Intramolecular Cyclization Assistance for Fast Degradation of Ornithine-Based Poly(ester amide)s

Caroline de Gracia Lux,[1] Jason Olejniczak,[2] Nadezda Fomina,[1] Mathieu L. Viger,[1] Adah Almutairi[1,3,4]

[1]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, California 92093
[2]Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92093
[3]Department of NanoEngineering, University of California at San Diego, La Jolla, California 92093
[4]Department of Materials Science and Engineering, University of California at San Diego, La Jolla, California 92093
Correspondence to: A. Almutairi (E-mail: aalmutairi@ucsd.edu)

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ABSTRACT: Inspired by the spontaneous cyclization of ornithine in peptides, polyesters containing protected ornithine (Orn) side chains along the backbone were synthesized and shown to degrade rapidly upon deprotection through intramolecular cyclization. A new ornithine-based poly(ester amide) PEA **1** and a lysine-based control PEA **2,** both bearing the light-sensitive protecting group *o*-nitrobenzyl alcohol (ONB), were synthesized. *Tert*-butyl carbamate (Boc)-protected versions **1-Boc** and **2-Boc** were also synthesized for proof of concept. GPC confirmed that **1-Boc** degrades over 40 times faster than **2-Boc** following deprotection into the designed intramolecular cyclization products. Finally, TEM visualization of particles made from **1** encapsulating iron oxide nanoparticles reveals complete disruption of nanoparticles and release of payload within a day upon UV irradiation. © 2013 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 3783–3790

KEYWORDS: biomaterials; controlled release; depolymerization; intramolecular cyclization; stimuli-sensitive polymers; photochemistry; polyester amide; self-immolative; triggered release

INTRODUCTION Over the past several decades there has been enormous progress in developing novel methods to synthesize and control assembly of polymers, less work has been done on designing novel mechanisms of controlled depolymerization or polymer disassembly. However, this trend is rapidly changing because of current technological needs in both the medical sector and "green" industry, which would be advanced by polymeric materials that can be disassembled in a controlled fashion on demand.[1] Such materials find applications in the electronics industry,[2,3] in patterning,[4] in self-healing[5] and reinforced composites,[6] in tissue engineering and implants,[7] tissue adhesives,[8] drug delivery[9–12] and biosensors.[13,14] Since the development of polyacetals used as photoresists,[3] designs of polymers intended to degrade by particular mechanisms have grown more diverse. Several self-immolative dendrimers and polymers[15] translate cleavage of a triggering group into degradation by intramolecular mechanisms, mainly by 1,6 and/or 1,4 elimination, cyclization-elimination, and hemiacetal elimination.[16–26]

We are interested in expanding the types of polymers that degrade by intramolecular cyclization, including polyesters and poly(ester amide)s. Currently, linear aliphatic polyesters[27] including poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and copolymers (PLGA) and poly(ε-caprolactone) (PCL), are widely used in biomedical applications. Due to their bulk and gradual degradation by hydrolysis of their ester linkages in physiological conditions,[28] they are particularly suitable for long term implants and controlled release applications.[29] However, this slow degradation also limits the range of applications for which they are useful; they could be made to degrade faster by chemical modifications allowing on-demand, stimulus-activated depolymerization.

Among synthetic biodegradable polymers, PEAs recently attracted interest as they combine the properties of amides and ester moieties. PEAs' amide-like properties include thermal stability (thermal stability results from strong intermolecular hydrogen bonding interactions between amide groups), mechanical strength, and potential for conjugation with drugs or bioactive compounds, while their polyester-like properties include degradability and flexibility.[30] PEAs may be functionalized on pendant amine or carboxylic acid functionalities. However, despite these advantages and their

Additional Supporting Information may be found in the online version of this article.

biocompatibility, there are still few reports of PEAs containing reactive side chains.[30–36] In addition, to our knowledge, the degradation process reported so far for such hydrophobic polymeric materials (mainly micelles and films) depends only on hydrolysis, driven by changes in solubility or enzymatic degradation of ester or amide linkages. A recent study using the amino acid 2,4-diaminobutyric acid as a self-immolative small molecule spacer in a PEA-based copolymer asserts backbone degradation by cyclization of pendant amine chains into a five-membered ring at 70 °C over a 2 week period.[37] Our current design of an amine-protected PEA degrades rapidly via lactamization of the polymer itself at physiologically relevant temperatures. Herein we characterize both the chemistry and the degree of degradation.

Ornithine is known to undergo spontaneous lactamization in peptides and activated esters and thus cannot be supported by tRNA synthesis.[38,39] Inspired by this we hypothesized that a related ornithine-based polymer bearing light-sensitive groups would degrade rapidly upon irradiation. As a control, we also synthesized lysine-based polymers; lysine's longer alkyl spacer between the amine and the acid makes it unreactive enough for inclusion in proteins, as the only lactam it could form would contain an unfavorable seven membered ring. This control demonstrates that cyclization occurs only in polymers with appropriately spaced amines and esters.

EXPERIMENTAL

Materials

Unless otherwise noted, all chemicals were obtained from commercial sources and were used without further purification. All reactions were carried out under a nitrogen atmosphere in oven dried glassware unless otherwise noted.

