Poly(trehalose): Sugar-Coated Nanocomplexes Promote Stabilization and Effective Polyplex-Mediated siRNA Delivery

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Supplementary Information

Contents

Materials

All reagents for synthesis were obtained at the highest possible purity from Fisher Scientific Co (Pittsburgh, PA) or Sigma-Aldrich Co (St. Louis, MO) and used as received unless noted otherwise. N(2-aminoethyl)methacrylamide was purchased from Polysciences (Warrington, PA). JetPEI was obtained from PolyPlus Transfections (Illkirch, France). Dialysis membranes were obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Dry methylene chloride, dimethyl formamide, methanol and tetrahydrofurane were obtained using an MBRAUN MB solvent purification system manufactured by M. Braun Inertgas-Systeme GmbH (Garching, Germany), using HPLC grade solvents obtained from Fisher Scientific Co (Pittsburgh, PA).

Thin layer chromatographies (TLC) were completed using aluminum-backed silicagel plates (silicagel 60, F_{254}) obtained from Merck (Darmstadt, Germany) and visualized using UV light (254 nm) or staining agents: ninhydrin solution in ethanol for the visualization of amines, *p*-anisaldehyde solution in H_2SO_4/acetic acid/ethanol for the visualization of carbohydrates.

Preparative chromatographies were performed using a Buchi Separcore chromatography system, (Buchi Labortechnik AG, Switzerland) using Buchi plastic chromatography cartridges or homemade glass columns manually packed with 60-200 mesh Premium Rf silicagel (Sorbent Technologies Inc., Atlanta, GA). All solvents used for preparative chromatography were HPLC grade obtained from Fisher Scientific Co (Pittsburgh, PA).

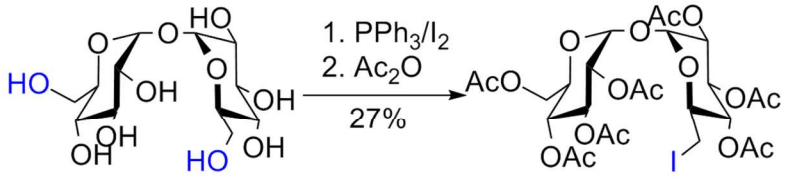
LC-MS data was obtained with an Agilent system, Agilent Technologies (Santa Clara, CA) with a time-of-flight (TOF) analyzer coupled to a Thermo Electron TSQ-LC/MS ESI mass spectrometer.

NMR spectra were recorded using 400MR Varian-400 Hz spectrometer in deuterated solvents, namely D_2O, d_4-MeOD, d_6-DMSO, $CDCl_3$, CD_2Cl_2. All deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). ^{1}H-NMR spectra were recorded at 399.7 MHz and ^{13}C-NMR spectra were recorded at 101 MHz. Spectra were analyzed using MNova software (version 7.0.1-8414, Mestrelab Research S.L. (Santiago de Compostela, Spain)).

SEC was conducted using 1.0 wt% acetic acid/0.1 M Na_2SO_4 as the eluent at a flow rate 0.3 mL/min on size exclusion chromatography columns [CATSEC1000 (7μ, 50×4.6), CATSEC100 (5μ, 250×4.6), CATSEC300 (5μ, 250×4.6), and CATSEC1000 (7μ, 250×4.6)] obtained from Eprogen Inc. (Downers Grove, IL). Signals were acquired using Wyatt HELEOS II light scattering detector (λ = 662 nm), and an Optilab rEX refractometer (λ = 658 nm). SEC trace analysis was performed using Astra V software (version 5.3.4.18), Wyatt Technologies (Santa Barbara, CA). Biological sample fluorescence was measured with GENios Pro luminometer (TECAN US, Research Triangle Park, NC). Quartz Crystal Microbalance (QCM) experiments were performed on Q-Sense E4 QCM (Q-sense; Vastra Frolunda, Sweden) instrument. Differential scanning calorimetery (DSC) was conducted on a TA Instruments Q100 under nitrogen.

Synthetic procedures

Synthesis of 6-iodo-6-deoxy-2,3,4,5,2',3',4',5',6'-O-acetyl trehalose (2)



1 **2**

Iodine (47.50 g, 0.1871 mol) was placed in a flame-dried 1.5 L round-bottom flask and 800 mL of dry dimethylformamide (DMF) was added. Triphenylphosphine (51.55 g, 0.1965 mol) was dissolved in 150 mL of dry THF and added to the iodine suspension. Anhydrous trehalose

(42.64 g, 0.1246 mol) was added via a solid addition funnel. The reaction was allowed to proceed at 80 °C for 4 h. Solvents were removed under reduced pressure yielding a thick yellow oil.

The oil was poured into a 1 L solution of sodium methoxide in methanol, the pH was adjusted to 9 using additional sodium methoxide, and the mixture was allowed to stir for 1h at 23 °C. Acidic resin DOWEX-2H (H^+ form) was added to neutralize the solution and subsequently filtered off. Methanol was removed under reduced pressure to yield a yellow oil.

The oil was poured in 600 mL of water via a funnel for liquids to precipitate triphenylphosphine oxide. This solution was stored at 4 °C for 12 h.

The aqueous solution was filtered and extracted with 2x50 mL of dichloromethane (DCM). Water was removed under reduced pressure, and the obtained oil was dried for 20 h over P_2O_5 at 500 mTorr.

The dry oil was dissolved in 800 mL of dry pyridine (Py) and the solution was placed in an ice-bath. Acetic anhydride (190 mL, 205 g (d=1.08 g/mL), 2.01 mol) was added over 15 min via an additional funnel. The reaction was allowed to stir at 23 °C for 14 h. The reaction mixture was poured on ice, and the reaction mixture was extracted with 4x200 mL of DCM. The organic extracts were combined, washed with a dilute sulfuric acid solution to remove Py and dried over Na_2SO_4 overnight. It should be noted that washing with NaCl(sat) solution leads to halogen exchange ($RCH_2I \rightarrow RCH_2Cl$) and must be avoided.

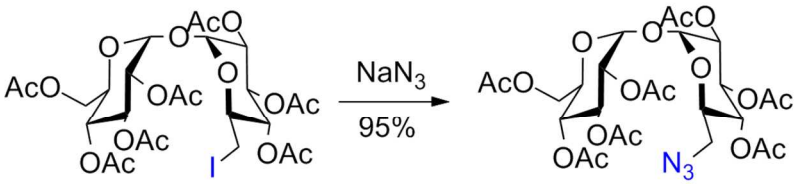
Na_2SO_4 was removed via filtration and DCM was removed under reduced pressure yielding a yellowish oil. Column chromatography was used to separate the mixture of per-O-acetylated 6-iodo-6-deoxy and 6,6´-diiodo-6,6´-deoxy trehalose using DCM/Et_2O=5/1 eluent.

This mixture of two products was separated by column chromatography using an eluent gradient DCM → DCM/Et_2O(5/1) to yield 22.3 g (24.0%) of **2**.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 5.56 – 5.45 (overlapping triplets, J = 10.2, 2H), 5.40 – 5.36 (d, 3.9 Hz, 1H), 5.36 – 5.33 (d, 3.9 Hz, 1H), 5.22 – 5.15 (dd, J = 10.3, 3.9 Hz, 1H), 5.10 – 5.00 (m, 2H), 4.94 – 4.84 (dd, J = 10.0, 9.2 Hz, 1H), 4.27 – 4.19 (m, 1H), 4.08 – 3.98 (m, 2H), 3.97 – 3.89 (ddd, J = 10.0, 9.0, 2.4 Hz, 1H), 3.30 – 3.20 (dd, J = 11.0, 2.6 Hz, 1H), 3.13 – 2.99 (m, 1H), 2.15 (s, 3H), 2.09 – 2.07 (m, 9H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ ppm: 170.67, 170.00, 169.94, 169.67, 169.58, 169.57, 92.21, 91.68, 72.46, 70.31, 70.11, 69.84, 69.56, 69.27, 68.70, 68.38, 68.05, 61.91, 25.70, 21.28, 20.79, 20.74, 20.68, 20.63, 2.61. IR: ν_{max} (cm^{-1}) 2956, 1744, 1595, 1432, 1367, 1211, 1168, 1137, 1033, 986, 957, 899, 804, 734, 653,

598, 564, 543, 488, 467. ESI-MS positive ion mode: calculated m/z $[M+NH_4]^+$ 764.1257, found m/z: 764.1297; calculated m/z $[M+Na]^+$ 769.0811, found m/z: 747.0832.

Synthesis of 6-azido-6-deoxy-2,3,4,5,2',3',4',5',6'-O-acetyl trehalose (3)



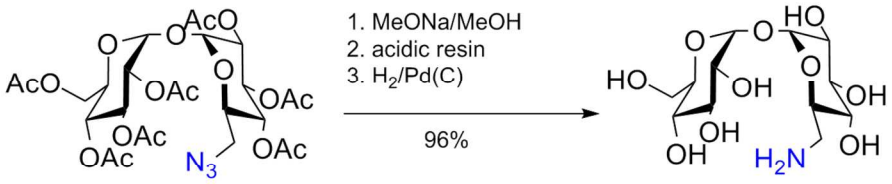
2 **3**

Compound **2** (16.56 g, 22.19 mmol) and NaN_3 (1.63 g, 25.07 mmol) were placed in a dry 500 mL flask equipped with a stir bar and capped with a septum. Next, 300 mL of dry DMF was added via cannula and nitrogen was bubbled through the solution for 20 min. The mixture was heated to 60 °C and stirred for 4 h. The reaction mixture was cooled down and concentrated under reduced pressure. The resulting solution was poured into 700 mL of H_2O at 0 °C and extracted with 3x150 mL of ethyl acetate. Organic extracts were combined, washed with brine, and dried over Na_2SO_4 overnight.

The solids were filtered off and the solvent was removed under reduced pressure yielding a white crystalline material. The product was recrystallized from ethylacetate/hexanes(1/3) to yield 11.94 g of **3** upon drying. The filtrate was placed in a freezer (-27 ^oC) resulting in precipitation of 1.62 g of **3**. The combined yield was 13.56 g (92.4%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 5.49 (ddd, J = 10.3, 9.3, 5.7 Hz, 2H), 5.35 – 5.30 (m, 2H), 5.04 (m, 4H), 4.25 (dd, J = 12.1, 5.6 Hz, 1H), 4.13 – 3.98 (m, 3H), 3.36 (dd, J = 13.3, 7.2 Hz, 1H), 3.17 (dd, J = 13.3, 2.5 Hz, 1H), 2.12 (s, 3H), 2.11 – 2.07 (2 overlapping singlets, 6H), 2.06 (s, 3H), 2.05(s, 3H), 2.04 (2 overlapping singlets, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ ppm: 170.57, 169.97, 169.68, 169.53, 169.51, 92.82, 92.43, 70.03, 69.88, 69.86, 69.77, 69.70, 68.54, 68.26, 61.77, 50.94, 20.67, 20.65, 20.63, 20.61. ν_{max} (cm^{-1}) 2959, 2099, 1745, 1683, 1435, 1368, 1288, 1213, 1170, 1135, 1072, 1035, 1017, 984, 959, 906, 841, 804, 656, 603, 571, 543, 489, 473, 447. ESI-MS positive ion mode: calculated m/z $[M+NH_4]^+$ 679.2318, found m/z: 679.2276; calculated m/z $[M+Na]^+$ 684.1872, found m/z: 684.1825.

Synthesis of 6-amino-6-deoxy-trehalose (4)

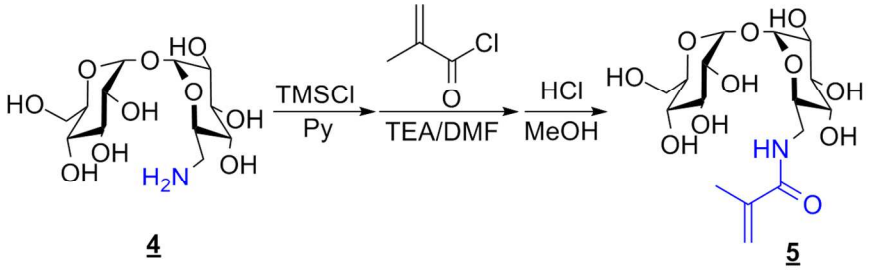


3 **4**

6-azido-6-deoxy-2,3,4,5,2',3',4',5',6'-O-acetyl trehalose **3** (11.81 g, 17.85 mmol) was dissolved in 450 mL of MeONa/MeOH (pH~9) and sonicated to assist dissolutionand stirred at 23 °C for 4h. The reaction progress was monitored by TLC/p-anisaldehyde stain (eluent: EtOAc/MeOH/H_2O=15/5/4). The solution was neutralized via the addition of DOWEX-50H resin. The resin was removed by filtration and the solvent evaporated to yield the crude produce, 6-azido-6-deoxy-trehalose as amorphous solid.

The crude 6-azido-6-deoxy-trehalose was dissolved in methanol and the mixture was deoxygenated by bubbling nitrogen for 30 min. Palladium on carbon (5% dispersion, 499 mg, 0.234 mmol) was added and hydrogen gas was bubbled through the solution for 20 h using a gas diffuser. Nitrogen was then bubbled through the solution to remove the remaining dissolved hydrogen. The Pd(C) was filtered off using Celite and the solvent was removed under reduced pressure to yield **4** (5.83 g, 95.8% yield). ^{1}H-NMR (400 MHz, D_2O) δ ppm: 5.10 – 4.97 (two doublets, J = 4.4 Hz, 2H), 3.73 – 3.54 (m, 6H), 3.54 – 3.44 (dd, J = 9.9, 3.8 Hz, 2H), 3.34 – 3.23 (t, J = 9.4 Hz, 1H), 3.21 – 3.10 (t, J= 9.4 Hz, 1H), 2.91 – 2.79 (dd, J = 13.7, 2.5 Hz, 1H), 2.64 – 2.53 (dd, J = 13.8, 7.8 Hz, 1H). ^{13}C-NMR (101 MHz, D_2O) δ ppm: 93.03, 92.91, 72.35, 72.30, 72.01, 71.26, 70.99, 70.89, 69.53, 60.35, 48.73, 41.32. ν_{max} (cm^{-1}) 3264, 2929, 2828, 1585, 1348, 1146, 1102, 987, 943, 803, 765, 642, 576, 519, 426. ESI-MS positive ion mode: calculated m/z $[M+H]^+$ 342.14, found m/z: 342.10.

Synthesis of 6-methacrylamido-6-deoxy trehalose (5)



4 **5**

2-Deoxy-2-aminotrehalose **4** (3.74 g, 11.0 mmol) was suspended in 200 mL of dry pyridine and cooled to 0 °C. Trimethylsilyl chloride (TMSCl, 10.0 g, 92.0 mmol, 1.2 eq per -OH) was added using an addition funnel. The reaction mixture was allowed to slowly warm to 23 °C and allowed to proceed overnight.

The reaction mixture was cooled down to ca. 0 ºC and poured into 600 mL of ice-cold carbonate buffer (pH=9). The aqueous suspension was extracted with 3x150 mL of hexanes. The extracts were combined and washed with water, followed by brine, and dried over Na_2SO_4.

