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One-pot Synthesis of Functional Poly(amino ester sulfide)s and Utility in Delivering pDNA and siRNA

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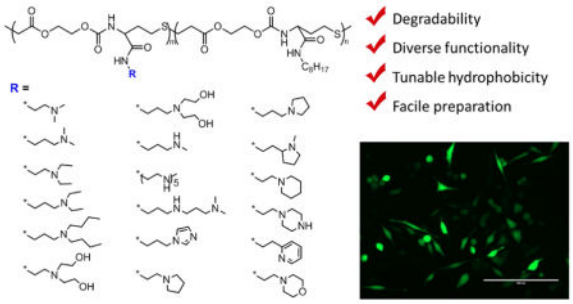
Abstract

The development of efficacious carriers is an important long-standing challenge in gene therapy. In the past few decades, tremendous progress has been made toward non-viral vectors for gene delivery including cationic lipids and polymers. However, there continues to be a need for clinically translatable polymer-based delivery carriers because they offer tunable degradation profiles and functional groups, diverse structures/morphologies, and scalability in preparation. Herein, we developed a library of 144 degradable polymers with varying amine and hydrophobic content via a facile method that involves thiobutyrolactone aminolysis and consequent thiol-(meth)acrylate or acrylamide addition in one-pot. The polymer platform was evaluated for pDNA and siRNA delivery to HeLa cells *in vitro*. Hydrophobically modified 5S, 2E1, 6CY1, 5CY2, and 2M1 grafted HEMATL polymers are capable of delivering pDNA depending on the chemical composition and the size of the polyplexes. Hydrophobically modified 5S and 2B grafted HEMATL and 5S grafted ATL polymers exhibit capability for siRNA delivery that approaches the efficacy of commercially available transfection reagents. Due to tunable functionality and scalable preparation, this synthetic approach may have broad applicability in the design of delivery materials for gene therapy.

Graphical Abstract



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1. Introduction

Gene therapy has shown great potential for the treatment of a wide range of serious acquired and inherited diseases during the past three decades [1–3]. Polymer-based carriers are an essential component of this treatment strategy. Between 1990 and 2013, numerous gene therapy clinical trials commenced worldwide [4, 5], of which > 60% were related to cancer therapy. The first commercial gene therapy product was approved for the treatment of squamous cell carcinoma in China in 2003 [6]. The delivery of pDNA encoding for functional proteins to replace mutated or down-regulated genes remains a promising strategy to treat a variety of diseases [7]. More recently, delivery of shorter nucleic acids that invoke the RNA Interference (RNAi) pathway has been shown to be a powerful way to regulate gene expression post-transcriptionally. In particular, strategies that silence oncogenes using synthetic short interfering RNA (siRNA) or restore endogenous microRNA (miRNA) that function as tumor suppressors, represent next generation therapeutic approaches to treat cancer [8].

One of the practical challenges for both classical gene therapy and RNAi therapeutics is to efficiently deliver DNA or RNA into cells. A number of difficult barriers must be overcome to facilitate effective delivery. These barriers include protection from degradation, localization to the diseased tissue or tumor, cellular uptake into targeted cells, and intracellular release [9–12]. Viral vectors are established carriers for gene delivery because of their high efficiency [13]. However, safety concerns and production costs have restricted their utility. Non-viral vehicles for gene delivery have attracted much attention because of reduced immune response, low cost, and highly tunable diversity in structure [13–15]. Typically, cationic materials are used to bind negatively charged nucleic acids and facilitate cellular uptake. Cationic lipids represent one of the most extensively investigated non-viral vectors [16–18], and are commercially available for use as *in vitro* transfection reagents (e.g. Lipofectamine 2000 and RNAiMax). Cationic lipid nanoparticles have been used in human clinical trials [19]. The other representative non-viral delivery carrier, cationic polymers, have attracted increasing attention because of the flexibility in their synthesis and structural modifications, as well as the relatively higher stability of polyplexes (spherical complex of nucleic acids and cationic polymers) [15, 20, 21].

Some commonly used cationic polymers for non-viral gene delivery, such as poly(ethylene imine) (PEI), can generate high cytotoxicity because polymers with strong positive charges can induce hemolysis, apoptosis, or autophagy [22, 23]. Therefore, more biocompatible and biodegradable cationic polymers have been prepared for gene delivery in the past two decades. A large number of carriers have been synthesized and summarized in various reviews [14, 24–31]. In the context of research reported herein, we were particularly interested in degradable cationic polymers for gene delivery. For example, (oligo)PEI or poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) was grafted to degradable polymer backbones (e.g. poly(carbonate)s and poly(caprolactone)s) to utilize the pH buffering amino groups on PEI and PDMAEMA and increase the biocompatibility of the copolymers [32–34]. Polyesters, including poly(amino ester)s have been extensively studied as degradable polymer carriers for gene delivery [20, 35–45]. Polyamides, e.g. poly(ketal amidoamine) or polypeptides [46–50], polycarbonate-based polymers [25, 51–53],

polyphosphoesters [54, 55], and bio-reducible poly(disulfide)s [33, 56–60] were also reported as gene delivery materials with varying physicochemical properties and distinct preparation procedures. However, classical synthesis of amino functional polyesters, polycarbonates, or polyamides sometimes involves multi-step protection/deprotection and/or ring-closure [25, 61, 62]. It has been shown that the development of combinatorial libraries of polymers is an effective way to discover efficacious nucleic acid carriers [63]. Because stringent polymerization conditions can limit the diversity of functional group incorporation, we aimed to synthesize a clinically-translatable platform with high structural diversity using a one-pot method.

In this report, we synthesized a poly(amino ester sulfide) library of 144 polymers with varying pKa and hydrophobic content using a one-pot method involving the reaction of amines with thiobutyrolactone (meth)acrylates or acrylamides. We were inspired by a recent report on an amine-thiol-ene polymerization strategy by Du Prez and coworkers [64], and decided to use a similar polymerization method to construct a polymer library. In this way, the resulting polymer library possesses a degradable backbone, diverse amine structures (modular apparent pKa), tunable hydrophobicity, and scalable preparation. *In vitro* screening of these polymers for pDNA and siRNA delivery to HeLa cells was performed to identify efficacious carriers. The binding of pDNA/siRNA with hit polymers and the size of the resulting polyplexes were comprehensively investigated in this paper to understand structure-function relationships. Due to tunable functionality and scalable preparation in one pot, this approach is a useful strategy to prepare polymer libraries for nucleic acid delivery.

