Anticancer drug delivery strategies are designed to take advantage of the differential chemical environment in solid tumors independently, or to high levels of reactive oxygen species (ROS) or to low pH, compared to healthy tissue. Here, the design and thorough characterization of two functionalizable “AND gate” multiresponsive (MR) block amphiphilic copolymers are reported, aimed to take full advantage of the coexistence of two chemical cues—ROS and low pH—present in the tumor microenvironment. The hydrophobic blocks contain masked pH-responsive side chains, which are exposed exclusively in response to ROS. Hence, the hydrophobic polymer side chains will undergo a change shift in a very relevant pH window present in the extracellular milieu in most solid tumors (pH 5.6–7.2) after demasking by ROS. Doxorubicin (DOX)-loaded nanosized “AND gate” MR polymersomes (MRPs) are fabricated via microfluidic self-assembly. Chemical characterization reveals ROS-dependent pH sensitivity and accelerated DOX release under influence of both ROS and low pH. Treatment of tumor-bearing mice with DOX-loaded nonresponsive and “AND gate” MRPs dramatically decreases cardiac toxicity. The most optimal “AND gate” MRPs outperform free DOX in terms of tumor growth inhibition and survival, shedding light on chemical requirements for successful cancer nanomedicine.
hydrophobic (in the bilayer) and hydrophilic drugs (in the center) with high loading capacity.[5] Block copolymers can be designed and synthesized such to provide “stealth” properties in circulation, to enable easy functionalization, and to fine tune the mechanical properties of the resulting polymersome.[6] In addition, block copolymers can be tailored to induce a change in physical chemical properties or be degradable in response to specific stimuli.[6b–d]

Abnormal metabolism in multiple pathologies, including neurodegenerative diseases, diabetes, and cancer results in the dramatic elevation of reactive oxygen species (ROS) levels. Furthermore, in cancerous tissues, rapid growth, accelerated metabolism, and insufficient blood supply result in a lack of oxygen establishing a hypoxic condition, generally followed by the production of high lactic acid amounts. This contributes to the formation of an acidic microenvironment where the extracellular pH ranges from 5.6 to 7.6 depending on the tumor aggressiveness.[7]

The involvement of ROS and pH in cellular signaling and disease states has motivated the construction of chemical tools for pH or ROS-specific detection systems,[8] as well as pH[8a–c] or ROS-responsive micromedicines or NMs.[8d,e,9] Besides single-responsive block copolymers and polymersomes, some multiresponsive (MR) copolymers and coassemblies have been developed to be responsive to both low pH and ROS through the combination of pH- and ROS-sensitive units.[10][8] Although proven effective, such dual-responsive systems are intrinsically sensitive to the chemical cues independent of one another. We hypothesized that a nanomedicine formulation based on a polymeric system exclusively responding to two coexisting tumor-associated chemical cues could result in more selective cancer drug delivery. To the best of our knowledge, no such “AND gate” multiresponsive formulation has been reported to date. Therefore herein, we aimed to develop “AND gate” MR amphiphilic block copolymers to produce drug containing MR polymersomes (MRPs), which take full advantage of the coexistence of two chemical cues—ROS and low pH—present in the tumor microenvironment (Figure 1A).

2. Results and Discussion

We based our design on the previously developed pH-responsive polymersomes composed of diblock copolymers (dBCs) with a hydrophilic poly(N-(2-hydroxypropyl)methacrylamide) (HPMA) block, which is reported to constitute a repellent polymeric shell facilitating long circulation,[9d,13] and a hydrophobic poly(2-(dissopropylamino)ethyl methacrylate) (PDPA) block, which in an environment with a pH below the pKₐ of the tertiary amines, the hydrophobic PDPA block will become protonated and charged, which destabilizes the polymersomes resulting in dictated accelerated cargo release.[8c,7] To transform this design into a multiresponsive “AND gate” system, we aimed to install an ROS-sensitive protecting group to mask the amine in the hydrophobic block. Therefore, as hydrophobic block we synthesized poly(2-(ethyl or isopropylamino)ethyl methacrylate) in which the secondary amines are masked as 4-(hydroxymethyl)phenylboronic acid pinacol ester carbamates (Scheme S1, Supporting Information). This block is tethered to an alkyl-azide-capped hydrophilic PHPMA block (1a MR-ethyl (MRE) and 1b MR-isopropyl (MRI) (Figure 1B; Scheme S2, Supporting Information). For synthesis and characterization, see Sections S1 and S8, Schemes S1 and S2, and Figures S1–S8 in the Supporting Information.