Characterization and Measurements

Flash column chromatography purification was performed using a Teledyne Isco Combiflash Companion with RediSep Rf prepacked silica or C18 columns. Thin layer chromatography was performed with EMD TLC Silica gel 60 F_{254} glass plates. ^{1}H NMR spectra were acquired using a Varian 400 MHz, a Bruker 600 MHz or JEOL 500 MHz NMR spectrometer and ^{13}C NMR spectra were acquired using a Varian NMR spectrometer at 100 MHz or a Bruker 150 MHz NMR spectrometer. Molecular weights were determined by gel permeation chromatography, performed with a Waters e2695 instrument with a series of Styragel HR4 and Styragel HR2 columns in DMF with 0.01% LiBr at 37 °C. The instrument was calibrated with monodisperse polystyrene standards. Irradiation with 350 nm UV light was performed using a Luzchem LZC-ORG photoreactor equipped with 8UV-A lamp (8W maximum intensity). Polymer degradation was monitored by gel permeation chromatography using an Agilent 1100 aqueous system with acetonitrile/phosphate buffer (0.2 M) (8/2). The instrument was calibrated with monodisperse polyethylene glycol standards. Particles were imaged by with a FEI Spirit TEM used at 120 kV. Mass determination and dimer degradation studies were performed with a HPLC-MS Agilent 160 Infinity (binary pump, UV-vis 1260 DAD, 6120 Quadrupole LC/MS ESI source) with a RP-18 column. HRMS measurements were done with an Agilent 6230 ESI-TOF MS.

Polymer Synthesis

Polymers **1** and **2** were synthesized from commercially available Fmoc-Orn-Boc or Fmoc-Lys-Boc in a four-step procedure (Fig. 1). A similar procedure was followed to synthesize the Boc versions; in both cases, monomers were polymerized by polycondensation of a diester amine bearing two functionalized α-amino acids, and a diacid chloride (Scheme S1). Synthesis and characterization of the Boc analogues are reported in the Supporting Information.

Compound 3a. Fmoc-Orn(Boc)OH (2.5 g, 5.5 mmol) was stirred in 5 mL of DCM/TFA mixture (1/1) for 1 h. The solvents were removed under reduced pressure. The residue was dissolved in 100 mL toluene and added to a heterogeneous mixture containing 4,5-dimethoxy-2-nitrobenzyl(4-nitrophenyl)carbonate (2.08 g, 5.5 mmol) and DIEA (20 mL) in 30 mL DMF. The reaction was stirred at room temperature overnight. The solvents were removed and the product was isolated by flash-chromatography on a reverse phase C18 column using a gradient of water/acetonitrile. Yield: 1.850 g (57%).

^{1}H NMR (600 MHz, DMSO-d_6, δ): 7.88 (d, J = 7.8 Hz, 2H; Ar H), 7.72 (d, J = 6.6 Hz, 2H; Ar H), 7.69 (s, 1H, Ar H), 7.61 (d, J = 7.2 Hz, 1H; NH), 7.47 (t, J = 6.0 Hz, 1H; NH), 7.41 (t, J = 7.2 Hz, 2H; Ar H), 7.32 (t, J = 7.2 Hz, 2H; Ar H), 7.17 (s, 1H, Ar H), 5.33 (s, 2H; CH_2), 4.28–4.20 (m, 3H; CH_2, CH), 3.97–3.90 (m, 1H; CH), 3.88 (s, 3H; CH_3), 3.86 (s, 3H; CH_3), 3.01 (dd, J = 12.6 Hz, J = 6.6 Hz, 2H; CH_2), 1.79–1.66 (m, 1H; CH), 1.62–1.55 (m, 1H, CH), 1.54–1.42 (m, 2H; CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 173.9, 156.2, 155.7, 153.4, 147.7, 143.9, 140.8, 139.3, 128.1, 127.7, 127.1, 125.3, 120.1, 110.4, 108.1, 65.7, 62.3, 56.2, 56.1, 55.0, 53.7, 46.7, 28.2, 26.3; HRMS (ESI, m/z): $[M+Na]^+$ calcd $C_{30}H_{31}N_3O_{10}Na$, 616.1902; found, 616.1900.

Compound 4a. 3a (0.4 g, 0.67 mmol), hexanediol (0.026 g, 0.22 mmol) and DMAP (0.027 g, 0.22 mmol) were dissolved in 3.8 mL of DCM and DMF (5/1) under argon atmosphere. A solution of DCC (0.153 g, 0.741 mmol) in 1 mL DCM was added to the reaction mixture dropwise. The reaction mixture was stirred overnight at room temperature. The solvent were removed and the product was purified by silica column using a linear gradient of DCM/methanol (100%/0%—10%/90%). Yield: 0.372 g (87%).

^{1}H NMR (600 MHz, DMSO-d_6, δ): 7.87 (d, J = 7.2 Hz, 4H; Ar H), 7.76 (d, J = 7.8 Hz, 2H; NH), 7.71–7.67 (m, 6H; Ar H), 7.45 (t, J = 5.4 Hz, 2H; NH), 7.39 (t, J = 7.2 Hz, 4H; Ar H), 7.31 (t, J = 7.2 Hz, 4H; Ar H), 7.16 (s, 1H; Ar H), 5.32 (s, 4H; CH_2) 4.28–4.20 (m, 6H; CH_2, CH), 4.00–3.90 (m, 6H; CH_2, CH), 3.87 (s, 6H; CH_3), 3.85 (s, 6H; CH_3), 3.02 (dd, J = 12.6 Hz, J = 6.6 Hz, 4H; CH_2), 1.75–1.67 (m, 2H; CH_2), 1.65–1.56 (m, 2H; CH_2), 1.54–1.41 (m, 8H; CH_2), 1.27–1.19 (m, 4H; CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 172.1, 156.5, 156.0, 155.6, 153.3, 147.7, 143.7, 140.6, 139.3, 127.8, 127.5, 126.9,

