3H]^{3+}$. HR-MS (ESI): calcd for $C_{52}H_{91}N_{13}O_{12}S$ *m/z* = 1122.670 $[M+H]^+$. Found *m/z* = 1122.669.

Biot-G1-(NHC(NH$_2$)NH$_2^+$)$_3$ (8)



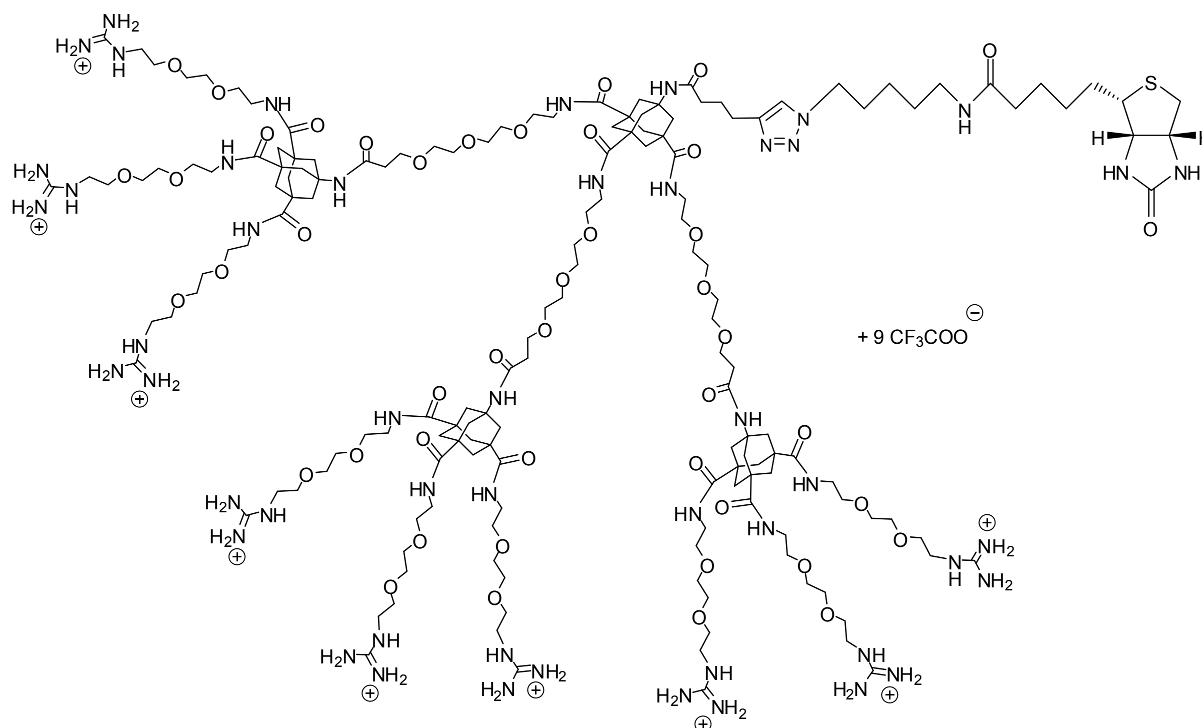
Biotin-N$_3$ (8.6 mg, 24.3 μmol), sodium ascorbate (963 μg, 4.86 μmol), and copper(II) sulfate pentahydrate (606 μg, 2.43 μmol) were added to a solution of G1-(NHC(NH$_2$)NH$_2^+$)$_3$ (30.0 mg, 24.3 μmol) in THF/H_2O (2 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Biot-G1-(NHC(NH$_2$)NH$_2^+$)$_3$ (8)** as a white solid (23.5 mg, 14.8 μmol, 61%). RP-HPLC$_{anal}$: t_r = 8.7 min, λ = 214 nm. ^{1}H NMR (CD$_3$OD, 400 MHz) δ: 7.75 (*s*, 1H, =C*H* triazole), 7.64 (br *t*, N*H*), 4.50 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CH$_2$S biot), 4.37 (*t*, *J* = 7.2 Hz, 2H, C*H$_2$*NN=N), 4.31 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CHS biot), 3.66-3.61 (*m*, 18H, 3x 3 C*H$_2$*O), 3.54 (*t*, *J* = 6 Hz, 6H, 3x C*H$_2$*O), 3.39 (*t*, *J* = 4.8 Hz, 12H, 3x C*H$_2$*NHC=O, 3x C*H$_2$*NHC=NH$_2$), 3.21 (*dd*, $^3J_1$ = 9.2 Hz, $^3J_2$ = 5.2 Hz, 1H, NHCHC*H*S biot), 3.16 (*t*, *J* = 6.8 Hz, 2H, C*H$_2$*NHCOCH$_2$), 2.93 (*dd*, $^2J_1$ = 12.8 Hz, $^3J_2$ = 4.8 Hz, 1H, NHCH(*H*CH)S biot), 2.75-2.68 (*m*, 3H, C*H$_2$*C=CH, NHCH(HC*H*)S biot), 2.24-2.16 (*m*, 4H, C*H$_2$*C=O, C*H$_2$*C=O biot), 2.09 (*s*, 6H, 3 C*H$_2$* ada), 1.98-1.83 (*m*, 10H, 3 C*H$_2$* ada, COCH$_2$C*H$_2$*, SCHC*H$_2$* biot), 1.79-1.38 (*m*, 10H, 5 CH$_2$C*H$_2$*CH$_2$ biot). FT-IR (neat, *v*/cm^{-1}): 3320, 3185, 2932, 2871, 1666, 1631, 1536, 1457, 1431, 1353, 1292, 1200, 1176, 1125. MS (ESI, *m/z*): 624.9 [M+2H]$^{2+}$, 416.9 [M+3H]$^{3+}$. HR-MS (ESI): calcd for $C_{55}H_{97}N_{19}O_{12}S$ *m/z* = 624.872 [M+2H]$^{2+}$. Found *m/z* = 624.876.

Biot-G2-(NH_3^+)$_9$ (9)



Biotin-N_3 (447 µg, 1.26 µmol), sodium ascorbate (50 µg, 0.25 µmol), and copper(II) sulfate pentahydrate (31 µg, 0.13 µmol) were added to a solution of G2-(NH_3^+)$_9$ (5.0 mg, 1.26 µmol) in THF/H_2O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Biot-G2-(NH_3^+)$_9$ (9)** as a white solid (2.9 mg, 0.67 µmol, 53%). RP-HPLC$_{anal}$: t_r = 7.9 min, λ = 214 nm. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.77 (*s*, 1H, =C*H* triazole), 7.66 (br *t*, N*H*), 4.50 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CH_2S biot), 4.38 (*t*, *J* = 6.8 Hz, 2H, CH_2NN=N), 4.31 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.4 Hz, 1H, NHC*H*CHS biot), 3.74-3.51 (*m*, 108H, 54 CH_2O), 3.42-3.36 (*m*, 24H, 12x CH_2NHC=O), 3.22 (*dd*, $^3J_1$ = 9.6 Hz, $^3J_2$ = 5.2 Hz, 1H, NHCHC*H*S biot), 3.19-3.12 (*m*, 20H, CH_2NHCOCH_2, 9x C$H_2$$NH_2$), 2.94 (*dd*, $^2J_1$ = 12.8 Hz, $^3J_2$ = 5.2 Hz, 1H, NHCH(*H*CH)S biot), 2.75-2.69 (*m*, 3H, CH_2C=CH, NHCH(HC*H*)S biot), 2.43 (*t*, *J* = 6 Hz, 6H, 3x OCH_2CH_2C=O), 2.24-2.16 (*m*, 4H, CH_2C=O, CH_2C=O biot), 2.09 (*s*, 24H, 12 CH_2 ada), 1.97-1.83 (*m*, 28H, 12 CH_2 ada, COCH_2CH_2, SCHCH_2 biot), 1.79-1.28 (*m*, 10H, 5 CH_2C$H_2$$CH_2$ biot). FT-IR (neat, *v*/cm^{-1}): 3330, 3078, 2927, 2876, 1674, 1633, 1536, 1454, 1429, 1354, 1292, 1200, 1178, 1125. MS (ESI, *m/z*): 1103.7 $[M+3H]^{3+}$, 828.0 $[M+4H]^{4+}$, 662.6 $[M+5H]^{5+}$. HR-MS (ESI): calcd for $C_{154}H_{271}N_{31}O_{45}S$ *m/z* = 827.747 $[M+4H]^{4+}$. Found *m/z* = 827.743.

Biot-G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (10)



Biotin-N$_3$ (408 µg, 1.15 µmol), sodium ascorbate (46 µg, 0.23 µmol), and copper(II) sulfate pentahydrate (29 µg, 0.12 µmol) were added to a solution of G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (5.0 mg, 1.15 µmol) in THF/H$_2$O (1 mL, 1:1) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere for 5h. After lyophilization, the crude product was purified by preparative RP-HPLC to obtain **Biot-G2-(NHC(NH$_2$)NH$_2^+$)$_9$ (10)** as a white solid (2.6 mg, 0.56 µmol, 49%). RP-HPLC$_{anal}$: t_r = 8.0 min, λ = 214 nm. ^{1}H NMR (CD$_3$OD, 400 MHz) δ: 7.77 (*s*, 1H, =C*H* triazole), 7.66 (br *t*, N*H*), 4.50 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 5.2 Hz, 1H, NHC*H*CH$_2$S biot), 4.37 (*t*, *J* = 6.8 Hz, 2H, C*H$_2$*NN=N), 4.31 (*dd*, $^3J_1$ = 8 Hz, $^3J_2$ = 4.8 Hz, 1H, NHC*H*CHS biot), 3.71-3.51 (*m*, 108H, 54 C*H$_2$*O), 3.42-3.36 (*m*, 42H, 12x C*H$_2$*NHC=O, 9 C*H$_2$*NHC=NH$_2$), 3.22 (*dd*, $^3J_1$ = 8.4 Hz, $^3J_2$ = 4.4 Hz, 1H, NHCHC*H*S biot), 3.17 (*t*, *J* = 6.4 Hz, 2H, C*H$_2$*NHCOCH$_2$), 2.94 (*dd*, $^2J_1$ = 12.8 Hz, $^3J_2$ = 5.2 Hz, 1H, NHCH(*H*CH)S biot), 2.75-2.69 (*m*, 3H, C*H$_2$*C=CH, NHCH(HC*H*)S biot), 2.42 (*t*, *J* = 6 Hz, 6H, 3x OCH$_2$C*H$_2$*C=O), 2.24-2.16 (*m*, 4H, C*H$_2$*C=O, C*H$_2$*C=O biot), 2.09 (*s*, 24H, 12 C*H$_2$* ada), 1.98-1.84 (*m*, 28H, 12 C*H$_2$* ada, COCH$_2$C*H$_2$*, SCHC*H$_2$* biot), 1.80-1.29 (*m*, 10H, 5 CH$_2$C*H$_2$*CH$_2$ biot). FT-IR (neat, ν/cm^{-1}): 3335, 3195, 2922, 2876, 1671, 1641, 1545, 1454, 1433, 1353, 1292, 1202, 1181, 1128. MS (ESI, *m/z*): 922.8 [M+4H]$^{4+}$, 738.4 [M+5H]$^{5+}$, 615.5 [M+6H]$^{6+}$. HR-MS (ESI): calcd for $C_{163}H_{289}N_{49}O_{45}S$ *m/z* = 738.038 [M+5H]$^{5+}$. Found *m/z* = 738.033.

High-Performance Liquid Chromatography Profiles (λ = 220 nm)

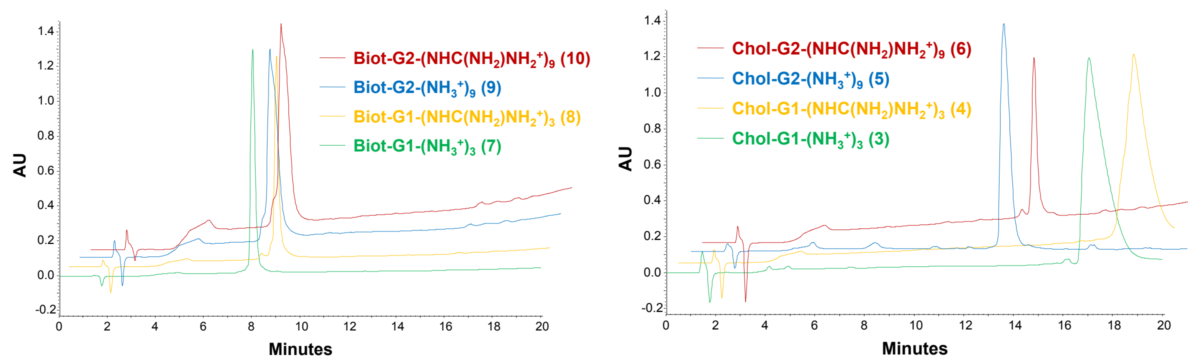


Figure S2. RP-HPLC chromatograms of the different functionalized *HYDRAmers*.