In the carbamate form, the hydrophobic block is not responsive to pH. Only after exposure to ROS, oxidation and self-immolative cleavage of the phenylboronic ester, the secondary amine will be exposed, rendering the hydrophobic block pH responsive (Figure 1C). This enables a hydrophobic to hydrophilic switch upon protonation in acidic environment. As nonresponsive (NR) control molecule (2, Figure 1D; Scheme S3, Supporting Information), we synthesized a dBC bearing side chains in the hydrophobic block as depicted in Figure 1D (Section S8, Figures S9–S11, Schemes S2 and S3, Supporting Information). The terminal azide on each polymer allowed further functionalization with cyclo-RGD peptide[10b,11c,d,12] and cyanine7 (Cy7) dye, for tumor targeting and in vivo biodistribution studies, respectively (3a,b and 4a,b, Figure 1E; Section S2 and Figure S12, Supporting Information). The cyclic arginyl-glycyl-aspartic acid (cyclo-RGD) peptide, the cyclized fragment of laminin, avidly binds to αvβ3 integrin receptors, which are overexpressed in neovascularization associated with various types of cancer, including T-cell lymphomas.[13] The weight-average molecular weight (Mₐ) of the synthesized dBCs was ≈16.8 kDa with reasonable dispersity D ≈ 1.37 for MRE (1a), D ≈ 1.19 for the MRI (1b), and ≈18.5 kDa for the NR dBC (2) (D ≈ 1.10) as determined by size-exclusion chromatography (Table 1; Section S1 and Figure S13, Supporting Information). The obtained hydrophilic/hydrophobic weight ratios of the dBCs (ϕ = volume fraction of the hydrophobic block = 10–40%) (Table 1) facilitate the preparation of polymersomes with high colloidal stability, almost null critical aggregation concentrations, and flexibility due to their mechanical and physical properties (Figure 1E).[5,9a,14] Furthermore, polymersomes enable the encapsulation of hydrophobic cargo in the interior, as well as hydrophobic cargo in the bilayer polymersomes wall, making our formulation amendable to a variety of biomedical and material science applications.

Having produced the dBCs, we first established the ROS-responsive capacity of the MRE (1a), MRI (1b), and NR (2) dBCs by H₂O₂ scavenging[15] (Figure 2A) assays, as well as by ¹H NMR (Section S1 and Figures S14–S18, Supporting Information), by quantification of the formation of p-hydroxymethylphenol in response to 1 × 10⁻¹ m H₂O₂[8e,9b,16] (Figure S19, Supporting Information). As expected, NR (2) is not sensitive to H₂O₂, while MRE (1a) and MRI (1b) are shown to be responsive to pathophysiologically relevant levels of H₂O₂ (≥1 × 10⁻⁶ m).[8e,9b,16] Interestingly, MRI (1b) seems more susceptible to reaction with H₂O₂ and subsequent unmasking of the secondary amine on the diblock copolymer than MRE (1a), while similar amounts of phenylboronic esters are incorporated into these two dBCs (Table 1). A larger fraction of the available 200 × 10⁻⁶ m H₂O₂ is consumed after 3 h incubation, as determined by the Amplex Red Hydrogen Peroxide/Peroxidase Assay (Figure 2A). Furthermore, the release kinetics and amplitude of p-hydroxymethyl phenol are ≈3 times higher for MRI compared to MRE as measured by ¹H NMR over time (Section S1 and Figure S19, Supporting Information). Next, to determine pH-responsive capability, potentiometric acid–base titration curves for 1.0 mg mL⁻¹ of dBCs solutions were determined in simulated physiological conditions at ionic strength, I = 0.15 mol L⁻¹ before and after treatment with
Figure 1. Nanosized “AND gate” multiresponsive polymersomes. A) Schematic presentation of “AND gate” multiresponsive drug delivery in a tumor environment with coexisting high ROS concentration and low pH. B) Structure of the “AND gate” multiresponsive diblock copolymers, composed of an alkyl-azide-capped (in black) PHPMA hydrophilic block (in blue) and an ROS-activatable pH-responsive hydrophobic block (in red). C) Mechanism of ROS-dependent pH responsiveness of the “AND gate” multiresponsive diblock copolymers. D) Structure of the side chain of the hydrophobic block of the nonresponsive diblock copolymer. E) Microfluidic self-assembly of the diblock copolymers into nanosized “AND gate” multiresponsive polymersomes.

