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# New class of biodegradable polymers formed from reactions of an inorganic functional group

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### **Abstract**

Although numerous small molecules have been synthesized with sulfenamide bonds ( $R_2N$ -SR), this is the first report of the synthesis of polysulfenamides. These polymers are readily synthesized at room temperature using secondary diamines and dithiosuccinimides. The dithiosuccinimides were readily synthesized in one step by the reaction of dithiols such as  $HS(CH_2)_6SH$  with N-chlorosuccinimide. The resulting dithiosuccinimides were either recrystallized or readily purified by chromatography on silica gel and required no special handling. The conversions of polymerization ranged from 95 to 98%, and the molecular weights of the polymer reached as high as 6,300 g mol $^{-1}$ . The sulfenamide bond was very stable in organic solvents, and no degradation was observed under atmospheric conditions in  $C_6D_6$  for 30 days. In contrast, the sulfenamide bond readily decomposed in less than 12 h in  $D_2O$ . Polysulfenamides were fabricated into micron-sized particles loaded with dye and endocytosed into JAWSII immature dendritic and HEK293 cells. Polysulfenamides represent a new class of polymers that are readily synthesized, stable in aprotic solvents, and readily degrade in water.

#### Introduction

Biodegradable polymers have shown wide utility in a variety of biomedical applications ranging from sutures, scaffolds for the growth of cells, and polymeric depots that provided sustained release of therapeutic agents. <sup>1–6</sup> Optimal use of these materials requires their molecular and macromolecular properties be tailored to the specific application for which the material is to be used. <sup>3,7–9</sup> For instance, polymeric particles loaded with drugs that are targeted to the endosomal compartments of cells should ideally be stable at physiological pH in the bloodstream, but readily break down at a lower pH to release its drug cargo in the endosome where the pH is approximately 5 to 6. <sup>10–13</sup> Biodegradable polymers are attractive in drug delivery applications because polymeric particles injected *in vivo* can accumulate in several organs including the liver, spleen, lungs, and heart and often result in toxic side effects if they do not break apart into smaller, easily excretable side products. <sup>14,15</sup> The degradation of these polymers is also the mechanism by which controlled release of drugs and other therapeutics is achieved. Most synthetic polymers in biomedical research are polyesters, polyamides, polyanhydrides, or some combination of these three functional groups. These polymers degrade to short polymers or small molecules at reasonable times

#### **Supporting Information**

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The synthesis of the water soluble sulfenamide in Figure 9, kinetic experiments, and degradation of sulfenamides are found in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

without the use of enzymes. <sup>16–18</sup> Thus, these polymers may be excreted from the body before toxic side-effects occur.

Integrating new functional groups into polymer chemistry allows the fabrication of polymers with new properties and the ability to tailor new materials. <sup>19,20</sup> Specifically, the introduction of a new functional group to synthesize biopolymers has the potential to open up new applications in this field and will allow more complex, potentially "smart" drug delivery vehicles to be synthesized. It is very challenging to design a polymer with a new functional group along its backbone that renders it stable at physiological pH but also allows it to be degraded at reasonable time scales in the body. Recent work has shown the promise of using biodegradable polymers linked with acetals or disulfides in medical applications that provide alternatives to functional groups based on carbonyls. <sup>21–24</sup> In this paper, we report the first synthesis of polymers with the sulfenamide functional group within the backbone of macromolecules, how these polymers degrade under different conditions, and how they can be fabricated into micron-sized particles loaded with fluorescent dye that are internalized into cells. Polysulfenamides are a new class of polymers that we believe will have real applications in medicine.

Sulfenamides are an understudied functional group with the general formula of RS-NR'R" that are used as protecting groups for amines (typically as (Ph<sub>3</sub>C)S-NR<sub>2</sub>),<sup>25</sup> vulcanizing agents in the rubber industry, <sup>26–28</sup> in the activation of C-H bonds, <sup>29</sup> as ligands on metals to promote O atom transfer, <sup>30,31</sup> as intermediates in the synthesis of sulfonamides, <sup>32</sup> as oxidants for alcohols, <sup>33,34</sup> and as ligands in the synthesis of inorganic coordination compounds.<sup>35–40</sup> In a recent article we reported on the synthesis of the first polydiaminosulfides based on the diaminosulfide functional group (R<sub>2</sub>N-S-NR<sub>2</sub>).<sup>41</sup> The diaminosulfide functional group differs from the sulfenamide functional group in its structure, reactivity, and reaction products. A loose analogy to explain the differences between diaminosulfides and sulfenamides is to compare this pair to esters and anhydrides. Structurally, an anhydride is an ester with an extra carbonyl. Similarly, a diaminosulfide is a sulfenamide with an extra amine. Much as the structural differences between an ester and anhydride lead to different reactivities and products of reaction, the structural differences between a sulfenamide and diaminosulfide lead to different reactivities and products of reaction. A diaminosulfide should not be confused for a sulfenamide any more than an ester is confused for an anhydride.

Some of the general properties of sulfenamides made them attractive functional groups to pursue for the synthesis of polymers. Prior work by others with small molecules containing sulfenamide bonds demonstrated that they were readily synthesized and possessed reasonable stabilities. 35,42–44 Molecules with sulfenamide bonds degraded into radicals at elevated temperatures which led to their applications as vulcanizing agents in the rubber industry. 26–28 Based on their properties as protecting groups for amines, it was known that sulfenamides were stable under neutral and basic conditions, but could be cleaved under acidic conditions. Furthermore, sulfenamides have been synthesized in high yields from activated thiols and amines at room temperature.

These properties were desirable for the development of new biodegradable polymers. We sought a functional group that was stable in organic solvents, would rapidly break down in acidic water, and would possess higher stability in water at neutral pH than at acidic pH. Additionally, prior reports of the synthesis of sulfenamides in high yields were promising because the development of a step growth polymerization requires that the yield of the reaction to form the polymer be high and typically exceed 95%.<sup>45</sup>

In this article we report the first synthesis of polysulfenamides. No prior examples of polymers containing this functional group have been reported so their synthesis was developed by us for the first time. Additionally, we report the stability of this functional group to different conditions and how they can be applied as new drug delivery vehicles.

