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ARTICLE

RAFT polymerization of ciprofloxacin prodrug monomers for the controlled intracellular delivery of antibiotics

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Debobrato Das[a], Selvi Srinivasan[a], Abby M. Kelly[a], David Y. Chiu[a], Bridget K. Daugherty[b], Daniel M. Ratner[a], Patrick S. Stayton[a], Anthony J. Convertine[a*]*

Prodrug monomers derived from the antibiotic ciprofloxacin were synthesized with phenolic or aliphatic esters linking the drug to a polymerizable methacrylate group. RAFT polymerization of these monomers exhibited linear pseudo-first-order kinetics and M_n vs. conversion plots, and low Đ values throughout the polymerization. Prodrug monomers were then copolymerized with polyethyleneglycol methacrylate to yield hydrophilic copolymers with narrow Đ values. A poly(O950) macroCTA was also synthesized and chain extended with the antibiotic monomers to form diblock copolymers. The resultant copolymers and diblock copolymers were characterized with ^{1}H and ^{19}F NMR and found to contain 16.5 and 30-35 wt.% ciprofloxacin, respectively. DLS measurements demonstrated that the copolymers remained unimeric between pH 5.6-7.4, while the diblock copolymers formed nanoparticles with diameters between 30-40 nm at physiological pH. Drug release kinetics were measured in human serum via HPLC analysis. Copolymers containing ciprofloxacin linked via phenolic esters showed faster hydrolysis rates with 50% drug released at 120h, whereas copolymers with the corresponding aliphatic ester linkages showed the same drug release over 22 d. Diblock copolymers with a discrete ciprofloxacin block and a poly(O950) stabilizing block self-assembled into micelles, and exhibited reduced hydrolysis rates for both ester linked drugs. *In vitro* toxicity measurements in RAW 264.7 cells showed the copolymers to be nontoxic up to 20 mg/mL following a 24 h incubation period. The polymer drugs were shown to be active against *Burkholderia thailandensis* in a bacteria-macrophage co-culture model of melioidosis with MIC values of 6.0 and 0.6 mM for the aliphatic and phenyl ester linked copolymeric prodrugs, respectively.

A Introduction

Respiratory melioidosis is an infectious disease found worldwide that is caused by the bacterium *Burkholderia pseudomallei*. Clinically, this disease presents as localized lung ulcerations with abscess formations and if left untreated can progress and disseminate into the bloodstream affecting all major organ systems. Mortality rates for these severe infections can be as high as 40% even with optimal antibiotic treatment.[4] Traditional therapies for acute melioidosis is limited by low therapeutic efficacy defined by a 10% infection recurrence rate, poor drug biodistribution, unfavorable pharmacokinetics, and systemic toxicity.[5-7] A prodrug pulmonary delivery vehicle could enable controlled antibiotic release at the target site of alveolar macrophage populations, and could help to lower dosing, decrease toxicity, and minimize bacterial resistance.[8] Various pharmaceutical carriers are currently being developed to increase the stability and pharmacokinetic properties of administered drugs (*e.g.* liposomal formulations of antibiotics).[9-12] For example, Wong et al. established that ciprofloxacin (Cipro) loaded liposomes administered via IV increased drug circulation lifetime, and resulted in enhanced antibacterial activity against *Francisella tularensis*. The liposome carrier also improved delivery to key tissue systems including the liver and spleen. [13]

[a] *Department of Bioengineering, University of Washington, Seattle WA, 98195*
[b] *Department of Biomedical Engineering, Tulane University, New Orleans LA, 70118.*

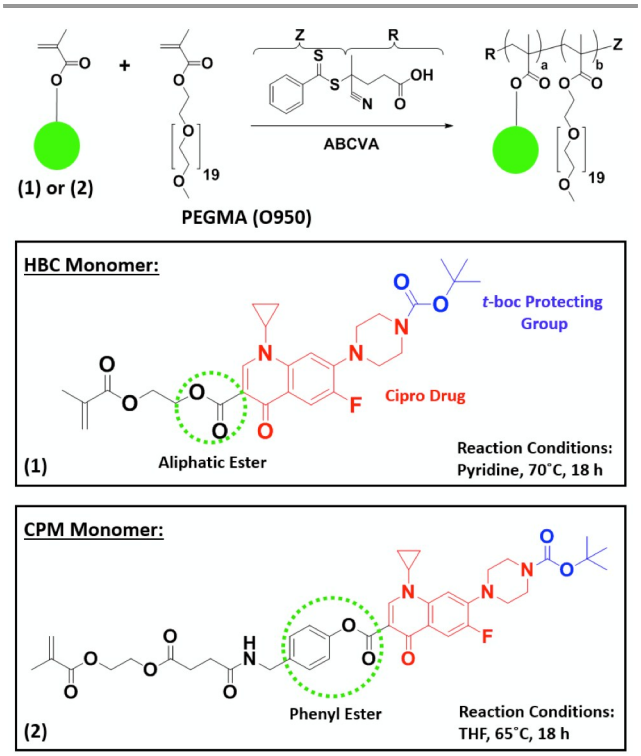
Electronic Supplementary Information (ESI) available: ^{1}H and ^{19}FNMR, mass spec, GPC chromatograms, and % cell viabilities of selected compounds and polymers. See DOI: 10.1039/x0xx00000x

Despite their wide-spread clinical use, liposomal delivery systems can sometimes be limited by lower drug loading efficiencies, rapid release of drug contents, and can require more complex formulation procedures that provide challenges to large-scale manufacturing.[4,7,8,14,15] An alternative to liposomes are polymeric carriers that can have poor drug conjugation efficiencies.[4,8] However, these polymer-drug can incorporate a range of sophisticated linkage chemistries or responsive self-immolation properties enabling the development of disease-specific release strategies.[16,17] A variety of polymer architectures have been evaluated for use as drug delivery scaffolds including linear polymers, hyperbranched structures (e.g. dendrimers and hyperbranched polymers), and polymer brushes.[18-24] With antibiotic drugs, Sanchez et al. prepared a library of linear poly-L-glutamic acid (PGA)-doxycycline conjugates through post-polymerization modifications of PGA carboxyl side chains into ester and amide functionalized drug linkages in efforts to investigate fibril deposits associated to familial amyloid polyneuropathy.[25] The authors demonstrated *in vitro* controlled release of drug from degradable ester modified PGA-drug conjugates with approximately 40% drug release within 16 days compared to the non-releasing amide alternative. Consequently, due to the stability of the amide linker employed, IV administration *in vivo* of the PGA-amide-doxycycline system was capable of having improved biodistribution compared to pure polymer negative controls.

Polymerizable prodrug monomers provide an attractive route by which drug conjugates can be prepared directly without the need for post polymerization conjugation reactions. In this strategy the therapeutic agent is linked to a polymerizable group via a hydrolytic or enzymaticly cleavable linkage. This approach has been employed by Dizman and coworkers to prepare copolymers containing methacrylated PEG and an antibacterial, Norfloxacin using conventional free radical polymerization.[26] The resultant copolymers demonstrated functional efficacy at a polymer concentration of 25 mg/mL in an *in vitro* planktonic setting with a 100% reduction of bacterial load compared to no treatment.

Reversible addition-fragmentation chain transfer (RAFT) polymerization is a versatile controlled living radical polymerization technique that enables the synthesis of polymers with predetermined molecular weights, complex architectures, and low molar mass dipsersities.[27] Moreover, RAFT technique has been employed by other groups to prepare polymeric prodrugs with controlled molecular weights and low heterogeneity.[28] Recently, Smith et al. utilized RAFT to copolymerize macromolecular prodrugs of the broad spectrum antiviral, Ribavirin, with the addition of poly(N-(2-hydroxypropyl) methacrylamide) (HPMA), as a therapeutic alternative against co-infections of HIV and the hepatitis C virus.[29] The authors observed fine control over the size and molecular weight distributions over varied target degree of polymerizations of their HPMA polymeric constructs with a maximum Ribavirin composition of 23 mol. %.