hydrogen peroxide (H₂O₂, 1 × 10⁻³ M) (Figure 2B; Section S1, Supporting Information).[8a,c] Before oxidation, hence with the phenylboronic ester mask in place, similar ζ-potential values were measured with negative charges marginally decreasing along the pH 3.0–10.0 gradient for all the dBCs. However, after H₂O₂ treatment, a charge-reversal effect was observed for MRE (1a) (pKₐ ≈ 5.8) and MRI (1b) (pKₐ ≈ 5.5), but not for NR (2). Based on these results we expect our diblock copolymers to be responsive at the lower end of the range of extracellular pH in most solid tumors (pH 5.6–7.2),[7] as has been reported for other synthetic NMs composed of block copolymers with a similar range of pKₐ and charge-reversal effect in xenograft mouse tumor models.[10b] Having characterized the chemical properties of the dBCs, we next set out to employ them to encapsulate doxorubicin (DOX) through assembly to nanosized polymersomes via hydrodynamic...
Figure 2. Characterization of nanosized “AND gate” multiresponsive polymersomes. A) H₂O₂ consumption. 0.1 mg of diblock copolymers was taken up in H₂O₂ (200 x 10⁻⁶ M). After 1 h incubation, the H₂O₂ concentration was determined by Amplex Red Hydrogen Peroxide/Peroxidase Assay. Poly(lactic acid)-block-poly(ethylene oxide) (PLA-b-PEO) was used as nonresponsive control (n = 3). B) ζ-potential over pH 3.0–10.0 gradient, before and after H₂O₂ (1 x 10⁻³ M) treatment (n = 3). C) Cryo-TEM images of NR and MR polymersomes in PBS buffer, scale bar = 100 nm. D) Number-weighted size distribution of the polymersomes under different simulated conditions after 24 h incubation (n = 3). E) In vitro DOX release assay under simulated ROS and pH conditions (n = 3).
flow focusing nanoprecipitation microfluidics (Figure 1E; Section S3 and Figure S20, Supporting Information). Microfluidics enables the reproducible production of polymersomes with comparable size, shape, cargo, and characteristics via optimized flow rate and organic solvents’ selection (Figure 1E; Section S3, Table S1, and Figure S20, Supporting Information). Homogeneous spherical polymersomes were obtained with all dBCs (Figure 2C).

