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pH Responsive, Functionalizable Spyrocyclic Polycarbonate: A Versatile Platform for Biocompatible Nanoparticles

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ABSTRACT: Polymeric nanoparticles are widely investigated to enhance the selectivity of therapeutics to targeted sites, as well as to increase circulation lifetime and water solubility of poorly soluble drugs. In contrast to the encapsulation of the cargo into the nanostructures, the conjugation directly to the polymer backbone allows better control on the loading and selective

triggered release. In this work we report a simple procedure to create biodegradable polycarbonate graft copolymer nanoparticles *via* a ring opening polymerization and subsequent post-polymerization modification strategies. The polymer, designed with both pH responsive acetal linkages and a norbornene group, allows for highly efficient post-polymerization modifications through a range of chemistries to conjugate imaging agents and solubilizing arms to direct self-assembly. To demonstrate the potential of this approach, polycarbonate-based nanoparticles were tested for biocompatibility and ability to be internalized in A549 and IMR-90 cell lines.

INTRODUCTION

In the past three decades, polymeric nanoparticles have emerged as a promising tool for targeted delivery. Their ability to increase the half life of cytotoxic drugs in the blood, impart aqueous solubility, minimize recognition and uptake by reticuloendothelial systems, and enhance structural stability has made them attractive to study as potential delivery vehicles.[1-5] In particular, polymeric conjugates, where the cargo is covalently linked to the polymer backbone, as opposed to encapsulated in the polymeric core, are especially attractive, as they allow predictable drug loading, enhanced stability at normal physiological conditions and afford a high level of control over the selective triggering of the release event which leads to enhanced therapeutic efficacy and minimizes potential side effects.[6] Furthermore, if the conjugated cargo can be released in response to a specific intracellular trigger (*i.e.* changes in pH, temperature, or the presence of redox linkers *etc.*), the delivery can be selectively directed to the targeted site.[7-9] Among these, many passive targeting strategies hinge on the fact that the extracellular pH of both primary and metastasized tumors is lower than the pH of normal tissues.[10-14] pH sensitive micelles can be triggered to release therapeutic agents in endosomes or lysosomes by hydrolysis or dissociation after uptake by cells *via* the endocytic pathway,[6, 15-17] as the pH experienced by the micelles once they enter cells *via* endocytosis can drop as low as 5.0-6.0 in endosomes and 4.0-5.0 in lysosomes.[18, 19] The fate of the endocytosed polymeric micelles typically relies on the efficiency of lysosomal escape, which has to be sufficiently fast to avoid degradation by lysosomal enzymes but prolonged enough to ensure particle disassembly.[20]

A number of chemistries have been utilized to produce micelles in which the release of the cargo is triggered by a drop in pH.[21] Hydrazone,[22-26] orthoester,[27-29] vinyl ether,[30, 31] cys-acotinyl[32, 33] and acetal[34-38] linkages have each been shown to release the conjugated drug much

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faster at a pH interval between 5.0 and 6.0, compared to physiological pH 7.4. Among them, polyacetals undergo a pH-dependent degradation but also produce biocompatible degradation products, such as alcohols and aldehydes. Many of the approaches used to prepare such polymeric conjugates however, rely on multi-step syntheses of amphiphilic di-block (hydrophilic-hydrophobic) or tri-block (hydrophilic-hydrophobic-hydrophilic) copolymers,[4, 14, 39-41] or composite architectures, such as dendrimeric/hyperbranched structures,[42-44] that have significant non-degradable fragments. Further innovative approaches have included multi-responsive polymers that lead to micelle disassembly to trigger release in cells.[14, 37, 45-48] In many micellar systems, the preference for the encapsulation of the cargo over covalent conjugation, difficult self-assembly approaches, and sometimes limited control over loading and product yields make these strategies less attractive for translation. In order to overcome these limitations, we herein report the synthesis of a novel pH responsive, biodegradable polycarbonate, synthesized *via* an easily accessible ring opening polymerization method. The introduction of the versatile norbornene moiety in the monomer structure affords the facile covalent attachment of multiple functionalities through a range of convenient routes. The self-assembled system was found to be highly biocompatible, as demonstrated by a viability > 95% in A549 (human lung cancer fibroblasts) and IMR-90 (human lung fibroblasts). Furthermore, the ability of the above cell lines to uptake such nanoparticles demonstrates their potential as drug delivery systems.