In this paper, we describe the use of RAFT polymerization to produce well-controlled polymeric prodrugs of Cipro, an antibiotic used to treat many Gram negative bacteria, including *Burkholderia pseudomallei*, from monomeric drug precursors. The ability to incorporate Cipro with varied drug linker designs, and in controlled composition and steric relation to other comonomers can result in notable delivery advantages. We demonstrate copolymer and diblock copolymer designs that exhibit high drug loading and interesting mechanisms for controlling drug carrier architecture, solubility, drug release kinetics, and antibacterial efficacy.



Scheme 1. Synthetic strategy for the preparation of (1) poly(O950-*co*-HBC) and (2) poly(O950-*co*-CPM) via RAFT polymerization. The resultant copolymers contain aliphatic (1) and phenyl (2) esters linking the Cipro prodrugs to the polymer backbone. Cleavage of the Boc protecting groups was accomplished by dissolving the copolymers in neat TFA for 2 h at 25 °C.

B Results and discussion

Synthesis of monomer prodrugs HBC and CPM

Synthetic illustration of methacrylate Cipro prodrug monomers HBC and CPM carrying aliphatic and phenyl ester linkages respectively, is given in Scheme S1 (ESI). Using HBC and CPM, the Cipro loading of the polymer prodrug can be controlled precisely by manipulating the molar ratio of the drug monomers during polymerization. Boc Cipro[44] was effectively conjugated to monomers via aliphatic or phenolic hydroxyl group using benzotriazole based uronium coupling reagent HBTU.

Kinetic evaluation of (hydroxyethyl)methacrylate-boc-ciproflxoacin (HBC)

Kinetic studies were conducted with HBC (see inset (1) in Scheme 1) to characterize the polymerization behavior of

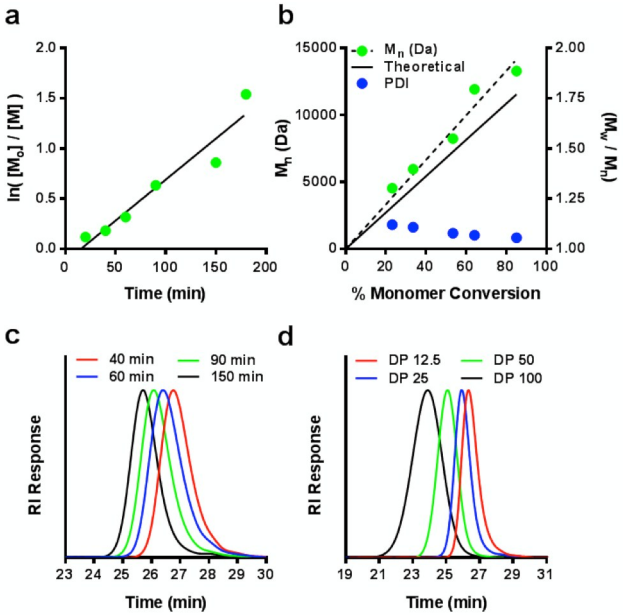


Fig. 1 Kinetic analysis of the RAFT polymerization of HBC. (a) Pseudo first order rate plot, (b) Mn and Đ vs. conversion, and (c) RI traces showing the evolution of molar mass with time for $[M]_o$:$[CTA]_o$:$[I]_o$ equal to 25:1:0.2 at 70 °C with CTP and ABCVA as the chain transfer agent and initiator respectively. (d) Molecular weight distributions for the RAFT polymerization of HBC conducted at initial $[M]_o/[CTA]_o$ ratios of 12.5, 25, 50, and 100 at a fixed initial $[M]_o$ and $[CTA]_o/[I]_o$ ratio 20 wt% and 10 respectively. M_n (Da) and Đ values for the series were determined to be: DP 12.5 (7650/1.09), DP 25 (12000/1.07), DP 50 (26300/1.08), and DP 100 (52400/1.15). Absolute molecular weight values were determined by SEC equipped with inline laser light scattering detectors. Monomer conversion was determined by ^{1}H NMR by comparison of the vinyl resonances normalized to the total ester region relative pre polymerization values.

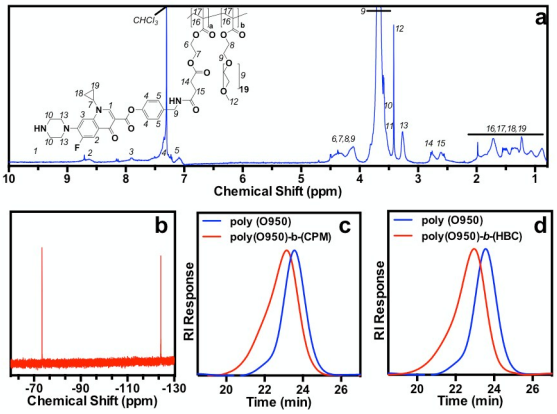


Fig. 2 Representative (a) ^{1}H NMR and (b) ^{19}F NMR spectrum of poly(O950-*co*-CPM) in $CDCl_3$ and C_2D_6SO respectively with assignment of the characteristic resonances associated with the comonomers. Copolymer composition was determined by comparing the CPM (9H) Boc resonances at δ = 1.52 ppm to the O950 (3H) methoxy resonance at δ = 3.4 ppm. ^{19}F NMR was conducted in C_2D_6SO using sodium trifluoroacetate ($C_2F_3NaO_2$) (0.11 μM) as an internal standard. Integration of the Cipro resonance at δ = -124.5 ppm (1F) relative to the internal standard at δ = -73.4 ppm (3F) was used to calculate the final polymer composition. SEC chromatograms supporting the formation of (c) poly(O950)-*b*-(CPM) and (d) poly(O950)-*b*-(HBC) from a poly(O950) mCTA (M_n of 17.5 kDa with a M_w/M_n of 1.12)

bulky methacrylate-based prodrug monomers. In these studies, HBC was polymerized at 70 °C in acetic acid using the dithiobenzoate-based RAFT agent, 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTP), and 4,4′-Azobis(4-cyanovaleric acid) (ABCVA) as the initiator. Following a short induction period (~20 min) polymerization proceeds with linear pseudo-first order kinetics (Fig. 1a) suggesting that radical termination reactions remain low throughout the course of the polymerization. Evaluation of the M_w/M_n and M_n vs. conversion plots illustrates the controlled nature of HBC polymerizations under these conditions. As shown in Fig. 1b, M_n versus conversion plot remains linear up to relatively high monomer conversion (~80%) with low molar mass dispersities and in good agreement with the theoretical molecular weights. For instance, polymerization of HBC for 40 min results in 23 % monomer conversion with experimental and theoretical molecular weights of 4.5 and 3.2 kDa, respectively, and a Đ of 1.12, while at 3 hours 85% conversion is reached with a M_n of 13.3 kDa ($M_{n\ theory}$ of 12.2 kDa) with a Đ of 1.06. Given the close agreement between the theoretical and experimental molecular weights under these conditions, it is likely that degradative chain transfer reactions are not occurring to a significant extent. Analysis of the MWDs shows that the peaks are unimodal and symmetric with a clear shift to lower elution volumes as a function of reaction time (Fig. 1c). Narrow and symmetric MWDs were also observed for the polymerization of HBC targeting a range of DPs between 12.5 and 100 with a slight increase in the molar mass dispersity (Đ ~1.15 at 64% conversion) for polymerizations targeting a DP of 100 (Fig. 1d). The lack of significant low molecular weight tailing in the MWDs coupled with low Đ values and subsequent blocking experiments provide strong evidence supporting the controlled nature of these polymerizations despite the steric bulkiness of the Cipro monomer.