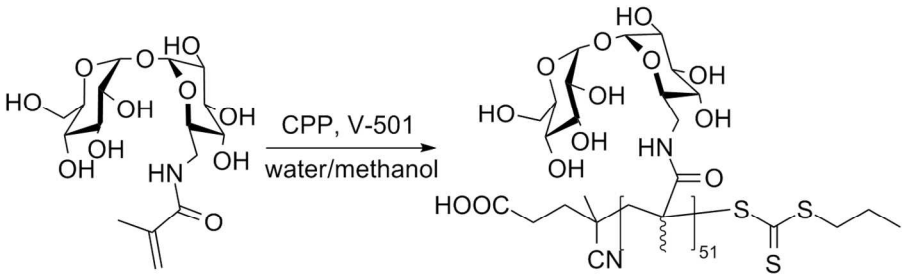
The solid was filtered off and hexanes were removed under reduced pressure. A white crystalline material was obtained upon evaporation and was then dried at 500 mTorr over P_2O_5 to yield 8.83 g (95.2%) of the 6-amino-6-deoxy-2,3,4,5,2′,3′,4′,5′,6′-nano-*O*-trimethylsilyl trehalose. ^{1}H-NMR (400 MHz, CD_2Cl_2) δ ppm: 4.87 – 4.74 (two overlapped doublets J = 3.2 Hz, 2H), 3.90 – 3.77 (td, J = 9.0, 3.2 Hz, 2H), 3.73 – 3.52 (m, 4H), 3.44 – 3.26 (m, 4H), 2.87 – 2.78 (dd, J = 13.2, 3.0 Hz, 1H), 2.68 – 2.57 (dd, J = 13.2, 5.4 Hz, 1H), 1.53 – 0.85 (bs, NH, 3H), 0.29 – -0.24 (m, 63H). δ ppm: 196.08, 120.68, 95.30, 95.21, 74.62, 74.11, 73.89, 73.50, 73.29, 73.25, 71.88, 71.90, 71.85, 62.56, 41.76, 18.22. ν_{max} (cm^{-1}) 2956, 1459, 1391, 1248, 1165, 1100, 1062, 1042, 1012, 965, 898, 872, 831, 746, 683, 666, 623, 590, 579, 517, 475, 454, 425.

The TMS protected aminotrehalose (1.687 g, 1.993 mmol) was dissolved in 2 mL of DCM and 35 mL of DMF. This solution was cooled to 0 °C and triethylamine (0.55 mL, 0.40 g (d=0.726 g/mL), 3.9 mmol) was added. Freshly distilled methacryloyl chloride (0.22 mL, 0.24 g (d=1.07 g/mL) 2.3 mmol) was dissolved in 5 mL of dry DCM and was added slowly. The reaction was allowed to proceed at 0 ºC for 1h and 3h at 23 °C.

The reaction mixture was cooled in an ice bath and poured into 200 mL of the ice-cold carbonate buffer (pH~8). The resulting suspension was extracted with 3x30 mL of hexanes. The organic extracts were combined and washed with water, followed by a brine, and dried over Na_2SO_4 overnight.

The solids were removed via filtration and the hexanes were removed under vacuum to yield a colorless oil. This oil was dissolved in 20 mL of dry methanol and cooled to 0 °C. Next, 0.2 mL of a ~1.25M HCl solution in methanol was added and the reaction was allowed to warm to 23 °C, and stirred for additional 10 min. Methanol was removed under reduced pressure yielding 0.765 g (93.3%) of the title compound (**5**) as a white solid foam. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm: 5.77 – 5.61 (t, J = 0.9 Hz, 1H), 5.40 – 5.35 (t, J = 1.4 Hz, 1H), 5.13 – 5.03 (dd, J = 16.1, 3.8 Hz, 2H), 4.65 – 4.55 (bs, NH, 1H), 3.98 – 3.90 (dt, J = 9.7, 4.8 Hz, 1H), 3.86 – 3.74 (m, 4H), 3.72 – 3.64 (dd, J = 11.9, 5.4 Hz, 1H), 3.57 – 3.52 (d, J = 4.8 Hz, 2H), 3.52 – 3.44 (td, J = 9.8, 3.8 Hz, 2H), 3.36 – 3.31 (dd, J = 9.8, 9.0 Hz, 1H), 3.20 – 3.13 (dd, J = 9.8, 9.0 Hz, 1H), 2.00 – 1.84 (m, 3H). ν_{max} (cm^{-1}) 3296, 2926, 2103, 1654, 1606, 1538, 1423, 1330, 1223, 1145, 1101, 1074, 1031, 984, 938, 843, 803, 573, 502, 433. ESI-MS positive ion mode: calculated m/z $[M+H]^+$ 410.17, found m/z: 410.20.

Synthesis of polytrehalose 6 ($pMAT_{51}$)



5 **6**, polytrehalose

6-Methacrylamido-6-deoxy trehalose **5** (0.765 g, 1.867 mmol, 65 eq) was dissolved in 7.00 mL of acetate buffer in D_2O. CPP (7.97 mg, $2.87x10^{-2}$ mmol, 1 eq) was dissolved in 645 µL of MeOD and added to the solution of **5**, followed by V-501 (0.805 mg, $2.87x10^{-3}$ mmol, 0.1 eq) in 244 µL of MeOD. Finally, 861 µL of MeOD was added to a final volume of 1.75 mL of MeOD. The reaction flask was sealed and connected to an NMR tube via cannula. The setup was deoxygenated by bubbling nitrogen for 45 min. At that time, 0.50 mL was transferred into the NMR tube. Polymerization was conducted in an NMR instrument at 70 °C while spinning the reaction tube at 20 Hz for 9 h. ^{1}H-NMR spectra were acquired at various time points. The remaining reaction mixture was stored at 4 °C.

The remaining reaction mixture was deoxygenated by bubbling nitrogen for 45 min. The flask was placed in an oil bath pre-heated to 70 ^oC for 6 h to yield 77% monomer consumption and a targeted degree of polymerization of 50. The reaction was stopped by removing the septum and

cooling the reaction mixture on ice. It was dialyzed against ultra-pure water (3500 Da MWCO) acidified to pH 4-5 with HCl. Water changes were performed every 8-10 h. After 3 d of dialysis, the polymer solution was lyophilized to yield 490 mg of white solid.

SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=21.0 kDa, dp_n=50.7~51, PDI=1.04.

^{1}H-NMR (400 MHz, D_2O) δ ppm: 5.76 – 5.28 (bs, 100H), 4.39 – 3.50 (m, 600H), 2.78 – 2.66 (bs, 2H), 2.53 – 0.68 (m, 264H).

^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.26 – 4.96 (bs, 86H), 4.00 – 3.04 (m, 516H), 2.35 – 2.21 (bs, 2H), 1.79 – 0.61 (m, 217H).

This above procedure was repeated to yield polymer necessary for the TEM experiments.

SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=17.7 kDa, dp_n=42.5~43, PDI=1.06.

a) b)

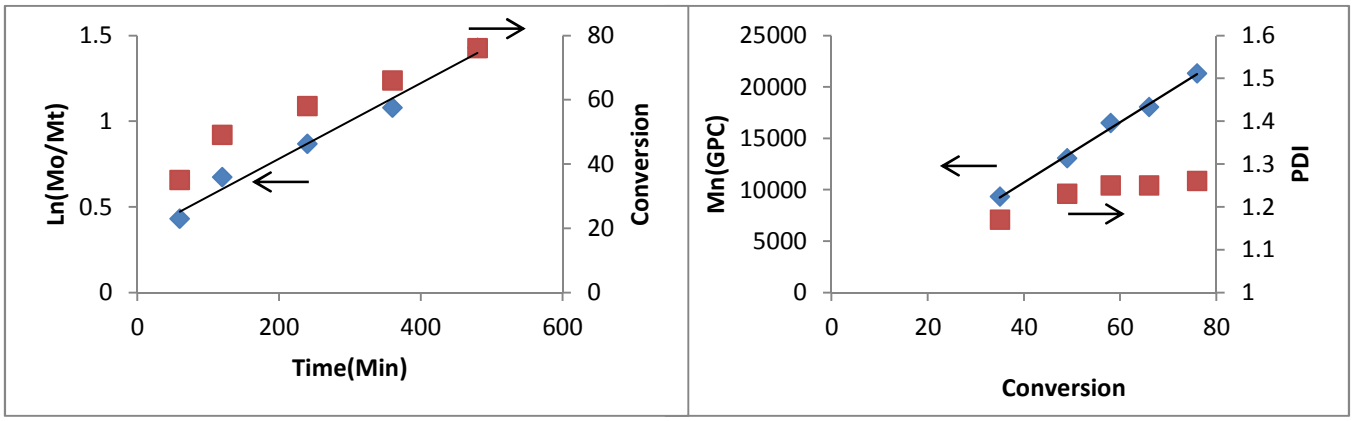
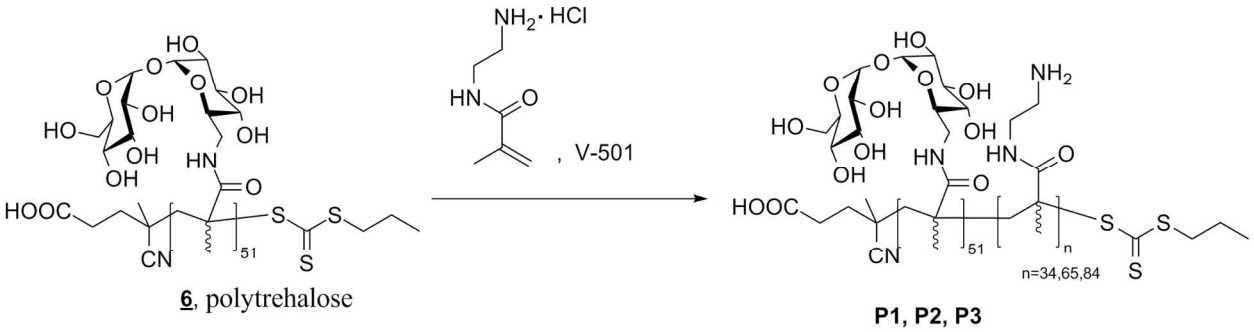


Figure S1. Kinetics of polymerization of **5**. a) Plot of $Ln(M_o/M_t)$ versus time (blue diamonds) and conversion versus time (red squares) determined using ^1H NMR. b) Plot of Mn(GPC) versus conversion (blue diamonds) and PDI versus conversion (red squares) determined using gel permeation chromatography and static light scattering.

Synthesis of diblock copolymers P1, P2 and P3



Polytrehalose **6** (327 mg, $1.56x10^{-2}$ mmol, 1.00 eq) was dissolved in 3.00 mL of acetate buffer and the resulting solution was placed in a flask that contained aminoethylmethacrylamide hydrochloride (AEMA·HCl, 311 mg, 1.89 mmol, 121 eq). To this, 0.780 mL of a solution containing V-501 (0.44 mg, $1.56x10^{-3}$ mmol, 0.10 eq) was added and the flask was sealed. The solution was deoxygenated by bubbling nitrogen for 45 minutes. The flask was placed in an oil bath preheated to 70 ^oC. Two 1.25 mL aliquots were removed with a syringe at 30 min (**P1**) and 60 min (**P2**). Each aliquot was immediately cooled on ice. After 90 min (**P3**), the reaction was halted by septum removal and placing the reaction flask on ice. All three samples were dialyzed (3500 Da

MWCO) against 3x4L of 0.5 M NaCl solution, followed by 3x4L 0.1 M NaCl and finally 6x4L of ultra-pure water. All dialysis media were acidified with HCl to pH 4-5.

Polymer solutions were lyophilized to yield white, flocculent powders. All experiments, with the exception of the TEM experiments, were completed using polymers P1-P3.

MAT_{51}-*b*-$AEMA_{34}$(**P1**), 113 mg. SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=23.4 kDa, PDI=1.04. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 100H), 3.94 – 2.94 (m, 740H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 242H).

MAT_{51}-*b*-$AEMA_{65}$(**P2**), 141 mg. SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=29.4 kDa, PDI=1.05. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 100H), 3.94 – 2.94 (m, 862H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 344H).

MAT_{51}-*b*-$AEMA_{84}$(**P3**), 181 mg. SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=31.8 kDa, PDI=1.06. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 100H), 3.94 – 2.94 (m, 960H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 417H).

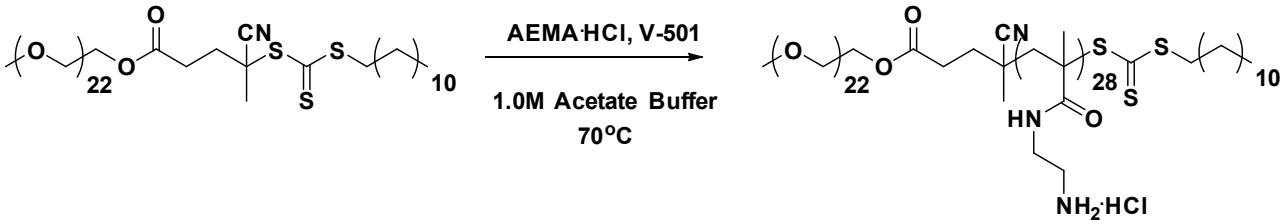
The polymerization procedure was repeated to yield additional polymer necessary for the TEM experiments to image the polyplexes.

MAT_{43}-*b*-$AEMA_{21}$(**P1***), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=20.4 kDa, PDI=1.07. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 86H), 3.94 – 2.94 (m, 600H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 192H).

MAT_{43}-*b*-$AEMA_{44}$(**P2***), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=23.3 kDa, PDI=1.07. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 100H), 3.94 – 2.94 (m, 692H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 261H).

MAT_{43}-*b*-$AEMA_{57}$(**P3***), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). M_n=25.0 kDa, PDI=1.08. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 5.18 – 4.97 (bs, 100H), 3.94 – 2.94 (m, 744H), 2.03-1.49 (m, backbone, under NaOAc resonance), 1.16 – 0.66 (m, 300).

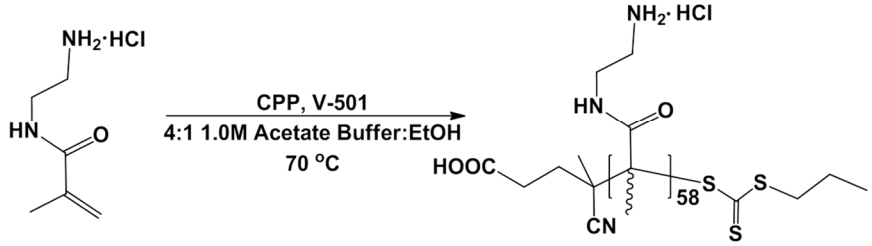
Synthesis of Control Polymer pPEG-*b*-AEMA



Poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate) (average Mn 1400) (34.8 mg, 2.50 x 10^{-2} mmol, 1.00 eq), and AEMA·HCl (412 mg, 2.50 mmol, 100 eq) were dissolved in 1.0M acetate buffer and charged into a roundbottom flask. V-501 (0.7 mg, 2.50 x 10^{-2} mmol, 0.10eq) and dissolved in 1 ml of 1.0 M acetate buffer and added to the round bottom flask giving a final concentration of 0.5 M. Following purging with nitrogen for 45 min, the flask was lowered into a preheated oil bath at 70°C for 30 min. The resulting polymer was purified via dialysis with 500 – 1000 Da molecular weight cut-off membrane against water (pH = 4-5) followed by ultra-pure water (pH = 7) to yield a white, fluffy solid after lyophilization.

p$\underline{PEG_{22}}$-$\underline{AEMA_{28}}$, SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). Mn=6.0 kDa, PDI=1.15. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 3.65 – 3.54 (m, 88H), 3.54 – 3.18 (m, 70H), 3.15 – 2.90 (m, 56H), 2.22 – 1.58 (m, backbone, under NaOAc resonance), 1.55 – 0.84 (m, 165H).

Synthesis of Control Polymer pAEMA



To synthesize an AEMA homopolymer, CPP (3.03 mg, 1.09x10^{-2}mmol, 1.00 eq), AEMA·HCl (108.3 mg, 0.658 mmol, 60 eq), and V-501 (0.614 mg, 2.19 x10^{-3} mmol. 0.20 eq) were dissolved in 3.29 mL of 1.0M acetate buffer (pH 5.2)/ ethanol (4:1) mix solvent in a 5mL round

bottom flask. The solution was purged with nitrogen for 45 min and the polymerization flask was submerged in a preheated oil bath at 70 ºC for 3 h before termination by exposure to air. The final product was purified by dialysis with 3500 Da molecular weight cut-off membrane against NaCl solution followed by water yielding a white, fluffy solid after lyophilization.