### **Results and Discussion**

#### Synthesis of polysulfenamides from disulfenamides

Because polysulfenamides were unknown prior to our work, the first step in this project was to develop their synthesis. In one approach two different dithiols were activated with sulfuryl chloride to yield intermediates with S-Cl bonds (Figure 1).<sup>35</sup> If left overnight exposed to air, these molecules formed disulfides. To prevent the formation of disulfides, these molecules were reacted with an excess of *N*-ethylmethylamine immediately after their formation. When molecule A was purified by chromatography on silica gel, the isolated yield was only 6%. When this molecule was purified by chromatography on basic alumina oxide, the isolated yield was 76%. This result was consistent with prior reports that described the decomposition of sulfenamides on acidic silica gel.<sup>32,46</sup> All subsequent purifications of sulfenamides were completed by vacuum distillation or by chromatography on basic alumina oxide.

Simple bimolecular reactions were studied to measure the kinetics of the transamination reaction shown in Figure 2 to determine the best conditions for polymerizations. Molecule B was synthesized and reacted with N-benzylmethylamine in  $C_6D_6$ , DMSO- $d_6$ , and  $CD_3OD$  at different temperatures. This reaction was very slow in  $C_6D_6$  with no conversion after 24 h at room temperature and only 11% conversion after 24 h at 60 °C. When DMSO- $d_6$  was used as the solvent, the reaction was faster and the conversion reached 7% after 24 h at room temperature and 32% after 24 h at 50 °C. The reaction proceeded rapidly in  $CD_3OD$  and reached a conversion of 40% after 3 h, and at longer periods of time it reached an equilibrium mixture of 50% of each sulfenamide. The rate constant for the reaction in  $CD_3OD$  was  $5.1 \times 10^{-4}$  M<sup>-1</sup> s<sup>-1</sup>. This reaction was most rapid in a polar, protic solvent which suggests a charged transition state that is stabilized by hydrogen bonding. It is believed that the reaction proceeded through an  $S_N2$  reaction mechanism when N-benzylmethylamine attacked the sulfur of the sulfenamide. It was possible that the hydrogen bond donating ability of  $CD_3OD$  lowered the energy requirement for N-ethylmethylamine to leave.

When the polymerization of a disulfenamide with a diamine was attempted, the results were not promising (Figure 3). Although the kinetics of reaction were rapid in methanol, the resulting polymer was insoluble in methanol which made this solvent a poor choice to conduct polymerizations. Polymerizations at high concentrations of monomers in solvent mixtures of  $(1/1 \ v/v) \ CD_3 OD/CDCl_3$  or  $(1.2/1 \ v/v) \ CD_3 OD/DMSO-d_6$  at 40 °C yielded oligomers even after 3 days. A polymerization was attempted in refluxing benzene for 24 h with the reaction vented to allow *N*-ethylmethylamine (boiling point of 36–37 °C) to boil off resulted in a low yield of a modest molecular weight polymer (Mw = 2,840 g mol<sup>-1</sup>). This method of polymerization was hindered by lack of solubility of the polymer in methanol and the slow kinetics for polymerization in aprotic solvents.

### Synthesis of polysulfenamides from dithiosuccinimides

To increase the rate of transamination and to allow the use of aprotic solvents in a polymerization, dithiols were activated with *N*-chlorosuccinimide (Figure 4). This reaction went to high yields at room temperature, and the dithiosuccinimides were stable to chromatography on silica gel. Furthermore, molecule C was readily purified by

crystallization. The dithiosuccinimides were stable at room temperature under ambient atmosphere, and they required no special handling.

The reaction of a thiosuccinimide (molecule D in Figure 5) with *N*-benzylmethylamine was much faster in aprotic solvents than the same reaction between molecule B and *N*-benzylmethylamine. In  $CD_2Cl_2$  at room temperature the reaction between molecule D and *N*-benzylmethylamine had a rate constant of  $8.2 \times 10^{-4} \, \mathrm{M}^{-1} \, \mathrm{s}^{-1}$  and reached 35% conversion after 4 h. This transamination reaction was approximately  $2\times$  faster than the reaction between molecule B and *N*-benzylmethylamine in  $CD_3OD$ . The difference in solvent was critically important because methylene chloride and chloroform were excellent solvents for both monomers and polymers in contrast to  $CD_3OD$  where the polymer precipitated. In addition, the reaction of a thiosuccinimide with *N*-benzylmethylamine went to >97% conversion at room temperature. The test polymerization shown in Figure 5b went to >95% conversion within 50 min in  $CDCl_3$  at room temperature. This polymerization method was used in all subsequent polymerizations.

A series of polymers were synthesized from dithiosuccinimides and secondary diamines (Table 1). The polymerizations were completed in methylene chloride or chloroform at room temperature for 24 h. The polymers had molecular weights up to 6,300 g mol<sup>-1</sup>, degrees of polymerization that ranged from 95% to 98%, and they were isolated in >80% yields by precipitation in methanol. Secondary diamines were chosen for this work because the synthesis of polymers using primary diamines (such as NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>) resulted in cross-linked, insoluble polymers. The cross-links were possibly due to the reaction of an amine with two equivalents of a thiosuccinimide to yield cross-links of the form RN(SR')<sub>2</sub>.

These polymers were well characterized by size exclusion chromatography (SEC). Entries 1 and 4 of Table 1 had strong peaks in both the multi-angle laser light scattering detector and the refractive index detector so absolute molecular weights were calculated. For entries 2, 3, and 5 the light scattering signal was too weak so the molecular weights were found by calibration with polystyrene standards. In all examples the polymers were analyzed by <sup>1</sup>H NMR spectroscopy to study the end groups. The peaks corresponding to the end groups integrated to very low values which supported the SEC data for the molecular weights and high conversions. An example of a SEC micrograph is shown in Figure 6.

### Degradation of sulfenamides in aprotic and protic solvents

Little data was available on the stabilities of sulfenamides in various solvents that could be used to anticipate the stabilities of polysulfenamides. To address this limitation, a sulfenamide was synthesized and its stability was studied in NMR tubes in  $CD_3OD$  and  $C_6D_6$  (Figure 7). After 30 days, the  $^1H$  NMR spectrum of the sulfenamide in  $C_6D_6$  was unchanged which demonstrated its stability in aprotic solvents. This molecule slowly degraded in  $CD_3OD$ ; after 33 days 15% of it degraded at room temperature and only 27% degraded when heated to 40 °C for the same period of time. These conditions indicated that sulfenamides possessed reasonable stabilities under neutral conditions in organic solvents. It should be noted that the polysulfenamides were synthesized in organic solvents under ambient conditions without any special handling.