RAFT copolymerization of HBC and ciprofloxacin-(phenol)methacrylate (CPM)

Shown in Scheme 1, is the synthetic strategy for the preparation of hydrophilic polyethyleneglycol methacrylate (950 Da) (PEGMA O950)-based copolymers with Cipro linked to the polymer backbone via aliphatic (HBC) and phenyl ester (CPM) groups. Based on the favorable kinetic profile for HBC the RAFT copolymerization of this monomer with O950 was conducted in pyridine at the same target $[M]_o/[CTA]_o$ and $[CTA]_o/[I]_o$ ratios (i.e. 25:1 and 5:1) (Scheme 1). A molar feed ratio of O950 to HBC of 74:26 (mol:mol) was selected to prevent the hydrophobic HBC residues from inducing self-assembly of the copolymers into nanoparticles at physiological pH values. After isolation of the polymer via precipitation in ether, the M_n and Đ were determined by GPC (Fig. S1a) to be 13.1 kDa and 1.08, respectively (Table 1). Copolymer composition was determined by both ^{1}H NMR (Fig. S2a) and ^{19}F NMR (Fig. S2b) with the latter technique yielding a molar composition of 72% O950 and 28% HBC (16 wt. % Cipro). Key copolymer resonances (Fig. S2a) include the characteristic (9H) Boc protective groups at δ = 1.52 ppm (sharp), the (3H) O950 methoxy residues at δ = 3.4 ppm (sharp), and the polymer backbone alkyl esters at δ = 4.1 ppm (sharp to broad). To produce the therapeutically active form of the polymeric prodrug, the Boc protecting groups were removed using neat TFA at 25 °C for 2 h. Quantitative Boc removal was confirmed

Table 1. Summary of composition, molecular weights, and molar mass dispersity for statistical copolymers of HBC and CPM with O950.

Poly. #	O950 (feed)	O950 (exp.)	HBC (feed)	HBC[1] (exp.)	CPM (feed)	CPM[1] (exp.)	M_n[2] (kDa)	dn/dc	Đ[2]	Drug (wt.%)
1	74	65	26	35	-	-	13.1	0.061	1.08	16
2	80	64	-	-	20	36	11.8	0.093	1.09	16.7

1. As determined by ^{19}F NMR in DMSO using a sodium tifluoracetate standard

2. As determined by size exclusion chromatography using Tosoh SEC TSK-GEL α-3000 and α-4000 columns PA) connected in series to an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, refractive index detector (Santa Barbara, CA). HPLC-grade DMF containing 0.1 wt% LiBr at 60 °C was used as the mobile phase at a flow rate of 1 mL min^{-1} .

by ^{1}H NMR by following the disappearance of the sharp (9H) resonance at δ = 1.52 ppm as shown as an example in Fig. 2a. The synthesis of poly(O950-*co*-CPM) was conducted under conditions similar to those employed for HBC except that the temperature was reduced to 65 °C to minimize the potential for cleavage of the more labile phenyl ester linkages. Because of the greater solubility of the CPM monomer in organic solvents it was possible to conduct polymerizations in THF. Similar to the polymerization of poly(O950-*co*-HBC), a feed ratio of 80:20 (mol:mol) O950:CPM was selected to prevent association of the hydrophobic CPM residues and subsequent formation of self-assembled nanoparticles. Copolymer composition (via ^{19}F NMR) was determined to be 64 mol % O950 and 36 mol % CPM (80 mol % O950 and 20 mol % CPM feed) (Fig. 2a-b) with M_n and Đ values, as measured by GPC (Fig. S1b), of 11.8 kDa and 1.09, respectively (Table 1).

Synthesis of poly(O950)-b-(HBC) and poly(O950)-b-(CPM)

Diblock copolymers were designed with the hydrophobic prodrug monomer residues localized in a discrete block and stabilized in solution by a hydrophilic poly(O950) segment. These diblock copolymers were prepared in order to establish the effect of this morphology on the resultant drug release profiles. This architecture could be advantageous in drug delivery applications because it potentially allows polymers with prodrug contents greater than 50 wt % to be synthesized, and provides a further tuning point for drug release kinetics. The diblock copolymers were synthesized by first preparing a poly(O950) macroCTA (M_n = 17.5 kDa, Đ = 1.12) (Table S1) from which HBC and CPM were polymerized targeting a DP of 25. The formation of the desired poly(O950)-*b*-(HBC) and poly(O950)-*b*-(CPM) diblock copolymers was confirmed by the clear shift in the MWDs to shorter elution volumes and lack of significant homopolymer impurity (Fig. 2c-d). Based on ^{19}F NMR, the block ratios for poly(O950)-*b*-(HBC) and poly(O950)-*b*-(CPM) were determined to be 18:56 (34 wt.% Cipro) (Fig. S3a) and 18:32 (30 wt.% drug) (Fig. S3b), respectively. Molecular weight, composition, and molar mass dispersity values for these materials are summarized in Table S1.

Aqueous solution studies for Cipro containing copolymers and diblock copolymers

The aqueous morphologies of both the copolymer and diblock copolymer architectures were evaluated under physiologically relevant conditions. At a pH of 7.4 and 7.0, where the deprotected Cipro residues should be predominately deprotonated and therefore hydrophobic in nature, hydrodynamic diameters of 40.7 ± 2.01 and 37.4 ± 1.85 nm are observed for poly(O950)-*b*-(HBC), respectively. Similar particle sizes were observed for poly(O950)-*b*-(CPM) (30.8 ± 2.14 at pH 7.4 and 29.5 ± 3.83 at pH 7.0) at these pH values. The apparent particle sizes are consistent with the formation of spherical core-shell nanoparticles where poly(O950) segments forming a hydrophilic corona around a dehydrated polymeric prodrug core.[30,31,32] In addition, the observed differences in size between the two diblocks at higher pH values (Fig. 3a) may be attributed to slightly longer HBC block relative to CPM (i.e. DP of HBC block of 56 vs. 32 for CPM) (Table S1).[33] Reduction of the solution pH to 5.2 results in a significant decrease in hydrodynamic diameter to around 5-8 nm for both diblock copolymers (Fig. 3a). This behavior is likely caused by an increase in the protonation state of the secondary amines present on Cipro residues in the polymer core. The resultant increase in positive charge along the polymer backbone destabilizes the micellar core via charge-charge repulsion while increasing the hydrophilicity of the core-forming segment. These sizes are consistent with molecularly dissolved unimers and do not change significantly upon further reduction of the solution pH (Fig. 3a). Zeta potential measurements for these materials at pH 7.4 were determined to be slightly positive with values of 5.24 ± 2.1 mV and 4.85 ± 2.5 mV observed for poly(O950)-*b*-(HBC) and poly(O950)-*b*-(CPM), respectively (Fig. 3b). Decreasing the pH to 5.2 increases the zeta potential to 26.56 ± 3.14 mV for poly(O950)-*b*-(HBC) and 22.96 ± 3.21 for poly(O950)-*b*-(CPM) (Fig. 3b) supporting an increase in the protonation state of the polymer at lower pH values.

Release kinetics of Cipro from copolymers and diblock copolymers quantified by HPLC

HPLC was used to quantify drug release by detecting free drug as a function of time normalized to amount of drug present during initial incubation. This in turn was standardized to the total available drug in the system as quantified by

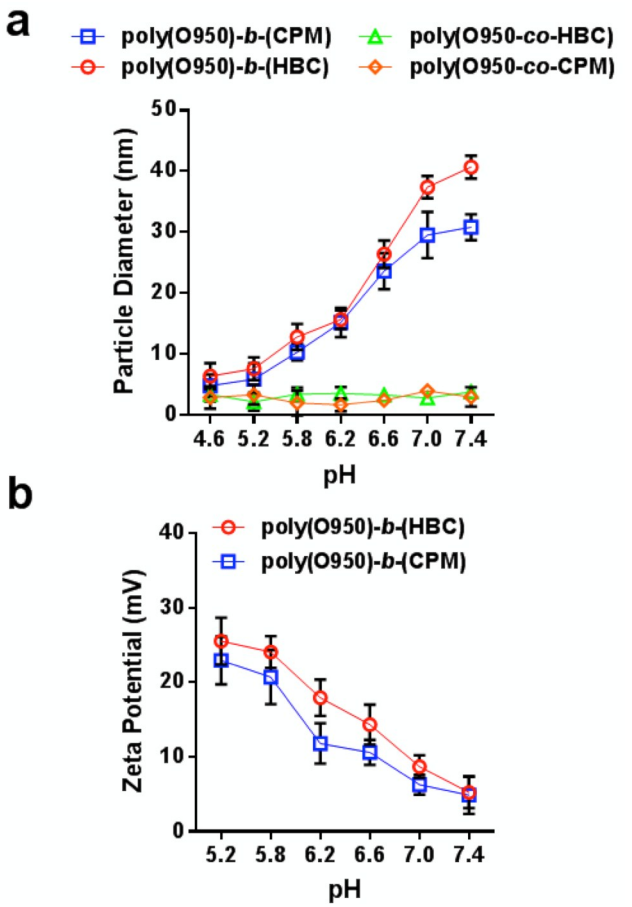


Fig. 3 Aqueous size and charge measurements for statistical copolymers and diblock copolymers containing Cipro prodrug residues. (a) Hydrodynamic diameter and (b) zeta potentials measurements as a function of pH. Buffers were prepared using 100 mM sodium phosphate or acetate with 150 mM NaCl for particle size measurements and 10 mM sodium phosphate for zeta potential measurements; all buffers were titrated to the appropriate pH. Polymer concentrations were made at 0.5 mg/mL and 1 mg/mL for DLS and zeta potential, respectively, and filtered using 0.22 µm filter before running experiments.