2. Experimental

2.1 Materials

DL-Homocysteine thiolactone hydrochloride (CTH), acryloyl chloride, methacryloyl chloride, 2-hydroxyethyl methacrylate (HEMA), 4-nitrophenyl chloroformate (p-NPC), dimethylphenylphosphine (DPP) and all amines were purchased from Sigma-Aldrich and used as received. All organic solvents were purchased from Fisher Scientific and purified with a solvent purification system (Innovative Technology). Milli-Q water was used throughout the experiments. siRNA against luciferase (sense strand: 5′-GAUUAUGUCCGGUUAUGUA[dT][dT]-3′; anti-sense strand: 3′-UACAUAACCGGACAUAAUC[dT][dT]-5′), Dulbecco's Modified Eagle Media (DMEM), and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. gWiz pDNA (GFP) was purchased from Aldevron. OptiMEM was purchased from Life Technologies. Lipofectamine 2000 (LF2000) and RNAiMax was purchased from Invitrogen and used following the supplier's recommended protocols.

2.2 Methods

The molecular weight of polymers was measured by Gel Permeation Chromatography (GPC) (Viscotek) equipped with RI detection and ViscoGEL I-series columns (Viscoteck I-MBLMW-3078) using DMF as the eluent at 0.75 mL/min and 45 °C. The instrument was calibrated with a series of 10 narrow polydispersity polystyrene standards (500 to 200,000 g/mol). The structure of monomers was characterized by ^{1}H NMR (Varian, 400 MHz) in

$CDCl_3$. Flash chromatography was performed on a Teledyne Isco CombiFlash Rf-200i chromatography system.

2.3 Synthesis of monomers

N-(2-oxotetrahydrothiophen-3-yl)acrylamide (ATL) was synthesized by the amidation of DL-homocysteine thiolactone hydrochloride with acryloyl chloride in dichloromethane (DCM) using triethylamine (TEA) as base (Scheme 1). 7.759 g (50 mmol) of CTH and 21.01 mL (150 mmol) of TEA were dissolved in 100 mL of DCM and the mixture was placed in a round bottom flask in an ice bath with stirring (600 rpm). 4.188 mL (50 mmol) of acryloyl chloride was added drop-wise into the mixture under N_2 using an addition funnel. After 24 h reaction at r.t, the salt was removed and the filtrate was washed with 50 mL of $NaHCO_3$ aqueous solution (10%) and 50 mL of HCl (0.1 N), dried with magnesium sulfate, and concentrated by rotary evaporation. ATL was purified by flash chromatography with a hexane/ethyl acetate gradient.

To synthesize *2-(((2-oxotetrahydrothiophen-3-yl)carbamoyl)oxy)ethyl methacrylate* (HEMATL), the hydroxyl group in HEMA was activated by reaction with p-NPC, then coupled with the primary amine in CTH. 6.253 mL (50 mmol) of HEMA and 8.405 mL (60 mmol) of TEA were added into 50 mL of dry tetrahydrofuran (THF) at 0 °C, followed by addition of 10.50 grams (50 mmol) of p-NPC (dissolved in 30 mL THF) under N_2. The mixture was kept stirring (600 rpm) at r.t. for 24 h. After removal of salt and THF, the raw intermediate was re-dissolved in 30 mL of DCM and added drop-wise into the mixture of 7.760 gram (50 mmol) of CTH and 15.40 mL (110 mmol) of TEA in 100 mL DCM. The reaction was kept at r.t. for 48 h. The pure HEMATL was isolated by flash chromatography using a hexane/ethyl acetate gradient.

2.4 Polymerization of monomers

To optimize the polymerization, varying temperature, solvents and ratios of monomer and were applied in the preliminary polymerizations. Dimethylphenylphosphine (DPP) was evaluated as a catalyst for thiol-(meth)acrylate Michael addition [65]. The polymer library with varying amines and octylamine percentage was prepared at the optimized conditions. The concentration of monomers was 0.5 M in all polymerizations. As a typical example, 17.1 mg (0.1 mmol) ATL, 1.1 equivalent dimethylamino-1-propylamine (DMP) (11.4 mg, 0.11 mmol) and 0.05 equivalent DPP (0.7 mg, 0.005 mmol) were added to 0.2 mL of DMSO, and the mixture was kept stirring at r.t. for 24 h.

2.5 pDNA delivery with functional poly(amino ester sulfide)s

HeLa cells (ATCC) were seeded into opaque white 96-well plates (10,000 cells/well) and cultured in complete DMEM with 5% FBS (37 °C, 5% CO_2) for *in vitro* pDNA transfection assays. After 24 hours, the media was replaced with fresh, FBS-containing media (200 μL/well). The polyplexes of pDNA and polymers were prepared at a polymer/pDNA ratio of 30:1 (wt/wt) in 10 mM phosphate buffer (pH 6.8) by vigorous pipette mixing in a 96-well plate. After incubation of 30 min at room temperature, 20 μL of polyplexes containing 200 ng of pDNA were added into each well. The cells were incubated with the polyplexes for 24 h, after which the medium was replaced with 100 μL PBS and the GFP fluorescence

intensity was measured on a plate reader (Tecan Infinite M200 Pro). Transfections were performed in triplicate.

2.6 siRNA delivery with functional poly(amino ester sulfide)s

HeLa cells stably expressing luciferase (HeLa-Luc) were derived from HeLa cells (ATCC) by stable transfection of the Firefly Luciferase gene using Lentiviral infection followed by clonal selection. HeLa-Luc cells were seeded into opaque white 96-well plates (10,000 cells/well) and cultured in complete, phenol red free DMEM with 5% FBS (37 °C, 5% CO_2) for *in vitro* siRNA transfection assays. After 24 hours, the media was replaced with fresh, FBS-containing media (200 μL/well). The polyplexes of siRNA and polymers were prepared at a polymer/siRNA ratio of 30:1 (wt/wt) in 10 mM citric acid/trisodium citrate buffer (pH 4.2) by vigorous mixing in a 96-well plate using pipette tips. 20 μL of polyplexes containing 50 ng of siRNA were added into each well. The cells were incubated with the polyplexes for 24 h, after which the cell viability and luciferase activity was analyzed using ONE-Glo + Tox assay kits (Promega). Transfections were performed in triplicate.

2.7 Fluorescence Microscopy

The complexes with selected polymers were added to HeLa cells using the same delivery protocol described above but using clear-bottom black plates. Fluorescence microcopy was performed on an AMG EVOS FL microscope.

2.8 pDNA/siRNA binding with poly(amino ester sulfide)s and polyplex size measurements

Polyplexes were prepared in the same way as that in the delivery assays for consistency. pDNA or siRNA binding was quantified using the Quant-iT RiboGreen Assay kit (Life Technologies). This assay measures free DNA/RNA and can therefore be used to quantify the binding efficiency of polyplexes. The size measurements were performed using dynamic light scattering (DLS, Malvern Zetasizer Nano ZS, He-Ne laser, $\lambda = 632$ nm). The number-weighted diameter (D_h) was the average result of 5 measurements.