An number-weighted size distribution of approximately 70 nm (polydispersity index (PDI) = 0.15) for NR, 74 nm (PDI = 0.13) for MRE, and 71 nm (PDI = 0.13) for MRI in phosphate-buffered saline (PBS) was determined by dynamic light scattering (DLS), crossection electron microscopy (cryo-TEM), and TEM (Figure 2C,D; Section S1 and Figure S21, Supporting Information), which falls within a size range known to be ideal for efficiently NM extravasations and tumor accumulation.18 DOX loading was determined to be 2.5% w/w (see Table S1 and Section S3 in the Supporting information). As a first step to characterize the responsiveness of the NMs, the effect of low pH (acetate buffer pH 5.3), ROS (1 × 10−3 M H2O2), and the combination thereof on the size of the polymersomes was determined (Figure 2D). The diameters of all NM formulations remained unchanged after 24 h incubation in PBS 7.4 or at pH 5.3, demonstrating high stability under these conditions and unresponsiveness to acidity as a single chemical cue as also evidenced by the DLS data (Figure 2D). ROS exposure (at pH 6.5) accelerates the DOX release for both responsive formulations, increasing the cumulative release to ≈50%. The DOX release is even further enhanced when the responsive NMs are in an environment in which ROS and pH 5.3 coexist, reaching a cumulative DOX release of 66.7 ± 2.8% for MRE-DOX and 78.4 ± 2.9% for MRI-DOX. In addition, the DOX release from single pH-responsive NMs manufactured exclusively containing the ethyl- or iso-unmasked pH-responsive side chains (Sections S1 and S4, Figures S21d,e, S22, and S23, Table S1, and Scheme S2, Supporting Information) was monitored (Figure S22, Supporting Information). DOX release kinetics at pH 5.3 were very similar independent of the presence of H2O2 reaching a cumulative release of ≈56%, which is most likely connected with the pH-switchable property of the ethyl- and iso-unmasked pH-responsive side chains (pKα ≈ 5.8, ethyl; pKα ≈ 5.5, iso, Figure 2B). Taken together, these data indicate that judged by the changes in appearance and the kinetics of DOX release in response to low pH and ROS alone or the combination of ROS and low pH, both MRI-DOX and MRE-DOX polymersomes respond in a multiresponsive fashion. Compared to MRE-DOX, MRI-DOX polymersomes seem to approach a more “AND gate” character under the conditions tested, possibly due to slightly higher pH sensitivity of MRI (1b) (Figure 2B).

Several phenyl boronic acid and ester ROS-responsive systems have been reported to release amino functionalities on polymer backbones in nanoformulations. For example, Deng et al. reported a phenyl boronic ester benzyl carbamate system which, after release of the primary amine, underwent vesicle bilayer cross-linking in response to ROS.19 Our DLS and NMR data show long-term existence of a positively charged secondary amine after phenyl boronic ester’s decomposition. Liu et al. described a boronic acid benzyl functionalized positively charged quaternary amine as a chelator for DNA delivery purposes. Upon ROS exposure, the benzyl boronic acid decomposes to generate an uncharged tertiary amine, resulting in the release of DNA, followed by water-mediated degradation of the side chain to release a carboxylic acid functionality on the polymer backbone.10b As outlined above, our data indicate that ROS-induced unmasking exposes a persisting pH sensor, which is effective (protonation/deprotonation) at a relevant pH window that is present in

Table 1. Synthetic parameters and molecular weight data of polymers prepared via reversible addition-fragmentation chain-transfer (RAFT) polymerization.

| (Co)polymer | Sample name | [Mn]/[CTA]/[I]p | Time [h] | Conversion [%] | $D (M_w/M_n)$ | $M_w$ SEC [g mol⁻¹] | $\phi$ |
|------------|-------------|-----------------|-----------|----------------|----------------|-------------------|-----|
| PHPMA      | P[(HPMA)25] | 100/2/10        | 18        | 36(1)         | 1.08(1)       | 3600(1)          | –   |
| NR         | P[(HPMA25)-b-(NR)18] | 100/2/10       | 24        | 90(5)         | 1.10(5)       | 9675              | 0.37|
| MRE        | P[(HPMA25)-b-(MRE)30] | 100/2/10       | 24        | 82(5)         | 1.37(5)       | 16 120            | 0.22|
| MRI        | P[(HPMA25)-b-(MRI)30] | 100/2/10       | 24        | 88(5)         | 1.19(5)       | 14 720            | 0.24|

(1) Weight fraction of the hydrophilic block (size exclusion chromatography (SEC)); (2) Conditions: tert-butanol, [Mn] = 3 m, 70 °C; (3) Determined by 1H NMR in D2O; (4) Determined by SEC in MeOH/acetate buffer, pH 6.5, 80/20 vol%; (5) Conditions: 1,4-dioxane/MeOH, vol% 60/40, [Mn] = 3 m, 70 °C; (6) Determined by 1H NMR in DMF; (7) Determined by SEC in DMF using poly(methyl methacrylate) (PMMA) as the standard.
they retain their hydrodynamic size over time. 