NMR Spectra

Figure S3. ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of **1**

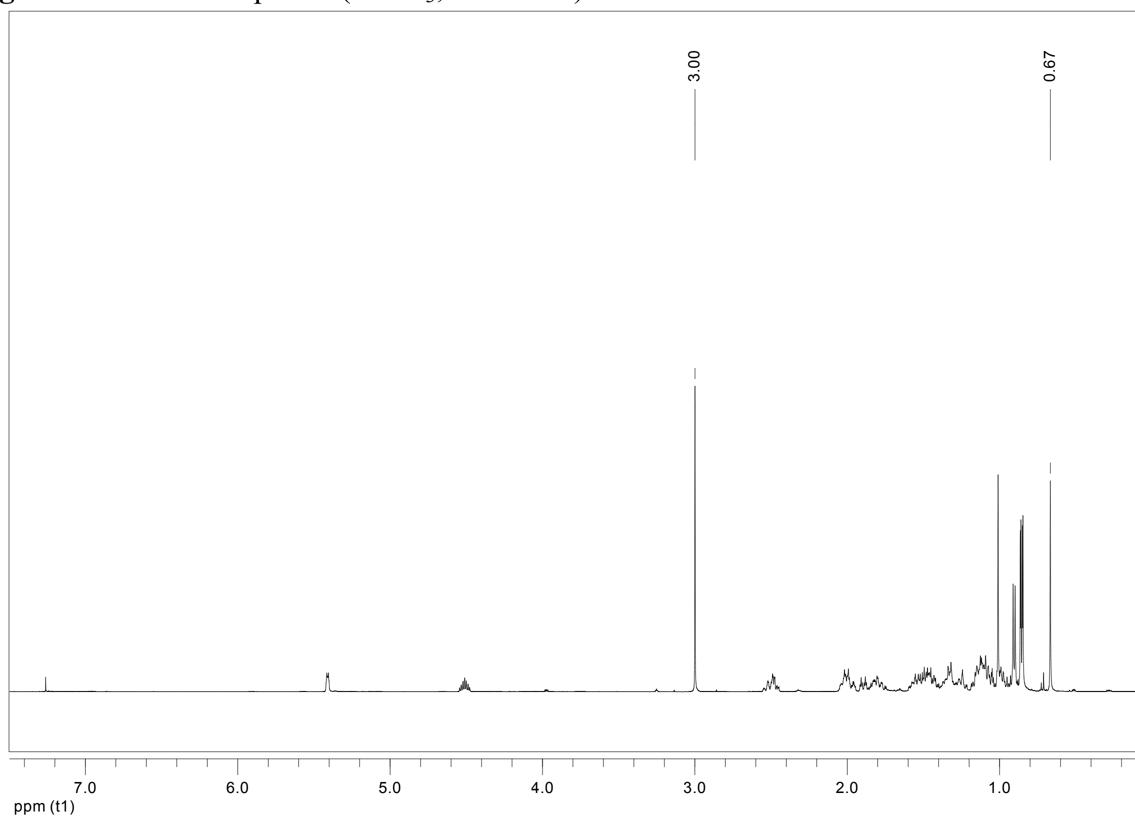
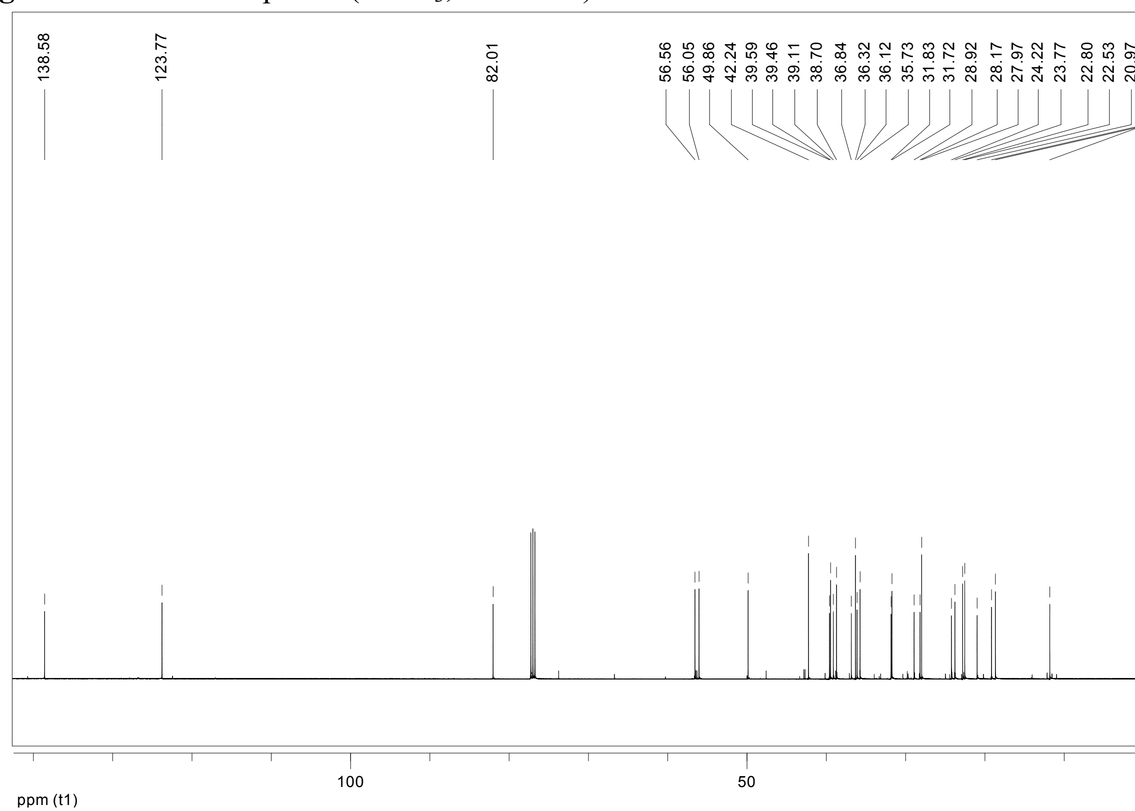