The kinetics and products of degradation of molecule E were studied under acidic conditions. First, molecule E was added to  $CD_3OD$  with five equivalents of benzoic acid. In contrast to the degradation of molecule E without benzoic acid (33 days to reach 15% degradation), the degradation of molecule E in the presence of benzoic acid reached 51% degradation within 4 min. When molecule E was exposed to two molar equivalents of p-toluenesulfonic acid (pKa < -2) or trifluoroacetic acid (pKa = 0.3) in  $CD_3OD$ , it degraded to

>90% within four minutes. Clearly, the decomposition of sulfenamides was catalyzed by acid as observed by others in prior work.  $^{32,46}$ 

The products of degradation of molecule E were studied under acidic conditions in D<sub>2</sub>O to understand how polysulfenamides would break down under these conditions. Molecule E was added to D<sub>2</sub>O with seven molar equivalents of acetic acid. Because molecule E was insoluble in D<sub>2</sub>O, the reaction mixture was vigorously stirred. After 41 h the organics were extracted and initially studied by <sup>1</sup>H NMR spectroscopy prior to isolation by column chromatography. The <sup>1</sup>H NMR spectrum of the extract before chromatography indicated that approximately 75% of the product was the thiosulfinate (molecule F in Figure 8a) with the remainder mostly composed of molecules G and H. The initial reaction of molecule E with water yielded a sulfenic acid, but sulfenic acids are transient intermediates that very rapidly condense as shown in Figure 8b. 40,47-49 Based on literature precedent, molecule F decomposed via multiple pathways to yield molecule G, molecule H, and a sulfinic acid (molecule I). 48,50–52 Thiosulfinate esters such as molecule F are known oxidation states for cysteine residues and found in nature products such as allicin (a component of garlic; Figure 8c). As such, thiosulfinates are already present in the body and are known to decompose at reasonable rates – the half-life of the decomposition of allicin at pH = 7.5 at 37 °C is approximately 2 days.<sup>8,50</sup>

To further study the degradation of sulfenamides in  $D_2O$ , a water soluble sulfenamide was synthesized (Figure 9). The decomposition of this molecule was studied in  $D_2O$  with eight molar equivalents of acetic acid, nine molar equivalents of KOH, or under neutral conditions without the addition of acid or base. The rates of decomposition were found under neutral ( $k = 4.7 \times 10^{-4} \, s^{-1}$ ) and basic conditions ( $k = 8.0 \times 10^{-5} \, s^{-1}$ ) but the decomposition was too rapid under acidic conditions to measure a rate constant. Because the sulfenamide completely decomposed within 4 min after addition to  $D_2O$  with acetic acid, only a lower limit for its rate of decomposition was found ( $1.6 \times 10^{-2} \, s^{-1}$ ). It is important to note that these rate constants are several orders of magnitude higher than the neutral and acidic degradation of esters. <sup>53</sup> Because of the rapid decomposition, we were unable to determine a value for the pKa of a protonated sulfenamide.

#### Fabrication and degradation of microparticles of polysulfenamides

Microparticles were fabricated from the polymer in entry 2 in Table 1 to study whether these polymers could form the basis of drug delivery vehicles. The microparticles were prepared using an oil-in-water single emulsion solvent evaporation methodology. In this approach, the polymer was dissolved in chloroform and then emulsified in surfactant containing water phase. The emulsion was stirred overnight until the chloroform evaporated. Drugs and fluorescent molecules such as rhodamine can be incorporated into the microparticles by mixing them in the polymer/chloroform mixture prior to emulsification. The average size of the microparticle was 2.6 microns with a PDI of 0.62. They were spherical in shape, had a smooth surface, and had a negative charge of -41 mV in PBS buffer at pH = 7.4 (Figure 10). The reason for the negative charge was understood in terms of prior experiments that demonstrated that sulfenamides slowly degraded to yield sulfinic acids that deprotonated and led to a negatively charged surface.

Microparticle degradation was studied at both physiological pH (7.4) and acidic pH (4.0) to learn whether these particles would decompose over time. Figure 10 shows the percentage weight loss of the samples at different points in time. After one week, a weight loss of 24% was observed for microparticles in acetate buffer as compared to a weight loss of 13% for particles in PBS. On day 35, the weight loss in acidic buffer was ~33% as compared to ~17% at physiological pH. Weight loss was significantly higher for particles in acetate buffer as compared to particles in PBS, indicating faster degradation of the microparticles in

acidic environments. SEM images of the acidic pH-degraded particles revealed a rough morphology of the polymer surface that was consistent with polymer degradation (Figure 10c). The differences in the rates of degradation of microparticles at pH 7.4 and 4.0 were expected based on the rapid degradation of molecule J under acidic conditions in  $D_2O$  and its slower degradation in neutral  $D_2O$ .

The overall rate of degradation of a microparticle depends on many factors other than the rate of degradation of the functional group along the backbone. For instance, microparticles fabricated from polycaprolactone lose less than 1% of their weight after 26 weeks in aqueous buffer, <sup>54</sup> but microparticles fabricated from poly(lactic-*co*-glycolic acid) with 50% lactic acid and 50% glycolic acid lose approximately half of their weight in two weeks in aqueous buffer. <sup>55</sup> This difference in rates of degradation of two similar polyesters is due to factors such as differences in crystallinity, molecular weight, molecular weight distribution, porosity, and hydrophobicity of the polymers. <sup>56</sup> Thus, although the degradation of the sulfenamide bond is rapid in water, microparticles fabricated from polysulfenamides will exhibit a wide range of degradation profiles based on the monomers used in their synthesis.

The uptake of these microparticles was studied in JAWSII and human embryonic kidney (HEK293) cells using confocal microscopy. JAWSII cells are immature dendritic cells derived from mouse bone marrow and are commonly targeted by particle based vaccines for generation of therapeutic immune responses. HEK293 cells are one of the most common cell lines used to evaluate novel gene therapies. Microparticles were loaded with rhodamine B during fabrication and were then exposed to the JAWSII and HEK293 cells. The microparticles were found to be located near the cytoplasm of the cell, indicating the phagocytosis of the microparticles by cells (Figure 11; see also the Supporting Information for an additional image). The uptake of microparticles by both cell lines suggests that this system has potential in immunological applications, especially in vaccine delivery and potential for gene therapy applications.