Having chemically characterized the dBCs and DOX-containing polymersomes, we set out to unveil their in vitro and in vivo chemotherapeutic potentials. First, to investigate uptake and cytotoxicity 4T1 (mouse mammary carcinoma) and B16F10 (mouse melanoma) cancer cell lines were exposed to NR–DOX, MRE–DOX, and MRI–DOX for 12 and 24 h. Similar particle uptake was determined for all formulations by measuring DOX fluorescence (Section S5 and Figure S24, Supporting Information), which is in line with their similar surface chemistry, size, shape, and charge (Figure 2B–D; Table S1, Supporting Information). All DOX-encapsulated NMs showed a dose-dependent cytotoxicity, whereas, importantly, the AND gate multiresponsive dBC formulation itself showed no effect on viability (Figure S25, Supporting Information). The serum stability of the NMs was monitored by evaluating their hydrodynamic size over time.\(^{{8f,21c}}\) Figure S26 (Supporting Information) shows the temporal stability of the polymersomes in 10% (v/v) diluted human plasma in PBS as a function of the incubation time (Section S1, Supporting Information). The size patterns of the polymersomes do not change within 24 h suggesting that the NMs are highly stable against aggregation in the simulated physiological media. The slight increase in hydrodynamic size (\(D_H = 8–10\) nm) should be attributed to the adsorption of a protein monolayer (corona) because the average size of the dissolved single proteins is \(\approx\) 8 nm.\(^{{8f,21c}}\)

With the aim to enhance antitumor efficacy, we introduced active cyclo-RGD-based tumor neovasculature targeting into our system. The influence of targeting av\(\beta\)3 integrin receptors on in vivo biodistribution and tumor accumulation was investigated using subcutaneous EL-4 (mouse lymphoma) tumor-bearing athymic nude foxn1nu mice. Coassemblies of MRE, MRI, and NR with or without cyclo-RGD, together with the respective Cy7-labeled dBCs (Sections S2 and S6, Figure S12, and Table S1, Supporting Information), were injected intravenously. Cy7 accumulation in the tumors was determined by noninvasive fluorescence imaging of live mice over a time course of 48 h (Figure 3A; Section S6, Supporting Information). Free dibenzocyclooctyne (DBCO)–Cy7 showed a steady decrease in fluorescence intensity from 2 h post injection onward. All polymersome formulations exhibited steady tumor accumulation or even increase up to 4–24 h after injection, after which a gradual decay in fluorescence intensity could be observed. Of the formulations tested, cRGD–MRE–Cy7 achieved the highest intensity at all time points. After 48 h, the ex vivo fluorescence intensity was measured in tumor, heart, kidneys, liver, lungs, and spleen (Figure S27, Supporting Information). Ex vivo tumor fluorescence corresponded with the final time point of noninvasive imaging. Overall, cyclo-RGD-equipped fluorescent coassemblies showed a higher accumulation in tumor, as well as kidneys, lungs, and liver compared to the nontargeted assemblies. In terms of tumor targeting, the biggest advantage of cyclo-RGD modification seems to be achieved for MRE (Figure S27a, Supporting Information).