Figure S4. ^{13}C NMR spectra ($CDCl_3$, 125 MHz) of **1**



Zoom 10-58 ppm of ^{13}C NMR spectra ($CDCl_3$, 125 MHz) of **1**

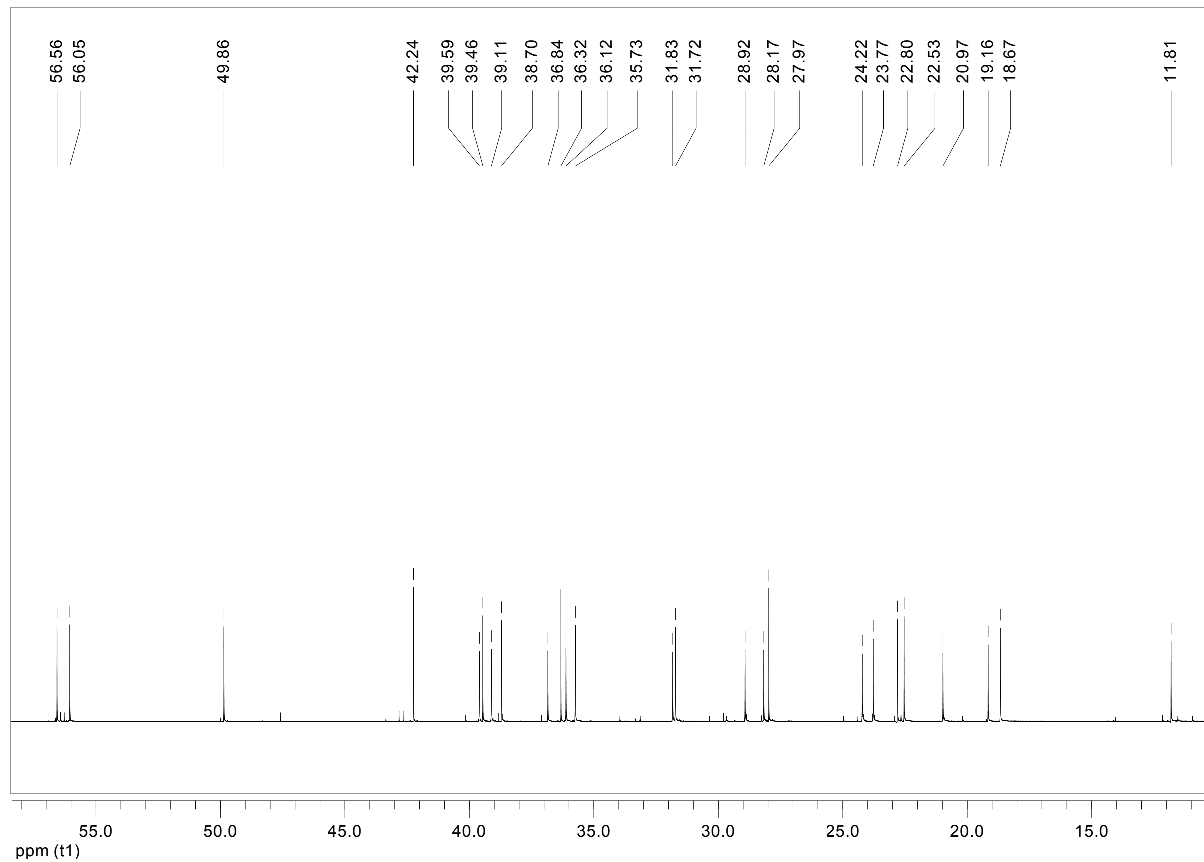


Figure S5. ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of **2**

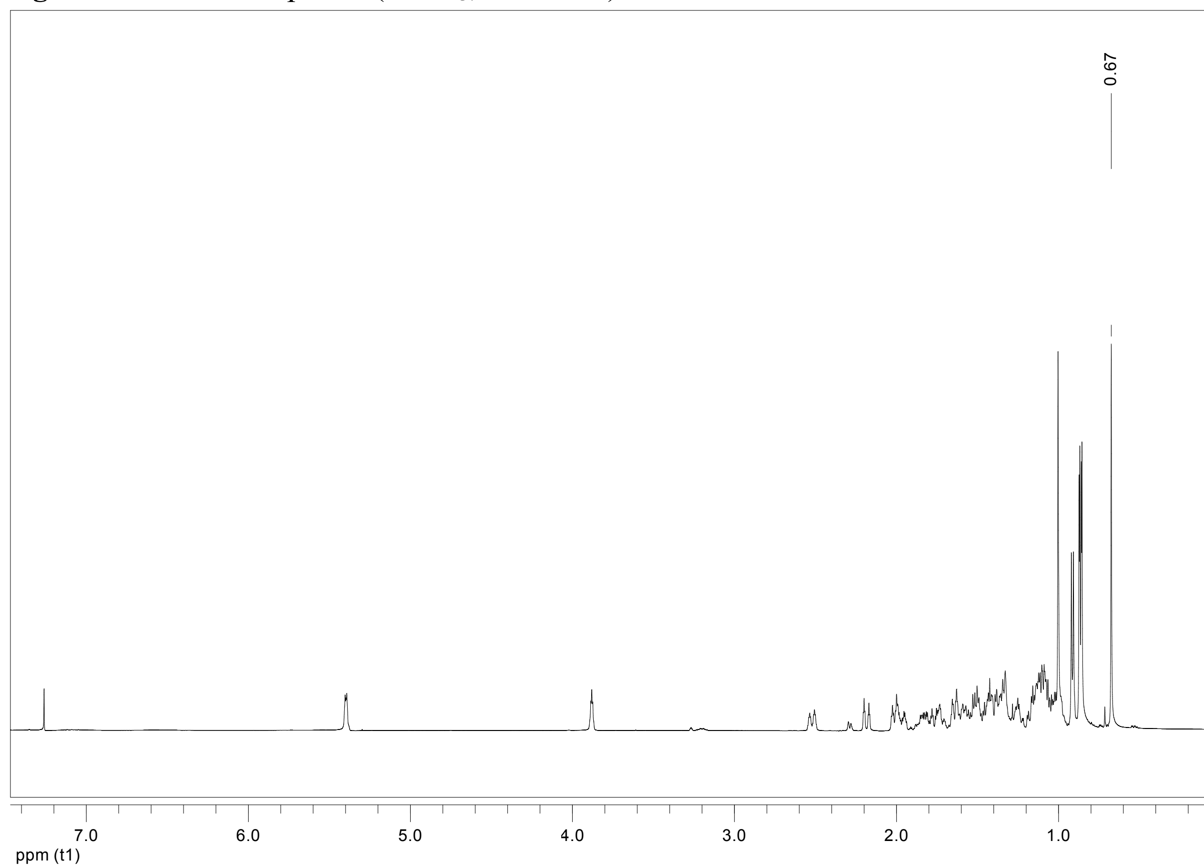
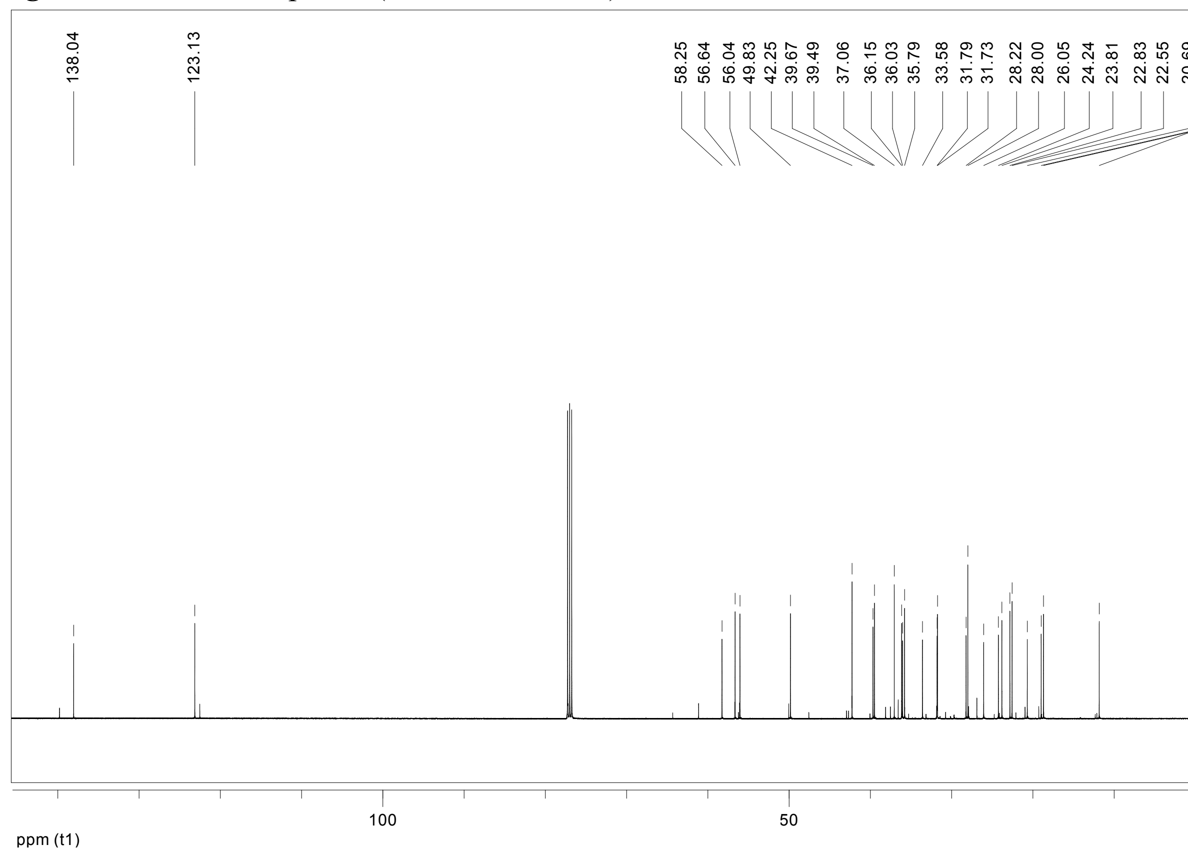