3. Results and Discussion

3.1 Synthesis of N-(2-oxotetrahydrothiophen-3-yl)acrylamide (ATL) and 2-(((2-oxotetrahydrothiophen-3-yl)carbamoyl)oxy)ethyl methacrylate (HEMATL)

The one-pot polymerization method involving thiobutyrolactone aminolysis and consequent thiol-(meth)acrylate addition is an attractive route towards functional, degradable polymers [64]. We designed new monomers that contained both (meth)acrylate and thiolactone groups to produce polymers containing ester, urethane, and sulfide linkages in the polymer backbone. In particular, we investigated the effect of changing acrylate, methacrylate, and acrylamide functionalities, and the spacer length between the thiolactone amide and the (meth)acrylate. Monomers containing both (meth)acrylate and thiolactone groups were synthesized via the reaction of CTH and acryloyl chloride or HEMAN (a p-NPC activated derivative of HEMA) (Scheme 2). The structures were confirmed by ^{1}H NMR spectra, as shown in Figure 1. The straightforward and easily scalable synthesis was achieved in 12.4 grams with ~72% yield and confirmed by ^{1}H NMR (Figure 1).

3.2 Preparation of a poly(amino ester sulfide) library

Before the combinatorial synthesis of the functional polymer library, the polymerization conditions were optimized with HEMATL and dimethylamino-1-propylamine (DMP) at varying temperature, solvent, and ratios of monomers and amines (Table S1, Figures S1 and S2). In general, the polymerization improved with increase of solvent polarity because more polar solvents are favorable for Michael addition reactions [65]. The aminolysis of thiolbutyrolactone could be completed in a few minutes [64]. The most polar organic solvent (DMSO) and DPP as a Michael addition catalyst was chosen for preparation of the polymer library. More trials in DMSO (Figure S2) show that polymerization is almost completed in 24 h and higher temperature is unfavorable. The addition of DPP promotes the polymerization of HEMATL and the amine at r.t (Figure S2a). Subsequently, the polymerization of the monomers was performed with 1.1 equivalent amines, 0.05 equivalent DPP, and a monomer concentration of 0.5 M in DMSO at r.t for 24 h. Figure 1 shows the GPC curve of a polymer that was prepared with HEMATL and DMP under these conditions and precipitated in diethyl ether.

3.3 pDNA delivery with poly(amino ester sulfide)s to HeLa cells

A library of 144 polymers was prepared under the optimized polymerization conditions with 0, 10, 30, and 50% octylamine (C8) by mole (Scheme 2). The polymers were diluted with DMSO and used in the preparation of polyplexes for delivery, binding, and DLS measurements to accelerate screening and identification of lead materials.

The successful delivery of pDNA (GFP) to HeLa cells induced the expression of green fluorescent protein leading to the increase of fluorescence intensity. Figure 3 plots fluorescence intensity after transfection with all 144 polyplexes. Nine polymers prepared with HEMATL and various amines produced a 2- to 7-fold increase in fluorescence intensity (insert in Figure 3). The transfection activity of those polyplexes was verified by the fluorescence microscopy (Figure 4). Side-by-side bright field and fluorescence images confirm the expression of GFP, which matches the average, relative expression measured in the plate reader.

To further examine the delivery efficacy, a 48 hour-incubation was applied for H-2E1-0.5C8, H-5S-0.1C8, and H-5S-0.3C8. Figure 5 shows an increase in relative fluorescence intensity and brighter green fluorescence in microscopy images, indicating greater transfection to cells under these conditions. It is worth noting that longer incubation with LF2000-pDNA complexes led to ~2 fold decrease in fluorescence intensity, perhaps due to higher toxicity under these conditions of longer incubation time.

As shown in Figure 3, HEMATL-based polymers modified with C8, combined with dimethylamine, diethylamine, or ethyleneimine exhibited distinct delivery activity for pDNA. To better understand these results, the binding ability of the polymers to pDNA and the size of polyplexes were measured (Figures 6 and 7). All of the active polymers efficiently bound pDNA, likely due to the high polymer to pDNA weight ratio (30:1) and the cationic nature of the active polymer structures enabling efficient electrostatic interactions with the anionic phosphate backbone of the pDNA. Characterization of these

polyplexes by DLS revealed that the hydrodynamic diameter varied greatly from 40 to 1000 nm depending on the polymer composition (Figure 7). For most of the polymer series, the size of polyplexes with pDNA decreases with an increase of octylamine percentage. That is most likely due to the hydrophobic octyl side groups, which results in more compact nanoparticles. Furthermore, the resulting nanoparticles with a smaller size and condensed structure contributes to the successful delivery of pDNA into HeLa cells. Figure 8 shows the size distribution of the most efficacious polyplexes (insert in Figure 3). Most of the polyplexes are smaller than 300 nm. None of the nanoparticles larger than 320 nm transfected pDNA to HeLa cells. Therefore, relatively small size is a necessary but not sufficient parameter for delivery. Several polymers (e.g. some in 5S and 6CY2 polymer series) formed smaller nanoparticles with pDNA but their polyplexes did not show good transfection activities. Polymerization control limitations did not allow us to study the effect of MW carefully in this paper, but a prior study of PBAEs for pDNA delivery indicated that the higher MW chains in a polymer mixture are solely responsible for pDNA delivery, which may also be the case with these polymers [40]. To summarize, all active polymers effectively bound pDNA. No conclusions could be made with respect to binding and pDNA efficacy. Correlating the results of size measurements with activity, increasing hydrophobic content of the polymer yielded smaller, more compact polyplexes that were generally more active in pDNA delivery.

3.4 siRNA delivery with poly(amino ester sulfide)s to HeLa cells

To extend the utilization of this degradable polymer platform, siRNA delivery capability was evaluated. HeLa cells, engineered to stably express a firefly luciferase reporter, were treated with polyplexes containing an siRNA against firefly luciferase (siLuc). As shown in Figure 9, a small number of siLuc polyplexes were capable of silencing luciferase expression at a dose of 50 ng siRNA per well. Focusing on the polymer hits, delivery efficacy was verified by a dose response experiment (Figure 10). Three polymers showed excellent dose response trends that approach (or exceed) the efficacy of RNAiMax at the siRNA doses above 50 ng. However, there was some cytotoxicity at the highest dose, an effect that could possibly be mitigated by decreasing the polymer:siRNA weight ratio. The binding with siRNA and the size of their polyplexes were measured (Figure 11). In comparison with pDNA delivery, the polymer hits do not necessarily show 100% binding with siRNA and the complex size is in a relatively wide range probably because the much shorter siRNA chain leads to less physical entanglement or van der Waals interactions between polymer chains and siRNA. Although most of the polymers were not effective, a few polymers approached the efficacy of RNAiMax. Further optimization of formulation and polymer structure is needed for better siRNA delivery in the future. Taken together, these results demonstrate the utility of polymer library synthesis and screening in the discovery and understanding of effective polyplexes for nucleic acid delivery.