$\underline{pAEMA_{58}}$, SEC (eluent: 1.0 wt% acetic acid/0.1 M Na_2SO_4). A flow rate of 0.3 mL/min, light scattering detector (λ = 662 nm) and refractometer (λ = 658 nm). Mn=7.3 kDa, PDI=1.09. ^{1}H-NMR (500 MHz, D_2O with 0.1 M NaOAc) δ ppm: 3.64 – 3.29 (bs, 118H), 3.26 – 2.92 (bs, 119H), 2.38 – 2.29 (bs, 2H), 2.02 – 1.65 (m, backbone, under NaOAc resonance), 1.20 – 0.73 (m, 175H).

Transmission electron microscopy (TEM) Imaging.

3.0μL of the polyplexes solutions (formulated with siRNA and polymers P1*, P2*, and P3*), prepared at NP value of 20 according to the method in detail below for cell culture, was applied onto a 300-mesh carbon coated copper grid (Ted Pella, Inc). The excess solution was wiped off with filter paper after 60s. Next a negative stain (3.0 μL 1% uranyl acetate solution) was applied in triplicate to the sample to facilitate visualization of the polyplexes in the TEM image. Imaging was operated in FEI Tecnai G2 Spirit BioTWIN transmission electron microscope at 120 kV. Images were recorded using EagleTM 2k CCD camera, and phase contrast was enhanced at 10-12 μm underfocus. Images were saved as TIFF files and analyzed in software ImageJ 1.46r.

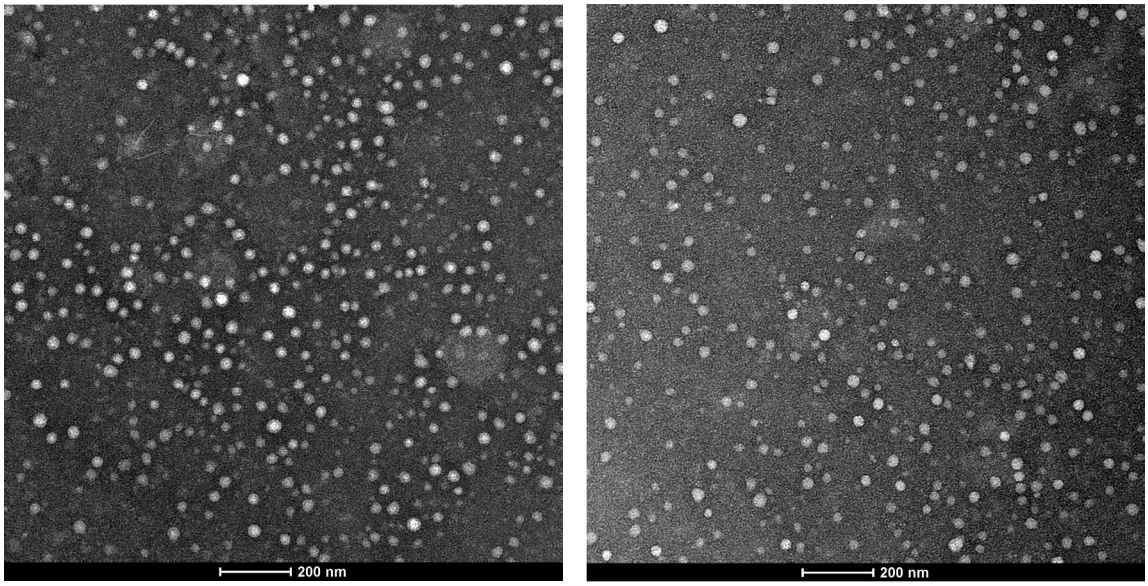
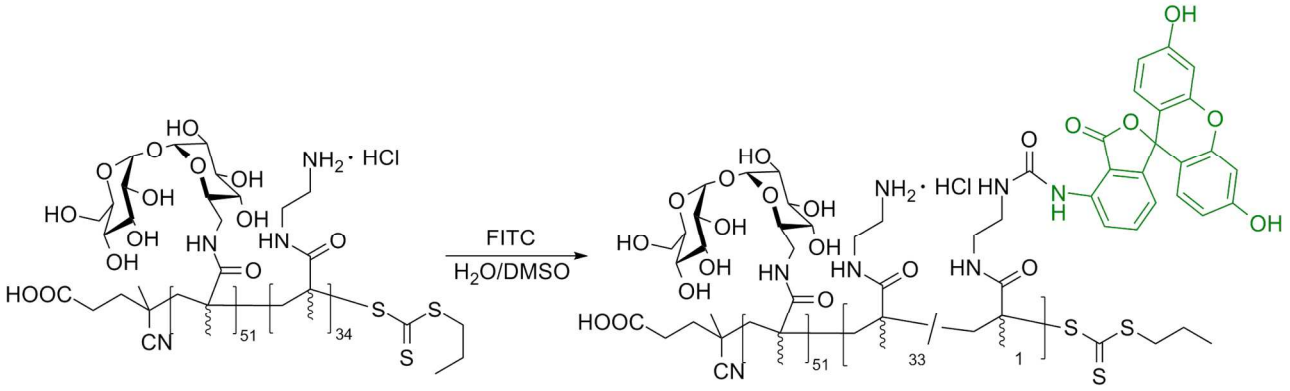


FIGURE S2. TEM images of siRNA polyplexes formed with polymer P2 (left) and P3 (right). TEM images of P1 polyplexes are shown in the main manuscript.

Synthesis of MAT_{51}-*b*-($AEMA_{33}$-*s*-AEMA-$fluorescein_1$) (P1-FITC)



P1 (MAT_{51}-*b*-$AEMA_{34}$·34HCl, 12.0 mg, 34 eq) was dissolved in 850 µL of deoxygenated 0.1 M $NaHCO_3$(bubbling N_2 for 30 min). The flask was placed in an ice bath and 20.0 µL of a DMSO solution containing fluorescein isothiocyanate (FITC, 0.18 mg, 1 eq) was added. The reaction mixture was allowed to slowly warm to 23 ^oC. After 12 H, it was dialyzed (3500 Da MWCO) for 8 h (in the dark) against each of the following aqueous solutions: 0.1 M NaCl (4 L x 2), 0.07 M NaCl (4L), 0.03 M NaCl (4L), H_2O (4 x 3). Upon lyophilization, **P1-FITC** (11.1 mg

(91.1%) recovered as a bright yellow powder.The fluorescence quantification of the P1-FITC polymer is shown in Figure S8 and Table S1.

Investigation of the material properties

Polyplex formulation with P1, P2, P3 and siRNA

To a 2 μM solution of siRNA in RNase-free an equal volume of polymer solution at an appropriate concentration was added via autopipette. The solution was mixed well by injecting/ejecting the solution with the pipette several times. The polyplexes were allowed to incubate undisturbed at 23 ^{o}C for 1 h prior to transfection.

Polyplex lyophilization

Polyplexes were prepared in 1-mL microfuge tubes according to the procedure described above using 35μL of a 2 μM siRNA solution and 35 μL of the corresponding polymer solution. Polyplexes were allowed to incubate for 1h at to 23 ^{o}C. Microfuge tubes containing solutions of polyplexes were cooled to -27 $^{\circ}$C for 2 h. Lyophilization was performed by placing the microfuge tubes in a 1L lyophilization jar at to 23 ^{o}C and 10-30 mTorr vacuum for 24 h. To re-dissolve polyplexes, 70 μL of RNase free water was added to lyophilized material and solution was allowed to incubate at to 23 ^{o}C for 1h.

Gel electrophoresis of polyplexes

A 0.6% w/w agarose gel was prepared by dissolving 0.3 g of agarose in 50 mL of TAE buffer while heating. The resulting agarose solution was allowed to cool to 40-45 ^{o}C and 3 μL of ethidium bromide solution was added.

Polyplex solutions (20 μL) were prepared by following the previously described procedure. Loading buffer (2 μL of Blue juice^{TM}) was added to each sample. The polyplex solutions (10 μL) were each loaded into the wells of the gel, and electrophoresis was performed at 60V for 45 min.

Images of gels were obtained using 312 nm UV light to detect ethidium bromide.

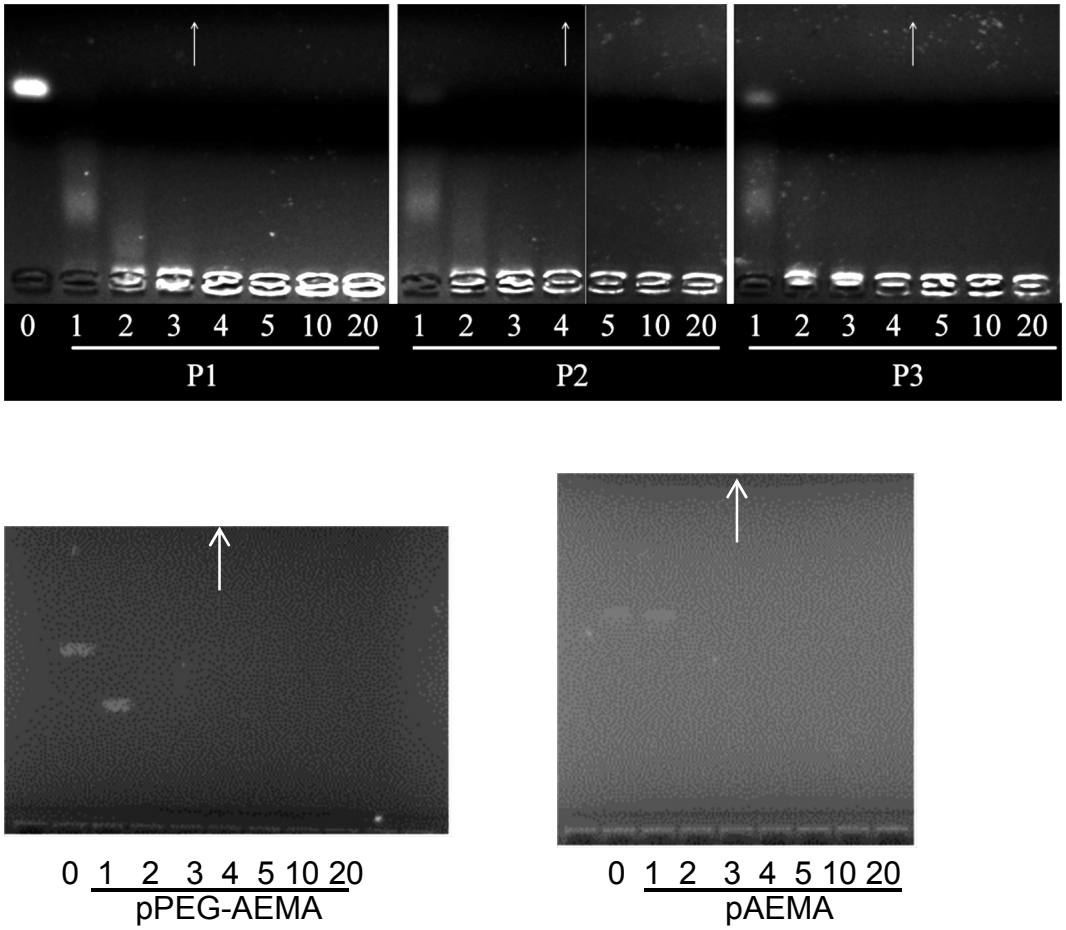
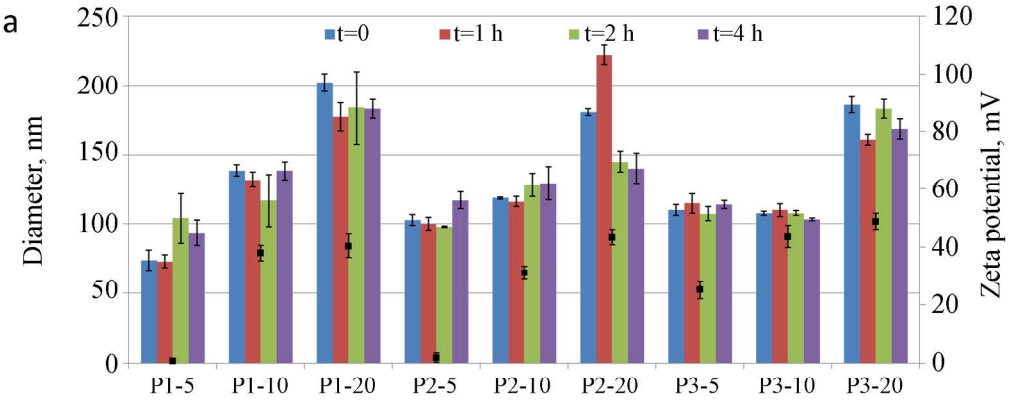


FIGURE S3. Gel electrophoresis assay for P1-P3 and the control polymers. Numbers under each well correspond to polymer amine/siRNA phosphate (N/P) ratio. Arrows point towards anode(+).

Dynamic light scattering measurements

Polyplexes were prepared according to the previously described procedure using 20 μL of a 2 μM siRNA solution and 20 μL of polymer P1, P2 or P3 solution of appropriate concentration to yield polyplexes at N/P ratios of 5, 10 and 20. After 1 h incubation at 23 ^{o}C, polyplex solutions were diluted with water, OptiMEM, or DMEM with 10% serum to a final siRNA concentration of 400 nM. Solutions were transferred into cuvettes and particle sizes were measured at 25 ºC. at various time points All measurements were taken at 633 nm on a Malvern Instruments Zetasizer Nano ZS (Westborough, MA) using a detection angle of 173º.

a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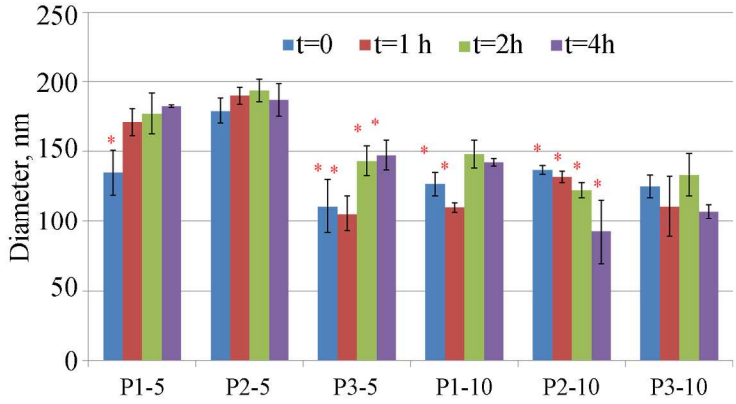
t=2 h
t=4 h
Diameter, nm
Zeta potential, mV
250
200
150
100
50
0
120
100
80
60
40
20
0
P1-5
P1-10
P1-20
P2-5
P2-10
P2-20
P3-5
P3-10
P3-20

b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t=1 h
t=2h
t=4h
Diameter, nm
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200
150
100
50
0
P1-5
P2-5
P3-5
P1-10
P2-10
P3-10

c

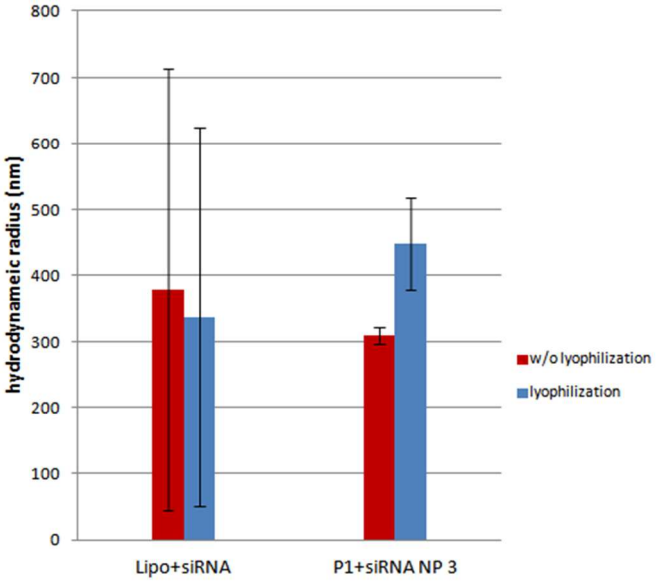

hydrodynameic radius (nm)
800
700
600
500
400
300
200
100
0
Lipo+siRNA
P1+siRNA NP 3
w/o lyophilization
lyophilization

FIGURE S4 Dynamic light scattering study to monitor P1-P3 polyplex stability at various conditions. **a.** Hydrodynamic radii and ζ-potentials of polyplexes in water over the period of 4 h. Labels signify "polymer_name-N/P" **b**. Hydrodynamic radii of polyplexes after lyophilization and resuspension in water. A star (*) denotes cases in which a second population of larger particles was present (500-1000 nm). Labels signify "polymer-N/P". **c.** Dynamic light scattering study to monitor complexation of siRNA with lipofectamine (at the transfection conditions) or P1 polymer at N/P=3 (at the point of P1 binding where little to no free polymer is present) before and after lyophilization in RNase-free water at 25 ^{o}C. Nanoparticle size (hydrodynamic diameter) was measured after complexation for an hour. For the measurement after lyophilization, the size was monitored an hour after being re-dissolved in RNase free water. All the experiments were completed in triplicate.