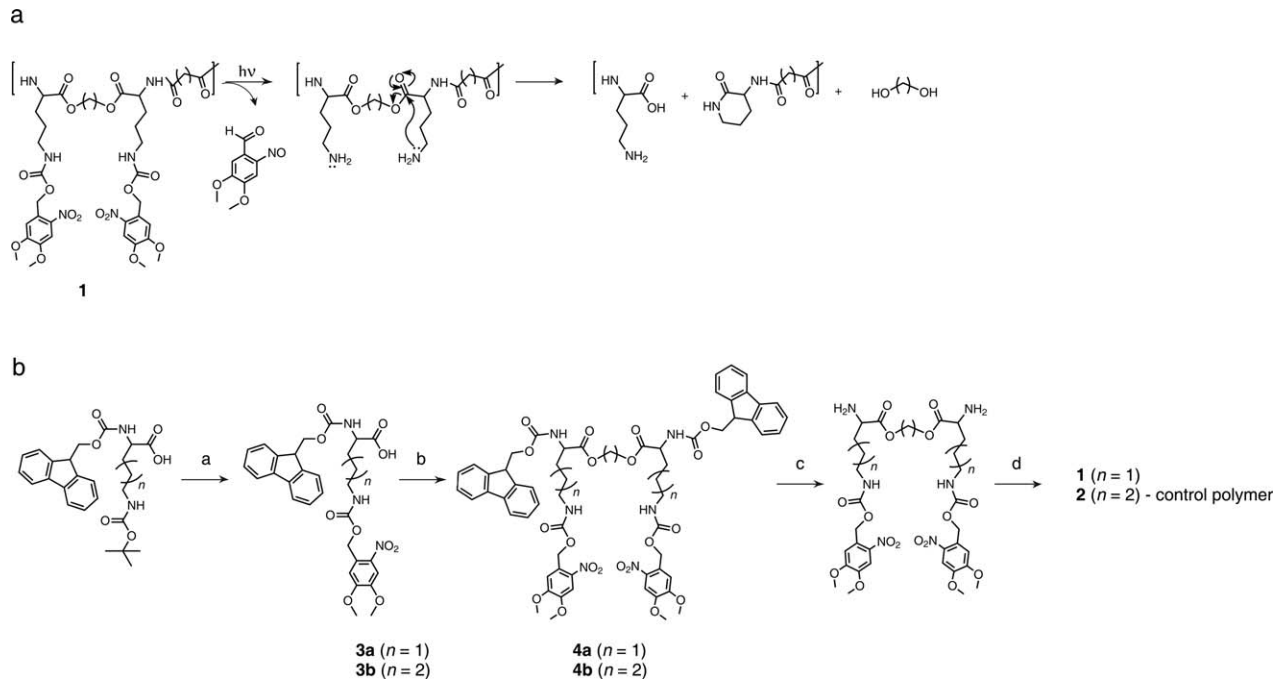

FIGURE 1 (a) Structure and mechanism of light-induced **1** degradation: removal of the light-sensitive groups followed by a combination of amino-assisted ester hydrolysis and aminolysis. (b) Synthesis of polymers **1** and **2**. (a) (i) TFA/DCM (1/1), (ii) 4,5-dimethoxy-2-nitrobenzyl (4-nitrophenyl) carbonate, DIEA, toluene, DMF (**3a,** 57%; **3b,** 78%), b) 1,6-hexanediol, DCC, DMAP, DCM (**4a,** 87%; **4b,** 80%), (c) piperidine, DMF, (d) adipoyl chloride, DCM (**1,** 48%; **2,** 60%). Control polymer **2** does not undergo backbiting cyclizations.

125.0, 119.9, 110.6, 108.2, 66.6, 64.2, 62.1, 56.1, 56.0, 53.7, 47.4, 46.6, 33.2, 27.8, 24.7, 24.3; HRMS (ESI, *m/z*): $[M+Na]^+$ calcd $C_{66}H_{72}N_6O_{20}Na$, 1291.4594; found, 1291.4685.

Polymer 1. **4a** (0.38 g, 0.299 mmol) was deprotected in 1.2 mL piperidine/DMF (5/95). The solvents were removed under reduced pressure. The residue was dissolved in 1.5 mL DCM under nitrogen atmosphere and pyridine (0.145 mL, 1.79 mmol) was added. Adipoyl chloride (0.043 mL, 0.299 mmol) was added to the reaction mixture dropwise. The reaction was stirred at room temperature overnight. The polymer was isolated by precipitation in 20 mL of chilled methanol. The low molecular weight fraction was removed by precipitating the polymer into methanol from DCM three times. Yield: 0.134 g (48%). Molecular weight (relative to polystyrene standards): M_w = 37 500 Da (PDI = 1.4).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.13 (d, J = 7.0 Hz, 2H; NH), 7.66 (s, 2H; Ar H), 7.45 (d, J = 6.5 Hz, 2H; NH), 7.14 (s, 2H; Ar H), 5.27 (s, 4H; CH_2), 4.18 (dd, J = 13.5 Hz, J = 8.0 Hz, 2H; CH), 4.02–3.92 (m, 4H; CH_2), 3.87 (s, 6H; CH_3), 3.84 (s, 6H; CH_3), 3.00 (d, J = 5.5 Hz, 4H; CH_2), 2.1 (s, 4H; CH_2), 1.71–1.63 (m, 2H; CH_2), 1.56-1.45 (m, 14H, CH_2), 1.25 (s, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 172.4, 172.3, 172.2, 155.7, 153.3, 147.7, 139.3, 139.2, 128.0, 110.4, 108.1, 108.1, 64.2, 62.3, 56.0, 56.2, 51.7, 34.7, 28.3, 28.0, 26.0, 24.9 ppm.