#### Toxicity of polysulfenamide microparticles

The *in vitro* toxicity of these microparticles was evaluated with HEK293 cells. In initial work, these cells were incubated with the microparticles at a range of concentrations of 1 microgram to 1,000 micrograms per mL of growth medium for 4 h. The cell viability was measured by standard techniques of measuring the metabolic activity of cells in a control without microparticles and the metabolic activity of cells that were exposed to the microparticles. The ratio of these concentrations was plotted as the "cell viability" in the y-axis of Figure 12a. No toxicity was observed at even the highest concentrations after 4 h, so a longer incubation time of 24 h was studied and shown in Figure 12a. At the expected working concentrations of microparticles minimal toxicity was observed. Interestingly, at the highest concentration of 1 mg of microparticles per mL of solution where the wells containing HEK293 cells were beginning to become saturated with microparticles, the cell viability was still 57 %. This suggests that polysulfenamides have low toxicity and are therefore suitable for drug, gene, and vaccine delivery applications.

The *in vivo* toxicity of the polysulfenamide microparticles were evaluated in BALB/c mice. Microparticles were administered via intraperitoneal (i.p.) injection because this route of administration is commonly utilized for evaluating microparticle based vaccines which are an intended application for this novel polymer and because the i.p route allows particles to enter the systemic circulation allowing for a better gauge of systemic toxicity than subcutaneous or intramuscular injection.. The mice were divided into five groups and were injected with either 5 mg, 10 mg, or 15 mg of polysulfenamide microparticles from entry 2 in Table 1; 10 mg of microparticles of poly(*D*,*L*-lactide-*co*-glycolide) as a control group; or a sterile PBS buffer solution. The mass of particles injected in these experiments was

significantly higher than the typical therapeutic mass of microparticles injected for vaccine based application. The mice were followed for 23 days and blood was drawn at 0, 2, 9, 16, and 23 days to test for the levels of liver enzymes. Specifically, alanine aminotransferease (ALT) and aspartate aminotransferase (AST) are liver enzymes (transaminases) that can be detected systemically as a result of hepatocellular damage (liver inflammation, necrosis, cellular degeneration and increased membrane permeability) and are approved FDA assays for determination of pre-clinical toxicity in new chemical entities.

The *in vivo* study showed that the polysulfenamide microparticles did not result in any significant increase in the serum ALT and AST levels over the study period, relative to either day 0 or poly(*D*,*L*-lactide-*co*-glycolide) microparticles, indicating lack of any liver damage (Figure 12b and 12c). In addition, mice were monitored daily and remained in good health as determined by the Body Condition Scoring technique. The vertebrae and dorsal pelvis were not prominent but palpable with slight pressure indicating a state of good health in the mice. No adverse injection site reactions such as infection, redness or wounding were observed. Furthermore, no mice died as a result of injections of polysulfenamide microparticles over the entire study period. In summary, the *in vivo* and *in vitro* studies showed little or no adverse effects from the polysulfenamides. Although these results are promising for an initial demonstration of toxicity, the toxicity of polysulfenamides will require a more thorough investigation in future studies to fully probe any toxicity resulting from the polymers or their degradation products (such as the sulfenic acids).

#### Conclusions

We report the first synthesis and characterization of polysulfenamides. These polymers are unique because they integrate a new functional group into polymer chemistry that has found some applications in small molecule chemistry. Two methods to synthesize polysulfenamides were reported and both polymerization reactions went to high conversions (up to 98%). It is notable that the synthesis of polysulfenamides from dithiosuccinimides proceeded at room temperature in methylene chloride; these reaction conditions are mild and suggest that more complex monomers can be used. Although the molecular weights of the polysulfenamides reported here did not reach 10,000 g mol<sup>-1</sup>, higher molecular weights are possible if the conversions of polymerization are increased or if higher molecular weight monomers are used. These polymers were stable in aprotic solvents and only slowly degraded in protic, organic solvents.

Polysulfenamides present new opportunities in macromolecules because they possess a functional group that was previously unknown in polymer chemistry. In this article we describe how polysulfenamides possess many of the right characteristics for applications in the delivery of pharmaceuticals. For instance, they were stable in organic solvents but degraded in water and degraded more rapidly in acidic water. Microparticles were fabricated from a polysulfenamide and these microparticles were readily internalized by two different cell lines. Other applications of polysulfenamides are envisioned that take advantage of the thermal lability of sulfenamide bonds, oxidation of the sulfurs, or their rapid degradation under acidic medium. Because many new opportunities for macromolecules arise when a new functional group is integrated into polymer science, we anticipate new applications for polysulfenamides.

## **Experimental procedures**

#### **Materials**

Phosphate buffer saline (PBS) is a product of Sigma-Aldrich (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Gibco BRL (Grand Island, NY).

LysoTracker® Green was obtained from Invitrogen (Eugene, Oregon, USA). Paraformaldehyde (16% solution, EM grade) was obtained from electron microscopy services (Hatfield, PA) and Vectashield mounting medium for fluorescence with DAPI was a product of Vector laboratories (Burlingame, CA). 1-Hexanethiol, 1,6-hexanedithiol, 2-mercaptoethyl ether, *N*-benzylmethylamine, *N*-ethylmethylamine, *N*,*N*'-dimethyl-1,6-hexanediamine, *N*-chlorosuccinimide, triethylamine, ethylenediamine, benzoic acid, *p*-toluenesulfonic acid, acetic acid, and sulfuryl chloride were purchased from Aldrich or Acros Organics at their highest purity and used as received. HPLC grade chloroform purchased from Acros Organics was used as the GPC solvent after filtering it through a glass frit. All other solvents were reagent grade and purchased from Acros Organics. Piperazine (99%) was purchased from Aldrich. It was purified by sublimation under vacuum at 130 °C. *N*,*N*'-Diethylethylenediamine (95%) was purchased from Aldrich and distilled under vacuum at 30 °C. Genduran silica gel 60 (230–400 mesh) and basic alumina Brockman activity I (60–325 mesh) were purchased from Fisher Scientific and used for column chromatography.