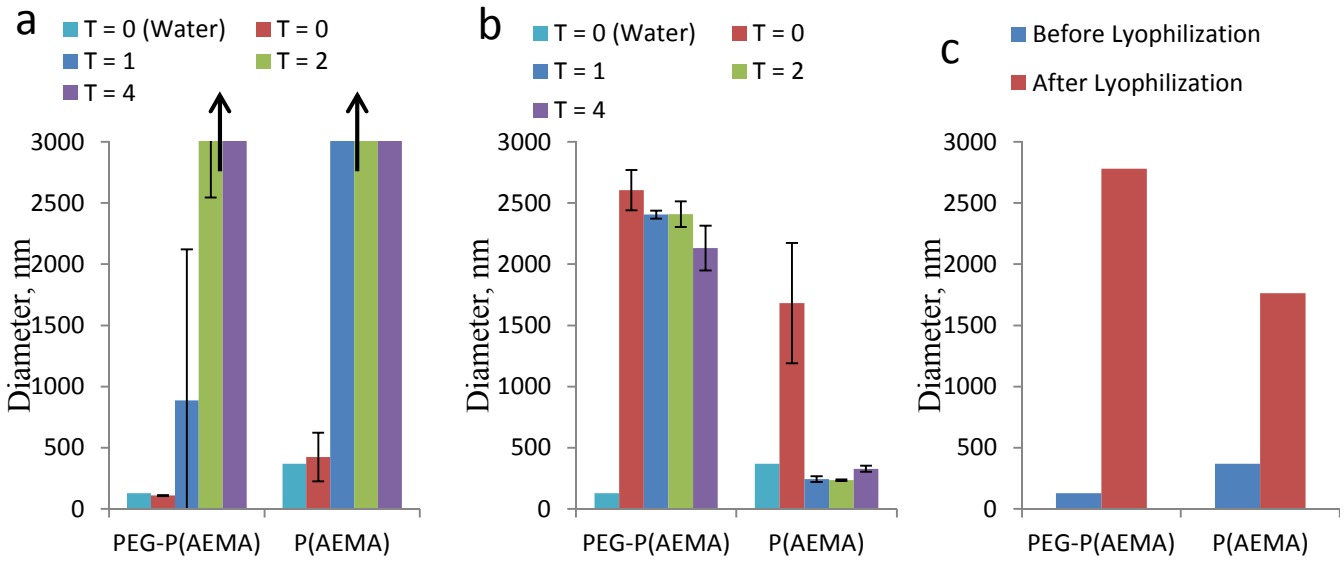


FIGURE S5. Hydrodynamic diameter (nm) of control polyplexes formulated with PEG-P(AEMA) or P(AEMA) N/P 20 in water (light blue bars). After 30 minutes, culture media was added. (a) Aggregation of control polyplexes after exposure to OptiMEM. (b) Aggregation of control polyplexes after exposure to DMEM (containing 10% serum). Experiments for (a) and (b) were analyzed at various timepoints of 0, 1, 2, 4 hours. (c) The control polyplexes were formulated in water (before lyophilization), lyophilized to dryness, and after lyophilization and reconstitution at time = 0. Arrows indicate measurement off of scale.

Evaluation of phase transitions of the polytrehalose and trehalose solutions with differential scanning calorimetry

Aqueous solutions of trehalose (0.58 mol%, 1.30 mol%, 2.21 mol%, 3.39 mol%, and 5.00 mol%) and polytrehalose (0.58 mol%, 1.30 mol%, 2.85 mol%, 3.39 mol%, 5.00 mol%, and 6.19 mol%) were prepared by weight using ultrapure water and trehalose dihydrate (obtained from Fisher Scientific, used as received) and polytrehalose correspondingly. Solutions were incubated for 12 h at 40 ºC in closed containers to ensure the complete dissolution of the materials. Solutions were cooled to 23 ^{o}C and 7-12 mg samples were immediately transferred into aluminum pans and hermetically sealed. An empty aluminum pan with a lid was used as a reference. Heating and cooling was done at 5 ºC/min. Isothermal conditioning was applied for 10 min at both highest temperature (70 ºC) and lowest temperature (-65 ºC). The data was recorded for the second heat/cool/heat cycle and analyzed using TA Instruments Universal Analysis 2000 software, version 4.5A (Waters Corporation, Milford, MA). To determine if the data for trehalose and poly(trehalose) was statistically different, an F-test (ANOVA Two-factor without replication) was performed for the two data sets (at alpha = .05) and found to be statistically significantly different.

Cell culture experiments

Diethylpyrocarbonate (DEPC)-treated water for experiments involving the use of siRNA was obtained from Fisher Scientific (Pittsburgh, PA). Propidium iodide, Lipofectamine™2000, UltraPure^{TM} Agarose-1000, trypsin, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffer saline (PBS), modified essential minimum eagle medium (Opti-MEM®) and Dulbecco's modified eagle medium (DMEM) were purchased from Invitrogen, Inc. (Carlsbad, CA). CellScrub™ Buffer was obtained from Genlantin, Inc. (San Diego, CA). Bovine albumin was purchased from Sigma-Aldrich (St. Louis, MO). The Luciferase Assay Kit and cell lysis buffer were obtained from Promega (Madison, WI). Bio-Rad DC Protein Assay Reagent A, Reagent B and Reagent S were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA). Anti luc2 luciferase siRNA (sense strand sequence 5′-GGACGAGGACGAGCACUUCUU-3′; antisense strand sequence 3′-UUCCUGCUCCUGCUCGUGAAG-5′) and Cy5-labeled anti Luc2 siRNA (sense strand sequence 5′-GGACGAGGACGAGCACUUCUU-Cy5-3′; antisense strand sequence 3′-UUCCUGCUCCUGCUCGUGAAG-5′) was purchased from Integrated DNA Technologies (Coralville, Iowa). Scrambled siRNA was obtained from Dharmacon, Inc (Lafayette, CO).

Luciferase expressing human glioblastoma cells U-87 MG-luc2 (U-87_luc2) were obtained from Caliper LifeSciences, Inc. (Mountain View, CA). Luciferase-expressing glioblastoma cells (U-87_luc2) were used for target gene (luciferase) down-regulation efficiency experiments, cellular uptake studies, and MTT assays for cell viability. The cells were grown in complete DMEM [supplemented with 10% (v:v) fetal bovine serum, 1% antibiotic-antimycotic solution (containing penicillin, streptomycin, and amphotericin B)] at 37 ºC and 5%.

Cellular uptake measurement by flow cytometry

Flow cytometry was performed to examine the cellular uptake of fluorescently-labeled siRNA within various formulations at 3 h post-transfection. In general, luciferase-expressing U-87_luc2 glioblastoma cells were seeded at 300,000/well in 6-well plates 24 h prior to transfection. To transfect, 66 μL of polyplex solutions were prepared following the protocol described above. Each polyplex solution was pipetted into 1584 μL of pre-warmed Opti-MEM to yield the final transfection solution, of which 500 μL was added to each well. After 3 h, the media was removed and cells were washed with 500 μL/well CellScrub™ Buffer for 15 min at 23 ^{o}C to remove any surface-bound polyplexes (and to ensure that the signal was coming only from internalized polyplexes). The CellScrub™ Buffer was then aspirated and cells were exposed to trypsin (0.05% (w/v), 500 μL/well) for 3 min to provide detachment from the plate, then complete DMEM (500 μL/well) was applied to inhibit trypsin. The cell suspension was collected and centrifuged at 1000 rpm for 10 min at 4^{o}C. The supernatant was removed and cells were washed twice with 0.5 mL PBS and centrifuged to remove any remaining the extracellular polyplexes. Finally, 1 mL PBS was added and the suspensions were kept on ice prior to flow cytometry analysis. Propidium iodide (1.0 mg/mL, 2.5 μL) was added prior to the analysis. The flow cytometer (FACSCalibur, Becton Dickinson, San Jose, CA) equipped with a helium-neon laser to excite Cy5 at 633 nm was used to count twenty thousand events for each sample. The threshold fluorescence level was defined by manually adjusting the positive region such that <1% of negative control cells were positive for fluorescence. Each treatment was performed in triplicate.

Luciferase assay and protein assay

Luciferase-expressing glioblastoma cells (U-87_luc2) were seeded at 50,000 cells/well in 24-well plates 24 h prior to transfection. The anti-luciferase (Luc2) siRNA, control (siCon) siRNA,

and polymer stock solutions were diluted with RNase-free water, and polyplexes were prepared following the previously described procedure. The polyplex solution was diluted to a desired siRNA concentration (1 nM, 3 nM, 10 nM, 30 nM, 100 nM) with pre-warmed Opti-MEM or DMEM with 10% FBS to yield the transfection solutions. Cells were washed with PBS before the addition of 200 µl of the transfection solution. The formation of siRNA-containing lipoplexes using Lipofectamine2000 was performed according to the manufacturer's protocol.

The cells were incubated with polyplex/lipoplex solutions for 4 h before complete DMEM was added. After 48 h, the cells were washed with 500 µL PBS and treated with 1x cell lysis buffer for 15 min at 23 ^{o}C. Aliquots (5 µl) of cell lysate were examined on 96-well plates with a luminometer for luciferase activity over 10 s with 100 µL of luciferase substrate added in each well immediately prior to relative light unit (RLU) determination. The average of duplicate fluorescence measurements was utilized for calculation.

The amount of protein (mg) in cell lysates was calculated using a standard curve generated with bovine serum albumin by following the protocol included in Bio-Rad DC protein assay kit. The relative light unit (RLU)/mg protein was then calculated and averaged across replicate wells for comparison. The protein and luciferase levels of non-transfected cells were used for normalizing the data and calculating the extent of gene knockdown. Each treatment was tested in triplicate in 24-well plates.

Assessment of toxicity via MTT assay

MTT reagent (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was used to estimate the cytotoxicty of the formulations. MTT can be reduced to purple formazan in living cells under the catalysis of mitochondrial reductase. Typically, U-87_luc2 glioblastoma cells were seeded at 50,000 cells/well in 24-well plates 24 h prior to transfection. The polyplexes were formed following the procedure described above. Transfection media (200 µL) was added to each well; 4 h later, complete DMEM was added at a concentration of 1 mL/well. Twenty four hours later, the media was aspirated and the cells were washed with PBS (500 µL/well). Serum-containing DMEM (1 mL) with 0.5 mg/mL of MTT was added to each well and the cells were incubated for 1 h. The media was then replaced with 600 µL of DMSO for 15 min at 23 ^{o}C. A 200 µL aliquot of the media was transferred to a well of a 96-well plate for analysis by colorimeter with wavelength at 570 nm.

Samples of non-transfected cells were used for the normalization. All the treatments were performed in triplicate.

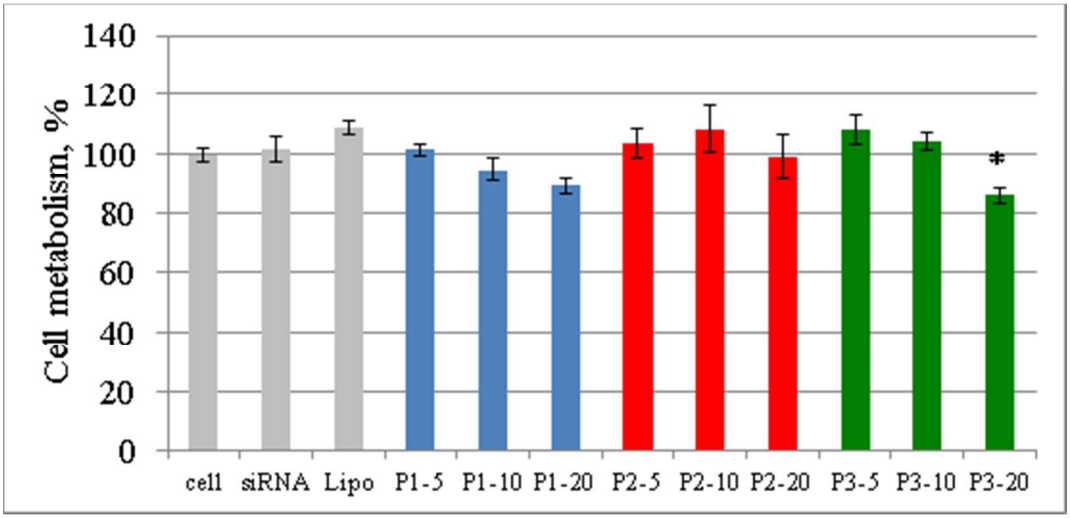


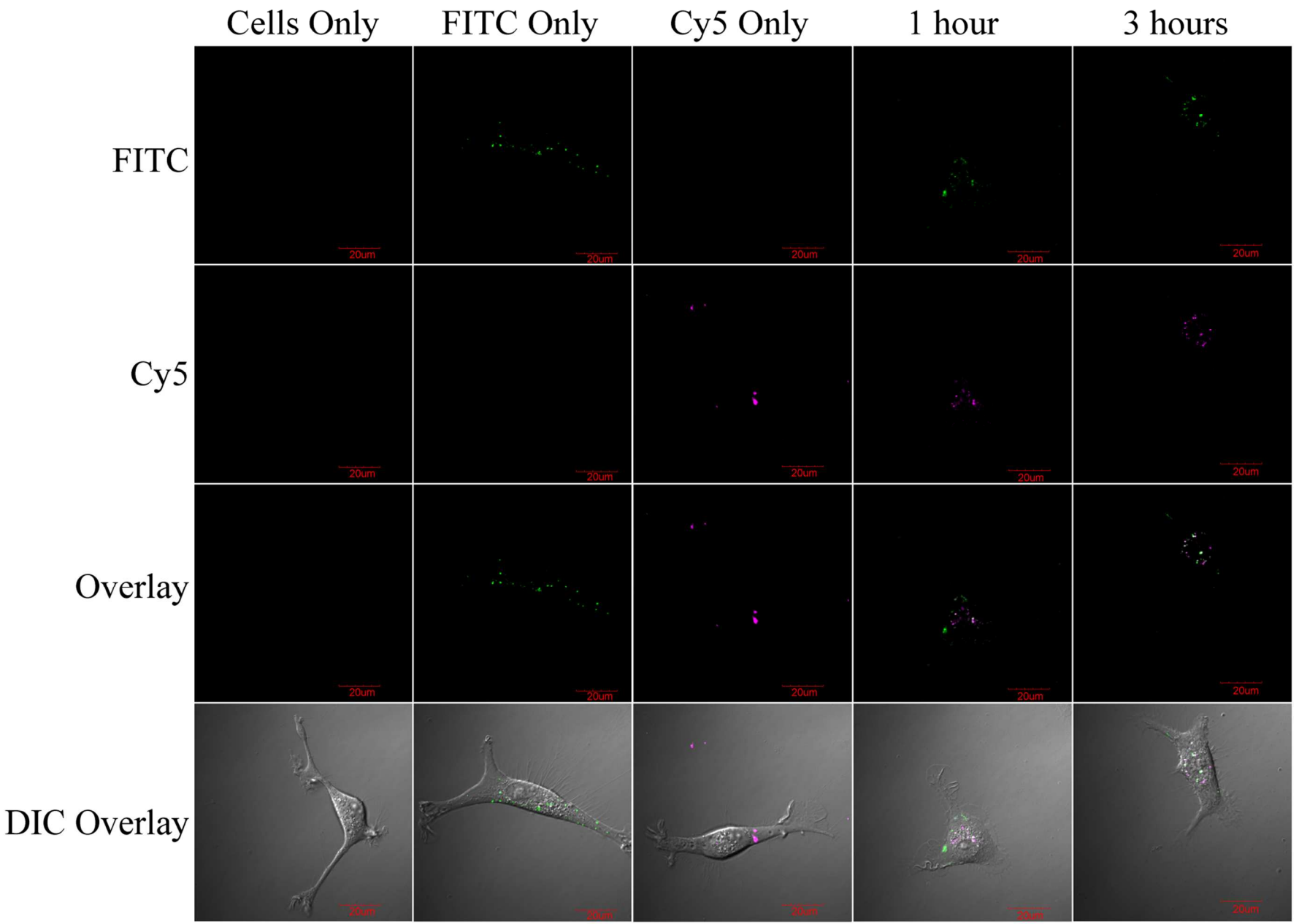
FIGURE S6. Results of MTT assay in U-87 cell line with various polyplex formulations. Bars indicate cellular metabolism (mean ±s.d., n=3). siRNA concentration was 100 nM in all cases. Labels signify "polymer–N/P ratio (e.g. P1-10). Note: '*' indicates statistically significant difference as compared to cell (at alpha level of 0.05).