Compound 3b. Fmoc-Lys(Boc)OH (1.56 g, 3.34 mmol) was stirred in 4 mL of DCM/TFA mixture (1/1) for 1 h. The solvents were removed under reduced pressure. The residue was dissolved in 60 mL toluene and added to a heterogeneous mixture containing 4,5-dimethoxy-2-nitrobenzyl(4-nitrophenyl)carbonate (1.26 g, 3.34 mmol) and DIEA (12 mL) in 20 mL DMF. The reaction was stirred at room temperature overnight. The solvents were removed and the product was isolated by flash-chromatography on a reverse phase C18 column using a gradient of water/ acetonitrile. Yield: 1.590 g (78%).

^{1}H NMR (600 MHz, DMSO-d_6, δ): 7.88 (d, J = 7.8 Hz, 2H; Ar H), 7.72 (d, J = 6.6 Hz, 2H; Ar H), 7.69 (s, 1H, Ar H), 7.61 (d, J = 7.2 Hz, 1H; NH), 7.47 (t, J = 6.0 Hz, 1H; NH), 7.41 (t, J = 7.2 Hz, 2H; Ar H), 7.32 (t, J = 7.2 Hz, 2H; Ar H), 7.17 (s, 1H, Ar H), 5.32 (s, 2H; CH_2), 4.27–4.21 (m, 3H; CH_2, CH), 3.90–3.88 (m, 1H; CH), 3.88 (s, 3H; CH_3), 3.86 (s, 3H; CH_3), 3.01 (dd, J = 12.6 Hz, J = 6.6 Hz, 2H; CH_2), 1.70–1.61 (m, 2H; CH_2), 1.42–1.34 (m, 4H; CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 173.8, 156.1, 155.6, 153.3, 147.7, 143.8, 140.7, 139.3, 127.9, 127.6, 127.0, 125.2, 120.1, 110.5, 108.1, 65.5, 62.2, 56.2, 56.1, 53.8, 46.6, 40.1, 30.5, 28.9, 22.8; HRMS (ESI, *m/z*): $[M-H]^-$ calcd $C_{31}H_{33}N_3O_{10}$, 606.22; found, 606.3.

Compound 4b. 3b (1.36 g, 2.24 mmol), hexanediol (0.088 g, 0.75 mmol) and DMAP (0.136 g, 10 %) were dissolved in 17 mL of DCM and DMF (7/1) under argon atmosphere. A solution of DCC (0.508 g, 2.46 mmol) in 3 mL DCM was added to the reaction mixture dropwise. The reaction mixture was stirred overnight at room temperature. The solvent were removed and the product was purified by silica column using a linear gradient of hexane/ethyl acetate (100%/0%—0%/100%). Yield: 0.790 g (80%).

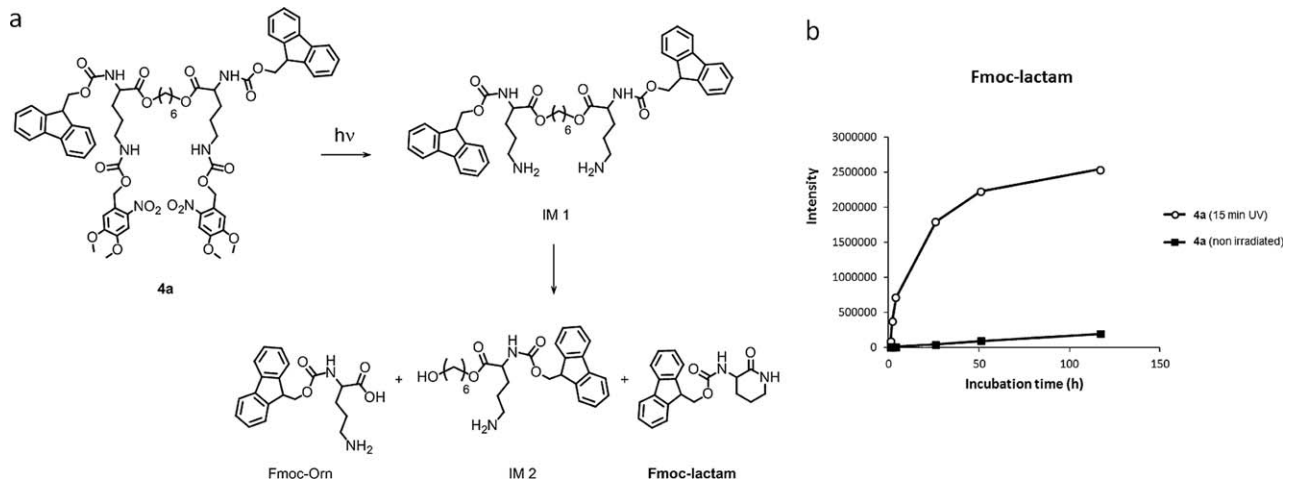

FIGURE 2 (a) Products formed by photolysis (15 min irradiation, 350 nm) of the light-cleavable monomer **4a** (n = 1) used in the synthesis of **1** in acetonitrile/phosphate buffer pH 7.4 (1/2). (b) Integration of the Fmoc-lactam product HPLC-MS peak of ornithine-based dimers as a function of incubation time, either unirradiated (red) or after 15 min UV irradiation (blue).