Figure S6. ^{13}C NMR spectra ($CDCl_3$, 125 MHz) of **2**



Zoom 10-59 ppm of ^{13}C NMR spectra ($CDCl_3$, 125 MHz) of **2**

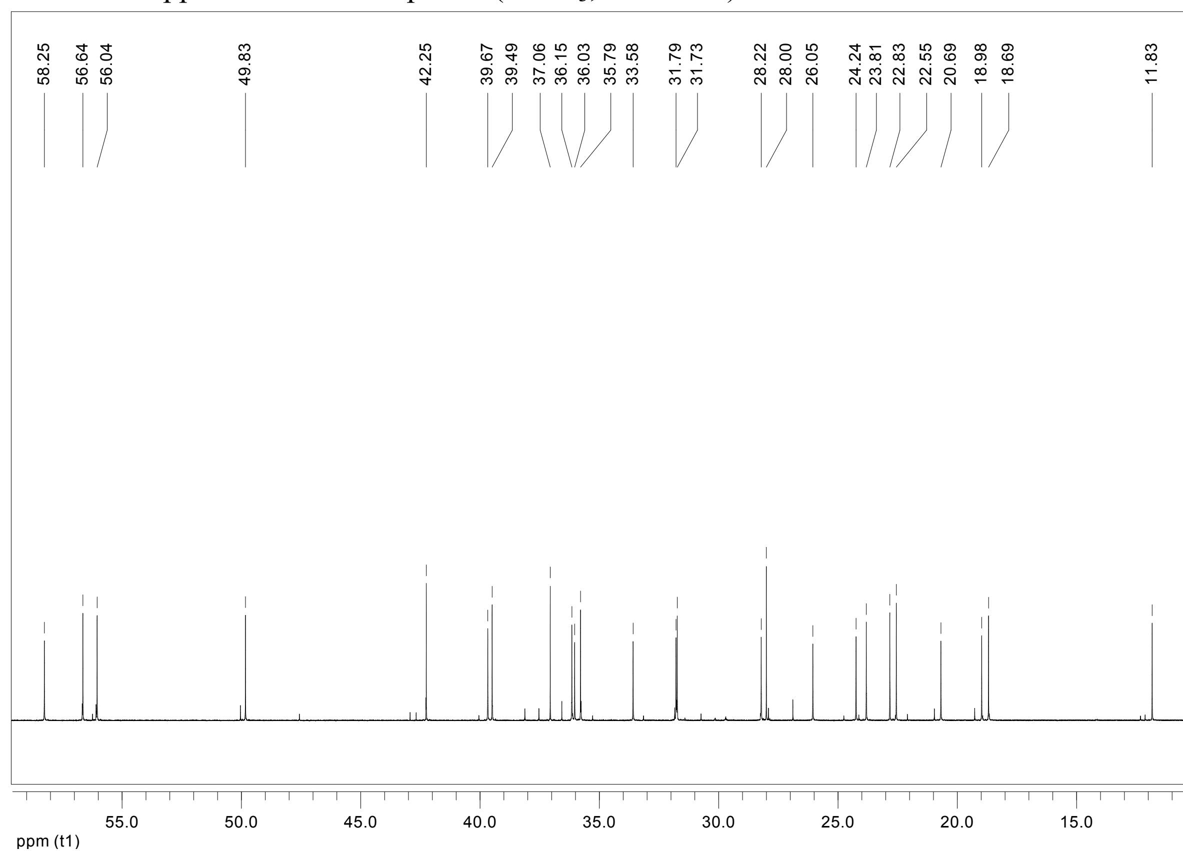


Figure S7. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **3**

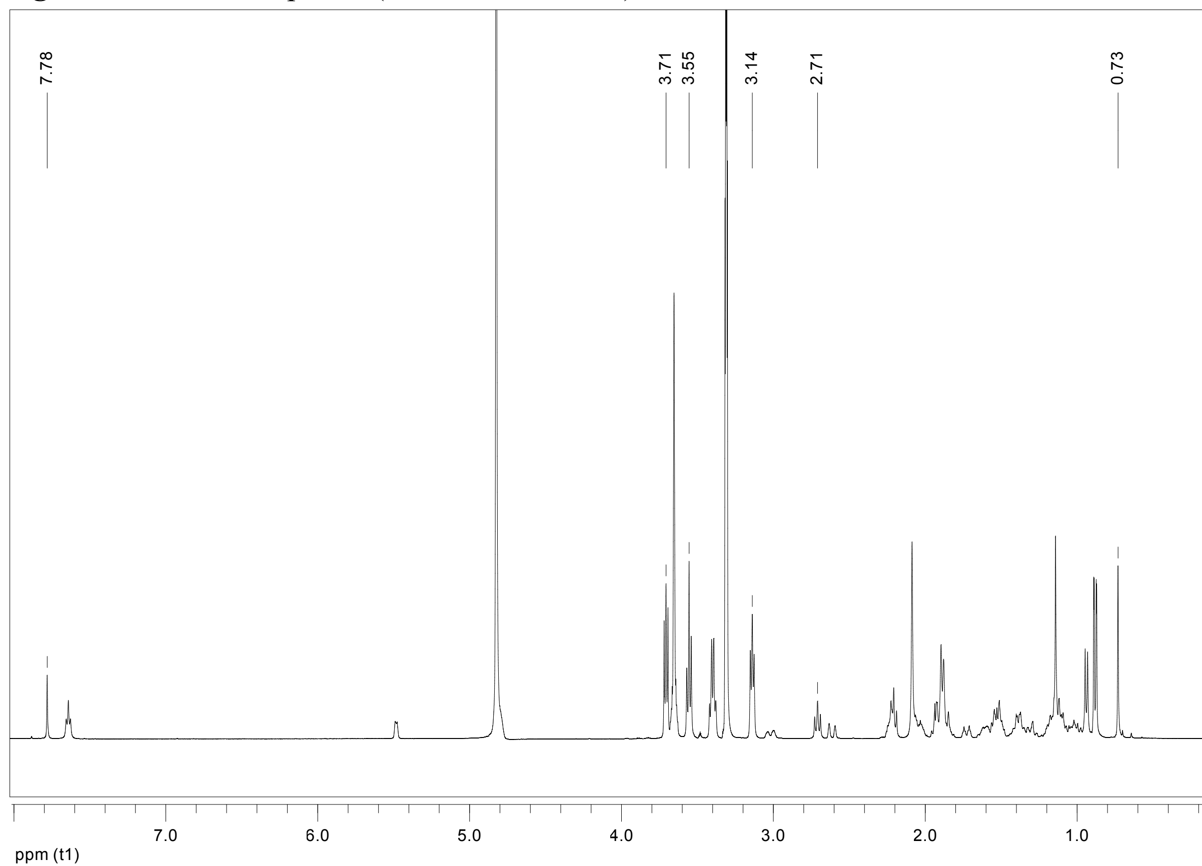


Figure S8. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **4**

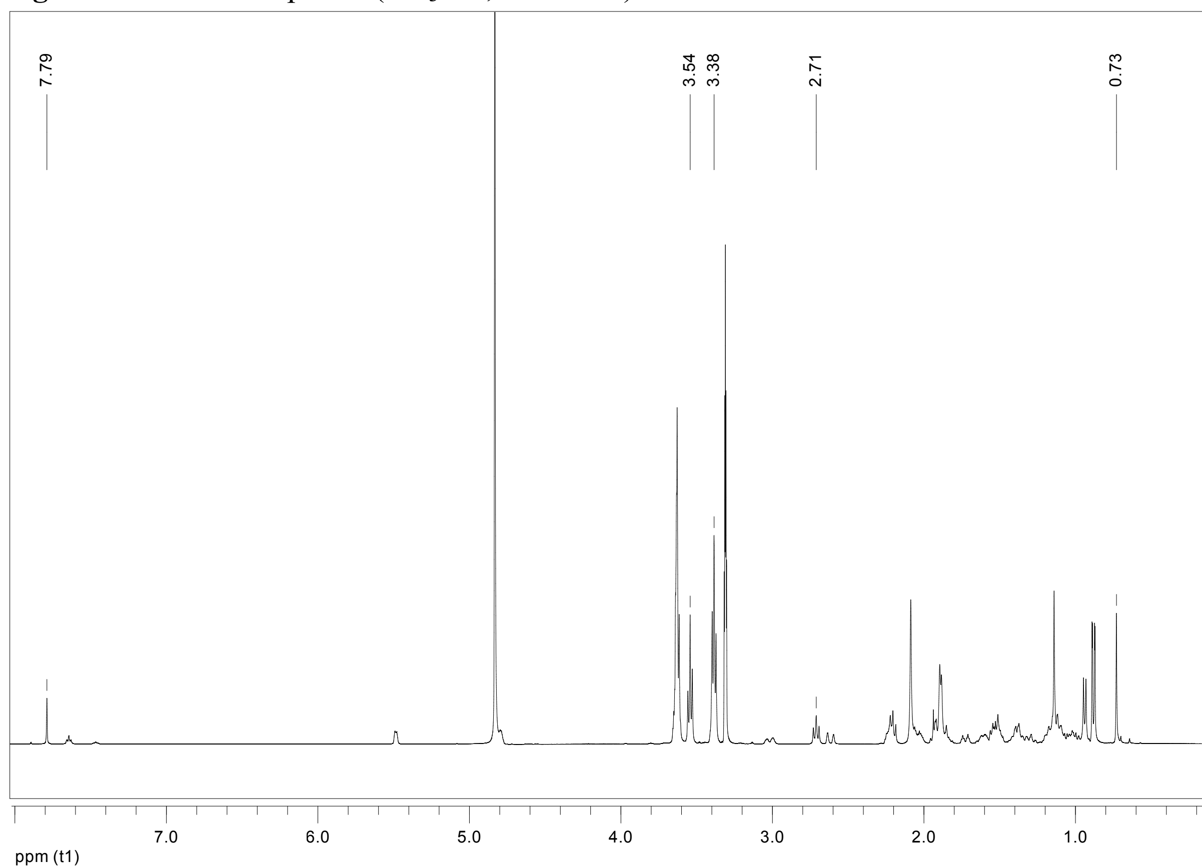


Figure S9. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **5**

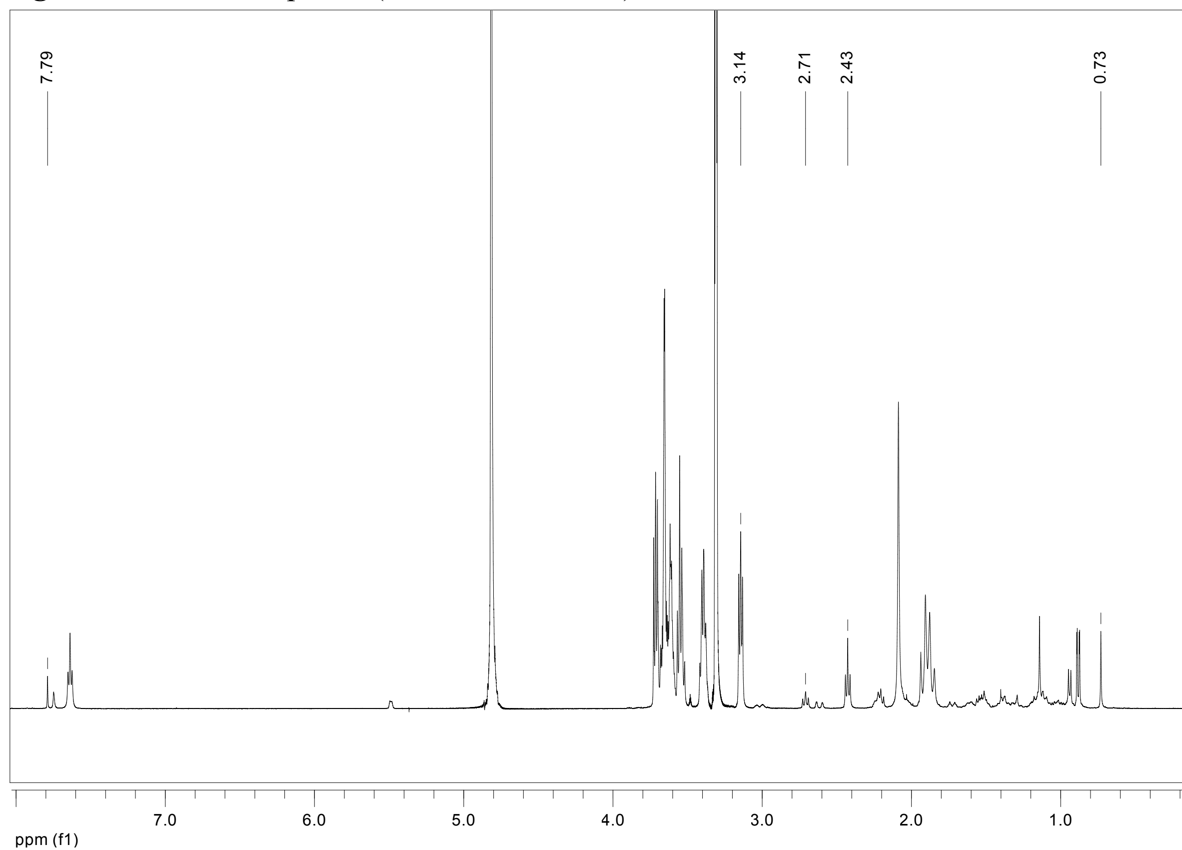