dissolving a known amount of polymer in 10% aq. H_2SO_4 for 48 h at 25 ˚C. Using a Cipro standard curve, the total amount of drug in the polymers was validated against compositional values obtained from ^{19}F NMR. In these studies, it was observed that free Cipro elutes at approximately 1.59 ± 0.04 min, as supported by representative tandem mass spectrometry (Fig. S4). Hydrolysis rates in human serum were determined for both of the deprotected monomers (ie. HBC and CPM) prior to their incorporation into copolymers as shown in Fig. 4a-b. These studies suggest that the respective rates of hydrolysis for the aliphatic (HBC) and phenyl (CPM) ester linked drugs are not significantly affected by the presence of serum proteins. A significant difference in the relative hydrolysis rates for these monomers was observed with CPM showing nearly 50 % drug release at 24 h while HBC required 120 h to reach a similar percent release (Fig. 4a). This apparent difference likely arises from the improved hydrolytic susceptibility of CPM's phenyl ester functionality, which is known to form a resonance-stabilized phenoxide as a leaving group.[34]

Butyrylcholinesterase (BChE) is a human enzyme generated by the liver that freely circulates in the blood to facilitate the breakdown of many drugs.[35,36] The addition of increasing concentrations of BChE to solutions of the prodrug monomer in buffer increases hydrolysis of both types of ester bonds as noted by the enhanced pseudo-first order release profiles for deprotected HBC and CPM monomers (Fig. 4a-b). Interestingly, without the added enzyme present, the release kinetics transition from an apparent first order kinetics to near zero-order for the deprotected HBC monomer suggesting that this particular monomer is more responsive to a natural esterase than the more labile phenolic ester monomer (CPM) (Fig. 4a-b). Although the release profiles of poly(O950-*co*-HBC) in serum and buffer are near zero-order, the kinetics of hydrolysis and subsequent release of free drug were observed to be faster in serum than buffer (Fig. 4c). In efforts to probe this observation, the copolymers were incubated in buffer with the addition of 50 mg/mL human serum albumin (HSA) and assayed for free drug as a function of time. In these studies, the inclusion of HSA was shown to increase release kinetics to rates similar to those observed in serum (Fig. 4c). This observation is hypothesized to arise from the association of the unimeric copolymer with proteins found in serum (e.g. human serum albumin) to produce polymer conformations with improved solvation of the pendent ester bonds. Slow release kinetics for end-linked Cipro-polymer conjugates have been observed by other groups.[37,38] For example, Sobczak et al. observed approximately 20-25% Cipro release over 35 days from multi-armed and star shaped homopolymers of poly(ϵ-caprolactone) and polylactide that were end-functionalized with 3-8 mol % of drug.[39]

The addition of BChE to poly(O950-*co*-CPM), in buffer resulted in a slight increase in the hydrolysis rate relative to buffer alone (Fig. 4d). This increase was, however, not as large as samples incubated in serum suggesting the importance of serum proteins in facilitating ester hydrolysis for polymer backbone-linked drugs. These findings suggest that the presence of enzyme alone may not be sufficient to significantly improve hydrolysis rates for esters found within low dielectric environments, such as the case with many polymer backbones.[39] In contrast, the large increase in drug release observed for monomers incubated with the enzyme (Fig. 4a-b) can be attributed to the lack of a polymerized chemical backbone, which may allow BChE to access the ester groups promoting faster cleavage rates than those observed in serum. Comparison of copolymers containing phenyl- (CPM) and aliphatic- (HBC) esters shows that poly(O950-*co*-CPM) hydrolyzed more rapidly than poly(O950-*co*-HBC) with approximately 50 % drug release observed at 120 h and 21 days respectively. In both cases however incorporation of the ester-linked drug into copolymers resulted in a substantial decrease in hydrolysis rates relative to the parent monomers (Fig. 4c-d).

The effect of polymer architecture on drug release behavior was also evaluated by synthesizing diblock copolymers consisting of a hydrophilic poly(O950) corona forming segment and a hydrophobic poly(HBC) or poly(CPM)