### Characterization of small molecules and polysulfenamides

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker DPX 300 at 300 MHz and 75 MHz respectively. CDCl<sub>3</sub> was used as NMR solvent with tetramethylsilane (TMS) as an internal standard. SEC was performed using chloroform as the mobile phase (1.00 mL min<sup>-1</sup>) at 40 °C. A Waters 515 HPLC pump and two Waters columns (Styragel HR4 and HR5E) were used in series. A DAWN EOS 18 angle laser light scattering detector from Wyatt Corp. to measure light scattering and a Wyatt Optilab DSP to measure changes in refractive index were used to measure absolute molecular weights of polymers. Polystyrene standards (1,260 g mol<sup>-1</sup>, 3,790 g mol<sup>-1</sup>, 13,000 g mol<sup>-1</sup>, and 30,300 g mol<sup>-1</sup>) were used to generate a calibration curve to measure relative molecular weights of some polysulfenamides as indicated in Table 1.

### Hexanesulfenyl chloride

Alkylsulfenyl chlorides were prepared according to a literature procedure. <sup>57</sup> Since they quickly returned to thiols after being exposed to air, alkylsulfenyl chlorides were used *in situ* for the preparation of sulfenamides. Sulfuryl chloride (6.28 g, 0.047 mol) was added dropwise to a solution of hexanethiol (5 g, 0.042 mol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) at 0 °C under nitrogen and stirred for 2 h 45 min. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.90 (t, 3H, J = 6.8 Hz), 1.39 (m, 6H), 1.78 (m, 2H), 3.11 (t, 2H, J = 7.1 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.03, 22.52, 27.83, 28.02, 31.31, 41.52.

#### 2,2'-Oxydiethanesulfenyl dichloride

2-Mercaptoethyl ether (3 g, 0.22 mol) was reacted with a solution of sulfuryl chloride (6.15 g, 0.046 mol) in  $CH_2Cl_2$  (30 mL) following the previous procedure. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  3.33 (t, 4H, J = 6.3 Hz), 3.88 (t, 4H, J = 6.2 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  40.92, 68.10.

#### N-Ethylmethyl-hexanesulfenamide (Molecule B)

A solution of hexanesulfenyl chloride (6.45 g, 0.042 mol) in  $CH_2Cl_2$  (30 mL) was slowly added to a solution of *N*-ethylmethylamine (7.50 g, 0.13 mol) in  $CH_2Cl_2$  (50 mL) at 0 °C under nitrogen and stirred for 7 h. The reaction was diluted with anhydrous  $Et_2O$  (130 mL) and washed with saturated NaCl solution in water (3 × 50 mL). The organic phase was dried over anhydrous magnesium sulfate and evaporated to give a brown oil. The product was isolated by vacuum distillation at 30 °C to yield a colorless oil (3.93 g, 53% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.89 (t, 3H, J = 6.8 Hz), 1.14 (t, 3H, J = 7.2 Hz), 1.35 (m, 6H), 1.54 (m, 2H), 2.65 (t, 2H, J = 7.2 Hz), 2.75 (s, 3H), 2.82 (q, 2H, J = 7.1 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  13.79,

14.09, 22.58, 28.27, 28.89, 31.59, 31.86, 46.82, 53.93. HRMS calcd for  $C_9H_{21}NS$ : 175.1395. Found: 175.1392.

### N,N'-Ethylmethyl-bis(2-mercaptoethyl)disulfenamide (Molecule A)

A solution of 2,2'-oxydiethanesulfenyl dichloride (4.49 g, 0.022 mol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was reacted with a solution of *N*-ethylmethylamine (7.70 g, 0.13 mol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) following the previous procedure. The extract was filtered through basic alumina to yield a colorless oil (3.29 g, 60% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.13 (t, 6H, J = 7.1 Hz), 2.74 (s, 6H), 2.82 (m, 8H), 3.67 (t, 4H, J = 7.1 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  13.70, 32.68, 46.89, 54.13, 70.01. HRMS calcd for C<sub>10</sub>H<sub>24</sub>N<sub>2</sub>OS<sub>2</sub>: 252.1321. Found: 252.1324.

## N-(Hexanethio)succinimide (Molecule D).58

Hexanethiol (3 g, 25.37 mmol) was added dropwise to a solution of *N*-chlorosuccinimide (3.36 g, 26.64 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (70 mL) at 0 °C under nitrogen and stirred for 20 min. The solution changed to a yellow-green color and immediately became cloudy. A solution of triethylamine (2.82 g, 27.91 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise to the reaction. After the mixture was stirred for 2.5 h, it was washed with a saturated NaCl solution in water (4 × 30 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to give a colorless oil. The product was isolated by column chromatography using silica gel and 20% ethyl acetate in hexanes to yield a colorless oil (4.73 g, 87% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.88 (t, 3H, J = 6.6 Hz), 1.28 (m, 4H), 1.40 (m, 2H), 1.54 (m, 2H), 2.85 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  13.99, 22.45, 27.90, 28.11, 28.61, 31.28, 37.62, 177.23. HRMS calcd for C<sub>10</sub>H<sub>17</sub>NO<sub>2</sub>S: 215.0980. Found: 215.0979.

### (2,2'-Oxydiethenthio)disuccinimide

2-Mercaptoethyl ether (10 g, 0.072 mol) was reacted with a solution of *N*-chlorosuccinimide (20.28 g, 0.15 mol) in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and a solution of triethylamine (16.10 g, 0.16 mol) in CH<sub>2</sub>Cl<sub>2</sub> (55 mL) following the previous procedure. The mixture was purified by column chromatography using 95% ethyl acetate in hexanes to yield a white solid (11.42 g, 48% yield).  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  2.78 (s, 8H), 2.95 (t, 4H, J = 5.9 Hz), 3.68 (t, 4H, J = 5.9 Hz).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  28.68, 36.93, 70.90, 177.29. HRMS calcd for C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub> + Na<sup>+</sup>: 355.0398. Found: 355.0387.