Figure S10. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **6**

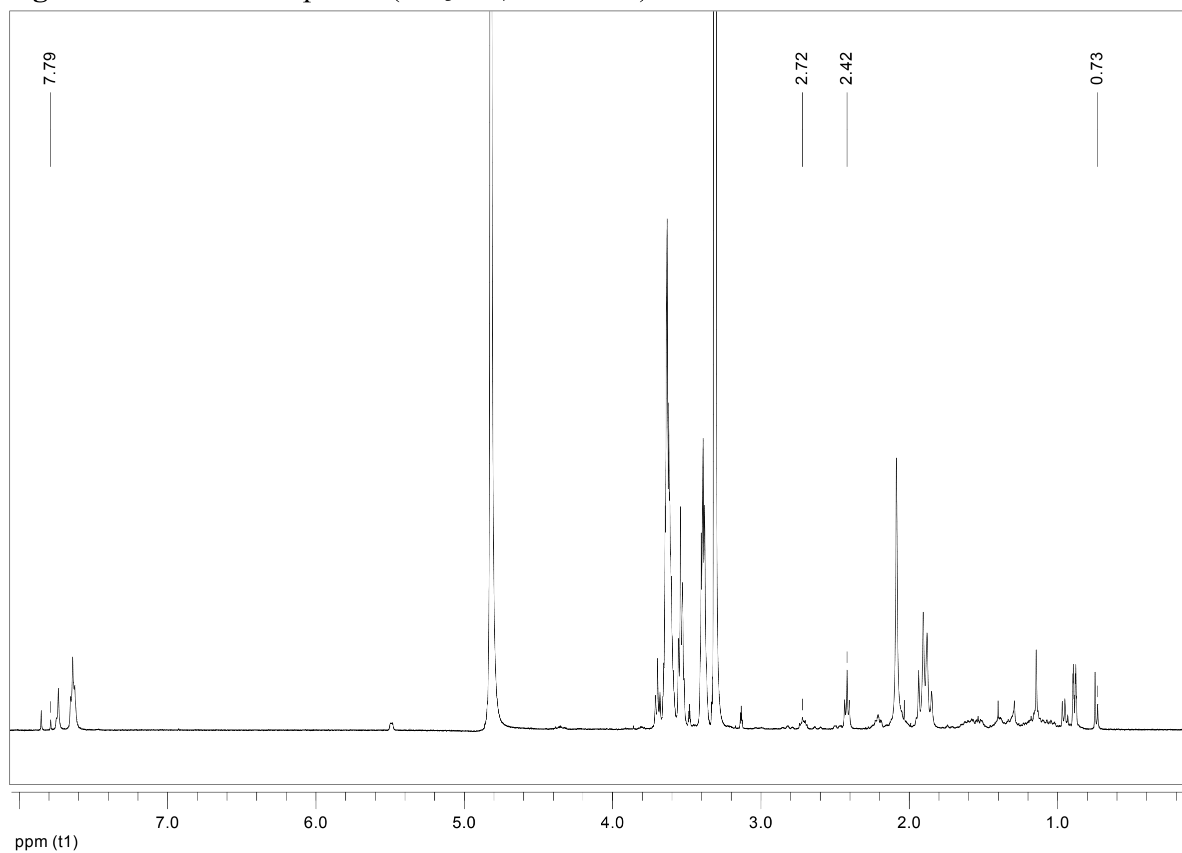


Figure S11. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **7**

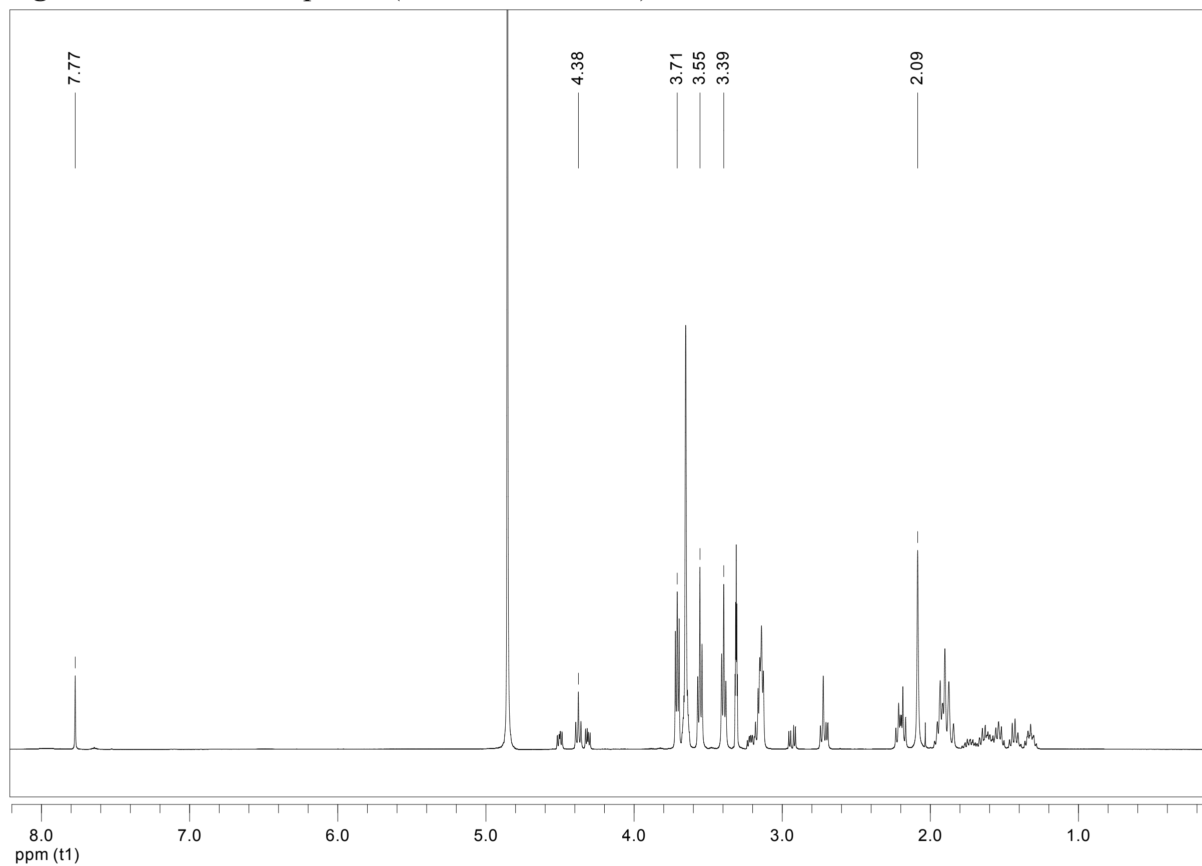


Figure S12. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **8**

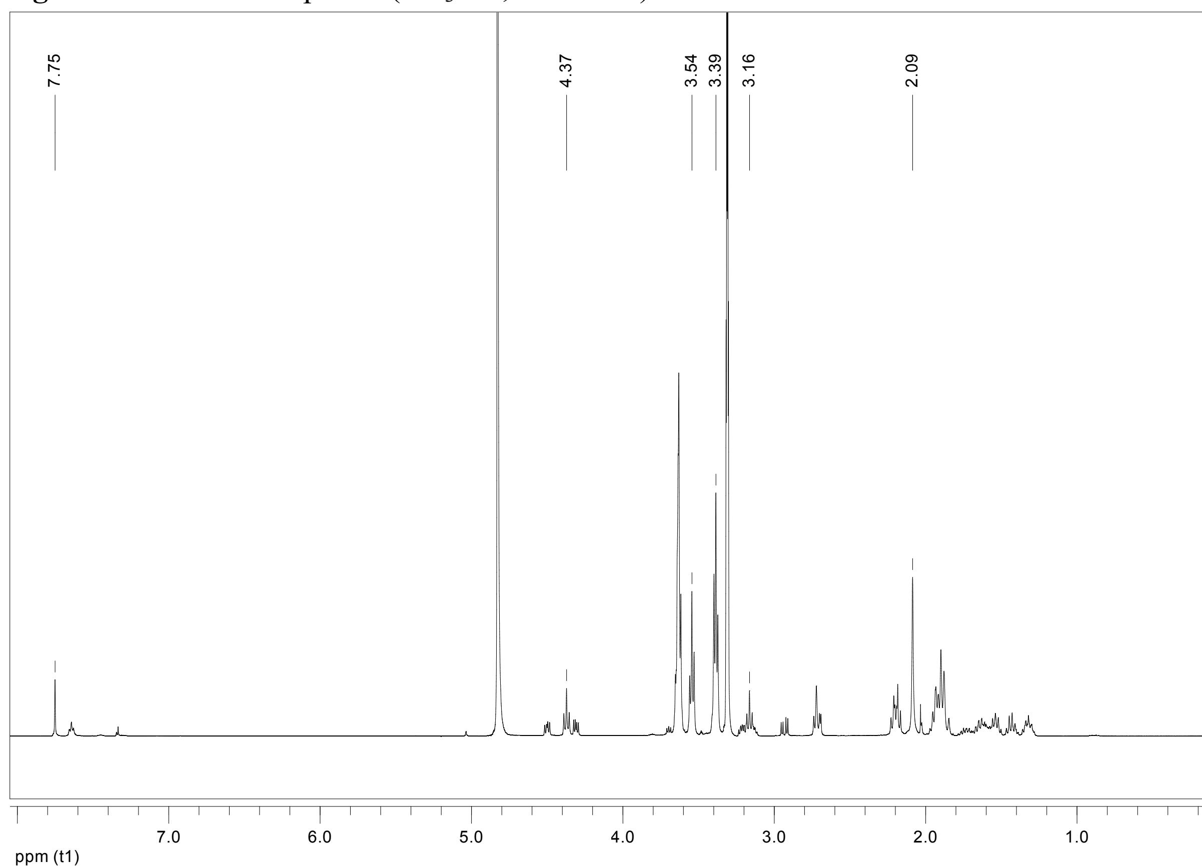


Figure S13. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **9**

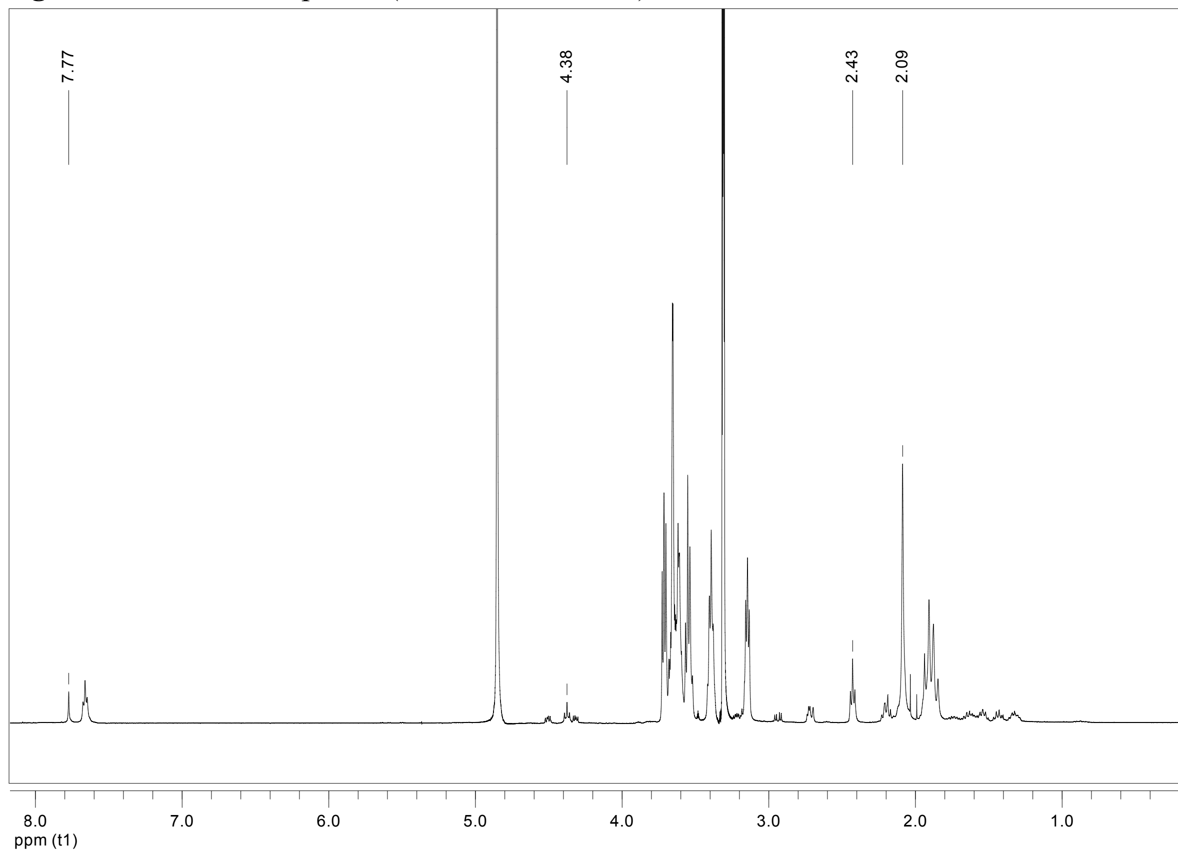
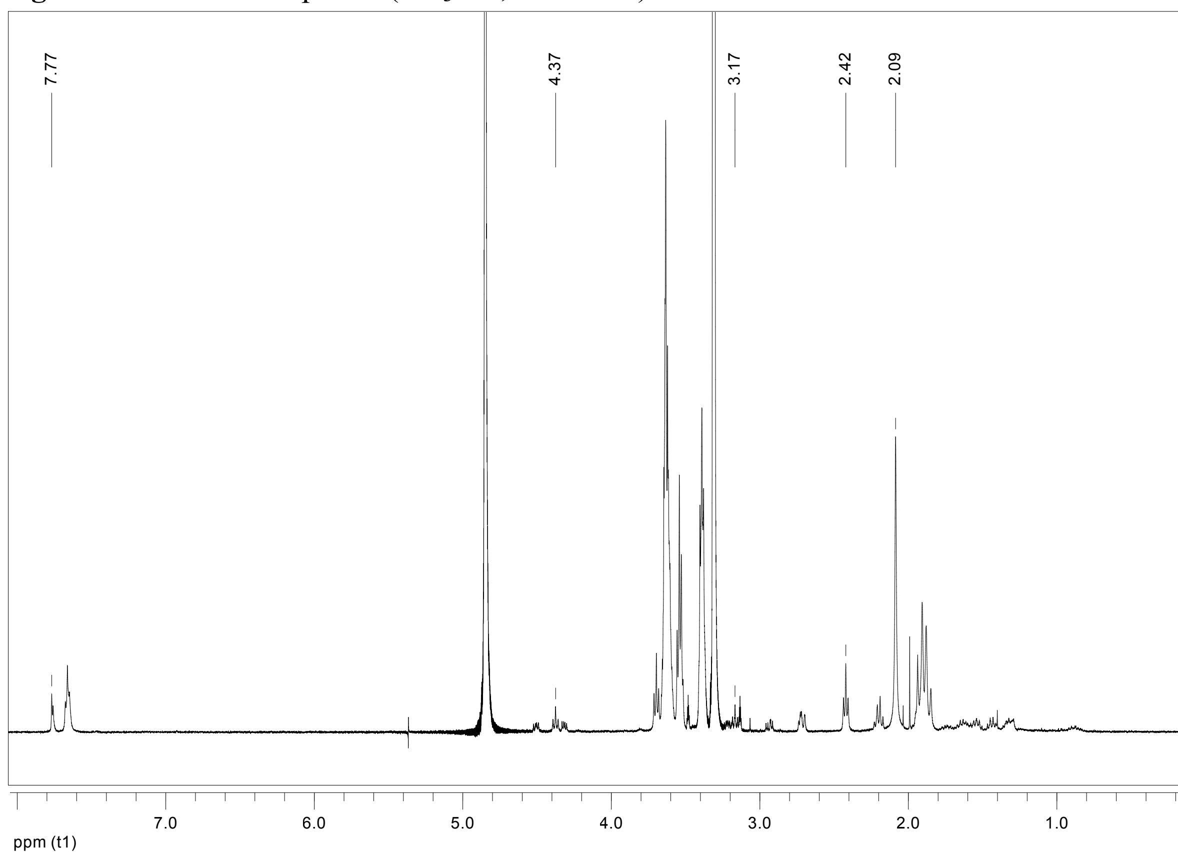