Live cell confocal microscopy

Live cell imaging experiments were performed at 1, 3, 6, 12, 24, 36 and 48 h post transfection. The cells were plated in 35 mm glass bottom dishes with coverslip number 1.5 (MatTek Corporation, Ashland, MA). The cells were plated at a density of 80000 cells/dish at 24 hours prior to transfection in 4 mL DMEM supplemented with 10 % FBS and 1 % AB/AM and incubated at 37 ^{o}C and 5 % CO_2. Polyplexes were formulated by adding 15 µL of FITC-polymer P1 at 10 N/P ratio to 15 µL of Cy5-siRNA at 2 µM in DEPC treated water and then incubated for 1h at 23 ^{o}C. Prior to transfection, the polyplex solution was diluted by adding 270 µL of OptiMEM® to make a final volume of 300 µL (final siRNA concentration of 100 nM). The cells were transfected by adding 300 µL/dish of diluted polyplex solution. The cells were allowed to transfect for 3 h at 37 ºC and 5 % CO_2. For the timepoints of 1 and 3 h, the cells were washed with PBS and 4 mL of phenol red-free DMEM was added to each dish and imaged directly. For time points 6, 12, 24, 36 and 48 h, the cells were washed with PBS and 4 mL of 10 % FBS DMEM was

added to the dish and further incubated for subsequent time points prior to imaging. In addition, for time points 36 and 48 h, the media was replaced with 4 mL of fresh 10 % FBS-containing DMEM at 24 hours post transfection. The controls were: (1) cells only, where the cells were not transfected, (2) FITC only, where the cells were transfected with polyplexes (where the only the polymer was labeled with FITC), (3) Cy5 only control cells were transfected with polyplexes where only the siRNA was labeled with Cy5.

Olympus FluoView FV1000 inverted confocal microscope was used for imaging. The image size was 1024*1024 pixels (12 bits). The sampling speed was 2.0 μs/pixel. The oil immersion objective used was PLAPON 60X O NA:1.42. The fluorophores used were FITC (fluorescein isothiocyanate) and Cy5 (Cyanine 5). The polymer was labeled with FITC in our laboratory as described above and the Cy5 labeled siRNA was ordered from Integrated DNA Technologies (Coralville, Iowa). The FITC fluorophore was excited by a 488 nm laser and the Cy5 fluorophore was excited by 633 nm laser and all the images were acquired at the same laser settings and analyzed using Olympus FLUOVIEW software.


Cells Only
FITC Only
Cy5 Only
1 hour
3 hours
FITC
Cy5
Overlay
DIC Overlay
20um

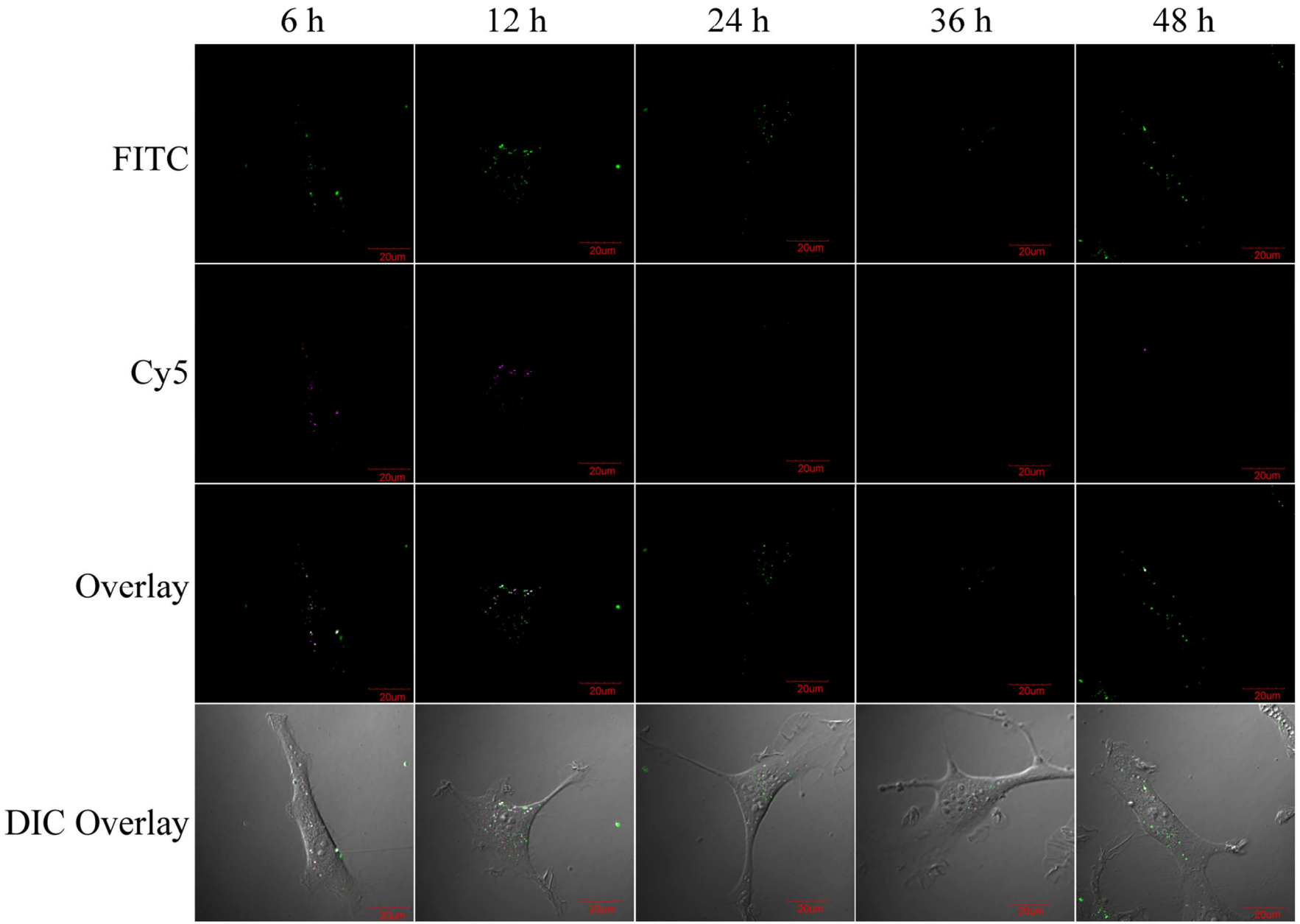

6 h
12 h
24 h
36 h
48 h
FITC
Cy5
Overlay
DIC Overlay
20um

FIGURE S7.| Confocal microscopy images obtained at various time points for cells transfected with polyplexes that were prepared with P1-FITC and Cy-siRNA at N/P ratio 10 and siRNA concentration 100 nM.

Fluorescence quantification of MAT_{51}-*b*-($AEMA_{33}$-*s*-AEMA-fluorescein$_1$) (P1-FITC):

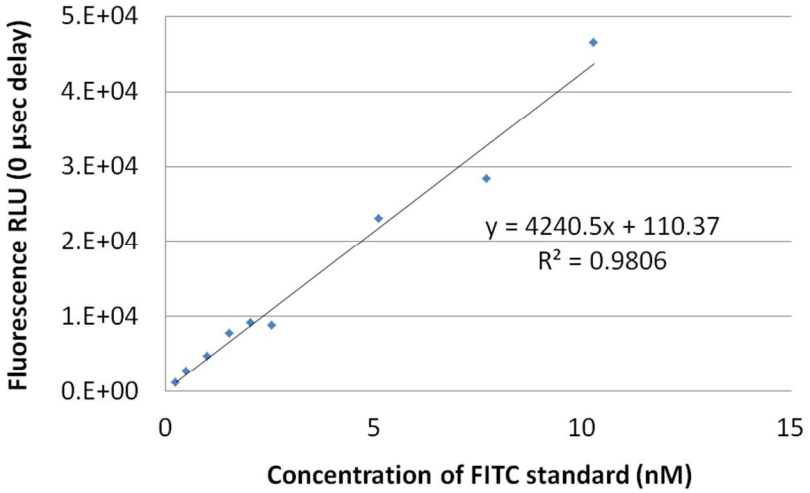


FIGURE S8. Fluorescent measurement of FITC with various concentrations (nM) to quantify the FITC labeling of the P1 polycation (P1-FITC). Using a 485/20 excitation filter in a TR-cassette and a 528/20 emission filter, excitation and emission wavelengths were selected to be 495 nm and 525 nm respectively on a Synergy^{TM} HT (BioTek Instrument, Inc. Winoosky, Vermont).

RLU of sample	Conc. Of FITC (nM)	Conc. Of Sample (ug/mL)	Ratio of loading
84635	16.97	50	0.90%
19302	3.90	10	1.04%
6822	1.41	5	0.75%

TABLE S1. Calculation of molar ratio of FITC labeling to P1 polymer. Three different mass concentrations of FITC labeled P1 polymers were measured for calculation. The molar mass of P1 is 26590 g/mol. Therefore the molar ratio of FITC labeling was found to be 0.90(±0.1)%.