EXPERIMENTAL SECTION

Materials and Methods. All reagents used for the monomer synthesis, polymerization reactions and coupling reactions were purchased from Sigma-Aldrich. All reagents were used without any further purification, except for 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and

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benzyl alcohol which were dried over calcium hydride and distilled under vacuum before polymerization. BODIPYC$_{10}$-SH,[49] 11-azidoundecanoic acid,[50] and PEG$_{550}$-SH,[51, 52] were synthesized following previously published procedures. NMR spectra were recorded on a Bruker HD-300 and HD-400 spectrometer at 293 K unless otherwise stated. Chemical shifts are reported as δ in parts per million (ppm) and referenced to the chemical shift of the residual solvent resonances (CHCl$_3$: ^{1}H δ = 7.26 ppm, ^{13}C δ = 77.16 ppm; DMSO: 6H δ = 2.50 ppm, ^{13}C δ = 39.52 ppm). Size exclusion chromatography (SEC) was used to determine the molecular mass and molecular mass distributions (dispersities, $Đ_M$) of the synthesized polymers. SEC in chloroform was conducted on a system comprised of a Varian 390-LCMulti detector suite fitted with differential refractive index (DRI), light scattering (LS) and ultra-violet (UV) detectors, equipped with a guard column (Varian Polymer Laboratories PLGel 5 mM, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5 mM, 300 × 7.5 mm). The mobile phase was chloroform with 5% triethylamine eluent at a flow rate of 1.0 mL min^{-1}, and samples were calibrated against Varian Polymer laboratories Easi-Vials linear poly(styrene) standards (162-2.4 × 105 g mol^{-1}) using Cirrus v3.3. Fluorescence microscopy images were obtained using fluorescence ORCM Hamamatsu camera, at ×20 magnification.

Synthesis of 2-norbornene-5,5-bis(hydroxymethyl)-1,3-dioxane (NHD): Following previously reported procedures,[53, 54] pentaerythritol (13.5 g, 99.2 mmol) was suspended in 100 mL of deionised water and heated to 80 °C under stirring until all the solid had dissolved. The solution was cooled down to ambient temperature before the addition of conc. HCl (330 μL, 3.26 mmol) with continual stirring for a further 15 min. 5-Norbornene-2-carboxaldehyde (10.0 g, 89.2 mmol) was added drop-wise to the acidified solution over 20 min and allowed to stir for a further

2 h. The mono-functionalised product formed an orange precipitate which was collected *via* vacuum filtration before further purification by silica plug, ethyl acetate as the eluent, and re-crystallization from hot toluene (30 mL) to yield white crystals (10.8 g, yield: 51 %). ^{1}H NMR (400 MHz; DMSO-d_6): δ 6.15 (dd, 1H, $^3J_{H-H}$ = 5.7, 3.0 Hz), 5.90 (dd, 1H, $^3J_{H-H}$ = 5.7, 3.0 Hz), 4.52 (t, 1H, $^3J_{H-H}$ = 5.4 Hz), 4.40 (t, 1H, $^3J_{H-H}$ = 5.2 Hz), 3.76-3.66 (m, 3H), 3.54 (d, 2H, $^3J_{H-H}$ = 5.2 Hz), 3.42 (m, 2H), 3.13 (d, 2H, $^3J_{H-H}$ = 5.2 Hz), 2.83 (s, 1H), 2.76 (s, 1H), 2.16 (ddd, 1H, $^3J_{H-H}$ = 12.8, 8.5, 4.0 Hz), 1.72 (ddd, 1H, $^3J_{H-H}$ = 12.8, 9.3, 3.8 Hz), 1.30-1.14 (m, 2H), 0.72 (ddd, 1H, $^3J_{H-H}$ = 11.9, 4.1, 2.6 Hz). ^{13}C NMR (101 MHz; DMSO-d_6): δ 137.26 (CH), 132.58 (CH), 105.41 (CH), 68.64 (CH$_2$), 68.46 (CH$_2$), 61.02 (CH$_2$), 59.57 (CH$_2$), 48.71 (CH$_2$), 43.19 (CH), 43.11 (CH), 41.62 (CH), 28.21 (CH$_2$).