### N,N'-(Hexanethio)disuccinimide (Molecule C)

1,6-Hexanedithiol (7 g, 0.047 mol) was reacted with a solution of *N*-chlorosuccinimide (13.06 g, 0.098 mol) in CH<sub>2</sub>Cl<sub>2</sub> (230 mL) and a solution of triethylamine (10.37 g, 0.102 mol) in CH<sub>2</sub>Cl<sub>2</sub> (35 mL) following the previous procedure to give a brown solid. The solid was recrystallized from methanol (350 mL) 3 times to yield a white solid (9.90 g, 62% yield).  $^1\mathrm{H}$  NMR (CDCl<sub>3</sub>):  $\delta$  1.47 (m, 8H), 2.83 (m, 12H).  $^{13}\mathrm{C}$  NMR (CDCl<sub>3</sub>):  $\delta$  27.62, 27.65, 28.62, 37.48, 177.26. HRMS calcd for C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub> + Na<sup>+</sup>: 367.0762. Found: 367.0757.

#### N-Benzylmethyl-hexanesulfenamide

*N*-Benzylmethylamine (1.11 g, 9.19 mmol) was added to a solution of molecule B (1.94 g, 9.01 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL) and stirred at room temperature for 19 h. The reaction was diluted with anhydrous Et<sub>2</sub>O (30 mL) and washed with water (4 × 20 mL). The organic phase was dried over anhydrous magnesium sulfate and evaporated to give a yellowish oil (1.7 g, 81% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.89 (t, 3H, J = 6.8 Hz), 1.34 (m, 6H), 1.56 (m 2H), 2.69 (m 5H), 4.01 (s, 2H), 7.28 (m, 5H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.07, 22.56, 28.26, 28.80, 31.56, 32.88, 45.97, 65.47, 127.25, 128.23, 128.68, 138.90. HRMS calcd for C<sub>14</sub>H<sub>23</sub>NS: 237.1551. Found: 237.1541.

### Polymer from entry 1 in Table 1

Piperazine (0.176 g, 2.05 mmol) was added to a stirred solution of (2,2'-oxydiethenthio)disuccinimide. (0.71 g, 2.05 mmol) in CHCl<sub>3</sub> (3.5 mL) and reacted at room temperature for 24 h. The polymer was precipitated into methanol (35 mL) and washed with methanol 3 times. The polymer was dried under vacuum to yield a white powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.86 (t, 4H, J = 6.8 Hz), 2.97 (s, 8H), 3.65 (t, 4H, J = 6.9 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  32.92, 57.28, 70.10.

### Polymer from entry 2 in Table 1

N,N'-(Hexanethio)disuccinimide (3 g, 8.71 mmol) in CHCl<sub>3</sub> (19 mL) was reacted with piperazine (0.75 g, 8.71 mmol) following the previous procedure to yield a white powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.40 (m, 4H), 1.56 (m, 4H), 2.69 (t, 4H, J = 7.5 Hz), 2.99 (s, 8H). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  28.62, 28.82, 32.17, 57.30.

### Polymer from entry 3 in Table 1

N,N'-(Hexanethio)disuccinimide (1 g, 2.90 mmol) in CHCl<sub>3</sub> (5 mL) was reacted with N,N'-diethylethylenediamine (0.34 g, 2.90 mmol) following the previous procedure. The polymer was precipitated into methanol (10 mL). The polymer was redissolved in a minimal amount of CHCl<sub>3</sub> and precipitated into methanol (2 mL) to yield a yellowish oil after drying under vacuum. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.14 (t, 6H, J = 7.1 Hz), 1.40 (m, 4H), 1.54 (m, 4H), 2.60 (t, 4H, J = 7.4 Hz), 2.90 (q, 4H, J = 7.1 Hz), 3.05 (s, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$ 13.99, 27.92, 28.91, 34.27, 53.23, 56.52.

### Polymer from entry 4 in Table 1

(2,2'-Oxydiethenthio)disuccinimide (0.625 g, 1.77 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was reacted with *N*,*N*'-diethylethylenediamine (0.21 g, 1.77 mmol) following the previous procedure. After evaporating the solvent, the polymer was precipitated into methanol (10 mL) and washed with methanol (15 mL). The polymer was dried under vacuum to yield a yellowish oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.14 (t, 6H, J = 6.9 Hz), 2.77 (t, 4H, J = 7.1 Hz), 2.90 (q, 4H, J = 7.0 Hz), 3.04 (s, 4H), 3.65 (t, 4H, J = 7.2 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.27, 35.14, 53.66, 56.79, 69.98.

#### Polymer from entry 5 in Table 1

N,N'-(Hexanethio)disuccinimide (1 g, 2.90 mmol) in CHCl<sub>3</sub> (6 mL) was reacted with N,N'-dimethyl-1,6-hexanediamine (0.42 g, 2.90 mmol) following the previous procedure. The polymer was precipitated into methanol (10 mL). The polymer was redissolved in a minimal amount of CHCl<sub>3</sub> and precipitated into methanol (7 mL) to yield a yellowish oil after drying under vacuum. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.31 (m, 4H), 1.41 (m, 4H), 1.55 (m, 8H), 2.64 (t, 4H, J = 7.4), 2.76 (m, 10H). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  26.87, 28.18, 28.40, 28.89, 31.68, 47.12, 59.87.

#### Polymer from a disulfenamide (Figure 3)

N,N'-Ethylmethyl-bis(2-mercaptoethyl) disulfenamide (1.34 g, 5.32 mmol) was reacted with N,N'-diethylethylenediamine (0.62 g, 5.32 mmol) in refluxing benzene (5 mL) for 24 h. After evaporating the solvent, the polymer was precipitated into methanol (2 × 15 mL). The polymer was dried under vacuum to yield a yellowish oil (0.12 g).  $^{1}$ H NMR (CDCl<sub>3</sub>):  $\delta$  1.14 (t, 6H, J = 6.9 Hz), 2.77 (t, 4H, J = 7.1 Hz), 2.90 (q, 4H, J = 7.0 Hz), 3.04 (s, 4H), 3.65 (t, 4H, J = 7.2 Hz).  $^{13}$ C NMR (CDCl<sub>3</sub>):  $\delta$  14.27, 35.14, 53.66, 56.79, 69.98.