Ultimately, the in vivo therapeutic efficacy of the AND gate multiresponsive DOX NMs was evaluated in subcutaneous EL-4 (mouse lymphoma) tumor-bearing C57BL/6N mice (Section S6, Supporting Information). Seven days after tumor inoculation, vehicle control, free DOX, cRGD–NR–DOX, cRGD–MRE–DOX, or cRGD–MRI–DOX were administered intravenously in three doses of 5 mg kg\(^{-1}\) DOX with 4 day intervals. All treatments with DOX led to tumor growth inhibition (Figure 3B) and survival benefit (Figure 3C) compared to the saline control. DOX and notably cRGD–MRE–DOX treatment induced temporary tumor reduction, which translated in the significant increase in overall survival for the mice treated with cRGD–MRE–DOX over all other treatment groups. Cardiac toxicity is considered a major side effect of DOX treatment.\(^{{22}}\) As a measure of cardiac toxicity, the enzymatic activity of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were determined in the serum of the mice 15 days after the start of the treatment (Section S7, Supporting Information).\(^{{22}}\) DOX treatment induced a dramatic increase in CPK activity in the serum, which could be reduced to levels close to those detected in the saline control groups by DOX polymersome formulation with our dBCs (Figure 3D). DOX-induced serum LDH activity could be reduced about 50% by administration with the cyclo-RGD-targeted polymersomes. Taken together, these results indicate that, of the formulations tested, the cRGD–MRE–DOX AND gate multiresponsive polymersomes deliver the most optimal therapeutic DOX efficacy with increased survival benefit and dramatically reduced cardiotoxic side effects. MRE was shown to be more pH sensitive compared to MRI after exposure to ROS exhibiting the biggest change in colloidal stability already apparent at pH 6.5 (Figure 2D). This, combined with sustained AND gate multiresponsive DOX release over time (Figure 2E), might form the basis for the therapeutic performance observed for cRGD–MRE–DOX.

3. Conclusion

In summary, we successfully developed functionalizable ROS and pH “AND gate” multiresponsive amphiphilic block copolymers, aimed to take full advantage of the coexistence of two chemical cues—ROS and low pH—present in the tumor microenvironment for cancer drug delivery applications. The hydrophilic blocks contain 4-(hydroxymethyl)phenylboronic acid pinacol ester carbamate masked pH-responsive side chains, which are exposed exclusively in response to ROS. Ethyl (MRE) and isopropyl (MRI) secondary amine side chains were synthesized as two versions of the pH-responsive entities, differing in charge-reversal pH and hydrophobicity, as well as a nonresponsive side-chain version. These blocks are linked to an alkyl-azide-capped hydrophilic block, which allowed further functionalization with cyclo-RGD for tumor av\(\beta\)3 integrin receptor targeting and Cy7 dye for in vivo biodistribution studies. Nanosized “AND gate” polymersomes loaded with DOX were fabricated via microfluidic self-assembly. Chemical characterization revealed ROS-dependent pH sensitivity of the polymersomes and accelerated DOX release under influence of both ROS and low pH, particularly within a relevant pH window that is present in the extracellular milieu in most solid tumors. Treatment of tumor-bearing mice with DOX-loaded nonresponsive and “AND gate” multiresponsive polymersomes dramatically decreased cardiac toxicity. Only one of these formulations, cRGD–MRE–DOX, outperformed free DOX in terms of tumor growth inhibition and survival, shedding light on chemical requirements for successful responsive cancer nanomedicine drug delivery.
**4. Experimental Section**

**Materials:** 3-Chloro-1-propanol (98%), sodium azide (NaN₃, ≥99.5%), 3-azido-1-propanol (≥96%), N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, crystalline) triphosgene (98%), 2-(ethylamino) ethanol (≥98%), triethylamine (TEA, ≥99%), H₂O₂ (30%/w solution in H₂O), 4-(hydroxymethyl)phenylboronic acid pinacol ester (97%), magnesium sulfate (MgSO₄), sodium sulfate (Na₂SO₄), silica gel (70–230 mesh), Sephadex LH-20, Sephadex A G-50, 1-hydroxybenzotriazole hydrate (HOBt, ≥97%), ethanolamine (≥99%), 4-(dimethylamino)pyridine (DMAP, ≥99%), dibenzocyclyctyne-N-hydroxysuccinimidyl ester (DBCO-NHS), o-phtaldialdehyde (OPA, ≥95%), 3-mercaptopropionic acid (MPA, ≥99%), potassium tetraborate tetrahydrate (≥99.5%), L-arginine hydrochloride (>99%), L-glycine hydrochloride (≥99%), L-aspartic acid (>99%), and ethylenediaminetetraacetate (EDTA) solution were purchased from Sigma–Aldrich (Czech Republic). The 4-isopropylphenylacetic acid was purchased from Santa Cruz Biotechnology (USA). Doxorubicin hydrochloride (>99%) was purchased from LC Laboratories (USA). DBCO–Cy7 was purchased from Lumiprobe (Germany). Cyclo(-RGDfK) peptide was purchased from MedChemExpress (USA). Solvents methanol (MeOH), N,N-dimethylacetamide (DMAc),