4. Conclusions

We developed a degradable functional polymer library with varying amines and hydrophobic degrees via a one-pot, combinatorial approach. 144 poly(amino ester sulfide)s were synthesized by the one-pot combination of thiolbutyrolactone aminolysis and the

consequent thiol-(meth)acrylate addition at room temperature. Polyplexes were prepared by direct pipette tip mixing and applied to HeLa cells to screen pDNA or siRNA delivery capability of the degradable polymer platform. *In vitro* delivery experiments and fluorescence microscopy show that hydrophobically modified 5S, 2E1, 6CY1, 5CY2, and 2M1 grafted HEMATL polymers are capable of delivering pDNA with 24 hour transfection and the reporter gene expression increases with longer incubation (48 hrs). Binding assays and DLS measurements indicate that the polymer series with similar chemical structure have identical binding efficacy but different polyplex sizes with pDNA which affects delivery. Small polyplexes (typically <300 nm in diameter) are favorable for delivery. But only small size is not sufficient for successful pDNA delivery; certain chemical composition (pKa and hydrophobicity) is also required. In addition, hydrophobically modified 5S and 2B grafted HEMATL and 5S grafted ATL polymers exhibit capability for siRNA delivery that approaches the efficacy of commercially available transfection reagents. It is worth noting that the ATL polymers contain amide linkages that are generally less degradable than ester linkages under physiological conditions. To increase the chemical diversity, ATL was explored and some ATL-based polymers were capable of delivering siRNA to HeLa cells. Due to tunable functionality and scalable preparation, this synthetic approach may have broad applicability in the design of delivery materials for gene therapy. Future exploration will focus on improving polymer structures and formulation procedures with the aim of enhancing gene delivery even further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- We developed a combinatorial library of 144 degradable polymers with varying amine and hydrophobic content via a facile method that involves thiobutyrolactone aminolysis and consequent thiol-(meth)acrylate or acrylamide addition in one-pot.
- Hydrophobically modified 5S, 2E1, 6CY1, 5CY2, and 2M1 grafted HEMATL polymers were capable of delivering pDNA depending on the chemical composition and the size of the polyplexes.
- Hydrophobically modified 5S and 2B grafted HEMATL and 5S grafted ATL polymers exhibited capability for siRNA delivery that approaches the efficacy of commercially available transfection reagents.
- Due to tunable functionality and scalable preparation, this synthetic approach may have broad applicability in the design of delivery materials for gene therapy.

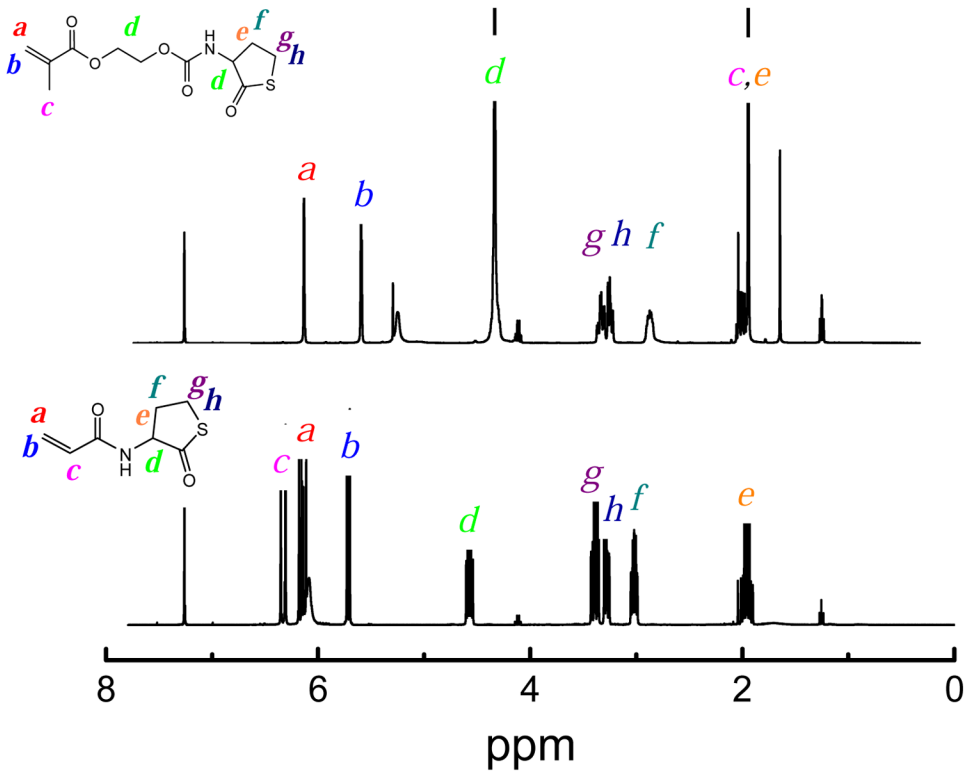


Figure 1.
^{1}H NMR spectra of ATL and HEMATL in $CDCl_3$.

