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Chiral Cationic Polyamines for Chiral Microcapsules and siRNA Delivery

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Polyethyleneimines (PEIs) are a well-studied class of polymers.[1,2] These polymers are synthesized commercially via the ring opening of aziridine (Scheme 1, Reaction 2),[3–5] although this process leads to highly branched polymers[6] with significant polydispersity indexes. The controlled synthesis of linear PEIs occurs via the cationic ring opening of oxazolines,[7–9] followed by hydrolysis of the resulting formamides (Scheme 1, Reaction 1). Using chiral oxazolines as substrates for the polymerization reaction provides straightforward access to homochiral PEIs,[10–12] with chiral centers at every polymer repeat unit.

The significant interest in PEIs is driven largely by various applications of PEIs in fields including chiral catalysis,[13–16] drug delivery,[17,18] and oligonucleotide complexation and delivery.[19,20] PEIs have also been covalently linked to form PEI-derived microcapsules,[21] which have been used for site-isolated catalysis.[22,23] In one example, the Lewis basic PEI catalyzed a reaction in the same reaction vessel as a Lewis acidic nickel catalyst, which was used to catalyze the second reaction.[24,25]

Use of the same PEI scaffold for multiple applications has rarely been reported, although such multi-purpose polymers would have significant operational advantages. Reported herein is the use of a single PEI scaffold for two purposes: the fabrication of covalently-linked chiral microcapsules, and the efficient delivery of siRNA to Huh7 cells.[26]

The chiral PEIs were synthesized via the cationic polymerization of 4-benzyl-2-oxazoline (**1a**) (both *R* and *S* configurations), followed by the hydrolysis of the initially formed polyformamide (Scheme 2). The resulting polymers were characterized by ^{1}H NMR spectroscopy, and the results were in agreement with literature-reported spectra.[11] Using this methodology, polymers with 13 and 30 repeat units were formed, with both *R* and *S* configured side chains.

Once synthesized, the homochiral PEIs were cross-linked to form homochiral microcapsules following the procedure developed by McQuade and co-workers.[22] Briefly, polymers **2a**

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were dissolved in methanol, and added to a solution of 2% Span 85, followed by the addition of 2,4-tolylene diisocyanate (TDI, compound **7**) (Equation 1), which cross linked the microcapsules to form a polyurea coating.[25] The resulting polyurethane-type structures have been shown to be stable in a variety of aqueous media.[27,28] After thorough solvent evaporation, chiral microcapsules were obtained.

The resulting microcapsules were imaged using transmission electron microscopy (TEM), and some images are shown in Figure 1. The diameters of the particles ranged from 57 nm–250 nm, with an average diameter of 141 nm (± 35 nm; 62 particles measured). These new supramolecular architectures contain narrow size distributions and uniform structures, in good agreement with literature-reported results for achiral microcapsule analogues.[21]

The newly formed microcapsules contain a variety of features that make them particularly amenable to supramolecular chiral catalysis, including: (a) multiple chiral centers, covalently confined in a small space; (b) multiple amino groups that can be protonated or deprotonated over a wide pH range;[29] and (c) a hydrophobic core resulting from the hydrophobic benzyl side chains.[30]

To investigate the effect of capsule formation on the resulting supramolecular chiral environment, the newly synthesized chiral microcapsules were used as catalysts for the transamination reaction of ketoacids to amino acids (Equation 2). Obtaining good enantioselectivities in such transamination reactions has been an ongoing research problem.[10] Preliminary results indicate that the microcapsule-catalyzed reactions proceeded with significantly higher enantioselectivities compared to the polymer-catalyzed reactions (up to 20% enantiomeric excess (*ee*) obtained for the synthesis of L-valine, under conditions where the polymer itself yielded 4% *ee*). Efforts to optimize the reaction conditions are in progress.

Interestingly, the chiral PEIs also functioned as efficient siRNA delivery agents. Although there are many reported examples of PEIs used for siRNA and DNA delivery,[31–33] many of these delivery vehicles suffer from high cytotoxicity.[34] The development of gene delivery agents that are both effective and less toxic remains a highly relevant research objective.

The following 4 polymers were investigated as potential siRNA delivery agents: *R*-**2a**-13; *S*-**2a**-13; *R*-**6**-13; and *S*-**6**-13, where the R/S designation refers to the chirality of the side chain and the number 13 refers to the number of repeat units in the polymers. The efficacy of these polymers in transfecting an Alexa488-labeled control siRNA sequence[35] to Huh7 cells[36] was measured by determining the intracellular fluorescence 24 hours post-transfection. The results obtained using the chiral polyamines were compared to results obtained using commercially available transfection reagents: Genjet siRNA Transfection Reagent (SignaGen Laboratories); HiPerFect Transfection Reagent (Qiagen Laboratories);[37] and Lipofectamine 2000 (Invitrogen Technologies).[38]

Figure 2 shows a graph of the intracellular fluorescence of Huh7 cells following their incubation with Alexa-labeled siRNA with various delivery reagents. The intracellular fluorescence obtained with compounds *S*-**6**-13 and *S*-**2a**-13 is substantially higher than the fluorescence observed with positive controls Lipofectamine and Genjet, indicating the polymers' ability to transfect siRNA efficiently (Table 1). More interestingly, compounds *R*-**2a**-13 and *R*-**6**-13, which are **identical** except for the three-dimensional configuration of the benzyl group, transfect siRNA with approximately the same efficiency as Lipofectamine and Genjet, and substantially lower than the "enantiomeric" polymers.