^{1}H NMR (600 MHz, DMSO-d_6, δ): 7.87 (d, J = 7.2 Hz, 4H; Ar H), 7.75 (d, J = 7.8 Hz, 2H; NH), 7.71–7.67 (m, 6H; Ar H), 7.45 (t, J = 5.4 Hz, 2H; NH), 7.39 (t, J = 7.2 Hz, 4H; Ar H), 7.31 (t, J = 7.2 Hz, 4H; Ar H), 7.16 (s, 2H; Ar H), 5.31 (s, 4H; CH_2) 4.27–4.20 (m, 6H; CH_2, CH), 4.00–3.91 (m, 6H; CH_2, CH), 3.87 (s, 6H; CH_3), 3.85 (s, 6H; CH_3), 3.00 (s, 4H; CH_2), 1.66–1.61 (m, 4H; CH_2), 1.48–1.24 (m, 16H; CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 172.4, 156.1, 155.6, 153.3, 143.8, 143.7, 140.7, 139.3, 127.9, 127.8, 127.0, 120.1, 110.5, 108.1, 99.5, 65.6, 64.2, 62.2, 59.8, 56.1, 56.0, 46.6, 30.3, 28.9, 27.9, 24.8, 22.8, 20.8.

Polymer 2. 4b (0.307 g, 0.237 mmol) was deprotected in 1.2 mL piperidine/DMF (5/95). The solvents were removed under reduced pressure. The residue was dissolved in 1.5 mL DCM under nitrogen atmosphere and pyridine (0.115 mL, 1.42 mmol) was added. Adipoyl chloride (0.035 mL, 0.237 mmol) was added to the reaction mixture dropwise. The reaction was stirred at room temperature overnight. The polymer was isolated by precipitation in 20 mL of chilled methanol. Low molecular weight fraction was removed by precipitating the polymer into methanol from DCM three times. Yield: 0.144 g (60%). Molecular weight (relative to polystyrene standards): M_w = 41 500 Da (PDI = 1.7).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.10 (d, J = 7.0 Hz, 2H; NH), 7.65 (s, 2H; Ar H), 7.42 (d, J = 6.5 Hz, 2H; NH), 7.14 (s, 2H; Ar H), 5.27 (s, 4H; CH_2), 4.19–4.10 (m, 2H; CH), 4.04–3.92 (m, 4H; CH_2), 3.87 (s, 6H; CH_3), 3.84 (s, 6H; CH_3), 3.04–2.93 (m, 4H; CH_2), 2.09 (s, 4H; CH_2), 1.64–1.26 (m, 24H; CH_2).

RESULTS AND DISCUSSION

Design and Strategy

Here, we report a new light-sensitive polymer: PEAs based on ornithine (PEA-Orn **1**) and lysine amino acids (PEA-Lys **2**). We synthesized both light-sensitive versions (**1, 2**), in which amine groups were protected with *o*-nitrobenzyl groups, and model Boc-protected polymers (**1-Boc, 2-Boc**) to study the mechanism of degradation. Light-sensitive polymer **1** was formulated into nanoparticles encapsulating model payloads to examine the kinetics of release. Polymer **2** serves as a control; only **1** should degrade by lactamization because the formation of a 6-membered ring is favorable while seven-membered rings have greater ring strain and thus are unfavorable. Accordingly, ornithine undergoes spontaneous cyclization, and thus cannot be supported on tRNA.[40,41]

Only Ornithine-Based Dimers 4a Degrade by Lactamization

Prior to investigation of polymer degradation, lysine- and ornithine-based dimers were exposed to UV light to test our hypothesis concerning the formation of lactam derivatives (Fig. 2). The presence of four different intermediates was confirmed over time by integrating the HPLC-MS peaks of the single ions (m/z = 791 (for IM 1, [M+H], z = 1), m/z = 455 (for IM 2, [M+H], z = 1), m/z = 355 [for Fmoc-Orn, [M+H], z = 1), and m/z = 359 (for Fmoc-lactam, [M+H+Na], z = 1) respectively]. All HPLC-MS graphs for both ornithine-based dimers (**4a**) and lysine-based dimers (**4b**) are presented in Supporting Information Figures S1–S2. The lactam derivative was formed from ornithine-based dimers and not from lysine-based dimers (Fig. S2, Supporting Information). Only ornithine-based dimers (4a) degrade by lactamization (Fig. 2). Non-irradiated dimers did not degrade.