NMR Spectra

^{1}H NMR of 2

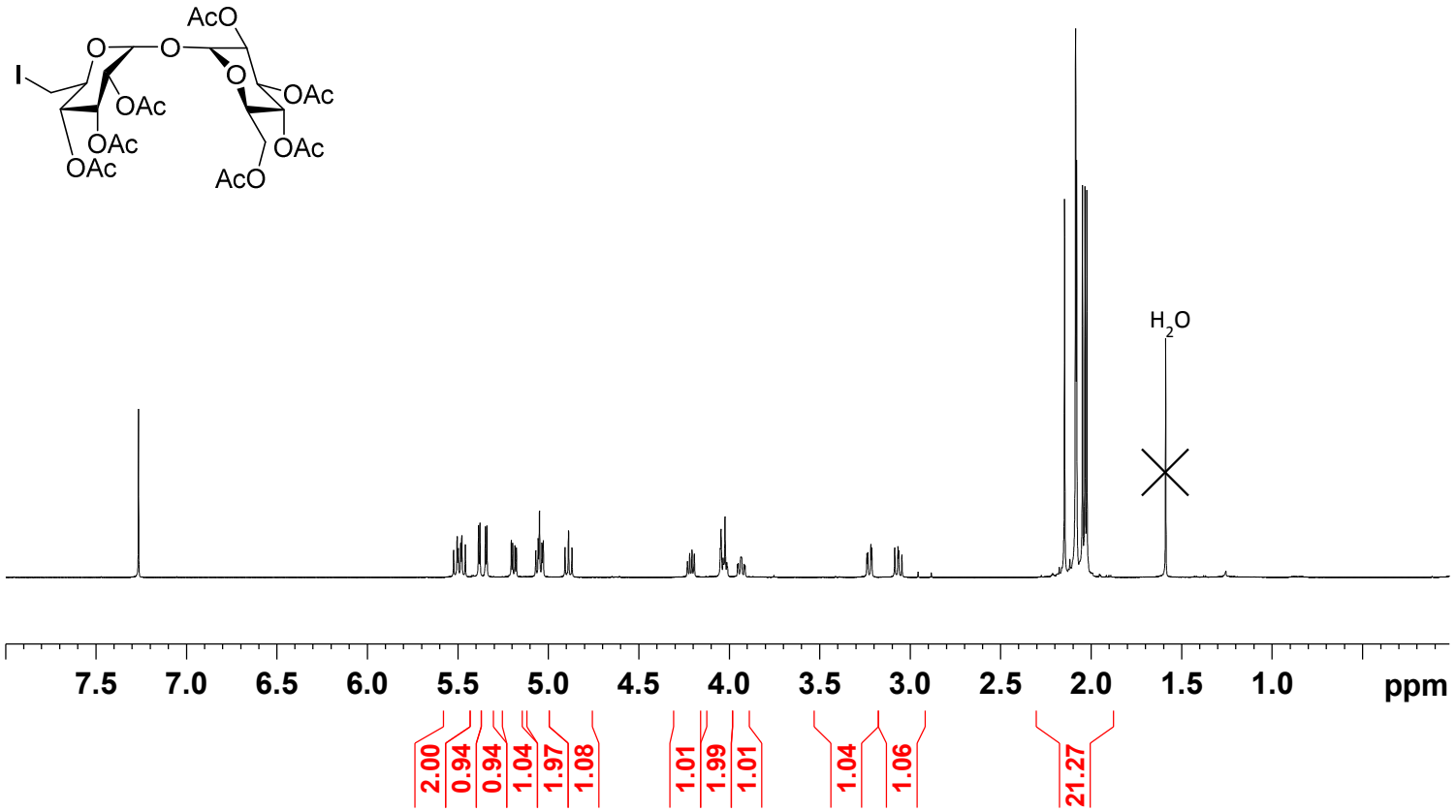


Figure S9. ^{1}H NMR of **2.**

2D COSY of 2

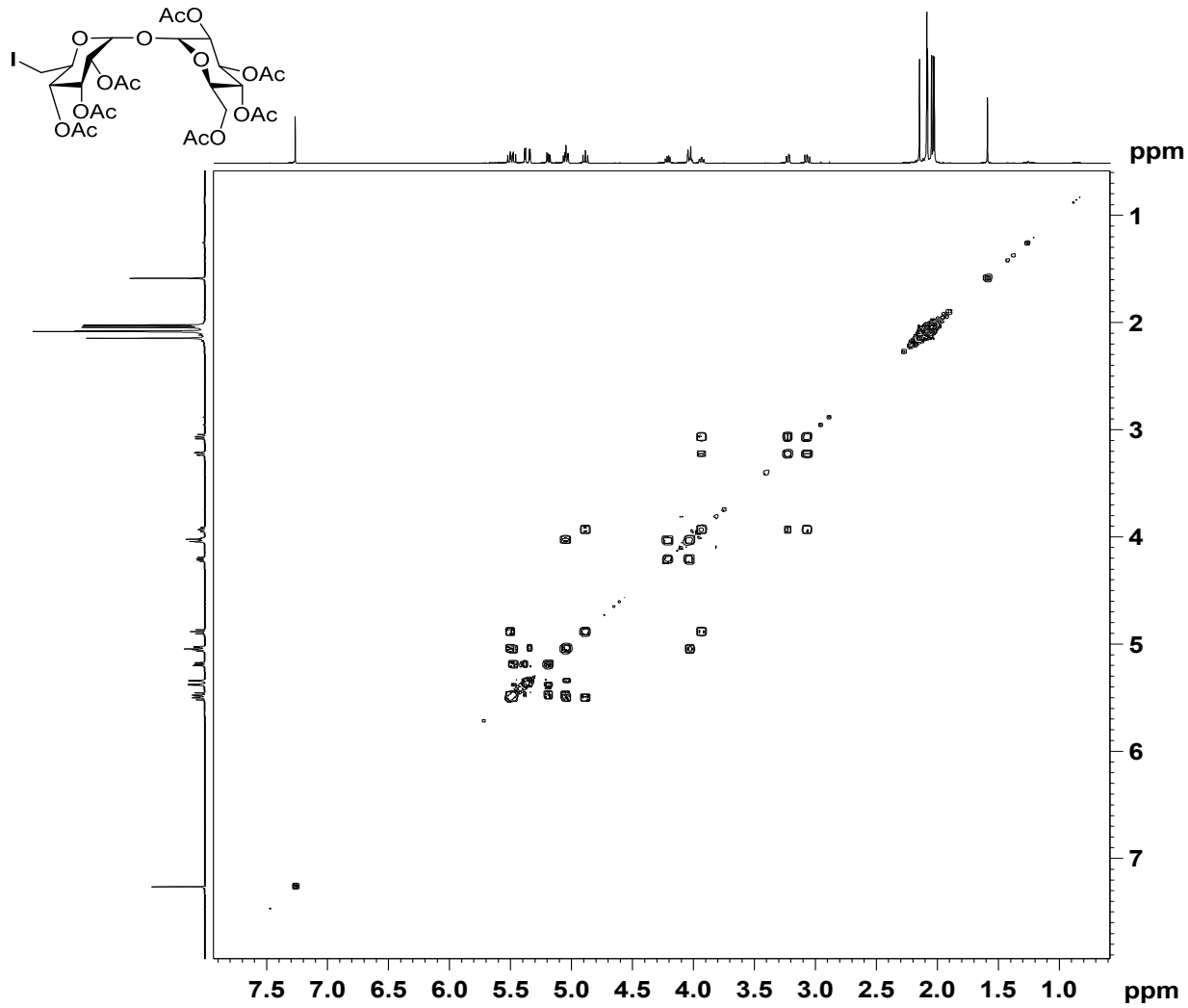


Figure S10. 2D COSY of **2.**

^{1}H NMR of 3

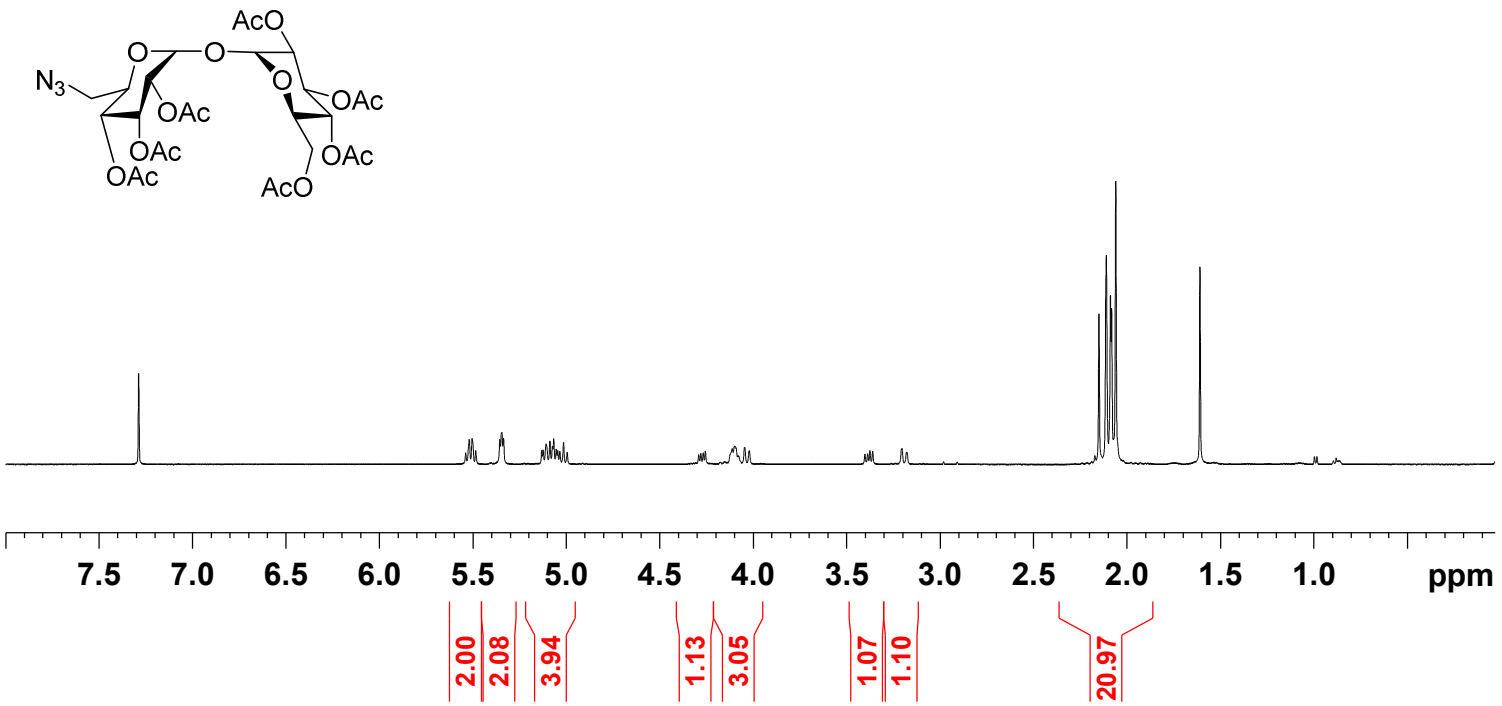


Figure S11. ^{1}H NMR of **3.**

^{1}H NMR of 4

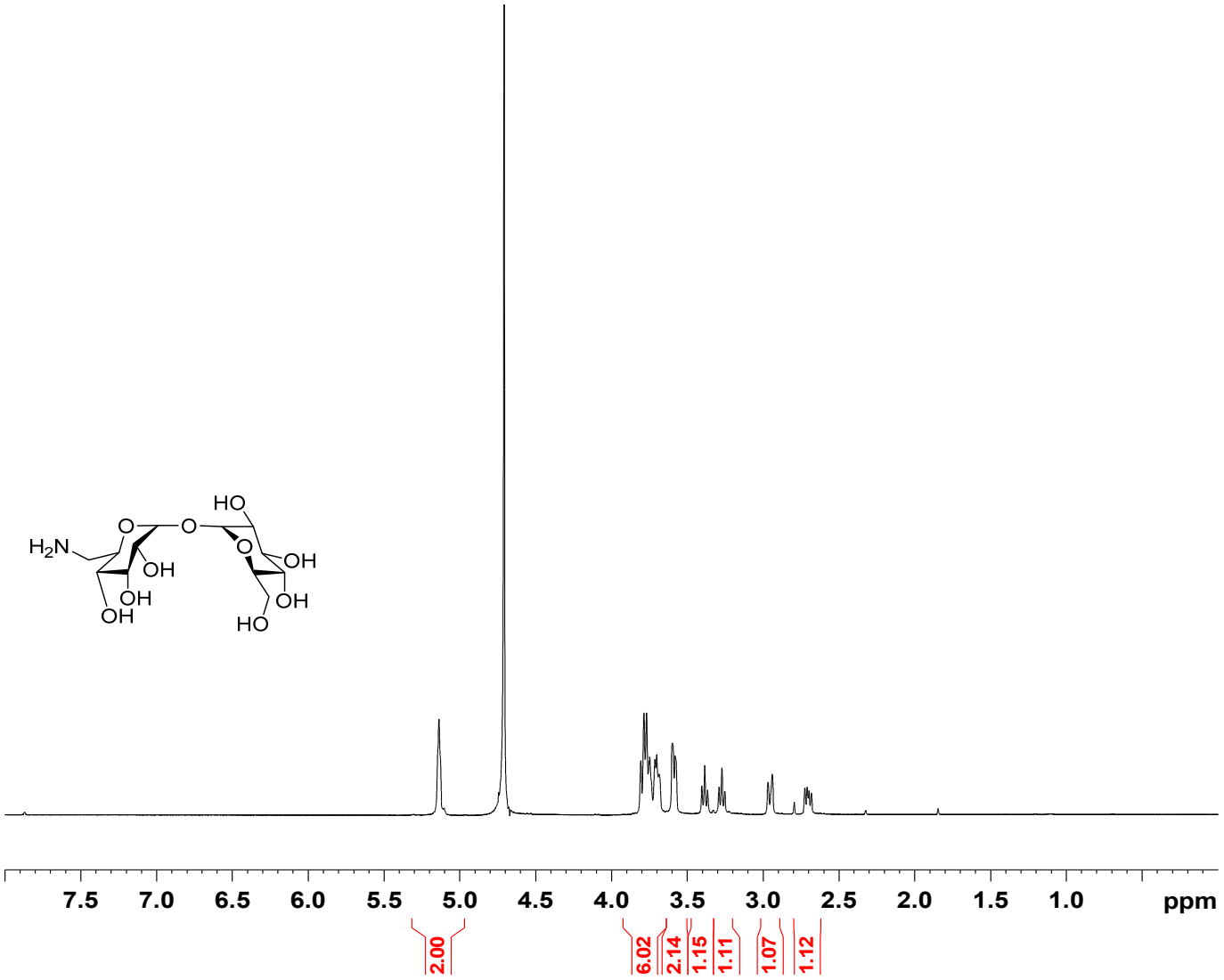


Figure S12. ^{1}H NMR of **4.**

^{1}H NMR of silylated intermediate between 4 and 5

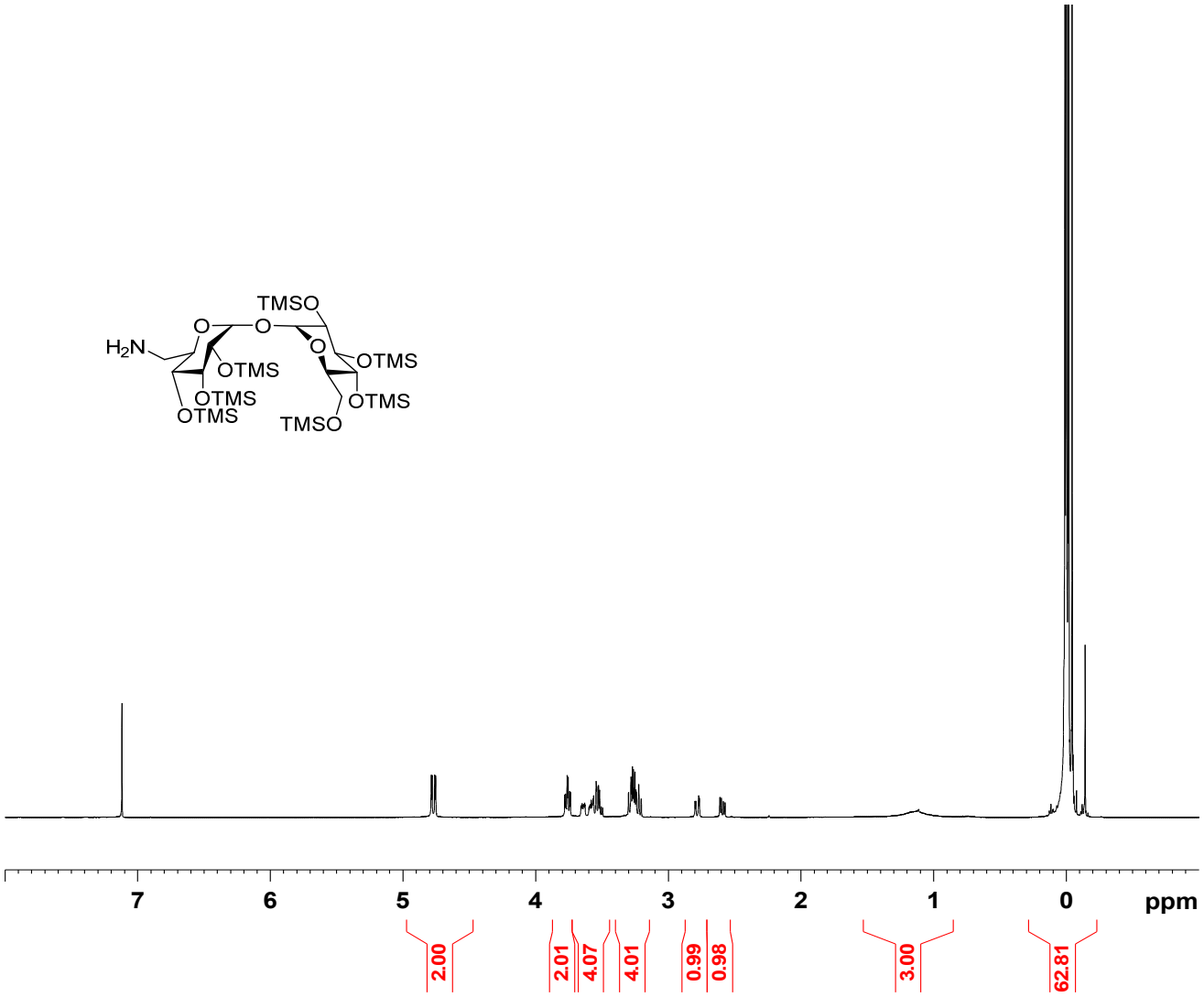


Figure S13. ^{1}H NMR of silylated intermediate between **4** and **5.**

^{1}H NMR of 5

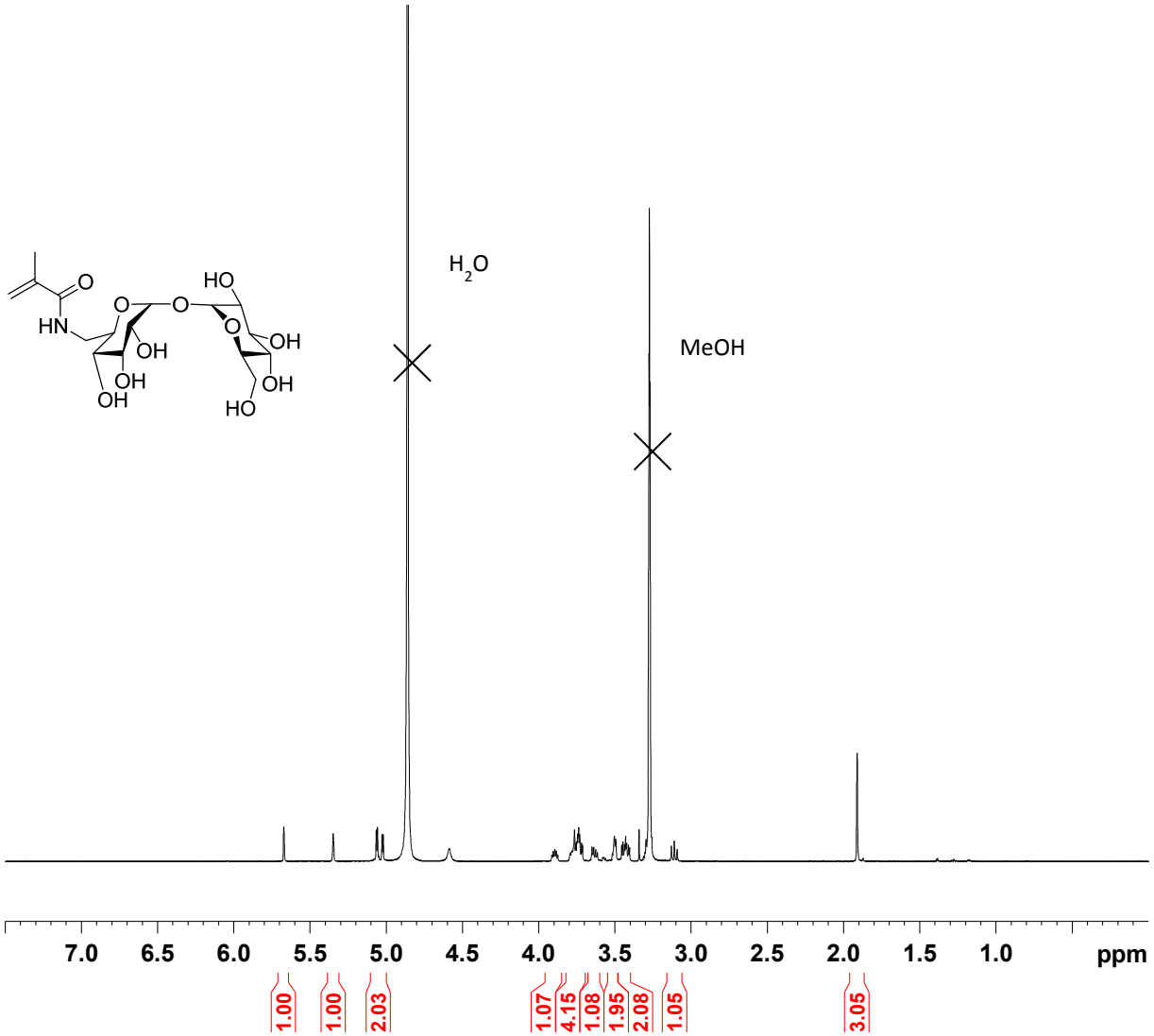


Figure S14. ^{1}H NMR of **5.**