Synthesis of 2-norbornene-5,5-bis(hydroxymethyl) trimethylene carbonate (NTC): In a dry 2-necked round bottom flask NHD (4.0 g, 16.7 mmol) was dissolved in 400 mL of THF and cooled to 0 °C using an ice-bath. Under a N$_2$ blanket, ethyl chloroformate (4.78 mL, 49.9 mmol) was slowly added and the solution stirred for 30 min. Triethylamine (6.95 mL, 49.9 mmol) was added drop-wise over 45 min and the reaction was stirred for 3 h while being allowed to warm to ambient temperature. The resultant salt formed during the reaction was removed *via* vacuum filtration and was further rinsed with THF (20 mL). The filtrate and washings were combined and solvent was removed *in vacuo* to yield off-white crystals. The crude product was purified by re-crystallization from hot cyclohexane/THF (30 mL) to yield white crystals. NTC was dried over phosphorus pentoxide and stored in the glovebox (2.6 g, yield: 59 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.15 (dd, $^3J_{H-H}$ = 5.7, 3.0 Hz, 1H), 5.93 (dd, $^3J_{H-H}$ = 5.7, 2.8 Hz, 1H), 4.59 (app. s, 2H), 4.05-3.83 (m, 3H), 3.96 (app. s, 2H), 3.57-3.52 (m, 2H), 2.93 (s, 1H), 2.83 (s, 1H), 2.27 (ddd,

$^3J_{\text{H-H}}$ = 12.8, 8.6, 3.9 Hz, 1H), 1.86 (ddd, $^3J_{\text{H-H}}$ = 12.8, 9.3, 3.8 Hz, 1H), 1.37-1.18 (m, 2H), 0.85 (ddd, $^3J_{\text{H-H}}$ = 11.9, 4.1, 2.6 Hz, 1H). ^{13}C NMR (300 MHz; CDCl$_3$): δ 153.73 (C=O), 137.92 (CH), 132.80 (CH), 128.65 (CH), 107.24 (CH), 68.67 (CH$_2$), 67.17 (CH$_2$), 49.39 (CH$_2$), 43.85 (CH), 42.22 (CH), 37.41 (CH), 28.51 (C). Mass spectrometry (ESI +ve); m/z = 267.12 (M$^+$). Elemental analysis; anal. calcd for C$_{14}$H$_{18}$O$_5$: C 63.15; H 6.18; N 0 %. Found: C 63.15, H 6.81, N 0.01 %.

General procedure for the organocatalysed ROP of NTC: All polymerizations were carried out using standard glovebox and Schlenk-line techniques. The ROP of NTC using 1 mol% DBU was carried out in dry CDCl$_3$ at ambient temperature using benzyl alcohol (BnOH) as the initiator. In a dry scintillation vial, NTC (66.5 mg, 2.5×10^{-1} mmol) was dissolved in 500 µL of CDCl$_3$ before the addition of freshly prepared stock solutions of DBU (4 µL, 2.5×10^{-3} mmol, 1 µL per 9 µL CDCl$_3$ stock) and BnOH (dependant on target chain length). The polymerisation was stopped by precipitation into hexanes and the polymer recovered *via* a silica plug. The crude material was loaded onto the silica plug in CH$_2$Cl$_2$. The residual monomer was eluted using CH$_2$Cl$_2$ (*R*f = 0.9) before a direct solvent switch to ethyl acetate was employed to elute the pure polymer (*R*f = 0.9), with the DBU catalyst remaining on the silica (*R*f = 0). DP20 homopolymer; ^{1}H NMR (400 MHz; CDCl$_3$): δ 7.36 (m, 5H), 6.15 (m, 20H), 5.93 (m, 20H), 5.15 (s, 2H) 4.54-4.33 (m, 40H), 4.08-3.71 (m, 110H), 3.66-3.42 (m, 40H), 2.92 (s, 20H), 2.81 (s, 20H), 2.30 (m, 20H), 1.81 (m, 20H), 1.44-1.15 (m, 40H), 0.82 (m, 20H). ^{13}C NMR (101 MHz; CDCl$_3$): δ 147.59 (C=O), 137.51 (CH), 132.45 (CH), 105.96 (CH), 70.70 (CH$_2$), 69.86 (CH$_2$), 67.79 (CH$_2$), 67.68 (CH$_2$), 48.70 (CH$_2$), 43.07 (CH), 42.97 (CH), 41.61 (CH), 28.11 (CH$_2$). M_n = 4.2 kg·mol^{-1}, $Đ_M$ = 1.10 (RI detection, CHCl$_3$ SEC).