Figure S14. ^{1}H NMR spectra (CD_3OD, 400 MHz) of **10**



ESI mode Mass Spectra

Figure S15. Mass spectra of **3**

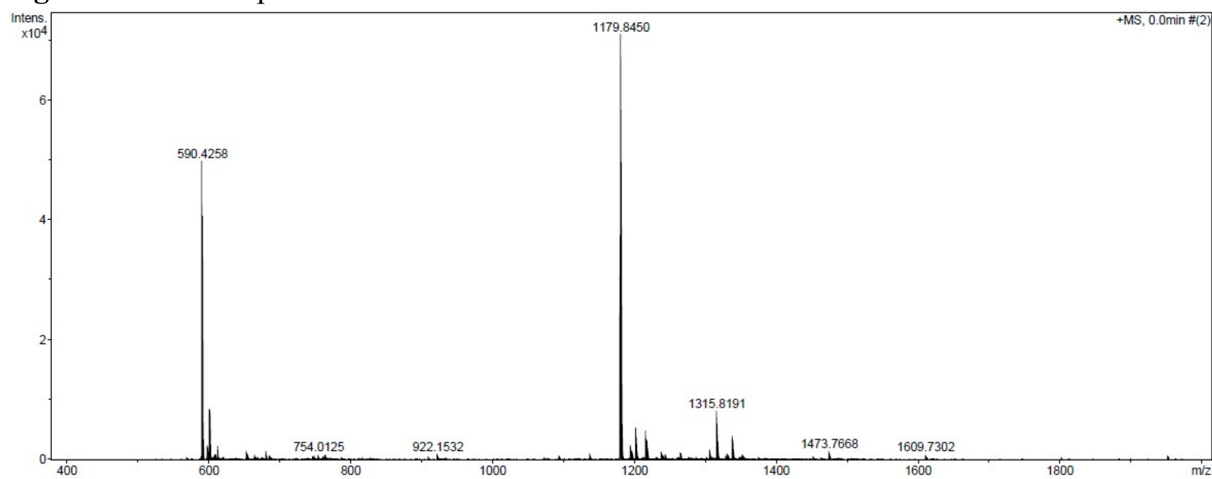


Figure S16. Mass spectra of **4**

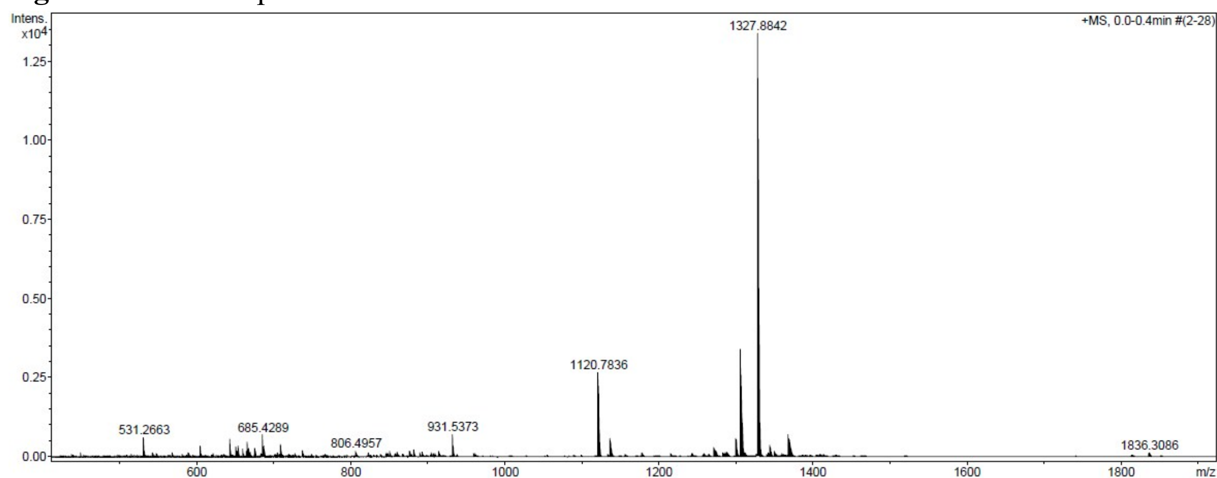


Figure S17. Mass spectra of **5**

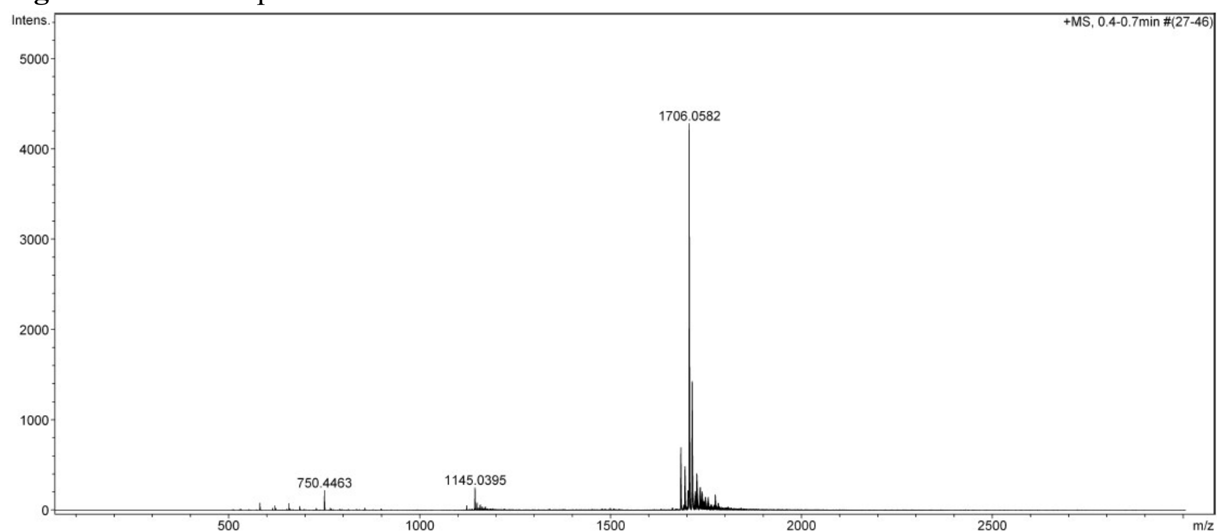


Figure S18. Mass spectra of **6**

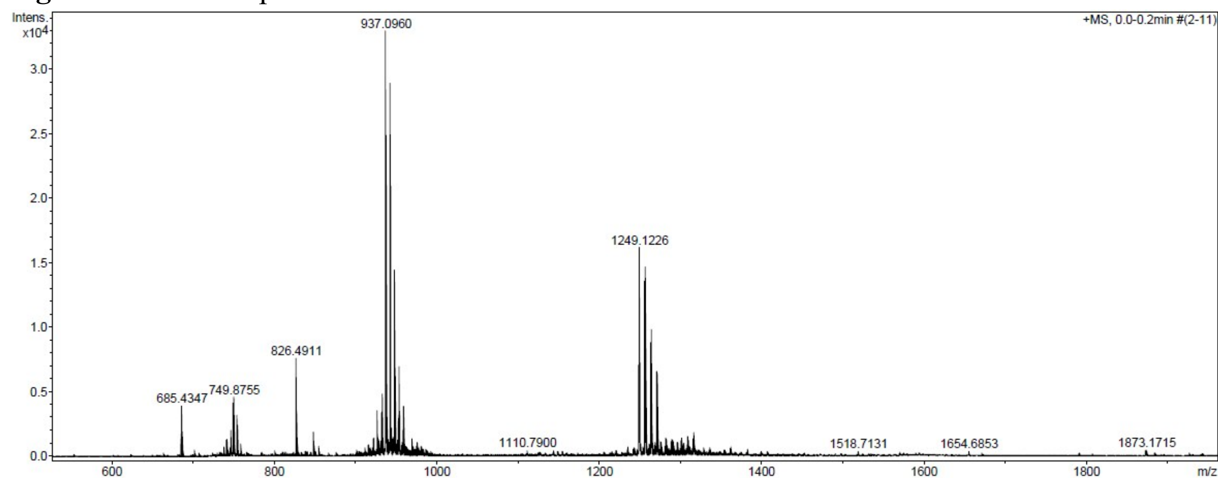


Figure S19. Mass spectra of **7**

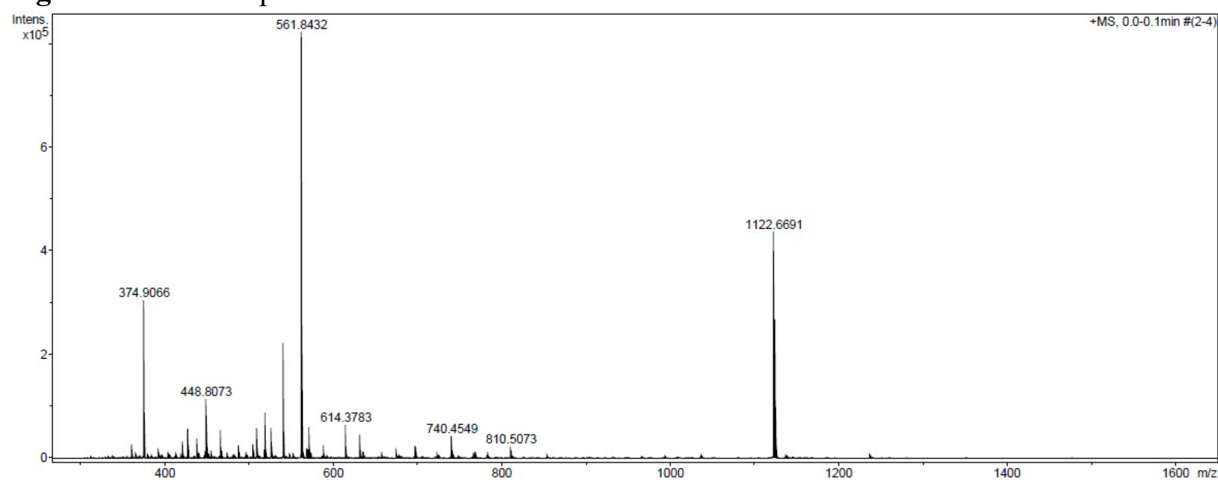


Figure S20. Mass spectra of **8**

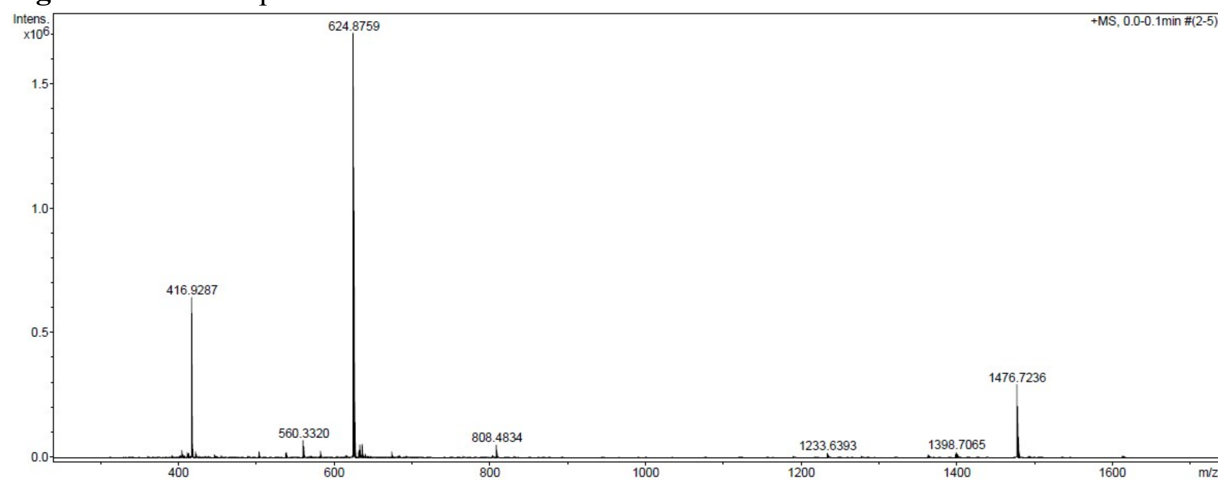


Figure S21. Mass spectra of **9**

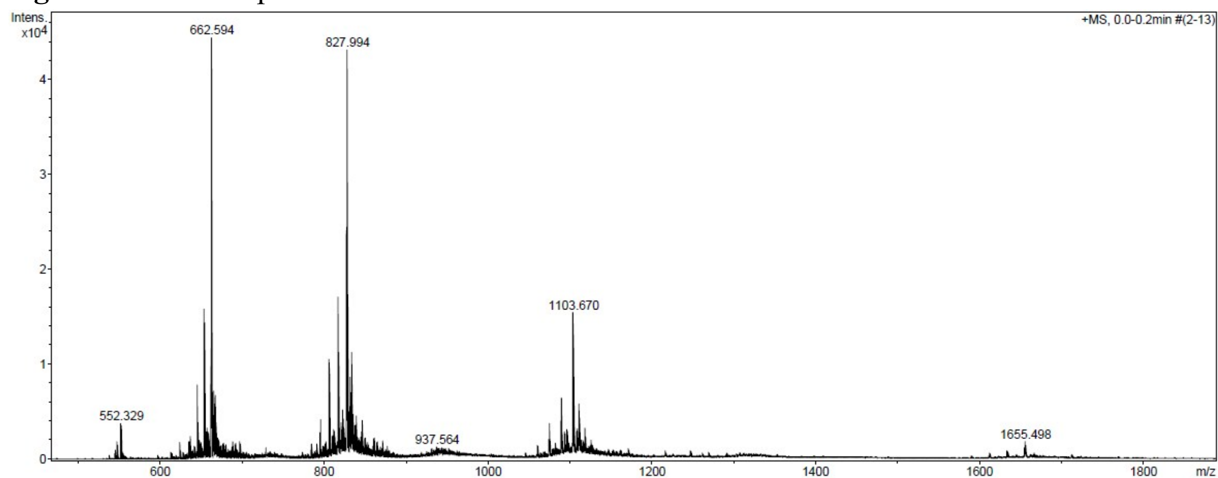
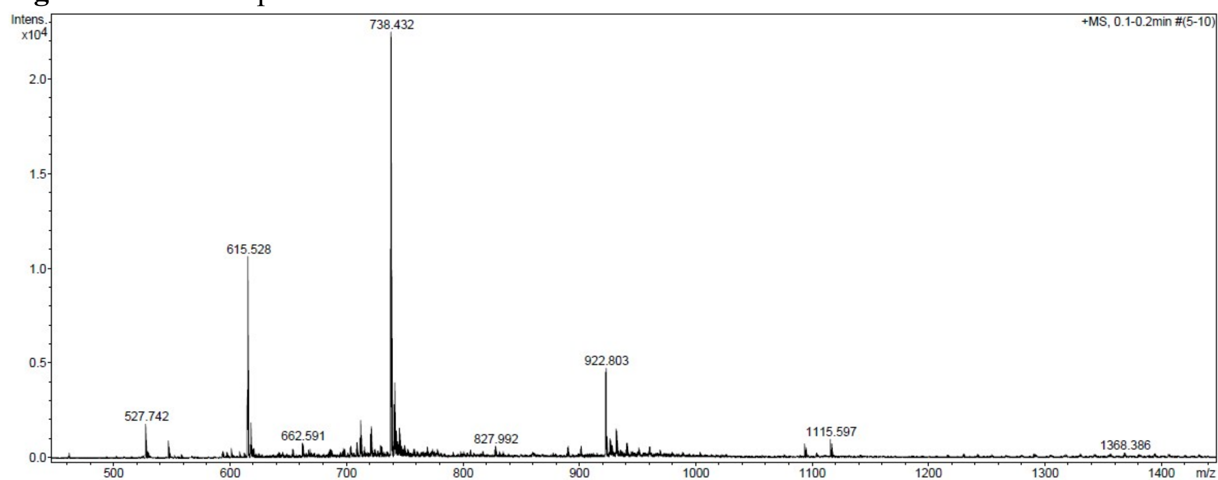