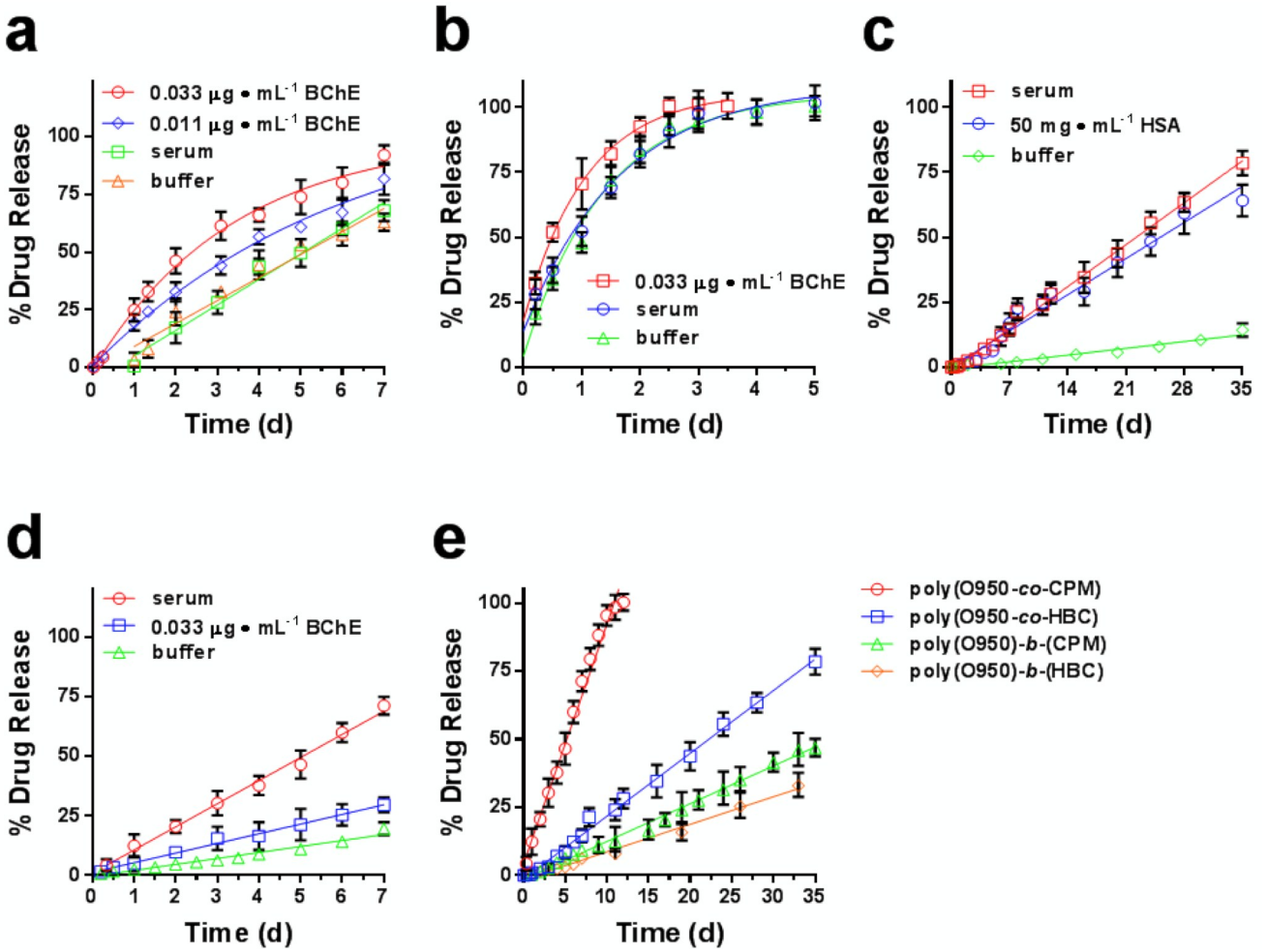


Fig. 4 Drug release kinetics measured by high-performance liquid chromatography (HPLC) as a function of time of (a) HBC monomer in the presence of varying amounts of Butyrylcholinesterase (BChE) and 100% human serum, (b) CPM monomer in the presence of BChE and serum, (c) poly(O950-*co*-HBC) in serum, pH 7.4 buffer, and human serum albumin, (d) poly(O950-*co*-CPM) in serum and in the presence of BChE, and (e) Cipro containing statistical copolymers and block copolymers in 100% serum. All drug release studies were conducted at 37 ˚C and free drug detection was quantified using an elution gradient profile at 277 nm. All studies were conducted with deprotected monomers and polymers. Free Cipro was extracted from serum samples using acetonitrile as an organic phase precipitation technique to remove proteins, and HPLC analysis was conducted using a mobile phase consisting of 2% aq. acetic acid:acetonitrile (84:16 v/v/).

core forming segment, as described above. As shown in Fig. 4e, sequestration of the prodrug residues to a hydrophobic core results in a significant decrease in ester hydrolysis rates relative to the molecularly soluble constructs. It is important to note that although the rate of drug release from the diblocks are slower than the copolymers, the total drug content for diblocks are greater (30 wt. % drug for poly(O950)-*b*-(HBC) and 34 wt. % drug for poly(O950)-*b*-(CPM) vs. 16 wt. % drug for poly(O950-*co*-HBC) and 16.7 wt.% drug for poly(O950-*co*-CPM)) (Table S1). Consequently, there is a larger quantity of drug released from the diblock copolymers over a longer period of time as compared to the copolymers.

In vitro polymer toxicity and efficacy

The cultured cell biocompatibility of the polymeric prodrugs was characterized in RAW 264.7 cells. Cells were incubated with varying concentrations of the copolymer and diblock copolymer prodrugs for 24 h. No notable (< 80% cell viability) toxicity was observed for both the poly(O950-*co*-HBC) and poly(O950-*co*-CPM) even at polymer concentrations of 20 mg/mL (Fig. 5a). In contrast, the diblock copolymer constructs demonstrated dose dependent toxicity with RAW cell viability falling below 80% at polymer concentrations exceeding approximately 1 mg/mL (Fig. S5). This toxicity is likely a result of interactions of the lightly charged polyCipro segments with cell membranes upon internalization and subsequent acidification in endosomal compartments. This phenomenon has been previously reported for other positively charged systems such as cationic polystyrene nanospheres (~40-50 nm) in RAW 264.7 cells.[40]

Based on the copolymer's lack of toxicity in RAW cells at elevated concentrations (Fig. 5a), poly(O950-*co*-HBC) and poly(O950-*co*-CPM) were selected for further studies to evaluate efficacy using a co-culture challenge assay with *Burkholderia thailandensis* infected RAW 264.7 cells (Fig. 5b). Here *B. thailandensis* was used as a surrogate model for the evaluation of *B. pseudomallei* infectivity.[41] The dose response curve of free Cipro was, consistent with literature minimum

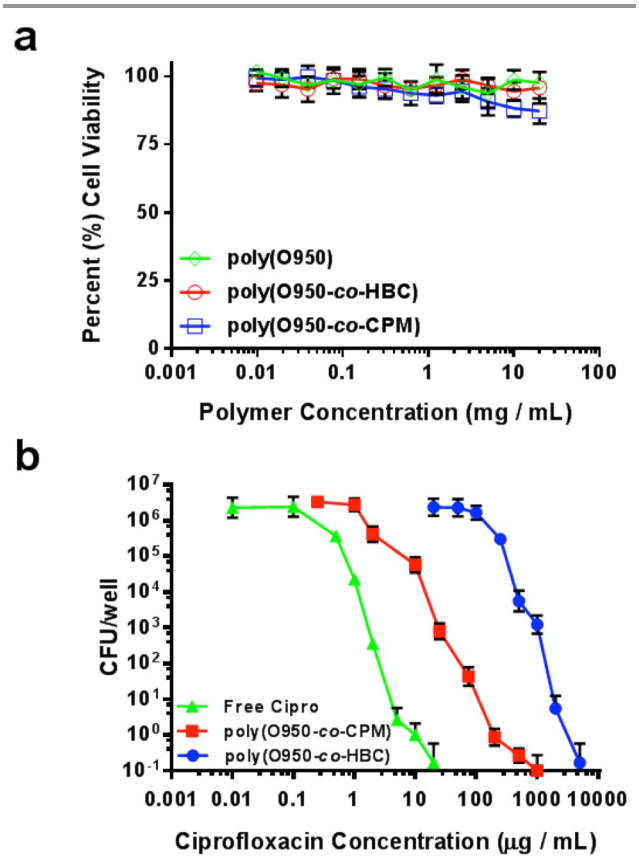


Fig. 5 *In vitro* toxicity and efficacy using RAW 264.7 cells representing (a) MTS results for varying concentrations of poly(O950-*co*-HBC) and poly(O950-*co*-CPM) compared to poly(O950) mCTA (negative control), and (b) co-culture assay with cells treated with varying concentrations of both copolymers and free ciprofloxacin (positive control) following infection with *B. thailandensis* to determine antibacterial efficacy. Polymer concentrations ranged from 20 mg/mL to 3.7 µg/mL, and toxicity was evaluated with the CellTiter 96AQueous One Solution Cell Proliferation Assay according to manufacturer's protocol.

inhibitory concentrations (MIC) of 10 µg/mL or 0.03 mM.[42, 43] Evaluation of poly(O950-*co*-HBC) in this assay yielded an MIC of 2000 µg/mL (6 mM) polymerized prodrug, which is consistent with hydrolysis studies where approximately 1-2% drug release is observed at 24 h in 100% serum (Fig. 4c). In contrast, co-culture studies conducted with poly(O950-*co*-CPM) resulted in a 10-fold reduction in the MIC to 200 µg/mL (0.6 mM) polymerized prodrug (Fig. 5b). These results are consistent with trends observed in the drug release studies and show that the phenyl ester linked Cipro releases more active drug over the timescale of this *in vitro* co-culture model as a result of higher drug cleavage rates (Fig. 4d).

C Experimental

Materials

Chemicals and all materials were supplied by Sigma-Aldrich unless otherwise specified. Sodium trifluoroacetate was purchased from TCI America. Recombinant human butyrylcholinesterase (BChE) was obtained from R&D systems. PEGMA 950 (Aldrich) (30 g) was purified as described previously.[29] Spectra/Por regenerated cellulose dialysis membranes (6-8 kDA cutoff) were obtained from Fisher Scientific. G-25 prepacked PD10 columns were obtained from GE Life Sciences. MTS cytotoxicity kits were obtained from Promega. Unless otherwise stated, RAW 264.7 cells, murine derived macrophages (ATCC), were maintained in Dulbecco's modified eagle medium (DMEM) containing L-glutamine (Gibco), 4.5g/L glucose, 10% fetal bovine serum (FBS, Invitrogen), and 1% penicillin-steptomycin (Gibco) at 37 °C and 5% CO_2.