Synthesis of PEG$_{550}$ thiol: Poly(ethylene glycol) methyl ether (550 g mol^{-1}), (10 g, 18 mmol) and 3-mercaptopropionic acid (3.82 g, 36 mmol) was dissolved in a mixture of benzene and toluene (1:1, 200 mL) and the mixture was heated to 80 °C. Two drops of H$_2$SO$_4$ were added and the solution was heated to reflux under Dean Stark conditions for 16 h. The solution was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in CH$_2$Cl$_2$ (100 mL) and washed with saturated NaHCO$_3$ solution (3 x 50 mL), brine (3 x 50 mL) and dried (MgSO$_4$). Charcoal (*ca.* 0.1g) was added and the solution was filtered through Celite® 545, evaporation of solvent yielded product as viscous yellow oil (Yield 9.65 g, 81%). ^{1}H NMR (400 MHz; CDCl$_3$): δ 4.18 (m, 2H), 3.73-3.38 (m, 52H), 3.28 (s, 3H), 2.71-2.58 (m, 4H), 1.62 (t, 1H, $^3J_{H-H}$ = 16). ^{1}H NMR spectroscopy indicated ca. 98% conversion of the hydroxyl group to mercaptopropionate group. M_n = 725 g·mol^{-1}, $Đ_M$ = 1.21 (RI detection, CHCl$_3$ SEC).

Functionalisation of Poly(NTC) (PNTC): *Benzyl azide*: In a dry vial fitted with a stirrer bar, PNTC (DP10) (50 mg, 2.11 × 10^{-5} mmol) was dissolved in 500 µL of 1,4-dioxane. Benzyl azide (28 µL, 2.11 × 10^{-4} mol) was added to the solution before being sealed and heated to 90 °C for 12 h with stirring. The functionalised polymer was recovered by precipitation into cold methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; CDCl$_3$): δ 7.45-7.16 (m, 62H), 5.13 (m, 2H), 4.91 (m, 10H), 4.69-2.99 (m, 223H), 2.82-2.51 (m, 17H), 2.39-0.66 (m, 116H). M_n = 3.8 kg·mol^{-1}, $Đ_M$ = 1.14 (RI detection, CHCl$_3$ SEC).

Dodecanethiol: In a dry vial fitted with a stirrer bar, PNTC (DP10) (50 mg, 2.11 × 10^{-5} mol) was dissolved in 500 µL of CHCl$_3$. Dodecanethiol (50 µL, 2.11 × 10^{-4} mol) and 2,2-dimethoxy-2-phenylacetophenone photoinitiator (54 mg, 2.11 × 10^{-4} mol) were added to the solution before being sealed and UV irradiated for 30 min. The functionalised polymer was recovered by

precipitation into cold methanol methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; CDCl$_3$): δ 7.35 (m, 5H), 5.14 (s, 2H), 4.58-4.05 (m, 34H), 4.05-3.23 (m, 100H), 2.86 (d, 22H), 2.66-1.05 (m, 194H), 0.97-0.67 (m, 28H). M_n = 4.6 kg·mol^{-1}, $Đ_M$ = 1.18 (RI detection, CHCl$_3$ SEC).