Figure S22. Mass spectra of **10**



Electrophoresis Gels

Gel retardation assay

0.2 µg of pDNA were complexed with *HYDRAmers* at different N/P ratios for 30 minutes at room temperature. The final volume was adjusted to 25 µl with diethylpyrocarbonate DEPC-treated water (RNase and DNase free) and 3 µl of DNA loading dye solution were added. The analysis was carried out by 1.0 % agarose gel electrophoresis containing GelRed for visualization. The gel was run for 30 minutes at 100 V before visualizing the pDNA shift by UV.

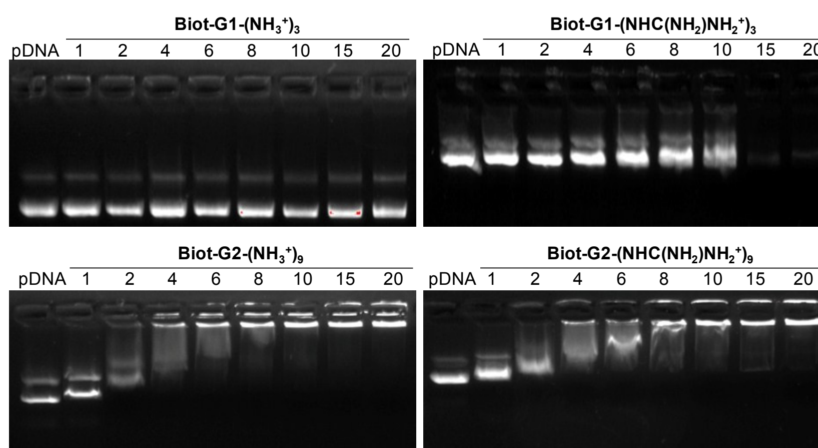


Figure S23. Gel electrophoresis images of pDNA migration in presence of biotinylated polycationic *HYDRAmers* at N/P ratios ranging from 1 to 20 (electrophoretic mobility from top to bottom). Control pDNA alone corresponds to the left lane of each gel.

Protection test of plasmid DNA from DNase I digestion

The protection test of pDNA from DNase I digestion was carried out according to the literature with minor modifications (Zhang X, et al. *Int. J. Pharm.* **2014**; 465: 444–54). Briefly, 0.2 µg of pDNA were complexed with *HYDRAmers* at different N/P ratios for 30 minutes at room temperature. The complexes were then incubated with 2 µL DNase I (0.1 U/µL, in 50 mmol/L Tris–HCl, 10 mmol/L MgCl, pH 7.4) at 37 ºC for 30 minutes. Then, 100 mM of EDTA was added to inactivate DNase I followed by incubation at 4 ºC for 10 minutes. Finally, the pDNA was released from the complexes by adding heparin at a ratio of 50 (heparin/pDNA, weight/weight). The final volume was adjusted to 25 µl with DEPC-treated water and 3 µl of DNA loading dye solution were added. The analysis was carried out by 1.0 % agarose gel electrophoresis containing GelRed for visualization. The gel was run for 30 minutes at 100 V before visualizing the pDNA shift by UV.

Heparin sulfate displacement assay

0.2 µg of pDNA were complexed with *HYDRAmers* at fixed N/P ratios (1 and 8) for 30 minutes at room temperature. The pDNA was released from the complexes by adding increasing amount of heparin ranging from 1 to 50 ratios (heparin/pDNA, weight/weight). The final volume was adjusted to 25 µl with DEPC-treated water and 3 µl of DNA loading dye solution were added. The analysis was carried out by 1.0 % agarose gel electrophoresis containing GelRed for

visualization. The gel was run for 30 minutes at 100 V before visualizing the pDNA shift by UV.

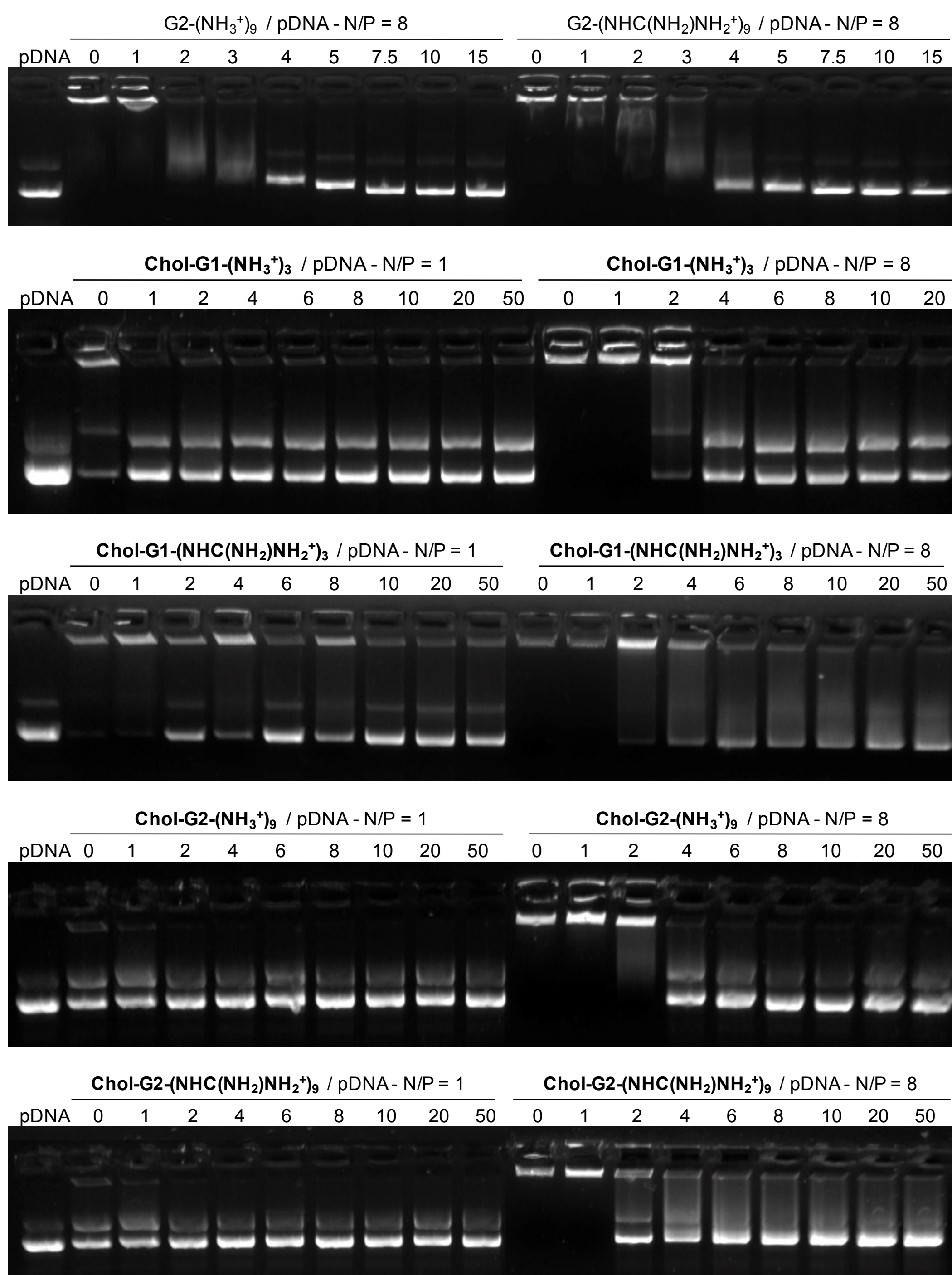


Figure S24. Gel electrophoresis images of pDNA displacement assay with increasing amount of heparin sulfate ranging from 1 to 50 ratios (heparin/pDNA, weight/weight) in presence of non-functionalized and cholesterylated polycationic *HYDRAmers* at fixed N/P ratios (1 and 8).

Surface Plasmon Resonance Experiments

Surface plasmon resonance analysis

BIACORE 3000 system (GE-Healthcare) was used to evaluate the binding of pcDNA3-EGFP plasmid to polycationic *HYDRAmers*. The sensor chip CM5, surfactant P20, amine coupling kit containing N-hydroxysuccinimide (NHS), EDC and 2-(2-pyridinyl-dithioethaneamine) (PDEA) were from GE-Healthcare (Upsala, Sweden). All biosensor assays were performed with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES)-buffered saline (HBS-EP) as running buffer (10 mM HEPES, 150 mM sodium chloride, EDTA 3 mM and 0.005 % surfactant P20, pH 7.4). Immobilization of **Biot-G1-(NH_3^+)$_3$**, **Biot-G1-($NHC(NH_2)NH_2^+$)$_3$**, **Biot-G2-(NH_3^+)$_9$** and **Biot-G2-($NHC(NH_2)NH_2^+$)$_9$** were performed through the biotin groups onto a pre-activated surface with streptavidin, which gave a response of 430, 1000, 4000 and 4000 RU, respectively. The different compounds were dissolved in the running buffer. All binding experiments were carried out at 25 °C with a constant flow rate (20 µl/min). Different concentrations of analytes were injected for 3 minutes, followed by a 3 minute dissociation phase. The sensor chip surface was regenerated after each experiment by injecting 10 µl of 10 mM HCl. The kinetic parameters were calculated using the BIAeval 4.1 software on a personal computer. Global analysis was performed using the simple Langmuir binding model 1:1. The specific binding profiles were obtained after subtracting the response signal from the channel control (biotin) and from a blank-buffer injection. The fitting to each model was judged by the reduced chi square and randomness of residue distribution.

HYDRAplex Transfection and Cytotoxicity Experiments

Cell culture and transfection protocol

For *in vitro* assays, HeLa and HEK293 cells from the American Type Culture Collection (ATCC) were used. Cells were maintained in RPMI 1640 medium, supplemented with 10 % fetal bovine serum (FBS) and 100 U/ml gentamycin. Cells were incubated at 37 °C and 5 % CO_2 and subcultured every 2–3 days.

For pcDNA3-EGFP transfection, 100,000 cells per well were seeded on 24-well plates and let them grow overnight. Medium was then discarded and *HYDRAplexes* containing 1 µg pDNA and diluted in 1 ml OPTI-MEM® were added. Transfection efficacy was determined at 48 hours by flow cytometry acquiring at least 10,000 events with a Gallios flow cytometer (Beckman Coulter, Villepinte-France).