Synthesis of 7-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquionoline-3-carboxylic acid (Boc Cipro)[44]. To 20 g (60 mmol) of ciprofloxacin in 350 mL of dioxane:water (1:1) was added 90 mL of 1N NaOH, followed by 20 g (91.6 mmol) of di-tert-butyl dicarbonate. The reaction mixture was stirred at room temperature for 17 h. The white precipitate obtained was filtered, washed with water and then with acetone. Boc Cipro obtained was dried under high vacuum overnight. Yield = 25.14 g (96.5 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (m, 2H), 1.40 (m, 2H), 1.49 (s, 9H), 3.29 (t, J = 5.0 Hz, 4H), 3.54 (m, 1H), 3.67 (t, J = 5.0 Hz, 4H), 7.37 (d, J = 7.1 Hz, 2H), 7.99 (d, J = 12.9 Hz, 1H), 8.73 (s, 1H).

Synthesis of (hydroxyethyl)methacrylate-boc-ciprofloxacin (HBC). Boc Cipro 10.35 g (24 mmol), N,N,N',N'-tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) 22.8 g (0.16 mol) and N,N-dimethylpyridin-4-amine (DMAP) 292 mg (2.4 mmol) were taken in 500 mL of CH_2Cl_2 and cooled to 0 °C. N,N-diisopropylethylamine 21 mL (0.12 mol) was added, followed by 2-hydroxylethyl methacrylate (HEMA) 11.7 g (90 mmol). After 10 min at 0 °C, the solution was stirred at room temperature for 16 h. The reaction mixture was washed with brine (2 X 200 mL) and the organic phase was dried over anhydrous sodium sulfate. After evaporation of the solvent, the product HEMA-Boc-Cipro (HBC) was precipitated in ether, and then purified by column chromatography using 5 % methanol in chloroform. Yield = 10.85 g (83.1 %).%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (m, 2H), 1.30 (m, 2H), 1.48 (s, 9H), 1.94 (s, 3H), 3.20 (t, J = 4.8 Hz, 4H), 3.42 (m, 1H), 3.64 (t, J = 4.8 Hz, 4H), 4.50 (m, 4H), 5.58 (s, 1H), 6.15 (s, 1H), 7.29 (d, J = 7.0 Hz, 1H), 8.0 (d, J = 13.1 Hz, 1H), 8.47 (s, 1H). MS (ESI, m/z): calc. for $C_{28}H_{34}FN_3O_7$ (M): 543.6, found: 544.5 $[M+1]^+$, 566.4 $[M+Na]^+$and 582.2 $[M+K]^+$ (Fig. S6).

Synthesis of butanoic acid, 4-[(4-hydroxyphenyl) methylamino]-4-oxo, 1-(2-methacryloyloxy)ethyl ester (SMA-phenol). Mono-2-(methacryloyloxy)ethyl succinate (SMA) 9.2 g (50 mmol) dissolved in 150 mL of CH_2Cl_2 was cooled to 0 °C. To this solution, *N*-hydroxysuccinimide 4.72 g (41 mmol) and *N-N'*-dicyclohexylcarbodimide 9.06 g (44 mmol) were added. After 15 min, the ice bath was removed and the reaction mixture was stirred at room temperature for 16 h. The byproduct dicyclohexylurea was filtered off, and the filtrate was concentrated to 40 mL by evaporating the solvent under reduced pressure. This solution containing the activated NHS ester was directly added to 6.15 g (50 mmol) of 4-

(aminomethyl)phenol pre-dissolved in 30 mL *N*,*N*-dimethylformamide, followed by 13.94 mL (0.1 mol) of triethylamine. After stirring for 6 h at RT, the reaction mixture was diluted with 200 mL of CH_2Cl_2, and washed with water (2 X 100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The thick residue obtained was treated with 100 mL ether, and vigorously stirred for 15 min. Then 75 mL hexane was added, and again stirred well for 10 min. The solvent was carefully decanted and the process was repeated one more time. The resulting SMA conjugated phenolic monomer was further purified by flash column chromatography using 5 % methanol in chloroform. Overall yield for two steps: 10.16 g (76 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93 (s, 3H), 2.50 (t, J = 6.7 Hz, 2H), 2.72 (t, J = 6.7 Hz, 2H), 4.30 (s, 4H), 4.34 (d, J = 5.6 Hz, 2H), 5.59 (m, 1H), 6.08 (t, J = 5.6 Hz, 1H), 6.12 (s, 1H), 6.30 (s, 1H), 6.75 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H). MS (ESI, m/z): calc. for $C_{17}H_{21}NO_6$ (M): 335.4, found: 358.8 $[M+Na]^+$ and 693.8 $[2M+Na]^+$.

Synthesis of ciprofloxacin-(phenol)methacrylate (CPM). Boc Cipro 2.15 g (5 mmol) and N,N-dimethylpyridin-4-amine (DMAP) 610 mg (5 mmol) were taken in 250 mL of CH_2Cl_2 and cooled to 0 °C. To this solution, N,N,N',N'-tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) 4.74 g (12.5 mmol) was added, followed by N,N-diisopropylethylamine 3.5 mL (20 mmol). After 10 min at 0 °C, the reaction mixture was stirred at RT for 30 min, and then cooled back to 0 °C. SMA-phenolic monomer 1.68 g (5 mmol) was then introduced and the reaction was continuously stirred at 0 °C for 20 min, and then at RT for 16 h. The reaction mixture was filtered and the filtrate was washed with water (100 mL) and brine (100 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residue was precipitated in ether, and then purified by column chromatography using 30 % tetrahydrofuran in chloroform containing 0.1 % triethylamine. Yield = 2.45 g (65.4 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (m, 2H), 1.35 (m, 2H), 1.5 (s, 9H), 1.94 (s, 3H), 2.52 (t, J = 6.7 Hz, 2H), 2.73 (t, J = 6.7 Hz, 2H), 3.33 (t, J = 4.9 Hz, 4H), 3.45 (m, 1H), 3.65 (t, J = 4.9 Hz, 4H), 4.32 (s, 4H), 4.44 (d, J = 5.7 Hz, 2H), 5.58 (m, 1H), 6.12 (s, 1H), 6.18 (t, J = 5.5 Hz, 1H), 7.15 (d, J = 8.5, 2H), 7.29 (two doublets merged, 3H), 8.05 (d, J = 13.1 Hz, 1H), 8.63 (s, 1H). MS (ESI, m/z): calc. for $C_{39}H_{45}FN_4O_{10}$ (M): 748.8, found: 750.1 $[M+1]^+$ and 771.8 $[M+Na]^+$ (Fig. S7).

Kinetic evaluation of HBC. Kinetic evaluation of HBC was conducted with 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTP) and 4,4′-Azobis(4-cyanovaleric acid) (ABCVA) as the RAFT chain transfer agent and initiator, respectively, in acetic acid at 70 °C. The initial monomer to CTA to initiator ($[M]_o$:$[CTA]_o$:$[I]_o$) ratio was 25:1:0.2. In order to understand the influence of the degree of polymerization (DP) on the evolution of molecular weight, RAFT polymerizations of HBC were conducted under similar reaction conditions with $[CTA]_o$:$[I]_o$ ratio of 5:1 and $[M]_o$:$[CTA]_o$ ratios of 12.5, 25, 50, and 100. Individual polymerization solutions were transferred to a septa-sealed vial and purged with nitrogen for 20 minutes. After the allotted time, the polymerization vials were transferred to a preheated water bath at 70 °C and allowed to polymerize for 2.5 h. Following polymerization, the individual vials were quenched by exposure to oxygen by opening the septa seal and immersing the vials in ice. The polymerizations were evaluated for monomer conversion via ^{1}H NMR in C_2D_6OS by comparing the HBC vinyl resonances at δ = 6.1 and 5.7 ppm to ester resonances at δ = 4.4 and 4.1 ppm.