PEG$_{550}$ thiol: In a dry vial fitted with a stirrer bar, PNTC (DP20) (100 mg, 2.11 × 10^{-5} mol) was

dissolved in 500 μL of THF. PEG$_{550}$SH (232 mg, 4.22 × 10^{-4} mol) and 2,2-dimethoxy-2-phenylacetophenone photoinitiator (106 mg, 4.22 × 10^{-4} mol) were added to the solution before being sealed and UV irradiated for 30 min. The functionalised polymer was recovered by precipitation into cold methanol methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; DMSO-*d6*): δ 7.38 (s, 9H), 5.13 (s, 3H), 4.68-3.58 (m, 460H), 3.55-3.30 (m, 1449H), 3.23 (s, 91H), 2.61 (m, 142H), 2.37-0.71 (m, 299H). M_n = 13.3 kg·mol^{-1}, $Đ_M$ = 1.10 (RI detection, CHCl$_3$ SEC).

Tetrazine: In a dry vial fitted with a stirrer bar, PNTC (DP10) (50 mg, 2.11 × 10^{-5} mol) was dissolved in 500 μL of 1,4-dioxane. 2,5-Dioxopyrrolidin-1-yl 6-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoate (76.6 mg, 2.11 × 10^{-4} mol) was added to the solution before being stirred at room temperature for 1 h. The functionalised polymer was recovered by precipitation into cold methanol methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; CDCl$_3$): δ 9.49-6.89 (m, 66H), 5.27 (s, 2H), 4.75-0.59 (m, 206H). M_n = 4.9 kg·mol^{-1}, $Đ_M$ = 1.2 (RI detection, CHCl$_3$ SEC).

Synthesis of 4-(11-azido-undecanamide)-*N*-methyl phthalimide: 11-Azido undecanoic acid (258 mg, 1.14 × 10^{-3} mol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (220 mg, 1.14 ×

10^{-3} mol) were dissolved in CH_2Cl_2 (3 mL). 4-Amino-*N*-methylphthalimide (20 mg, 1.14×10^{-4} mol) was then added and the reaction was stirred at 0 ˚C for 1 h, and for the following 24 h at rt. After this, the organic layer was diluted with CH_2Cl_2 (50 mL) and washed with 1 M HCl (3 × 50 mL) and 0.1% $NaHCO_3$ (3 × 50 mL). The organic layer was then dried with $MgSO_4$ and the solvent removed *in vacuo*. The product was purified *via* flash chromatography (CH_2Cl_2/Acetone) and obtained as a pale yellow solid (yield = 63%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.47 (s, 1H), 8.07-8.03 (dd, $^3J_{H-H}$ = 3 Hz, $^3J_{H-H}$ = 9 Hz, 1H), 7.99 (d, $^3J_{H-H}$ = 3 Hz, 1H), 7.74 (d, $^4J_{H-H}$ = 9 Hz, 1H), 3.22 (t, $^3J_{H-H}$ = 15 Hz, 2H), 3.13 (s, 3H), 2.43 (t, $^3J_{H-H}$ = 15 Hz, 2H), 1.71-1.67 (m, 2H), 1.59-1.50 (m, 2H), 1.24 (m, 12H). ^{13}C NMR (300 MHz; CDCl$_3$): δ 171.88 (C=O), 168.30 (C=O), 168.13 ((C=O), 143.64 (C), 133.90 (C), 126.93 (CH), 124.59 (C), 123.77 (CH), 114.03 (CH), 51.60 (CH$_2$), 37.96 (CH$_2$), 29.51 (CH$_2$), 29.43 (CH$_2$), 29.33 (CH$_2$), 29.22 (CH$_2$), 28.95 (CH$_2$), 26.81 (CH$_2$), 25.47 (CH$_3$), 24.13. Mass spectrometry (ESI +ve); m/z = 315.3 (M$^+$). Elemental analysis anal. calcd for $C_{15}H_{17}N_5O_3$: C 62.32; H 7.06; N 18.17. Found: C 61.99; H 7.03; N 17.71 %.