Mechanism of Polymer Degradation

Our strategy for the degradation of these new polymers is not to rely only on protonation of pendant amine groups to increase hydrophilicity and facilitate hydrolysis of the polymer, but to tune the chemical structure of the materials to enable additional, rapid mechanisms of degradation. The length of the alkyl spacer between the neighboring protected amine and the polymer backbone dictates the possible mechanism and rate of degradation. Degradation of polymers **1**

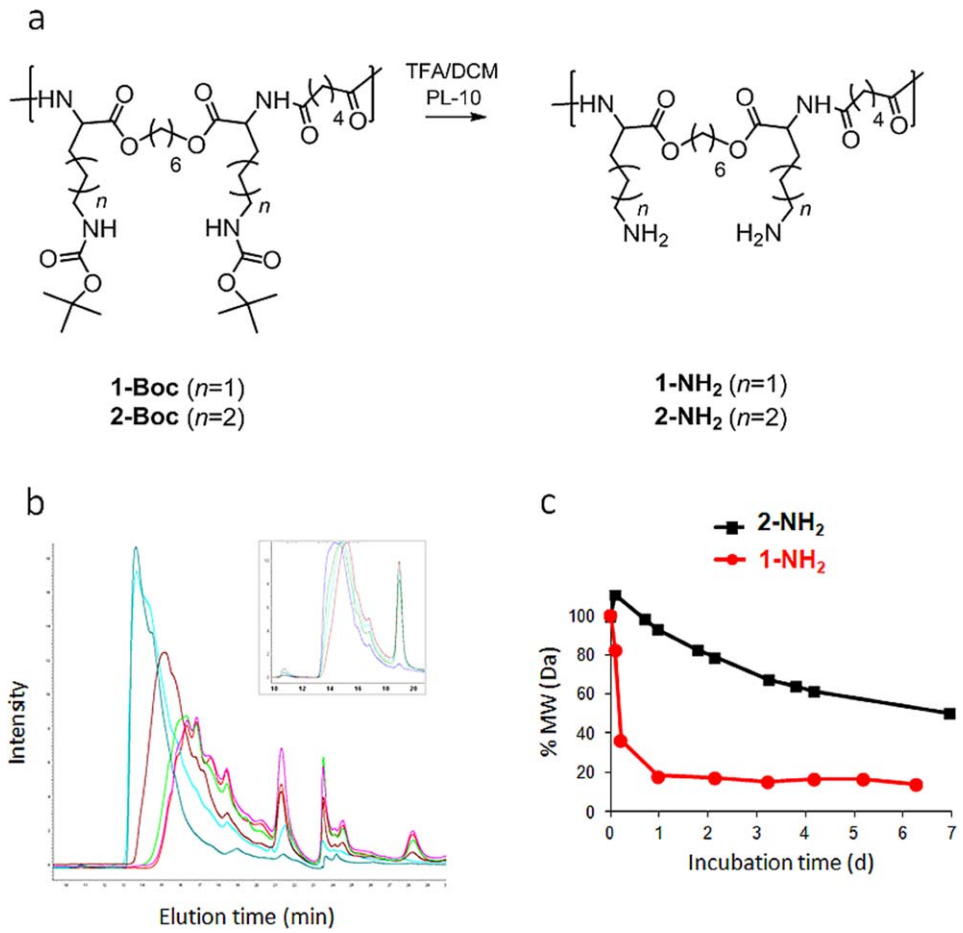

FIGURE 3 (a) Deprotection procedure to unmask the amine: exposure to TFA/DCM (1:1) mixture and purification on a PL-10 desalting column. (b) GPC traces of **1-NH$_2$** (main graph) and **2-NH$_2$** (inset) after 0, 2, 5, 24, 50 and 150 h of incubation at pH 7.4. (c) Loss of MW (relative to PEO standards) over time.

upon deprotection of the amine moieties includes intramolecular general base catalysis, general acid-specific base catalysis (assisted by intramolecular H-bonding), and intramolecular nucleophilic cyclization[40,41] (Fig. 1), while degradation of polymer **2** does not involve intramolecular cyclization.

Ornithine-Based Polymer 1-Boc, and Not Lysine-Based Polymer 2-Boc, Degrades Rapidly

To determine whether lactamization affects the polymer degradation rate, we first monitored the degradation of Boc-deprotected polymers (**1-Boc** and **2-Boc**) at pH 6.0 and 7.4 by GPC (Fig. 3). As expected, degradation was faster at neutral versus slightly acidic pH, as both lactamization (intramolecular aminolysis) and NH_2-catalyzed hydrolysis are more favorable at pH 7.4. Boc-protected rather than light-sensitive polymers were chosen for proof of concept because of the ease of complete removal of Boc protecting groups throughout the backbone, eliminating the effect of deprotection efficiency. At pH 7.4, $t_{1/2}$ (**2**-NH_2) $\approx$ 140 h, while $t_{1/2}$ (**1**-NH_2) $\approx$ 3 h, revealing that lactamization allows faster degradation than ester hydrolysis. Similar experiments at pH 6.0 show that $t_{1/2}$ (**1-Boc**) $\approx$ 150 h, while $t_{1/2}$ (**2-Boc**) is estimated at $\approx$150 days. After Boc deprotection, **2** is much more stable than **1** at both pH values, so lysine-based polymers can be considered control polymers regardless of the protecting group. A solution of **1-Boc** in 0.2 M phosphate buffer/acetonitrile (3.5/6.5) was also incubated at 37 °C without being deprotected. Unfortunately, 1-Boc is not soluble in aqueous solution and needs a mixture of organic and aqueous solvents to be dissolved. However, aliquots removed periodically did not show any loss in molecular weight, thus validating the controlled intramolecular depolymerization mechanism (Fig. S3a–b, Supporting Information).