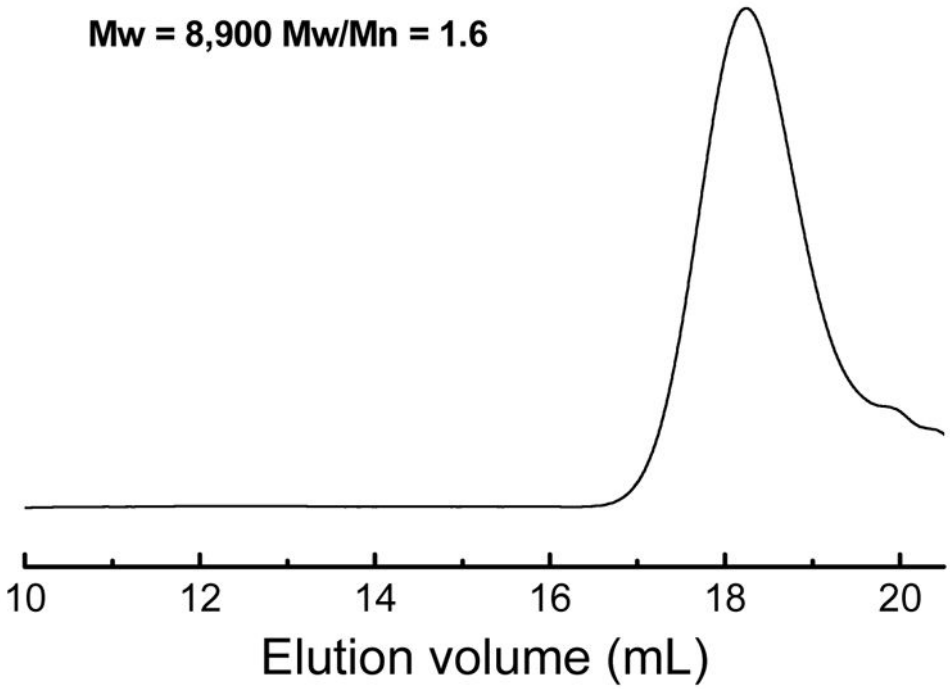


Figure 2.
GPC curve of the HEMATL-DMP polymer after precipitation in diethyl ether.

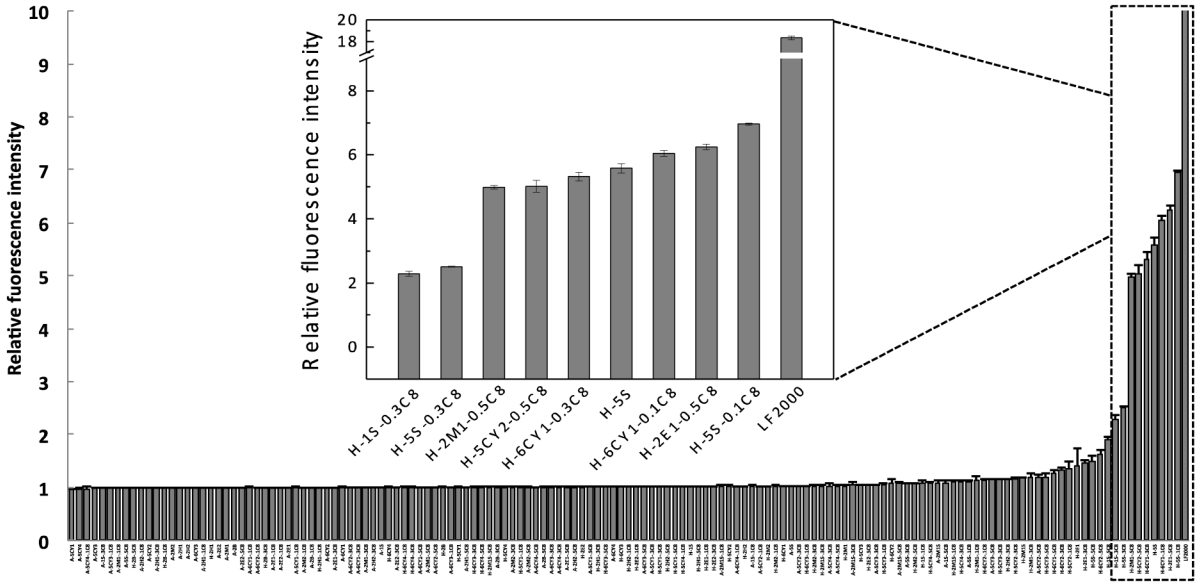


Figure 3.

Relative fluorescence intensity after treatment with pDNA (GFP)-polymer polyplexes for 24 h. LF2000 was used as a positive control for pDNA delivery. The fluorescence intensity was normalized to untreated cells.

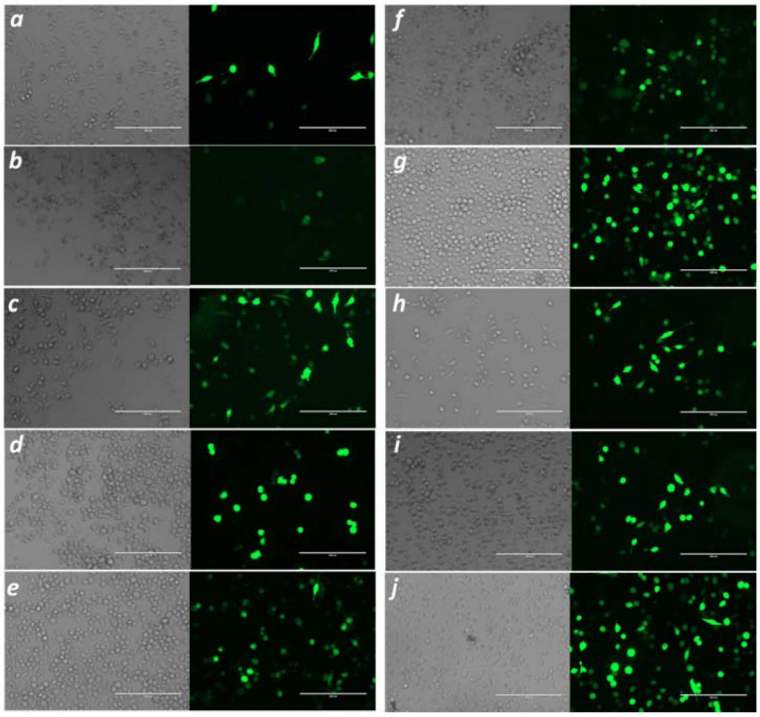


Figure 4.

Fluorescence and bright field images of cells after 24 hour-treatment with complexes of pDNA and H-1S-0.3C8 (**a**), H-5S-0.3C8 (**b**), H-2M1-0.5C8 (**c**), H-5CY2-0.5C8 (**d**), H-6CY1-0.3C8 (**e**), H-5S (**f**), H-6CY1-0.1C8 (**g**), H-2E1-0.5C8 (**h**), H-5S-0.1C8 (**i**), and LF2000 (**j**). The scale bar is 200 μm.