Synthesis of PNTC-*g*-BODIPY-*g*-PEG conjugate: In a dry vial fitted with a stirrer bar, PNTC (DP20) (50 mg, 1.09×10^{-5} mol) was dissolved in 500 µL of CHCl$_3$. BODIPYC$_{10}$-SH (10 mg, 2.4×10^{-5} mol) and 2,2-dimethoxy-2-phenylacetophenone (6 mg, 2.4×10^{-5} mol) were added to the solution before being sealed and UV irradiated for 30 min. The functionalised polymer was recovered by precipitation into cold diethyl ether methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; CDCl$_3$): δ 7.37 (m, 5H), 6.15 (m, 20H), 6.05 (s, 1H), 5.93 (m, 20H), 5.16 (m, 2H), 4.42 (m, 40H), 3.97-3.81 (m, 89H), 3.57 (m, 22H), 3.31 (m, 2H), 2.93-2.81 (m, 40H), 2.51-2.41 (m, 8H), 2.30 (m, 23H), 1.87-1.80 (m, 28H), 1.58 (m, 11H), 1.38-1.21

(m, 20H), 0.97 (s), 0.95 (s, 3H with δ 0.97), 0.90-0.81 (m, 20H). M_n = 4.4 kg·mol^{-1}, $Đ_M$ =1.17 (RI detection, CHCl$_3$ SEC), M_n = 4.3 kg·mol^{-1}, $Đ_M$ =1.17 (UV detection, 475 nm, CHCl$_3$ SEC).

PNTC-g-BODIPY (53 mg, 1.2×10^{-5} mol) was then re-dissolved in 500 µL of CHCl$_3$. PEG$_{550}$-SH (145 mg, 2.2×10^{-4} mol) and 2,2-dimethoxy-2-phenylacetophenone (56 mg, 2.2×10^{-4} mol) were added to the solution before being sealed and UV irradiated for 30 min. The functionalised polymer was recovered by precipitation into cold diethyl ether methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (400 MHz; CDCl$_3$): δ 7.35 (m, 5H), 6.03 (s, 1H), 5.13 (m, 2H), 4.38-4.23 (m, 90H), 3.92 (m, 72H), 3.62 (m, 810H), 3.48-3.42 (m, 118H), 3.35 (m, 50H), 2.76 (m, 24H), 2.61 (m, 27H), 2.48-2.39 (m, 9H), 2.28-2.19 (m, 20H), 2.00 (m, 20H), 1.64 (m, 30H), 1.26 (m, 10H), 0.90-0.81 (m, 16H). M_n = 17.3 kg·mol^{-1}, $Đ_M$ = 1.35 (RI detection, CHCl$_3$ SEC), M_n = 16.2 kg·mol^{-1}, $Đ_M$ = 1.35 (UV detection, 475 nm, CHCl$_3$ SEC).

Synthesis of PNTC-g-Phthalimide-g-PEG conjugate: In a dry vial fitted with a stirrer bar, PNTC (DP20) (50 mg, 1.09×10^{-5} mol) was dissolved in 500 µL of CHCl$_3$. Phthalimide-azide (10.4 mg, 3.3×10^{-5} mol) was added and the solution was stirred overnight at 90 °C. The functionalised polymer was recovered by precipitation into cold methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (500 MHz; CDCl$_3$): δ 8.03-7.37 (m, 26H), 6.15-5.91 (m, 35H), 4.42 (m, 45H), 3.93 (m, 111H), 3.13-1.98 (m, 120H), 1.69-1.32 (m, 94H). M_n = 4.4 kg·mol^{-1}, $Đ_M$ = 1.35 (RI detection, CHCl$_3$ SEC); M_n = 3.9 kg·mol^{-1}, $Đ_M$ = 1.41 (UV detection, 375 nm, CHCl$_3$ SEC). PNTC-g-Phthalimide (70 mg, 1.61×10^{-5} mol) was then re-dissolved in 500 µL of CHCl$_3$. PEG$_{550}$-SH (79.3 mg, 1.8×10^{-5} mol) and 2,2-dimethoxy-2-phenylacetophenone (31 mg, 1.2×10^{-4} mol) were added to the solution before being sealed and UV irradiated for 30 min. The functionalised polymer was recovered by precipitation into cold