Cellular viability determination

The cytotoxicity of the *HYDRAplexes* was tested in both HeLa and HEK293 cells by flow cytometry. For this purpose, cells were treated as above explained. 48 hours after the addition of the complexes cells were tripsinized and stained with both APC-Annexin V (AnnV; BD Pharmingen 550475) and propidium iodide (PI, 0.2 µg·ml-1; Sigma-Aldrich) in a calcium containing buffer. DMSO (20%) was used as death positive control. A negative staining for both AnnV and PI is shown in viable cells. Early apoptosis is demonstrated by AnnV positive and PI negative staining, whereas double positive cells are considered to be late apoptotic or death.

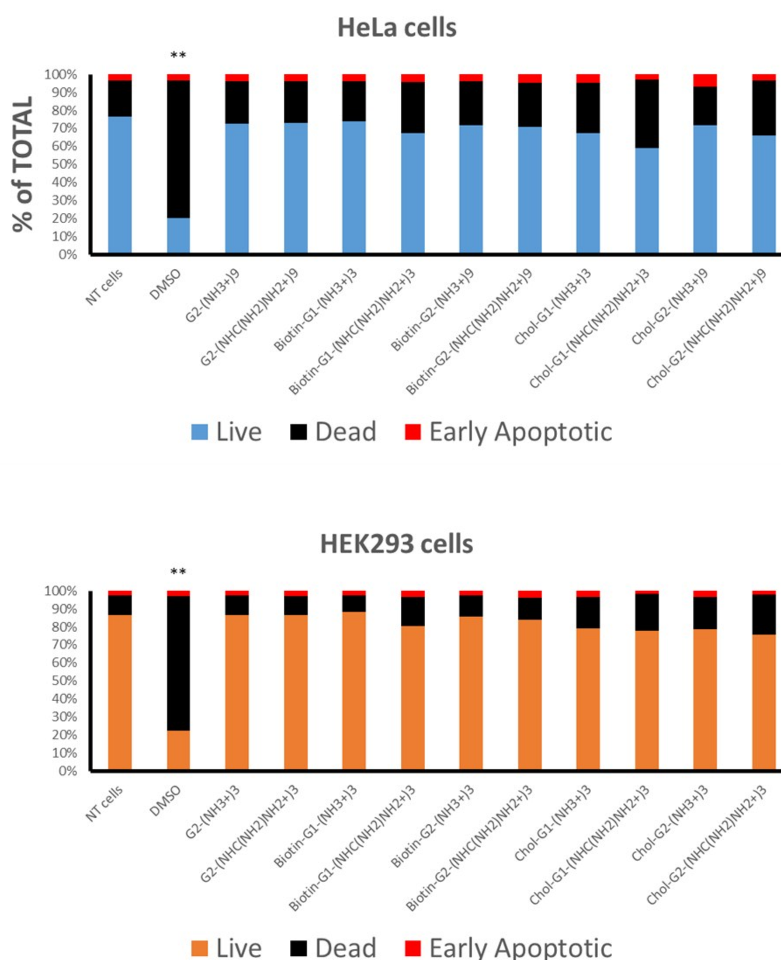


Figure S25. Analysis of cellular viability in HeLa (top graph) and HEK293 (bottom graph) treated for 48 hours with different *HYDRAplexes* containing 1 µg pDNA at 4 N/P ratio. $^{**}p < 0.01$ Vs. Non-treated cells (NT cells).

Cryo-TEM Experiments

Cryo-TEM observations

Five microliters of solution of the *HYDRA*plex between cholesterylated G2-$(NHC(NH_2)NH_2^+)_9$ (**6**) and pDNA at 4 N/P ratio in diethylpyrocarbonate (DEPC)-treated water (RNase and DNase free) were deposited onto a freshly glow discharged (Elmo, Cordouan Technologies) carbon lacey grid (Ted Pella). The grid was mounted onto a homemade vitrification machine. The temperature and the humidity were controlled and fixed at 23°C and 85% of relative humidity. The excess of liquid was blotted from both sides with filter paper and the grid plunged rapidly into liquid ethane cooled by liquid nitrogen. The grid was mounted onto a Gatan 626 cryo holder and observed under low dose conditions in a Techai G2 TEM (FEI) operating at 200 kV. The images were taken with a slow scan CCD camera (2k2k Eagle, FEI).

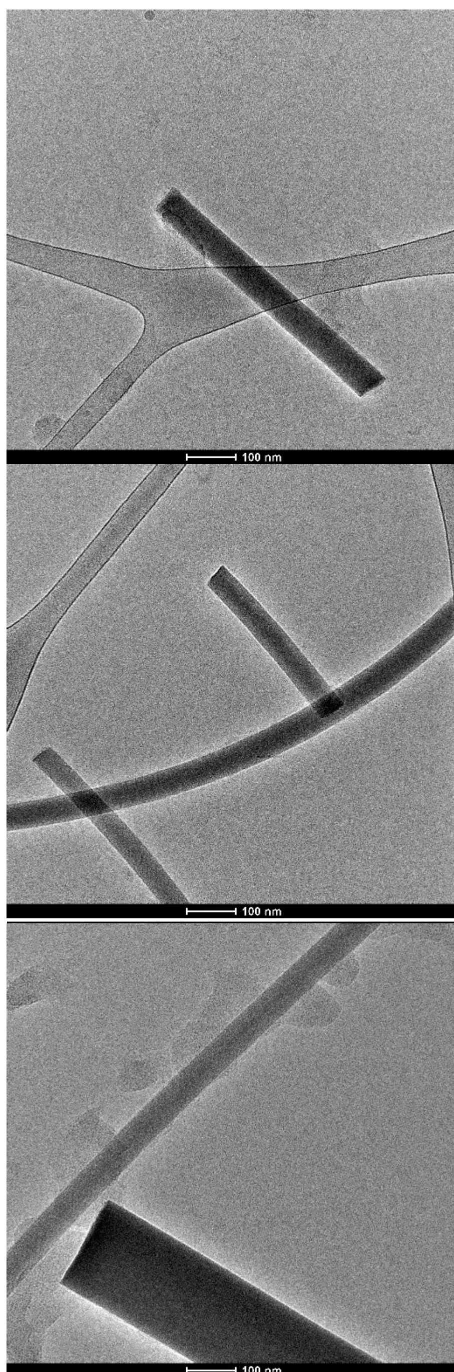


Figure S26. Cryo-TEM images of a solution *HYDRA*plex between cholesterylated G2-(NHC(NH_2)NH_2^+)$_9$ (**6**) and pDNA at 4 N/P ratio, incubated for 3 hours before acquisition.

***HYDRAplex* Size, Zeta Potential and Morphology Experiments**

Dynamic light scattering measurements

The hydrodynamic diameters were determined *via* DLS measurements using a Malvern nanoZS apparatus. *HYDRAplexes* diluted in DEPC-treated water were prepared as described for electrophoresis gel experiments. Self-assembled *HYDRAmers* were also diluted in DEPC-treated water then left for 30 minutes at room temperature. The detection angle was fixed at

173° backscatter and low-volume quartz batch cuvette (ZEN2112) was used for each sample. The measurements were performed in triplicate with automatic run duration at 25 °C. Data were analyzed using the multimodal number distribution software included with the instrument. Zeta potentials were measured with the same apparatus and with the following specifications: 20 measurements in triplicate per sample using disposable capillary cell (DTS1070); dielectric constant = 78.5; temperature = 25 °C; beam mode F(Ka) = 1.5 (Smoluchowski model).

Table S1. DLS measurements of *HYDRAplex* hydrodynamic diameters and zeta potentials at N/P ratios ranging from 1 to 8.

N/P ratio:	1			2			4			8		
HYDRAplexe	d (nm)[a]	PDI[a]	ζ (mV)[b]	d (nm)[a]	PDI[a]	ζ (mV)[b]	d (nm)[a]	PDI[a]	ζ (mV)[b]	d (nm)[a]	PDI[a]	ζ (mV)[b]
G2-$(NH_3^+)_9$	488.5	0.505	-1.1	226.6	0.279	-3.1	193.5	0.204	10.7	148.8	0.123	11.5
G2-$(NHC(NH_2)NH_2^+)_9$	337.0	0.287	-40.9	231.8	0.260	-15.5	192.2	0.306	9.1	153.4	0.260	6.8
Chol-G1-$(NH_3^+)_3$	594.7	0.698	-40.7	399.5	0.679	-6.9	185.7	0.709	10.8	132.2	0.470	14.5
Chol-G1-$(NHC(NH_2)NH_2^+)_3$	608.9	0.933	3.4	297.6	0.758	20.3	109.4	0.366	22.2	83.0	0.341	18.9
Chol-G2-$(NH_3^+)_9$	703.2	0.732	-25.3	362.3	0.749	-17.1	282.8	0.559	17.9	92.8	0.342	19.2
Chol-G2-$(NHC(NH_2)NH_2^+)_9$	390.5	0.482	-8.4	330.2	0.479	19.6	134.4	0.337	21.9	73.7	0.332	24.1

[a] d: average diameter of *HYDRAplexes* with the corresponding polydispersity (PDI)

[b] ζ: zeta potential of *HYDRAplexes*

Table S2. DLS measurements of self-assembled *HYDRAmer* hydrodynamic diameters with the same concentration of dendron as used for the corresponding *HYDRAplex* formation at N/P ratio equal to 8.

HYDRAmer	d (nm)[a]	PDI[a]
G2-$(NH_3^+)_9$	0	-
G2-$(NHC(NH_2)NH_2^+)_9$	0	-
Chol-G1-$(NH_3^+)_3$	465,2	0,572
Chol-G1-$(NHC(NH_2)NH_2^+)_3$	386,4	0,287
Chol-G2-$(NH_3^+)_9$	230,1	0,390
Chol-G2-$(NHC(NH_2)NH_2^+)_9$	316,8	0,249

[a] d: average diameter of *HYDRAmers* with the corresponding polydispersity (PDI)

Transmission electron microscopy

TEM analysis was performed on a Hitachi H7500 microscope with an accelerating voltage of 80 kV. *HYDRAplexes* diluted in DEPC-treated water were prepared as described for electrophoresis gel experiments. Self-assembled *HYDRAmers* were also diluted in DEPC-treated water then left for 30 minutes at room temperature. Ten µl of each sample was then deposited on carbon-coated copper TEM grids (Formvar–Carbon film on 300 mesh square copper grids from Electron Microscopy Sciences) and air-dried. The complexes were deposited on TEM grids without staining at the same concentrations of gel electrophoresis and DLS experiments.

Chol-G1-$(NH_3^+)_3$ Chol-G1-$(NHC(NH_2)NH_2^+)_3$ Chol-G2-$(NH_3^+)_9$ Chol-G2-$(NHC(NH_2)NH_2^+)_9$

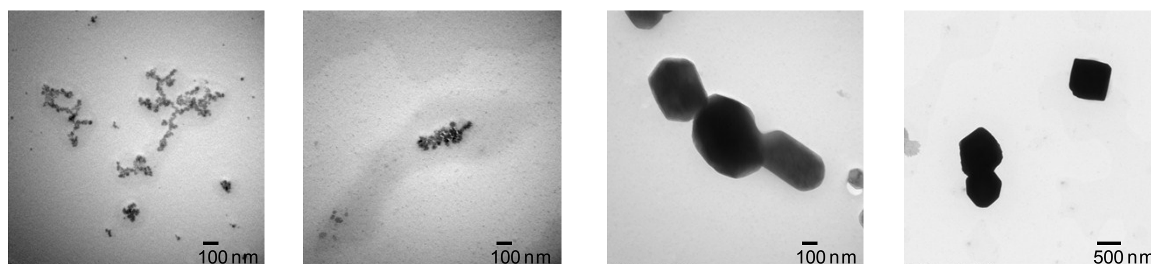


Figure S27. TEM images of self-assembled cholesterylated *HYDRAmers* with the same concentration of dendron as used for the corresponding *HYDRAplex* formation at N/P ratio equal to 8.

Synthesis Procedure References

[1] M. Grillaud, J. Russier and A. Bianco, *J. Am. Chem. Soc.*, 2014, **136**, 810−819.

[2] G. Lamanna, M. Grillaud, C. Macri, O. Chaloin, S. Muller and A. Bianco, *Biomaterials*, 2014, **35**, 7553−7561.