1-Boc, and Not 2-Boc, Degrades by Lactamization

Depolymerization of **1-Boc** and **2-Boc** was also observed by NMR in deuterium phosphate buffer (0.2 M) at pH 7.4. NMR spectra confirmed degradation rates and the identity of the by-products (Fig. 4). New chemical shifts appear around 4.2 and 3.3 ppm following irradiation of **1,** but not of **2,** indicating the formation of lactam derivatives only for **1**. The chemical shift at 3.5 ppm, found for both **1** and **2,** indicates cleavage of ester bonds to form alcohols (ester hydrolysis). After 96 h, almost 80% of the amine and ester functions of polymer **1** undergo lactamization and hydrolysis,

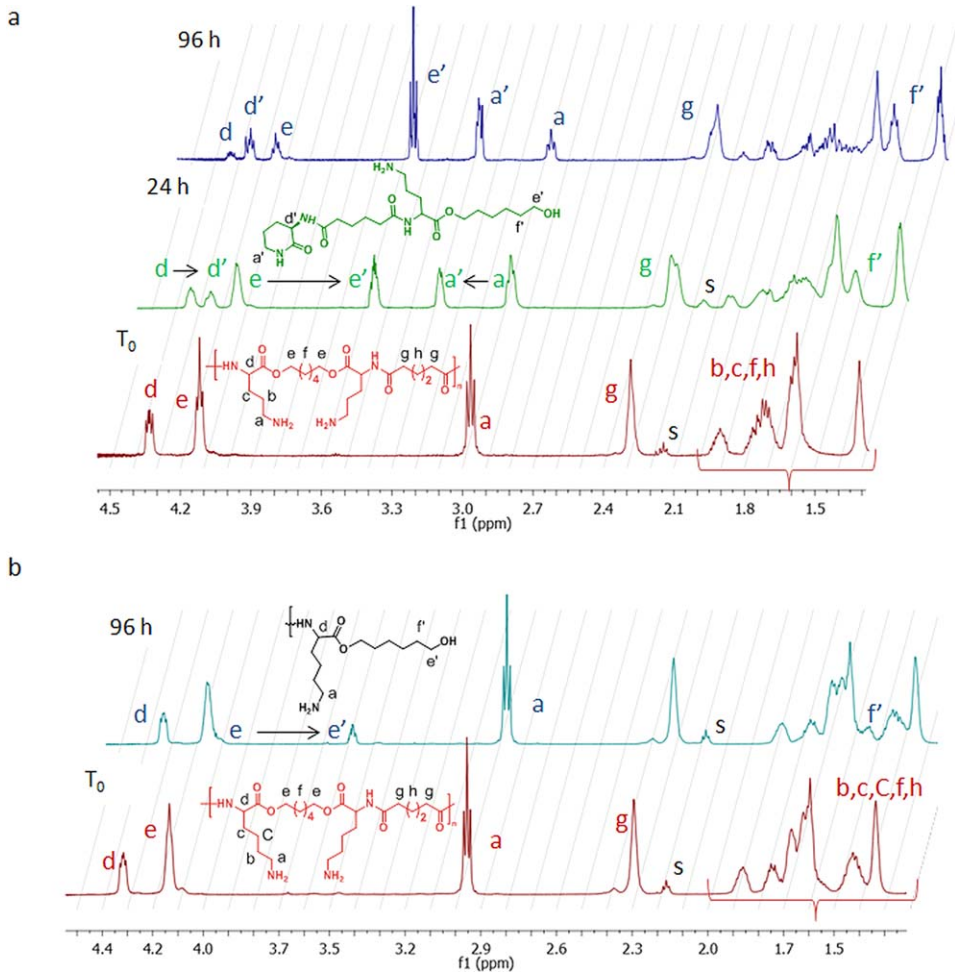

FIGURE 4 ^{1}H NMR spectra of deprotected polymer **1-Boc** (a) and **2-Boc** (b) in deuterium phosphate buffer (0.2 M) at pH 7.4, as a function of incubation time (37 °C).

respectively. For the same incubation time, hydrolysis of **2** does not exceed 15%. Complementary MALDI (Fig. S4, Supporting Information for **1**-NH_2 at pH 7.4 after 24 h of incubation) validated that the by-products obtained when the polymer is partially degraded were the expected lactam. However, no degradation products were detected for **2**-NH_2, suggesting that 24 h incubation was not sufficient to form any significant amount of low molecular weight oligomers.

Nanoparticles of Polymer 1 Fall Apart Upon Irradiation; Remain Intact While Unirradiated

We formulated particles from polymer **1** encapsulating 10 nm super paramagnetic iron oxide (Fe_3O_4) nanoparticles (SPIONs) by electrospray as **1** did not form nanoparticles by single emulsion because of its low solubility in organic solvent. SPIONs were chosen as payload to allow tracking of release by TEM. This formulation method employs high voltage to inject charge into DMF/$CHCl_3$ solution containing polymer **1** and SPIONs, which causes the liquid to break into a jet of very fine aerosol nanodroplets propelled toward the glass slide collector. Dense, solid particles are generated as the solvent evaporates in flight. Optimal encapsulation efficiency obtained by this technique was confirmed by TEM: no SPIONs remain unencapsulated.