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**Figure 3.** Tumor accumulation, chemotherapeutic tumor challenge model, and assessment of cardiac toxicity. A) Noninvasive imaging of Cy7 tumor accumulation in EL-4 (mouse lymphoma) tumor-bearing athymic nude FoxN1 Nju mice injected intravenously (i.v.) with the indicated Cy7 formulations over 48 h (n = 3 mice per group). Representative image with tumor demarcated (left) and graph of tumor fluorescence intensity over time (right). B–E) C57BL/6N mice were inoculated subcutaneously with EL-4 tumor cells. Tumors were allowed to develop for 7 days, after which the mice were treated i.v. with three doses with 4 day interval of saline, free DOX, or cyclo-RGD functionalized NMs (n = 6–8 mice per group). B) Tumor growth of T-cell lymphoma EL-4. C) Kaplan–Meier survival plot of mice after treatment. D) Serum creatine kinase and E) lactate dehydrogenase (LDH) levels after treatment in mice bearing EL-4 lymphoma tumors. The arrows in panels (B) and (C) indicate the days of DOX or equivalent administrations. Statistical significance: *p < 0.05, one-way ANOVA.
diethyl ether, 1,4-dioxiane, ethanol (EtOH), tetrahydrofuran (THF), N,N-dimethylformamide (DMF), dichloromethane (DCM), toluene, ethyl acetate, petroleum ether, and hexane were purchased from Lachner (Czech Republic) and dried over molecular sieves (3 Å). Methacryloyl chloride (≥97%) was distilled before use. Deuterated solvents (CDCl₃, d₇-DMF, d₆-dimethyl sulfoxide (d₆-DMSO), CD₃OD) were purchased from Euriso-top (France). Azobisisobutyronitril (AIBN, ≥99%) and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (chain transfer agent (CTA), ≥97%) were purchased from Sigma–Aldrich and recrystallized from methanol prior to use.

Section S1 (Supporting Information) describes the methods used for the preparation of the monomers, block copolymers, and nanomedicines, as well as the characterization (size exclusion chromatography, ¹H NMR and ¹³C NMR, UV–vis spectrophotometry, Fourier transform infrared attenuated total reflection spectroscopy, amino acid analysis, dynamic and electrophoretic light scattering, pH titration, transmission electron microscopy, and cryogenic transmission electron microscopy). Section S2 (Supporting Information) describes the coupling of the cyclo-RGD peptide and the Cy7–DBCO fluorescent dye to the block copolymers. Section S3 (Supporting Information) discusses the microfluidics design rationale and fabrication of the nanomedicines. Section S4 (Supporting Information) describes the in vitro DOX-release experiments. Section S5 (Supporting Information) describes the in vitro cell experiments (cell culture, cellular uptake, and viability assay), and Section S6 (Supporting Information) describes the in vivo studies (biodistribution and antitumor activity). Section S7 (Supporting Information) describes the creatine phosphokinase (CPK) assay, and Section S8 (Supporting Information) describes the in vivo studies (biodistribution and antitumor activity). Section S9 (Supporting Information) describes the characterization (size exclusion chromatography, ¹H NMR and ¹³C NMR, UV–vis spectrophotometry, Fourier transform infrared attenuated total reflection spectroscopy, amino acid analysis, dynamic and electrophoretic light scattering, pH titration, transmission electron microscopy, and cryogenic transmission electron microscopy).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