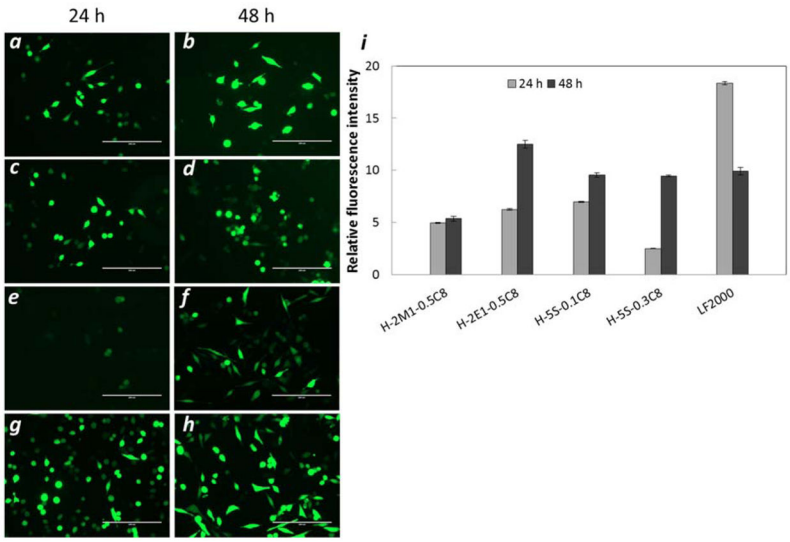


Figure 5.

Fluorescence images of cells after 24 or 48 hour treatment with polyplexes of pDNA and H-2E1-0.5C8 (**a, b**), H-5S-0.1C8 (**c, d**), H-5S-0.3C8 (**e, f**), and LF2000 (**g, h**), and relative fluorescence intensity normalized to untreated cells (**i**). The scale bar is 200 μm.

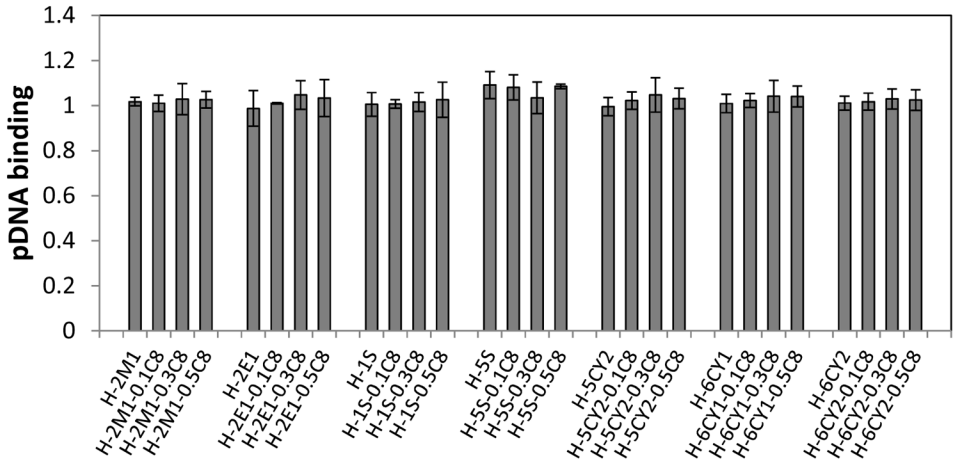


Figure 6.

pDNA binding with polymer series prepared with HEMATL and amines 2M1, 2E1, 1S, 5S, 5CY2, 6CY1, and 6CY2. The polyplexes were prepared under the same condition as in delivery assays. The binding fraction is shown, indicating no free pDNA at a polymer/pDNA ratio of 30:1 (wt/wt).

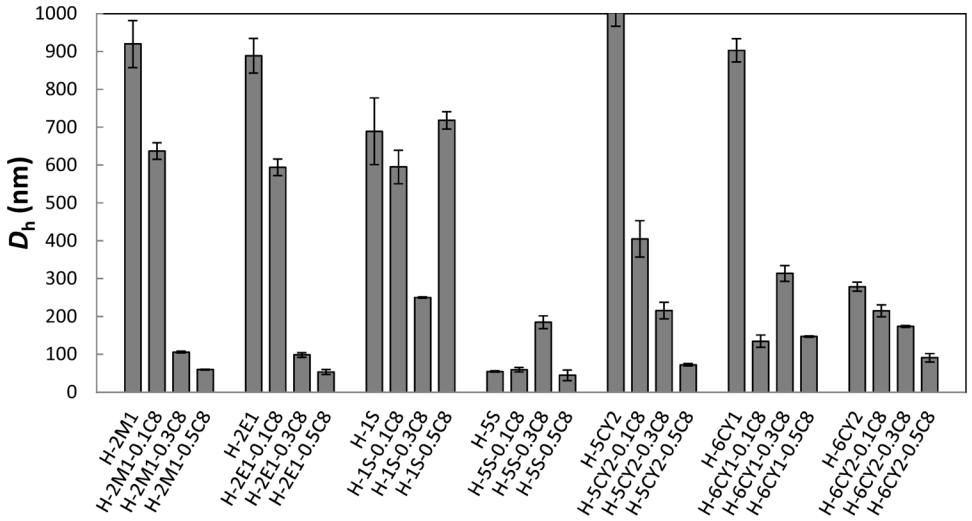


Figure 7.

The hydrodynamic diameters (D_h, number weighted) of pDNA polyplexes from Figure 6.

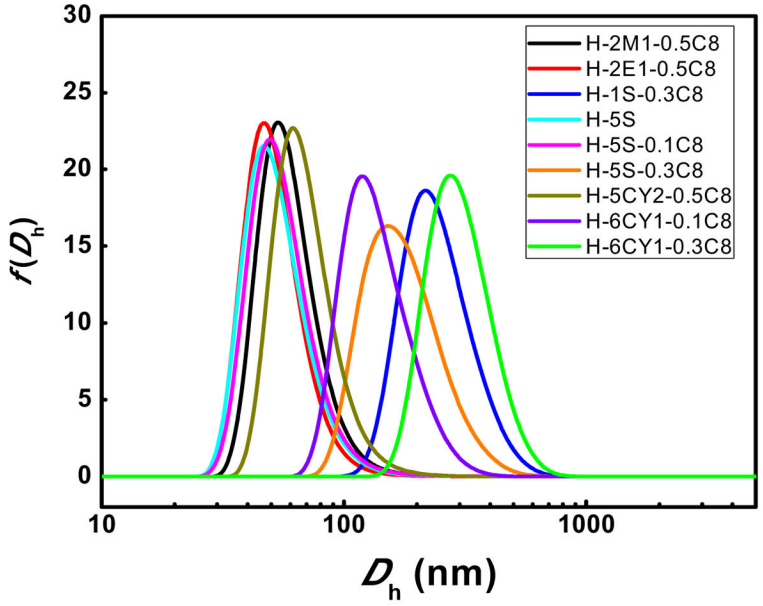


Figure 8.
D_h distribution of the most efficacious pDNA polyplexes.