### Preparation of microparticles

Blank microparticles were prepared by oil-in-water (o/w) single emulsion, solvent evaporation technique. Briefly, 300 mg of polymer was dissolved in 3.5 ml of chloroform in a heated water bath (55 °C). This organic phase was then emulsified with 35 ml of 1% (w/v) mowiol® at 13,500 rpm for 1 min using an IKA Ultra-Turrax T25 basic homogenizer (IKA, Wilmington, NC). The mixture was stirred overnight to evaporate the organic phase. The microparticles were then washed three times with deionized water (5000 rpm for 10 min) and lyophilized (Labconco FreeZone 4.5, Kansas City, MI). Microparticles were stored at -20 °C until use.

### Surface morphology

Microparticle morphology was assessed by scanning electron microscopy (SEM, Hitachi S-4000). Air dried microparticles were placed on silicon wafers mounted on SEM specimen stubs. The stubs were coated with ~5 nm of gold by ion beam evaporation followed by imaging using SEM operated at 3 kV accelerating voltage.

### Particle size and zeta potential

Microparticle size and zeta potential was measured using the Zetasizer Nano ZS (Malvern, Southborough, MA). The microparticles were suspended in deionized water at a concentration of 1 mg ml<sup>-1</sup>. The zeta potential was measured using folded capillary cell in automatic mode and the size was measured using a disposable sizing cuvette (DTS0012). The size was measured at 25 °C at a 173° scattering angle.

### In vitro analysis of microparticle degradation

For *in vitro* analysis, ~85 mg of the microparticles was suspended in either phosphate buffer saline (PBS, pH 7.4) or acetate buffer (pH 4). The samples were agitated at 37 °C, 150 rpm in a horizontal shaker (C24 incubator shaker, New Brunswick Scientific, Edison, NJ). At specific time points, the samples were centrifuged for 10 min at 7000 rpm. The pellet was washed three times with deionized water and lyophilized. The dry weight of the pellet was recorded and percentage weight loss was calculated for each sample. In addition, the samples were also analyzed by scanning electron microscopy to observe change in surface morphology of microparticles.

#### Particle uptake

Uptake of rhodamine labeled microparticles was studied in JAWSII cells and HEK293 cells. Chambers were initially coated with 300  $\mu$ l of poly L-lysine (0.1% w/v) overnight. Following coating, ~ 1 × 10<sup>4</sup> cells were seeded per well and incubated overnight at 37 °C in a humidified 5% CO<sub>2</sub>-containing atmosphere. Rhodamine labeled particles were added to the media at a concentration of 50 micrograms per well. The particles were incubated with the cells for ~ 18 h. Cells were washed three times with sterile PBS and fixed with 4% paraformaldehyde. The nucleus was stained with DAPI. Vectashield mounting medium was added onto slide and sealed with coverslip. The samples were imaged using LSM710 confocal microscope (Carl Zeiss MicroImaging).

## **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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HS 
$$O$$
 SH  $O$  CIS  $O$  SCI  $O$  SCI  $O$  SCI  $O$  Molecule A  $O$  Molecule A

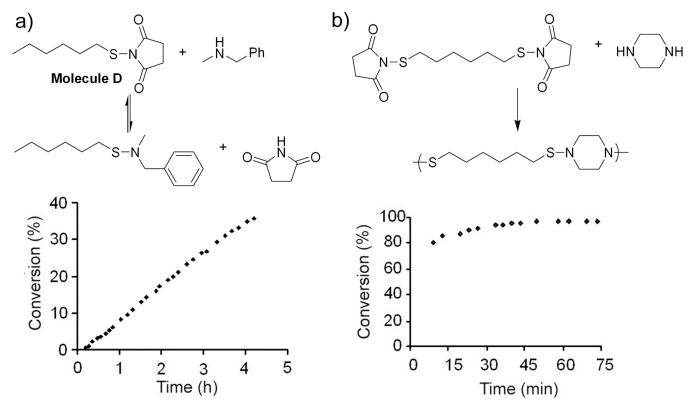
**Figure 1.** Two different disulfenamides were synthesized in one pot from dithiols.

**Figure 2.** The kinetics of the transamination reaction in a) was studied by <sup>1</sup>H NMR spectroscopy in CD<sub>3</sub>OD as shown in b). The conversion in b) refers to the conversion of molecule B to the indicated product. The NMR tube was sealed to prevent the release of *N*-ethylmethylamine.

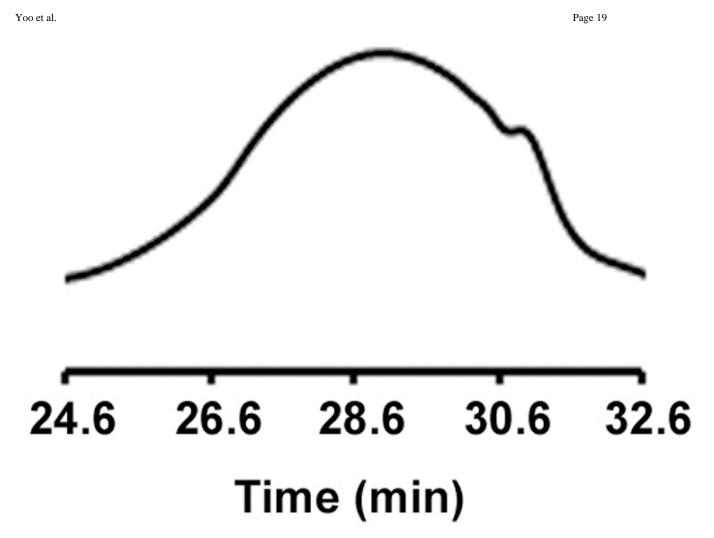
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**Figure 3.** The polymerization of a disulfenamide with a secondary diamine in benzene yielded a low molecular weight polymer.

**Figure 4.** Dithiosuccinimides were readily synthesized from *N*-chlorosuccinimide and triethylamine.



**Figure 5.**a) The reaction of a thiosuccinimide with a secondary amine in CD<sub>2</sub>Cl<sub>2</sub> is shown. The yield of the reaction was >97% after extended reaction times. b) The kinetics of a typical polymerization to demonstrate that the polymerizations were rapid and went to quantitative conversions at reasonable concentrations. The starting materials in b) were three times more concentrated than the starting materials in a).