Electrosprayed particles formulated from polymer **1** encapsulating SPIONs were characterized by transmission electron microscopy (TEM) upon degradation (Fig. 5). Unirradiated particles or particles irradiated for 5 min with 350 nm UV light were examined after 1 day incubation at 37 °C in dilute triethylamine/water solution (pH 8.0; used instead of PBS to minimize washing necessary to remove phosphate salts). A disruption in the morphology of the nanoparticles is observed by TEM after irradiation.

Irradiated particles appear only as free SPIONs and chunks of aggregated material [Fig. 5(b) and Fig. S6, Supporting Information). In contrast, when samples are not irradiated, no evidence of degradation nor aggregation is observed; only intact particles are visible [Fig. 5(a) and Fig. S5, Supporting Information). In parallel, particles were also examined after 15 min irradiation and 1 or 4 days incubation in order to mimic the NMR degradation study. Similar results were obtained concerning both integrity and degradation (Figs. S7–S8, Supporting Information). Complete particle

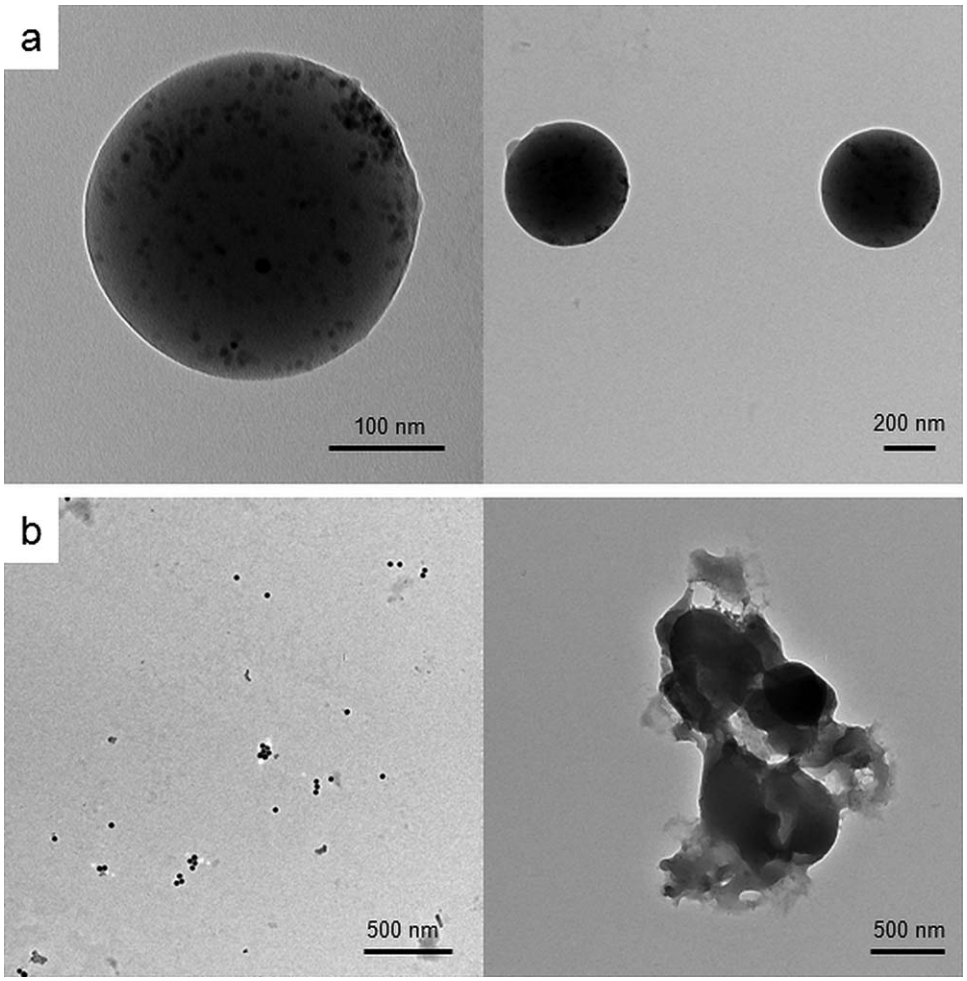

FIGURE 5 Representative TEM images of nanoparticles; either unirradiated (a) or irradiated for 5 min with 350 nm UV light (b) after 1 day incubation at 37 °C in dilute triethylamine/ water solution (pH 8.0).

degradation within 1 day, much faster than the 4 days required to observe 80% of lactam derivatives by NMR, implies that the carrier is falling apart by other processes in addition to cyclization and hydrolysis. This is not surprising; the huge change of hydrophilicity upon removal of the photolabile group is likely to contribute to particle breakdown.

CONCLUSIONS

A novel stimuli-responsive PEA containing protected ornithine self-immolative side chains was synthesized and employed to prepare nanoparticles that degrade upon irradiation. ^{1}H NMR and GPC reveal the degree of degradation through intramolecular cyclization forming lactam derivatives. As expected, once formulated into light-sensitive nanoparticles, both degradation and release occur only upon irradiation, with a synergistic effect of cyclization and solubility switch. Our polymer degrades completely and rapidly at physiologically relevant temperatures. This is in contrast to recently published studies on similar polymers that require heating to 70 °C for a period of 2 weeks to degrade.[37] This difference stems from our polymer design that allows each monomer to be degradable on deprotection. Furthermore, these polymers switch solubility in aqueous solution, which speeds cyclization reactions as observed in the faster degradation at physiologically relevant temperatures. This work helps open up new possibilities for rapid release upon triggered degradation of poly(ester amide)s.

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