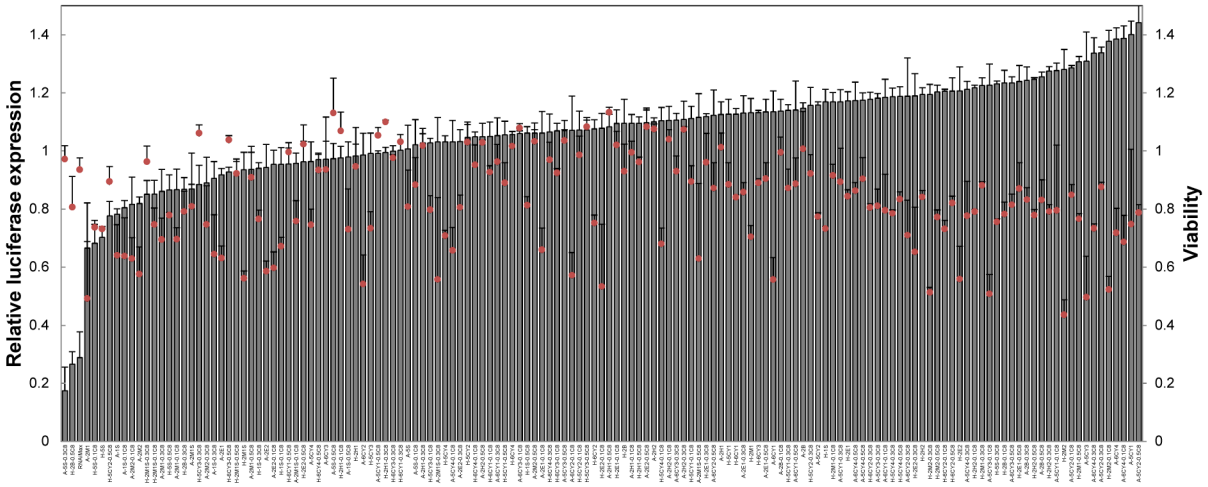


Figure 9.

in vitro screening of the polymer library for luciferase siRNA delivery to HeLa cells. Gray bars and red circles indicate relative luciferase activity and cell viability, respectively.

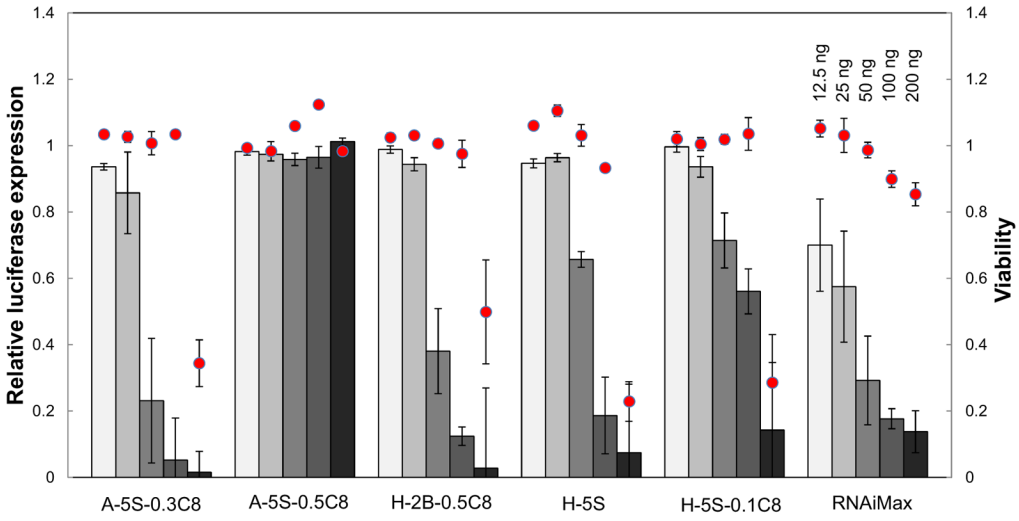


Figure 10.

siRNA dose response curve for three active and one inactive polymer. RNAiMax was used as the positive control to benchmark the efficacy. The same polyplexes were prepared as those in Figure 9 and different amount was added to the cells depending on the desired dosage. The dose range varied from 12.5 to 200 ng (4.55 to 53.3 nM) per well. Red dots denote the cell viability and grey bars indicate relative luciferase expression compared to untreated cells.

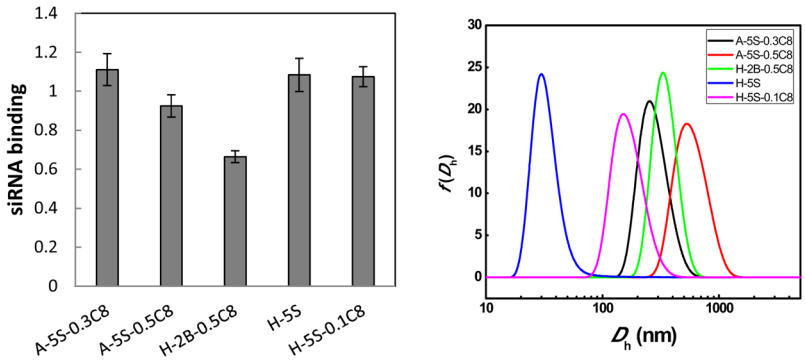
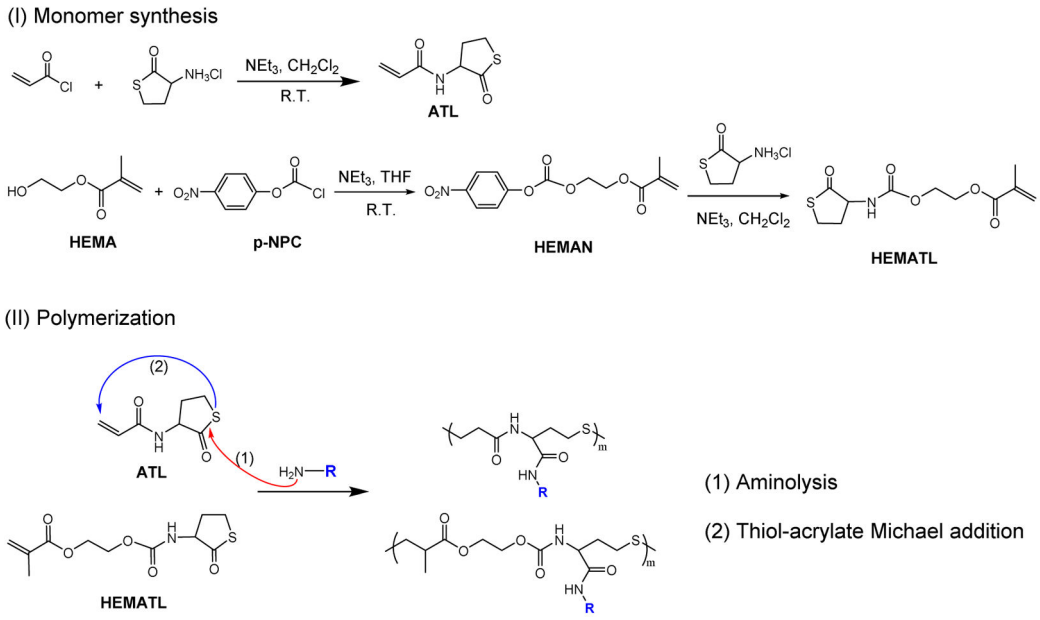
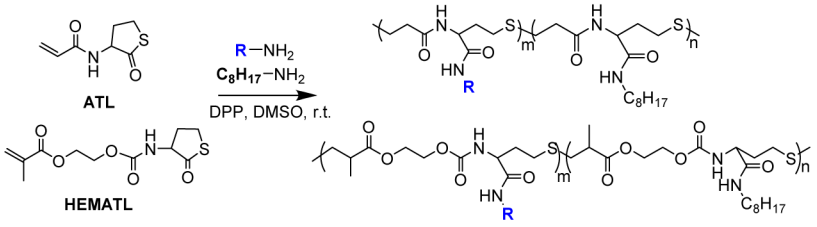