The chirality of the side chains of the PEIs thus has a **direct** and **measurable** effect on the ability of PEIs to transfect siRNA efficiently: *S* chiral centers (compounds *S*-**6**-13 and

S-**2a**-13) transfect siRNA more efficiently than the R analogues. Such a result may seem intuitive: that the interaction of two chiral macromolecules (chiral PEI and chiral siRNA) depends on the three-dimensional configuration of both molecules. This intuition is borne out by the results of this study, which is the first direct proof that the chirality of a polyamine directly impacts its transfection efficiency. Similar effects of the chirality on transfection efficiency were recently observed for the lipid delivery agent 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP).[39] In that report, the *R* enantiomer performed better than either the *S* enantiomer or the racemic DOTAP mixture.

The toxicity of the newly synthesized PEIs was tested using an MTT assay.[40] After 24 hours of incubation, the absorbance of the cells was quantified and the cell viability was calculated. Using 1000 nM of *S*-**2a**-13 reduced the cell viability to 88%, and 1000 nM of *S*-**6**-13 reduced it to 82%. By comparison, Lipofectamine reduced cell viability to 89%, and compounds *R*-**2a**-13 and *R*-**6**-13 reduced viability to 78% and 71%. Thus, the toxicity of the chiral PEIs, like the transfection efficiency, depends on the three-dimensional configuration of the benzylic side chains.

The differences in transfection efficiency and toxicity mean that the *S*- and *R*-configured PEIs likely have fundamentally different three-dimensional architectures. The relationship between the chirality of individual stereocenters and the overall polymer configuration has been studied for related polymers using circular dichroism spectroscopy.[41–43] These differences in chirality affect the polymers' solubility[44] and their interactions with DNA,[45] and as shown here, their transfection efficiencies.