^{1}H NMR of pMAT$_{43}$

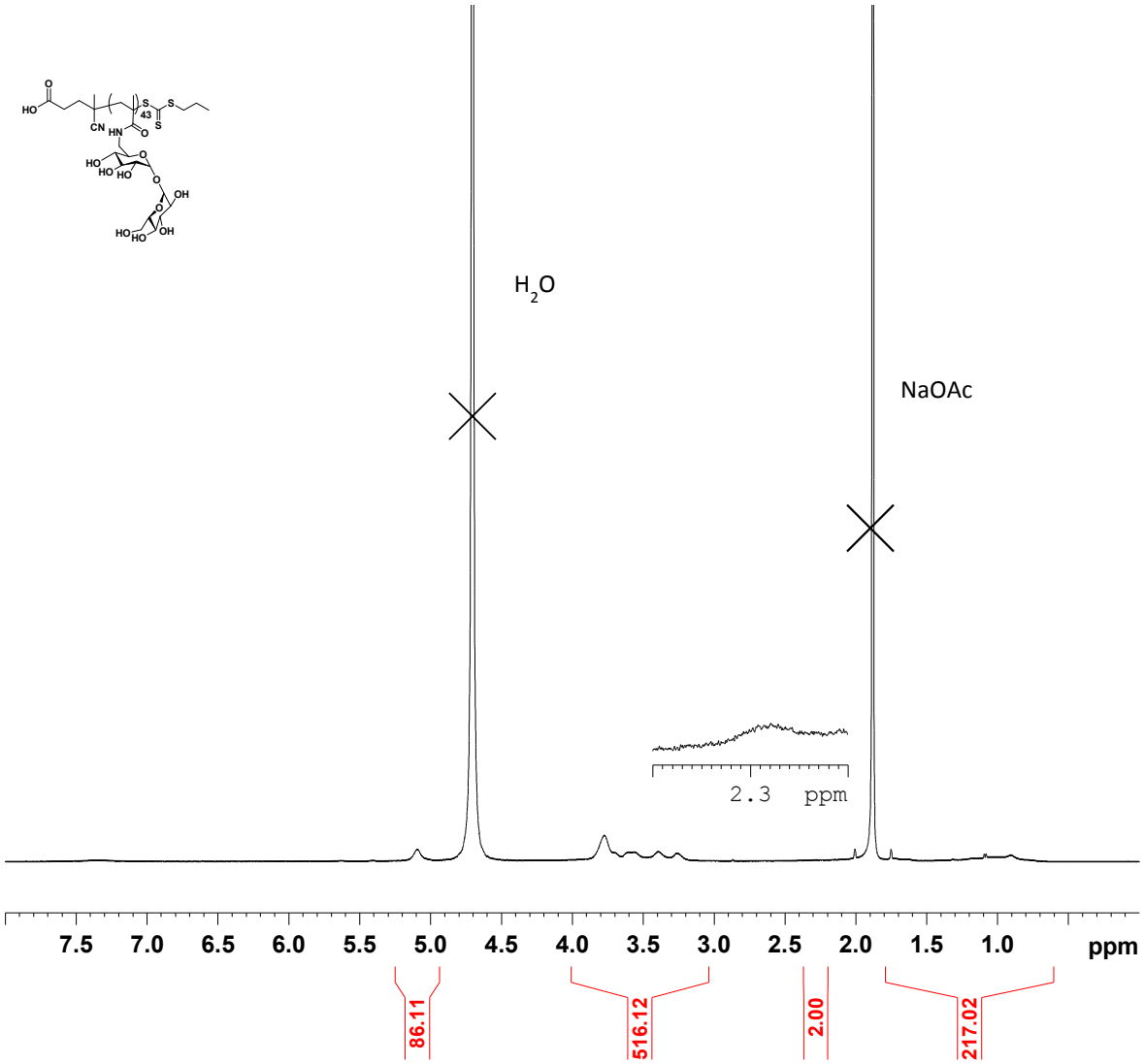


Figure S15. ^{1}H NMR of pMAT$_{43}$

^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{34}$

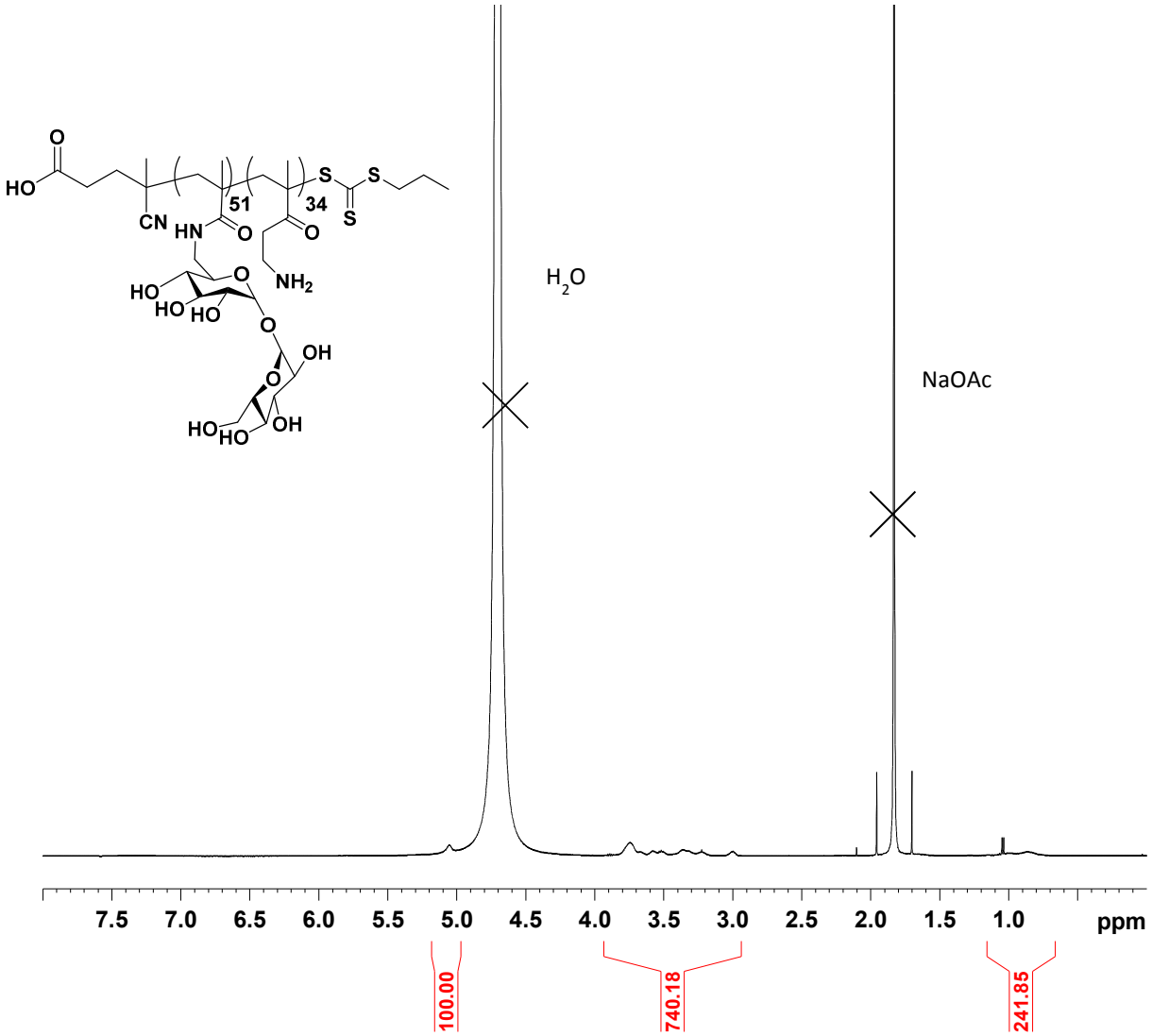


Figure S16. ^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{34}$

^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{65}$

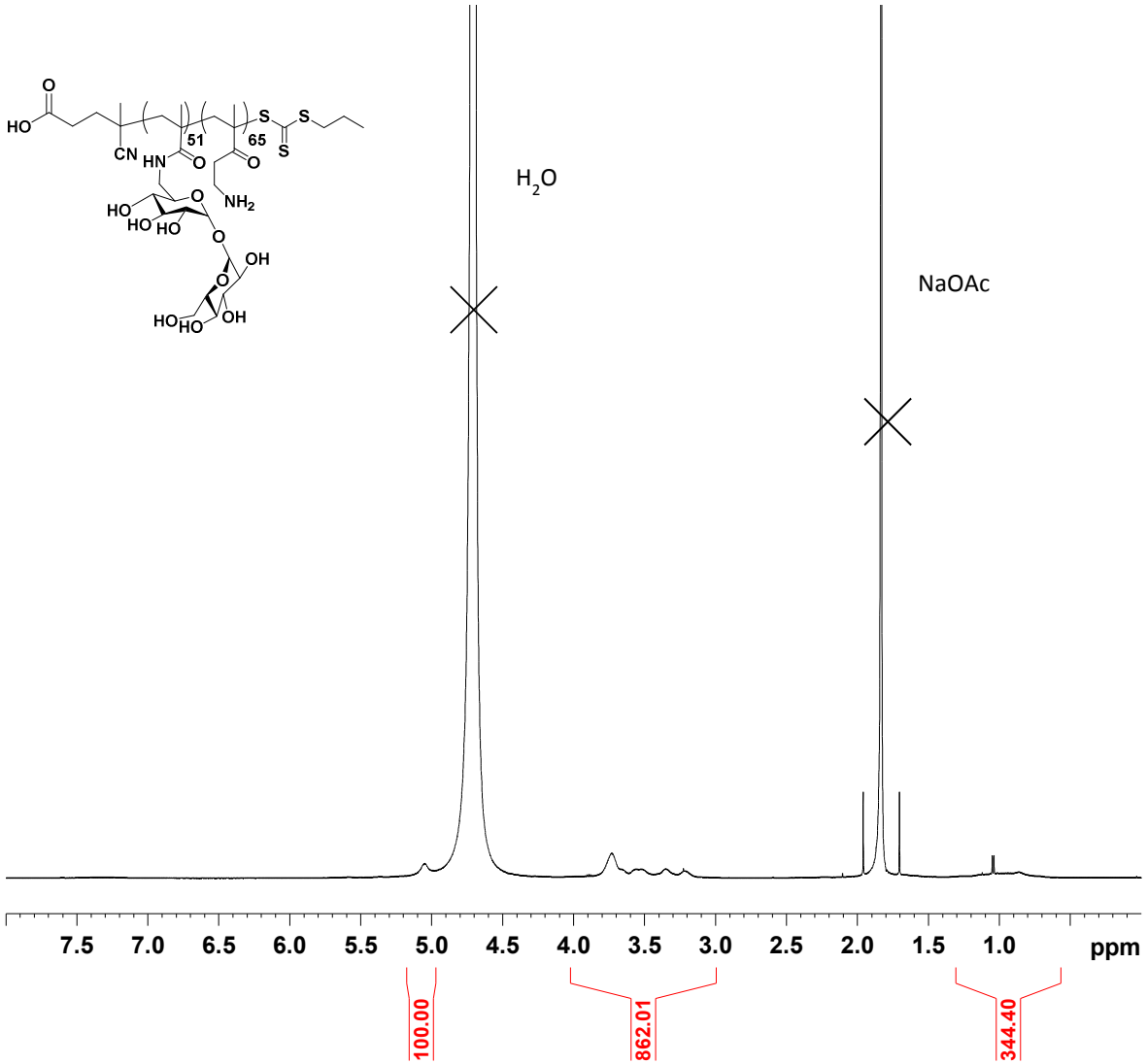


Figure S17. ^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{65}$

^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{84}$

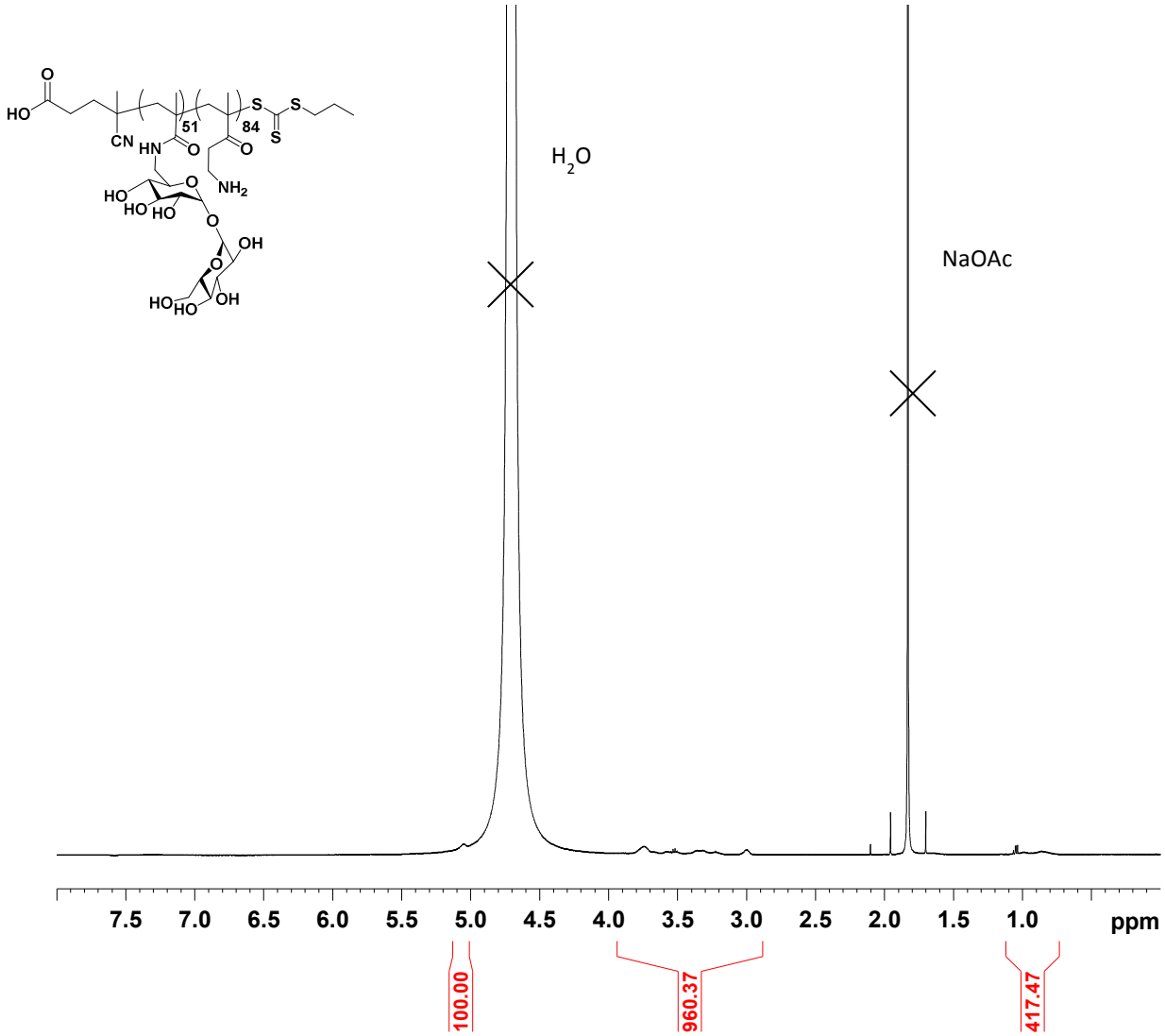


Figure S18. ^{1}H NMR of pMAT$_{51}$-*b*-AEMA$_{84}$

^{1}H NMR of pPEG$_{22}$-AEMA$_{28}$.