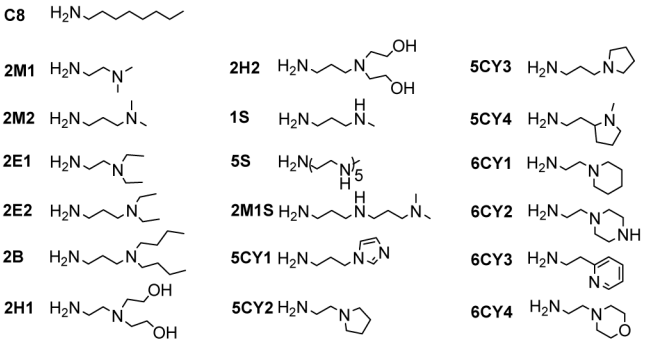
Figure 11.
siRNA binding and the sizes of the siRNA polyplexes.



Scheme 1.

Synthesis of monomers ATL and HEMATL and preparation of functional poly(amino ester sulfide)s via combination of thiolbutyrolactone aminolysis and thiol-(meth)acrylate addition.





	Polymers with ATL				Polymers with HEMATL			
2M1	A-2M1	A-2M1-0.1C8	A-2M1-0.3C8	A-2M1-0.5C8	H-2M1	H-2M1-0.1C8	H-2M1-0.3C8	H-2M1-0.5C8
2M2	A-2M2	A-2M2-0.1C8	A-2M2-0.3C8	A-2M2-0.5C8	H-2M2	H-2M2-0.1C8	H-2M2-0.3C8	H-2M2-0.5C8
2E1	A-2E1	A-2E1-0.1C8	A-2E1-0.3C8	A-2E1-0.5C8	H-2E1	H-2E1-0.1C8	H-2E1-0.3C8	H-2E1-0.5C8
2E2	A-2E2	A-2E2-0.1C8	A-2E2-0.3C8	A-2E2-0.5C8	H-2E2	H-2E2-0.1C8	H-2E2-0.3C8	H-2E2-0.5C8
2B	A-2B	A-2B-0.1C8	A-2B-0.3C8	A-2B-0.5C8	H-2B	H-2B-0.1C8	H-2B-0.3C8	H-2B-0.5C8
2H1	A-2H1	A-2H1-0.1C8	A-2H1-0.3C8	A-2H1-0.5C8	H-2H1	H-2H1-0.1C8	H-2H1-0.3C8	H-2H1-0.5C8
2H2	A-2H2	A-2H2-0.1C8	A-2H2-0.3C8	A-2H2-0.5C8	H-2H2	H-2H2-0.1C8	H-2H2-0.3C8	H-2H2-0.5C8
1S	A-1S	A-1S-0.1C8	A-1S-0.3C8	A-1S-0.5C8	H-1S	H-1S-0.1C8	H-1S-0.3C8	H-1S-0.5C8
5S	A-5S	A-5S-0.1C8	A-5S-0.3C8	A-5S-0.5C8	H-5S	H-5S-0.1C8	H-5S-0.3C8	H-5S-0.5C8
2M1S	A-2M1S	A-2M1S-0.1C8	A-2M1S-0.3C8	A-2M1S-0.5C8	H-2M1S	H-2M1S-0.1C8	H-2M1S-0.3C8	H-2M1S-0.5C8
5CY1	A-5CY1	A-5CY1-0.1C8	A-5CY1-0.3C8	A-5CY1-0.5C8	H-5CY1	H-5CY1-0.1C8	H-5CY1-0.3C8	H-5CY1-0.5C8
5CY2	A-5CY2	A-5CY2-0.1C8	A-5CY2-0.3C8	A-5CY2-0.5C8	H-5CY2	H-5CY2-0.1C8	H-5CY2-0.3C8	H-5CY2-0.5C8
5CY3	A-5CY3	A-5CY3-0.1C8	A-5CY3-0.3C8	A-5CY3-0.5C8	H-5CY3	H-5CY3-0.1C8	H-5CY3-0.3C8	H-5CY3-0.5C8
5CY4	A-5CY4	A-5CY4-0.1C8	A-5CY4-0.3C8	A-5CY4-0.5C8	H-5CY4	H-5CY4-0.1C8	H-5CY4-0.3C8	H-5CY4-0.5C8
6CY1	A-6CY1	A-6CY1-0.1C8	A-6CY1-0.3C8	A-6CY1-0.5C8	H-6CY1	H-6CY1-0.1C8	H-6CY1-0.3C8	H-6CY1-0.5C8
6CY2	A-6CY2	A-6CY2-0.1C8	A-6CY2-0.3C8	A-6CY2-0.5C8	H-6CY2	H-6CY2-0.1C8	H-6CY2-0.3C8	H-6CY2-0.5C8
6CY3	A-6CY3	A-6CY3-0.1C8	A-6CY3-0.3C8	A-6CY3-0.5C8	H-6CY3	H-6CY3-0.1C8	H-6CY3-0.3C8	H-6CY3-0.5C8
6CY4	A-6CY4	A-6CY4-0.1C8	A-6CY4-0.3C8	A-6CY4-0.5C8	H-6CY4	H-6CY4-0.1C8	H-6CY4-0.3C8	H-6CY4-0.5C8
	0 C8	0.1 C8	0.3 C8	0.5 C8	0 C8	0.1 C8	0.3 C8	0.5 C8

Scheme 2.

Structure of amines and the percentage of octylamine in feed for the polymers.