In summary, chiral polymers **6** and **2a** were synthesized via straightforward, well-precedented procedures. These polymers were used for two novel applications: the fabrication of chiral, covalently-linked microcapsules, and the transfection of siRNA to Huh7 cells. The chiral microcapsules can be used for a number of potential applications in supramolecular chiral catalysis and in supramolecular enantiomer separations. The chirality-dependent siRNA transfection also provides an intriguing platform for further investigation. In particular, polymer *S*-**2a**-13 demonstrated good transfection efficiency and limited toxicity, and will be used for further biochemical investigations. The results of these and other experiments will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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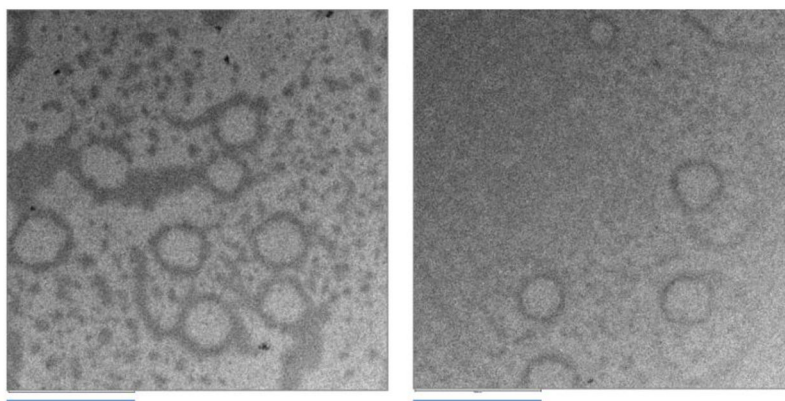
Figure 1.
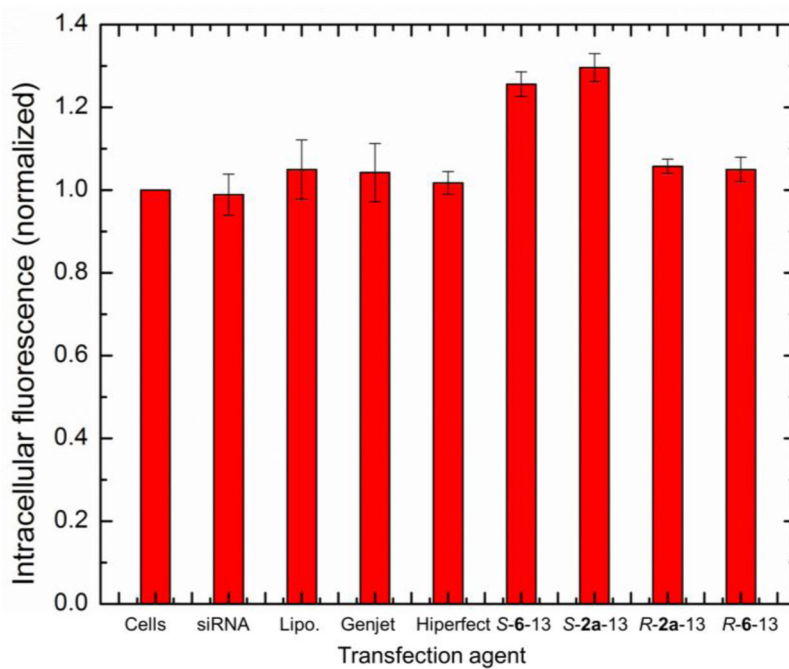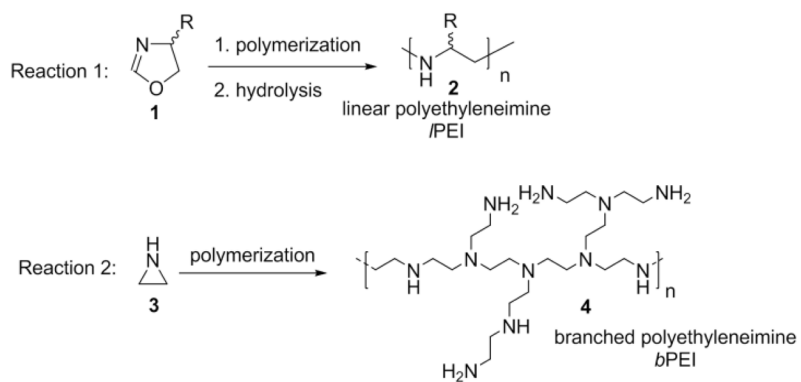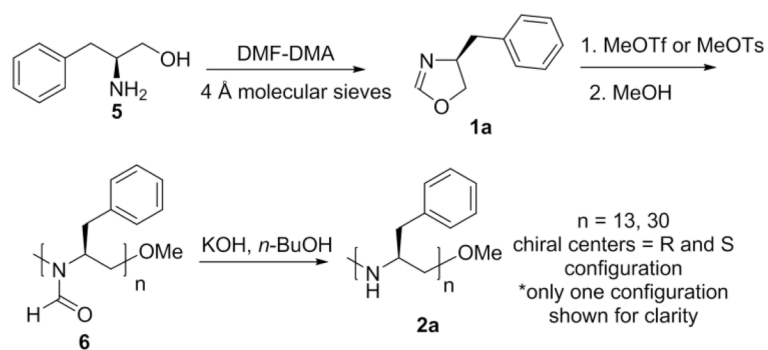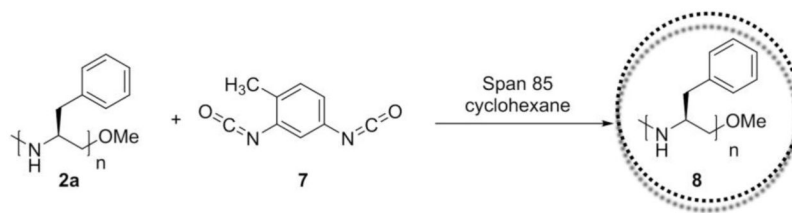TEM images of chiral microcapsules **8** (Blue line represents a 500 nm scale)

Figure 2.
Chart of the intracellular fluorescence of Huh7 cells after transfection with siRNA (all PEIs were used at a 1000 nM final concentration)

Scheme 1.
General synthetic methods for linear and branched polyethyleneimine (PEI)

Scheme 2.
Synthesis of chiral polymers **2a**

Equation 1.
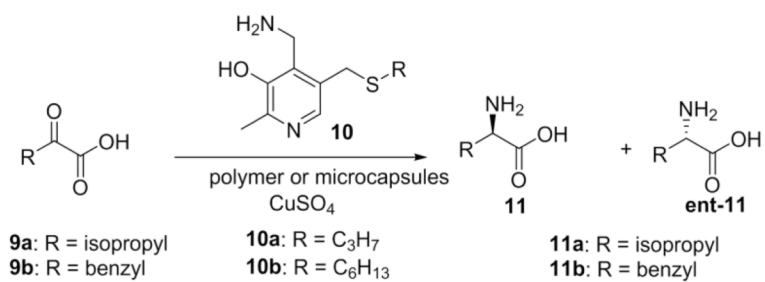Synthesis of chiral covalently-linked microcapsules

Equation 2.
Enantioselective transamination of ketoacids **9** to amino acids **11**

Table 1

Transfection efficiencies of chiral PEIs and commercial transfection agents

Transfection agent	Intracellular fluorescence (normalized to 1.00 for cells alone)
S-**6**-13	1.26
S-**2a**-13	1.30
Lipofectamine	1.05
Genjet	1.04
R-**2a**-13	1.06
R-**6**-13	1.05