RAFT copolymerization of PEGMA 950 (O950) and HBC. The RAFT copolymerization of poly(O950-*co*-HBC) was conducted in pyridine with CTP and ABCVA as the CTA and initiator, respectively, with $[M]_o$:$[CTA]_o$ and $[CTA]_o$:$[I]_o$, equal to 25:1 and 10:1. To a 100 mL round-bottom flask was added CTP (112.6 mg, 403 µmol), ABCVA (11.3 mg, 40.3 µmol), HBC (1.25 g, 2.62 mmol), O950 (7.08 g, 7.46 mmol), and pyridine (40.67g). The solution was then septa sealed and purged with nitrogen for 60 minutes. The round-bottom flask was then transferred to a preheated water bath at 70 °C and allowed to polymerize for 18 hours. The polymeization solution was then precipitated in ether and the resultant polymer was dried in vacuo for 48 h. The final molecular weight and Đ, as measured by GPC, and molar composition of poly(O950-*co*-HBC) was 13.1 kDa, 1.08, and 72:28 O950:HBC (74:26 feed), respectively. This corresponded to a 16 wt. % Cipro incorporation. Copolymer compositions was determined by both ^{1}H NMR and ^{19}F NMR. Briefly, analysis by ^{19}F NMR used sodium trifluoroacetate ($C_2F_3NaO_2$) as an internal standard where 1.5 uL of a 10 mg/mL solution of $C_2F_3NaO_2$ was added to 1 mL of a 20 mg/mL polymer solution in C_2D_6OS and molar composition was determined by comparing the three fluorine resonances from the internal standard at δ = -73.4 ppm against the single fluorine resonances from Cipro containing copolymer at δ = -124.5 ppm. Molar compositions were also analyzed by ^{1}H NMR in $CDCl_3$ by comparing the HBC (9H) Boc resonances at δ = 1.52 ppm to the O950 (3H) methoxy at δ = 3.4 ppm. Both methods of drug quantification were in good agreement and resulted in similar drug composition. For the purpose of this study, values obtained by ^{19}F NMR are being reported for all polymer systems.

RAFT copolymerization of O950 and CPM. The RAFT copolymerization of poly(O950-*co*-CPM) was conducted in THF with CTP and ABCVA as the CTA and initiator respectively with $[M]_o$:$[CTA]_o$, $[CTA]_o$:$[I]_o$, equal to 25:1 and 10:1, similar to the polymerization of poly(O950-*co*-HBC). To a 25 mL round-bottom flask was added CTP (38.8 mg, 139 µmol), ABCVA (3.89 mg, 13.9 µmol), CPM (1.00 g, 1.34 mmol), O950 (2.03 g, 2.14 mmol), and THF (14.15g). The solution was then septa sealed and purged with nitrogen for 30 minutes. The round-bottom flask was then transferred to a preheated water bath at 65 °C and allowed to polymerize for 18 hours. The polymerization solution was then precipitated in ether and dried in vacuo for 48 h. The final molecular weight and Đ, as measured by GPC and molar composition of poly(O950-*co*-CPM) was 11.8 kDa, 1.09, and 64:36 O950:CPM (80:20 feed), respectively (16.7 wt % Cipro in the final copolymer). Similar to poly(O950-*co*-HBC),

the copolymer composition of poly(O950-*co*-CPM) was determined by both ^{19}F NMR and, independently, by ^{1}H NMR, as previously described above.

Synthesis of poly(O950) via RAFT. The synthesis of a poly(O950) macroCTA was conducted in DMSO with CTP and ABCVA as the CTA and initiator respectively with $[M]_o$:$[CTA]_o$, $[CTA]_o$:$[I]_o$, equal to 25:1 and 10:1.[29] To a 50 mL round-bottom flask was added CTP (82.34 mg, 2.95 µmol), ABCVA (8.26 mg, 29.5 µmol), O950 (7.00 g, 7.37 mmol), and DMSO (28 g). The solution was then septa sealed and purged with nitrogen for 60 minutes. The round-bottom flask was then transferred to a preheated water bath at 70 ^{o}C and allowed to polymerize for 18 hours. The transparent solution was then precipitated in ether six times, solvent decanted, and product collected and dried in vacuo. The resulting polymer had a molecular weight and Đ of 17.5 kDa and 1.12, respectively.

Synthesis of poly(O950)-b-(HBC) and poly(O950)-b-(CPM) via RAFT. The RAFT polymerization of poly(O950)-*b*-(HBC) from a poly(O950) macroCTA (17.5 Da, 1.12 Đ) was conducted in acetic acid with $[M]_o$:$[mCTA]_o$, $[mCTA]_o$:$[I]_o$ equal to 25:1 and 5:1. To a 25 mL round-bottom flask was added O950 mCTA (644 mg, 36.8 µmol), ABCVA (2.06 mg, 7.36 µmol), HBC (0.5 g, 0.92 mmol), and acetic acid (2.64 g). The solution was then septa sealed and purged with nitrogen for 30 minutes. The round-bottom flask was then transferred to a preheated water bath at 70 ^{o}C and allowed to polymerize for 2.5 hours. The solution was then precipitated in ether for six times, solvent decanted, and product collected, dried in vacuo, and lyophilized over 48 h. The final molecular weight, Đ, and composition of poly(O950)-*b*-(HBC) was 48 kDa and 1.27, respectively, corresponding to DPs for each blocks of 18 and 56 respectively (34 wt. % ciprofloxacin in the final copolymer). Similar to the copolymers, the diblock compositions were determined by both ^{1}H NMR and ^{19}F NMR. Briefly for ^{19}F NMR analysis, 3 uL of a 10 mg/mL solution of $C_2F_3NaO_2$ was added to 1 mL of 20 mg/mL diblock polymer solution in C_2D_6OS and molar composition was determined again by comparing the three fluorine resonances from the internal standard at δ = -73.4 ppm against the single fluorine resonances from Cipro diblock polymer at δ = -124.5 ppm. In addition, ^{1}H NMR in $CDCl_3$ was used to again compare the HBC (9H) Boc resonances at δ = 1.52 ppm to the O950 (3H) methoxy at δ = 3.4 ppm. Analogous to the copolymers, values obtained from ^{19}F NMR are being reported for both diblocks.

The RAFT polymerization of poly(O950)-*b*-(CPM) utilized the same O950 mCTA homopolymer (Mn = 17.5 kDa, Đ = 1.12) as the one used in the synthetic strategy for the polymerization of poly(O950)-*b*-(HBC), as noted above. This reaction was conducted in THF with $[M]_o$:$[mCTA]_o$, $[mCTA]_o$:$[I]_o$ equal to 25:1 and 10:1. To a 10 mL round-bottom flask was added O950 mCTA (455 mg, 26.7 µmol), ABCVA (0.75 mg, 2.67 µmol), CPM (0.5 g, 0.67 mmol), and THF (2.55 g). The solution was then septa sealed and purged with nitrogen for 30 minutes. The round-bottom flask was then transferred to a preheated water bath at 65 ^{o}C and allowed to polymerize for 18 hours. The final copolymer was subsequently isolated as detailed above. The final molecular weight, Đ, and composition of poly(O950)-*b*-(CPM) was 41.8 kDa and 1.35, respectively, corresponding to DPs for each blocks of 18 and 32 respectively (30 wt. % Cipro in the final copolymer).

Deprotection and purification of copolymer and diblock systems. Post polymerization removal of the Boc protecting groups, present on HBC and CPM residues, was conducted in neat trifluoroacetic acid (TFA) and 1:1 $CHCl_3$:TFA, respectively at a polymer concentration of 50 mg/mL . The reaction was allowed to proceed at 25^{o}C for 2h after which time the solution was precipitated in ether. The product was collected and dried in vacuo for 48 h. In order to remove any TFA salts attached to the secondary amine group on Cipro after deprotection and precipitation, the polymers were redisolved in molecular grade water and dialyzed against first 250 mM and then 10 mM NaH_2PO_4 at pH 7.4 with repeated buffer changes (2-3x) over two days. The polymers were then frozen and lyophilized before further purification via PD-10 desalting column (GE Life Sciences) followed by lyophilization for an additional 48 h.

Gel permeation chromatography (GPC). Absolute molecular weights and polydisperity indices were determined using Tosoh SEC TSK-GEL α-3000 and α-e4000 columns (Tosoh Bioscience, Montgomeryville, PA) connected in series to an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, refractive index detector (Santa Barbara, CA). HPLC-grade DMF containing 0.1 wt.% LiBr at 60 ^{o}C was used as the mobile phase at a flow rate of 1 ml/min.