**Figure 6.** The SEC micrograph of the polymer from entry 1 in Table 1.

**Figure 7.**The stability of molecule E was studied under three different conditions.

Figure 8.

a) The first degradation product of the decomposition of a sulfenamide in D<sub>2</sub>O was molecule F. This molecule further decomposed to yield molecules G and H. Molecules F, G, and H were observed by <sup>1</sup>H NMR spectroscopy. Molecule I and hexanethiol were not observed but were predicted by literature precedent. b) Sulfenic acids were the first product of degradation of molecule E but they were known to rapidly react to yield thiosulfinates as shown. c) The structure of allicin.

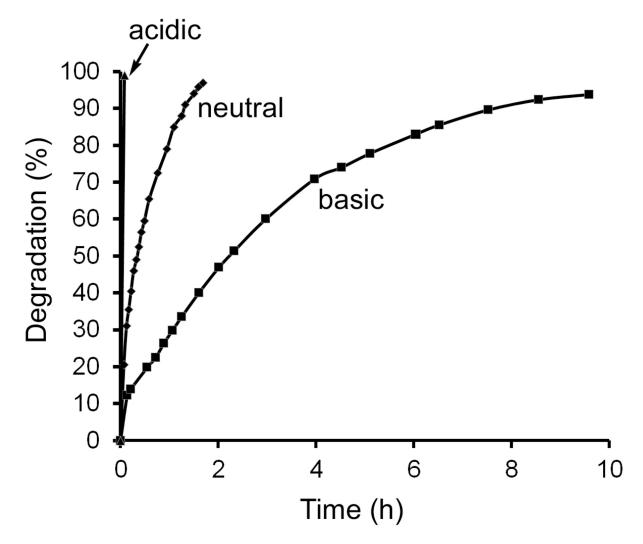
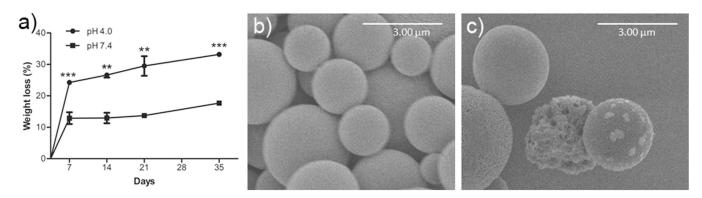


Figure 9. The kinetics of decomposition of a sulfenamide was studied by  $^1H$  NMR spectroscopy in  $D_2O$  with either nine molar equivalents of NaOH, eight molar equivalents of acetic acid, or no added acid or base.



**Figure 10.** Microparticles with the structure of entry 2 in Table 1 were studied for their degradation and surface reactions. a) Time dependent weight loss of the polymer incubated at acidic pH (4.0) and physiological pH (7.4). Data represents the percentage weight loss as mean of three samples. b) Scanning electron microscopy (SEM) image of microparticles as prepared. c) SEM image of microparticles after incubation in acetate buffer (pH 4.0) for 14 days. Microparticles at various stages of degradation can be observed.

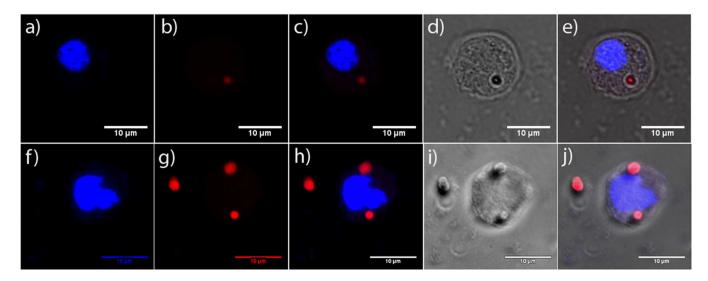
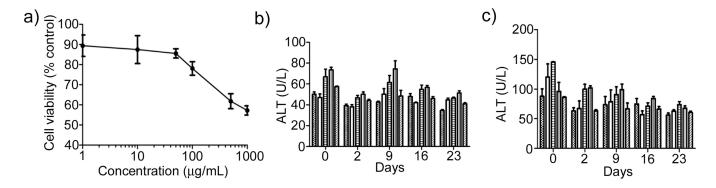


Figure 11.

Uptake of microparticles fabricated from entry 2 in Table 1 and loaded with rhodamine and studied using confocal microscopy. a) – e) The cells are JAWSII immature dendritic cells and in f)–j) they are HEK293 cells. The nucleus of the cell was stained with DAPI to appear blue and the rhodamine appeared red. In d) and i) the transmitted light images indicate the periphery of the cells and the location of the microparticles. In e) and e) merged images clearly outline the periphery of the cell, the location of the nucleus, and the microparticles to clearly demonstrate that the microparticles were found on the interior of the cells. The images were taken at different sections to ensure that the microparticles were in the same focal plane as the planes taken through the cells. The microparticle in the far left of images e0–j) was not found in a cell and was used to show a free microparticle.



**Figure 12.**a) Cytotoxicity of polysulfenamide microparticles fabricated from entry 2 in Table 1 towards HEK293 cells. Cell viability was determined by MTS assay and the expressed as percentage of control without microparticles. b) and c) These graphs show the *in vivo* ALT and AST levels in BALB/c mice following i.p. injection of microparticles. There are five sets of data for each day in the graphs in b) and c). The data for each day are presented in order of mice injected with PBS buffer, 10 mg of PLGA microparticles, 5 mg of polysulfenamide microparticles, 10 mg of polysulfenamide microparticles, and 15 mg of polysulfenamide microparticles.

**Table 1** Synthesis of polysulfenamides from dithiosuccinimides.

Entry	Composition	$ m M_w$ $(g\ mol^{-1})^a$	Conversion (%)b	PDI
1	(S^O_S-N_N+	3,700	97	1.42
$2^c$	+s~~~N_N+	6,300	98	1.22
3 <sup>c</sup>	(s \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	3,700	97	1.06
4	(s\\^O\\_s\^N\\_N\	2,300	95	1.60
5 <sup>c</sup>	(s) (s) (N) (N)	2,900	96	1.13

aThe absolute  $M_W$  and PDI were measured with multi-angle laser light scattering and refractive index detectors unless otherwise noted.

b The conversion was calculated based on the molecular weight of the polymer and monomers according to the equation Xn = 1/(1-P).

 $<sup>^{\</sup>it C}{\rm The}~{\rm M_W}$  and PDI were measured versus polystyrene standards using a calibration curve.