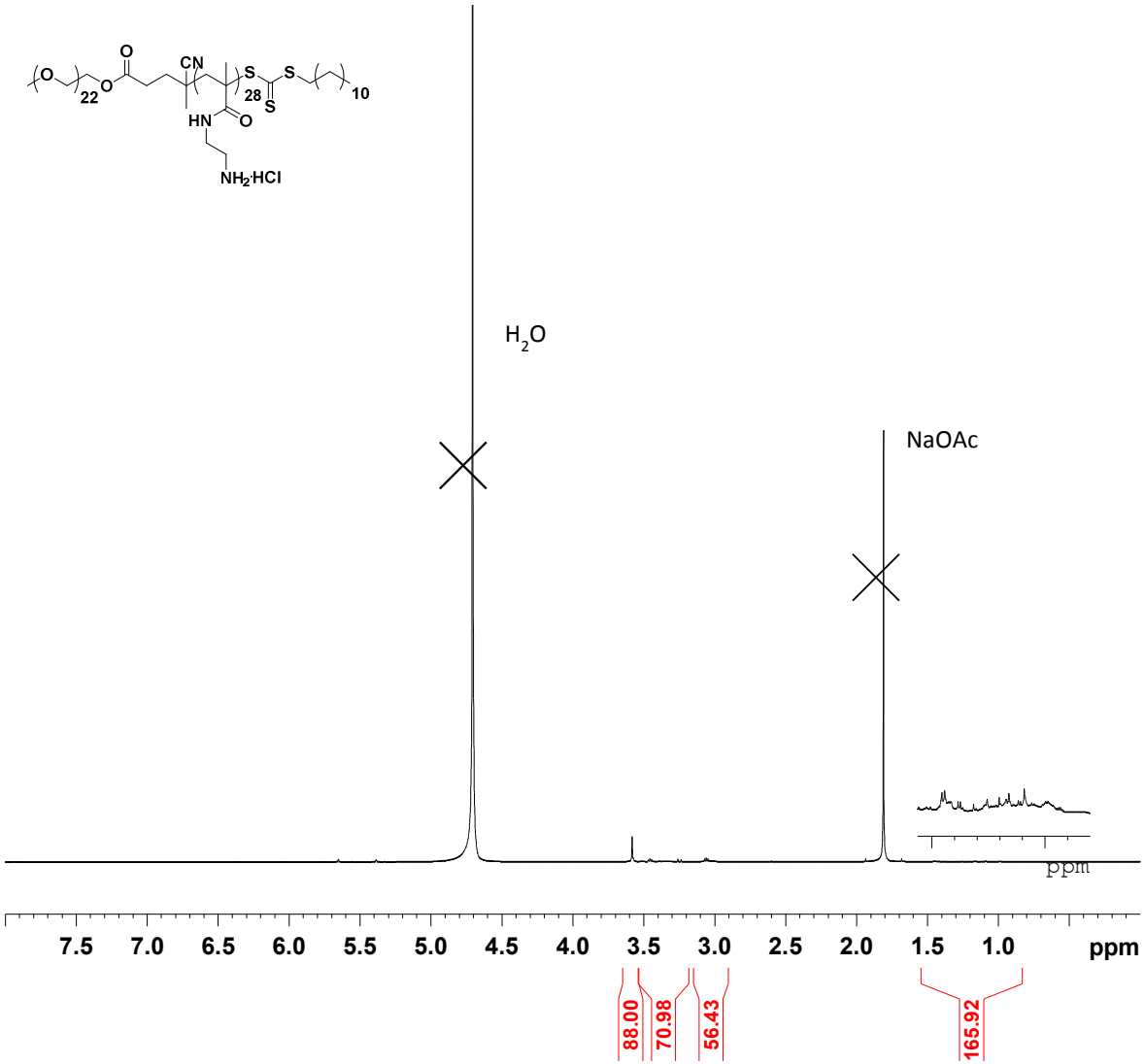


Figure S19. ^{1}H NMR of pPEG$_{22}$-*b*-AEMA$_{28}$.

^{1}H NMR of pAEMA$_{58}$

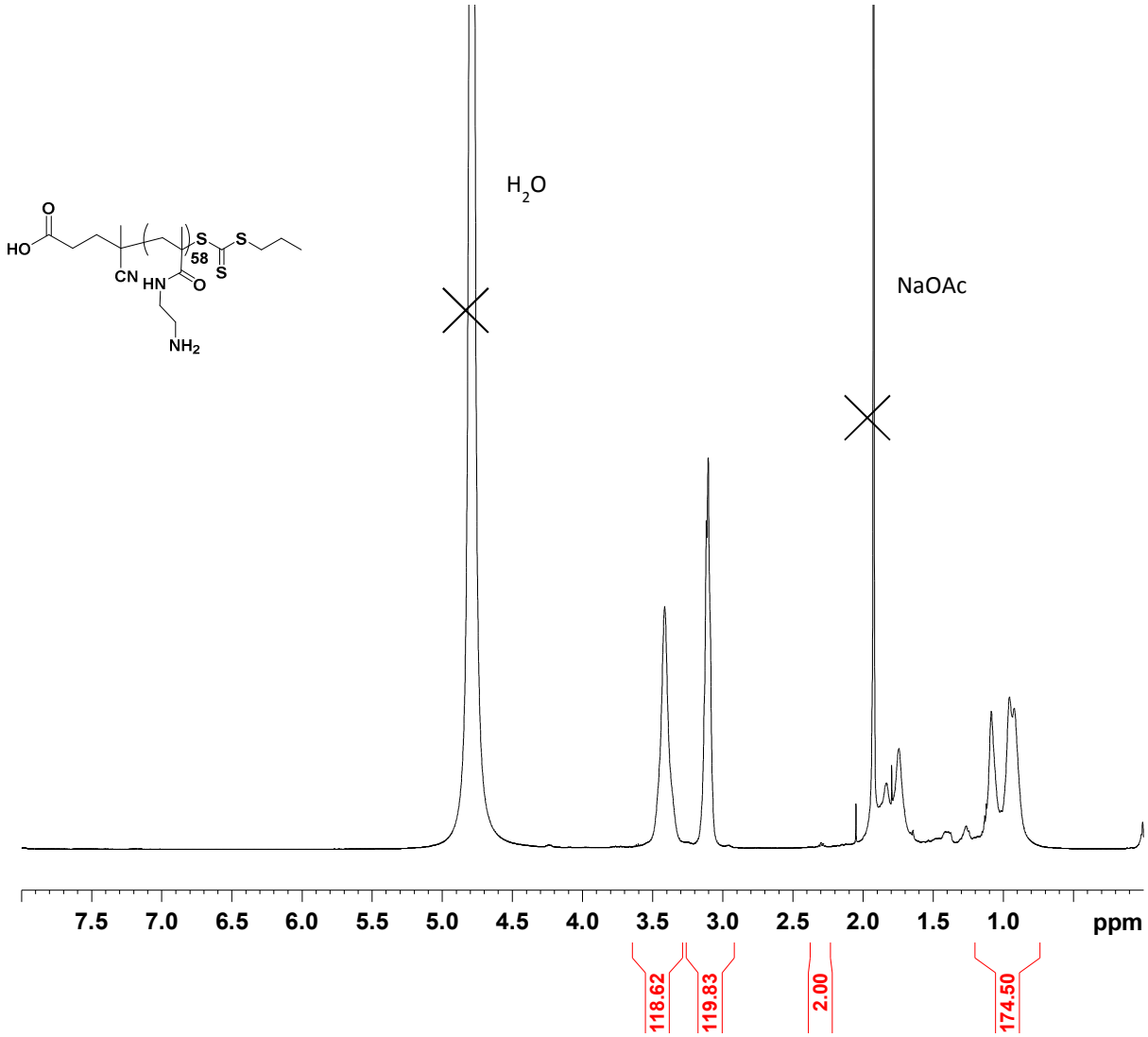


Figure S20. ^{1}H NMR of pAEMA$_{58}$.

Luciferase gene knockdown via anti-luciferase siRNA facilitated lyophilized lipoplexes and polyplexes

P1, PEG-AEMA and P(AEME) at N/P ratio 5, 10 and 20

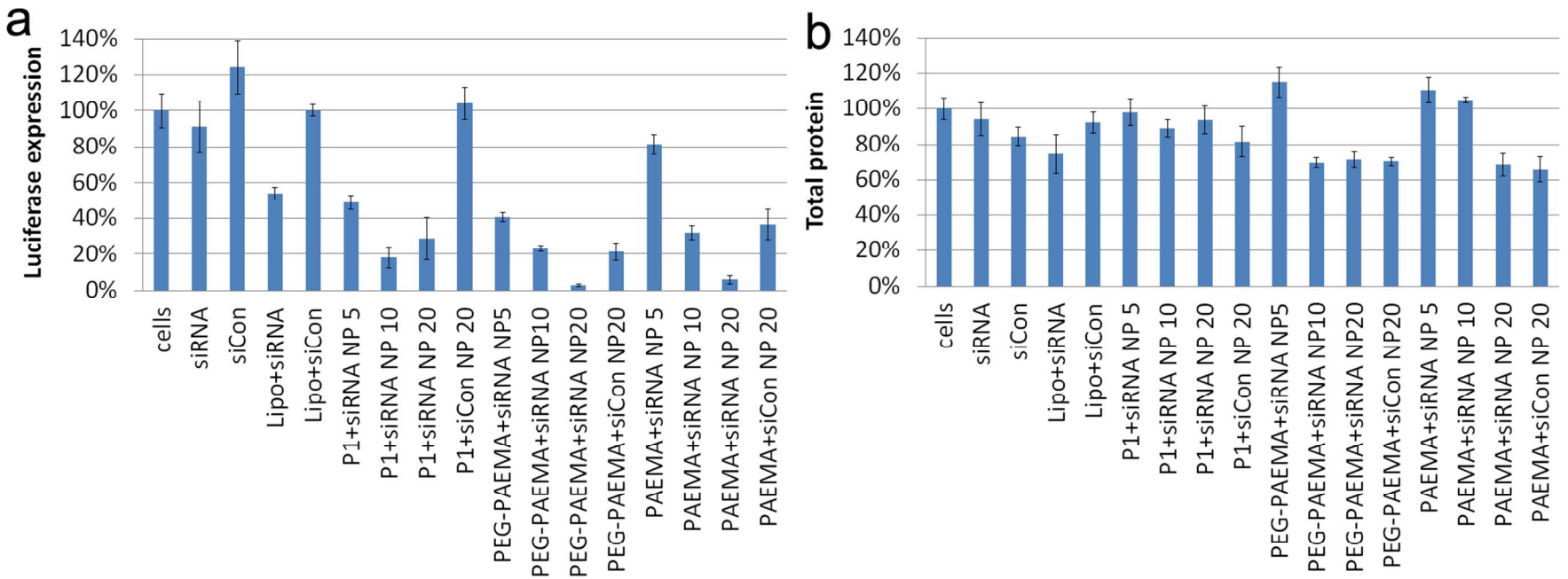


Figure S21. Luciferase gene knockdown via anti-luciferase siRNA facilitated by P1, PEG-P(AEMA) and P(AEME). a. Comparison of target gene down-regulation in luciferase-expressing U-87 cells between freshly-prepared P1 polyplexes, and controls: PEG-P(AEMA) and P(AEMA) polyplexes. b. Protein assay to estimate the cytotoxicity of the polyplexes formed with P1, PEG-P(AEMA) and P(AEMA) at N/P=5, 10 and 20. Transfections were performed at an siRNA concentration of 100 nM in OptiMEM (mean ±s.d., n=3). Labels signify "polymer name N/P ratio", Lipo=Lipofectamine, siCon=siRNA with scrambled sequence (negative control), "cells" = no treatment was applied to cells (negative control), PAEMA=P(AEMA).

P1 at N/P ratio 3

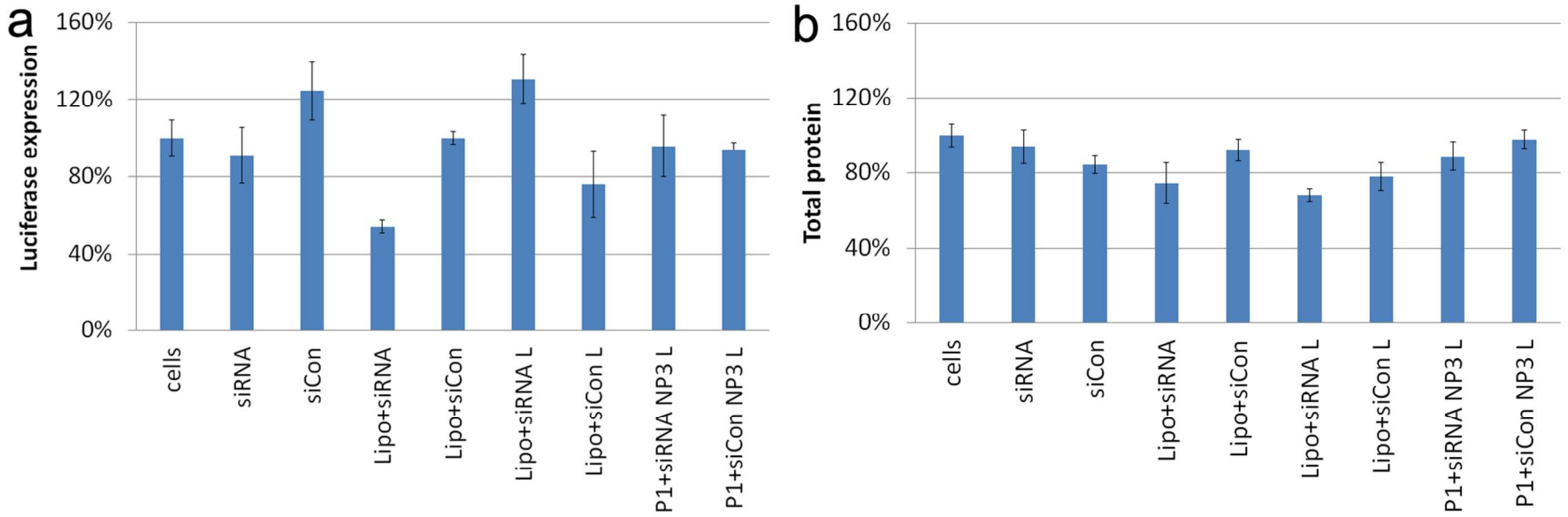


Figure S22. Luciferase gene knockdown via anti-luciferase siRNA facilitated with lyophilized lipoplexes and polyplexes. a. Examination of target gene down-regulation in luciferase-expressing U-87 cells with lyophilized lipoplexes and lyophilized P1 polyplexes at N/P=3. b. Protein assay to estimate the cytotoxicity of lyophilized lipoplexes and P1 polyplexes at N/P=3. Transfections were performed at an siRNA concentration of 100 nM in OptiMEM (mean ±s.d., n=3). Labels signify "polymer name, RNA type, NP ratio" and L=lyophilized, Lipo=Lipofectamine, siCon=siRNA with scrambled sequence (negative control), "cells" = no treatment was applied to cells (negative control).