Characterization of copolymer and diblock micelles. Dynamic light scattering (DLS) studies of the block copolymers were conducted using a Malvern Instruments Zetasizer Nano series instrument equipped with a 22 mW He-Ne laser operating at 632.8 nm. Solutions of the copolymer and diblock were prepared in the pH range capturing the endosomal trafficking pathway (7.4, 7.0, 6.6, 6.2, 5.8, 5.2, and 4.6) with either 100 mM sodium phosphate or acetate buffer with 150 mM NaCl at a polymer concentration of 0.5 mg/mL. The resulting solutions were filtered with 0.22 µm filters prior to measurement, and mean diameter was defined as the ± half peak width. All measurements were performed in triplicate comparing the copolymers to the diblocks. The polymer micelles were analysed for zeta potential, using a ZetaPALS detector, at 1 mg/mL polymer concentration as a function of pH (7.4, 7.0, 6.6, 6.2, 5.8, and 5.2) with either 10 mM sodium phosphate or acetate buffer.

Analysis of Cipro by high-performance liquid chromatography (HPLC). The HPLC analysis of Cipro was carried out with an Agilent 1260 Quaternary HPLC Pump, Agilent 1260 Infinity Standard Automatic Sampler, Agilent 1260 Infinity Programmable Absorbance Detector, and Agilent ChemStation

software for LC system (Palo Alto, CA). Both ciprofloxacin hydrochloride and liquid Sera Human from AB blood donor were purchased and used as received. The analyte was separated at ambient temperature using a Zorbax RX-C_{18} (4.6 x 150 mm; 5 µm) analytical column (Agilent Technologies, CA).

The UV detector was operated at 277 nm, and the mobile phase consisted of 2% aqueous acetic acid and acetonitrile (84:16) v/v, as described elsewhere.[45] The flow rate was set at 1.0 mL/min and sample injection volume at 20 µL. A stock solution of Cipro was prepared in deionized water at 10 mg/mL. Working solutions of Cipro for standard curves were diluted from stock solution using the mobile phase to the listed concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL, and 1.56 µg/mL.

Each listed solution above was diluted with a 1:1 v/v ratio of either mobile phase:deionized water or mobile phase:human serum to create a final Cipro standards of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL, 1.56 µg/mL, and 0.78 µg/mL for pharmaceutical and biological analysis, respectively. Both non-serum (mobile phase:deionized water) and serum standards were subsequently treated with 50% acetonitrile (v/v) to promote protein precipitation. Serum standards were centrifuged at 12,000g for 15 minutes and supernatants were collected and filtered using a 0.45µm low protein binding filter before HPLC analysis. Non-serum standards were analysed without the need for centrifugation. All standards were processed using a gradient HPLC elution profile, where the mobile phase transitioned to 100% acetonitrile over 15 minutes, followed by 10 minutes of column washing with acetonitrile and water and 5 minutes of equilibration with mobile phase.

Drug release from polymeric prodrugs. The drug release from polymer conjugates was carried out in serum at 37 ˚C at a polymer concentration of 6 mg/mL. Sample time points were collected on a regular basis. Quantification of total Cipro in the polymer conjugates was measured by taking 6 mg/mL of polymer and dissolving it in 10% aq. H_2SO_4 for 48 h at 25 ˚C, denoted by Peak(H_2SO_4). The HPLC with a gradient elution profile was used to quantify amount of drug released using the same instrument parameters set forth for drug standards. A 1:1 dilution of serum sample to 2% aqueous acetic acid and acetonitrile (84:16) v/v was conducted, followed by another 1:1 dilution with acetonitrile. The resulting samples were vortexed and centrifuged at 12,000g for 15 minutes. Supernatants were collected and filtered using a 0.45µm low protein binding filter before running on the HPLC. Percent (%) drug released was subsequently quantified using the formula: % Drug Released = [Peak(t_x) – Peak(t_0)]/[Peak(H_2SO_4)], where t_x and t_0 are the peaks resolved by the HPLC at time x and zero, respectively.

***In vitro* cytotoxicity measurements.** The cytotoxicity of the prodrug copolymers and diblock copolymers were evaluated in RAW 264.7 cells using the CellTiter 96AQueous One Solution Cell Proliferation Assay (MTS) (Promega Corp., Madison, WI). RAW cells were seeded in DMEM (Gibco, Life Technologies, Grand Island, NY) containing 1% pen/strep and 10% fetal bovine serum (FBS) at a density of 50,000 cells/well in 96-well plates and allowed to adhere for 18 h at 37 °C with 5% CO_2. After incubation, polymers diluted in supplemented DMEM at a concentration of 40 mg/mL total polymer were added to cells in triplicate wells in a 1:1 dilution, then serially diluted down the plate (20 mg/mL-9.77 µg/mL), and cells were incubated for 24 hours. After the allotted time, cells were evaluated using the CellTiter MTS assay according to the manufactures instructions. The absorbance at 490 nm was evaluated using a Tecan Safire 2 microplate reader. MTS reagent alone was used as a negative control and all treatments were compared to untreated cells as a positive control to acquire percentage viability. All experiments were carried out in triplicate wells on duplicate days.

***In vitro* co-culture activity using a *B. thailandensis* infection model.** To evaluate the *in vitro* efficacy of the polymer systems, RAW 264.7 murine macrophage cells were seeded into 48 well plates at a density of 500,000 cells/mL in 250 µL of antibiotic free DMEM (Gibco) + 10% FBS and allowed to adhere for 18 h at 37 °C with 5% CO_2. After 18 hours, RAW cells were infected with *Burkholderia thailandensis* (E264) at early log phase (OD_{600}=0.2) at a MOI of 5, and incubated for 1 hour. Growth media was then replaced with fresh DMEM containing 10% FBS and 250 µg/mL Kanamycin to remove extracellular bacteria and cells were incubated for another hour. Media was then replaced with unsupplemented DMEM containing varying concentrations of HBC copolymer (20-3000 µg/mL), CPM copolymer (1-2000 µg/mL), or free drug (0.01-100 µg/mL) into triplicate wells per treatment. Cells were incubated an additional 22 hours. After incubation, cell media was aspirated, cells were washed three times with 1x PBS, and lysed with 100 µL of PBS + 0.1% Triton X-100 (Sigma Aldrich, St. Louis, MO). Lysates were pooled by treatment, serially diluted, and plated onto triplicate LB agar plates at multiple10x dilutions, and incubated for 24 hours. After 24 hours colony forming units (CFU) were counted. Data represented as CFU/well vs. Cipro dose. All experiments were repeated on duplicate days.

Conclusions

Methacrylate-based prodrug monomers were synthesized from the antibiotic Cipro and then incorporated into copolymers and diblock copolymers using RAFT polymerization. Linear pseudo first order kinetics were observed for the homopolymerization of HBC and both monomers showed narrow and symmetric molecular weight distributions over a range of target DPs between 12 and 100. Prodrug monomers were then either copolymerized with polyethyleneglycol methacrylate (O950) to yield hydrophilic copolymers or chain extend from poly(O950) macroCTAs to yield diblock copolymers. The resultant copolymers and diblock copolymers contained 16 and 34 % drug respectively. DLS and zeta potential measurements were employed to evaluate the pH-dependent aqueous solution properties of these constructs. At physiological pH values the diblock

copolymer constructs yielded hydrodynamic diameters that are consistent with micelles, which disassembled upon a reduction in the solution pH to 6.6. In contrast, the copolymers formed molecular dissolved unimers with particle sizes that were largely independent of the solution pH. Moreover, copolymers containing Cipro linked via phenolic esters showed faster hydrolysis rates with 50% drug released at 120 h, whereas copolymers with the corresponding aliphatic ester linkages showed similar percent drug release over 22 d. Diblock copolymers with a discrete Cipro block showed greatly reduced hydrolysis rates for both ester linked drugs. In addition, *in vitro* toxicity measurements in RAW 264.7 cells showed the copolymers to be nontoxic up to 20 mg/mL following a 24h incubation period. Co-culture efficacy was determined using *Burkholderia thailandensis* where an MIC of 6.0 and 0.6 mM of polymerized antibiotic were determined for the aliphatic ester and phenyl ester linked polymeric prodrugs respectively. In conclusion, here we show that polymer architecture and drug linkage chemistry can influence drug release kinetics and be tuned to yield a richly controllable delivery system.

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