methanol (15 mL) before being filtered and dried *in vacuo*. ^{1}H NMR (500 MHz; CDCl$_3$): δ 8.20-7.36 (m, 20H), 4.41 (m, 45H), 4.24 (m, 39H), 3.95 (m, 76H), 3.64 (m, 415H), 3.36 (s, 25H), 3.12-2.28 (m, 90H), 2.27-1.90 (m, 55H), 1.82-1.60 (m, 44H), 1.24 (m, 51H), 0.83 (m, 14H). M_n = 17.6 kg·mol^{-1}, $Đ_M$ = 1.29 (RI detection, CHCl$_3$ SEC); M_n = 15.9 kg·mol^{-1}, $Đ_M$ = 1.48 (UV detection, 375 nm, CHCl$_3$ SEC).

Cleavage of acetals: In a dry vial fitted with a stirrer bar, PNTC (DP10) (50 mg, 2.11×10^{-5} mol) was suspended in 1 mL of ethanol. 0.01 M HCl (10 μL) was added to the solution before being sealed and stirred for 16 h. The cleaved polymer was recovered by precipitation into hexanes methanol (15 mL) before being filtered and dried *in vacuo*. M_n = 4.2 kg·mol^{-1}, $Đ_M$ = 1.32 (RI detection, CHCl$_3$ SEC).

Self-assembly and multi-angle light scattering analysis: PNTC-*g*-PEG$_{550}$ polymers were self-assembled by solvent switch method. A solution of polymer (5 mg) was dissolved in THF (1 mL) at a concentration of 5 mg mL^{-1} and stirred overnight. 9 mL of DI H$_2$O was then added slowly (0.6 mL h^{-1}). The micelles' solution was then dialysed for 2 days to remove the THF, with frequent changes of water (MWCO = 3.5 kDa). The micelles were then freeze dried, resuspended in water at a concentration of 1 mg mL^{-1}, and stirred at room temperature overnight before being filtered (0.45 μm filters) and analysed *via* DLS and SLS.

Transmission electron microscopy (TEM) analysis: Samples for TEM analysis were prepared by drop casting 7 μL from a solution of micelles dialysed in water (0.5 mg mL^{-1}) onto a carbon/formvar-coated copper grid placed on filter paper. Samples were stained with a 1%

uranyl acetate solution to facilitate imaging of the thin organic structures unless specified. Samples were also prepared on graphene oxide support films[55] to negate the necessity for staining. Imaging was performed on a Jeol 2000 transmission electron microscope operating at 120 kV.

Viability studies on A549 and IMR-90 cell lines: A549 and IMR-90 cells were cultured in F12K and DMEM, respectively, with addition of 10% FBS and 100U mL^{-1} pen/strep. Cells were seeded on 12 well plates at 2000 cells cm^{-2} and left adhere and proliferate for 72 h. The medium was then replaced with PNTC-*g*-PEG$_{550}$ in a concentration range from 0 to 2 mg mL^{-1}. Briefly, a solution of micelles in water (50 mg mL^{-1}) was sterile filtered through a 0.22 µm filter. This solution was then diluted with cell culture medium (with the addition of 10% FBS and 100U mL^{-1} pen/strep) to a final concentration of 10 mg mL^{-1}. This stock solution was then used to prepare the dilutions directly on the well plates containing cells. After 72 h the solution was removed, cells were washed with PBS (1 mL × 3) and incubated with 10% PrestoBlue viability assay following the supplier instructions. The fluorescence intensity (FI) was detected in a BioTek Plate Reader (λ_{ex} = 530 nm, λ_{em} = 590 nm). Cell data are reported as viability % in comparison with control sample. Experiments were performed in triplicate.

Internalization studies on A549 and IMR-90 with PNTC-*g*-BODIPY-*g*-PEG: Cells were seeded on 12 well plates at 2000 cells cm^{-2} and left to adhere for 72 h. The medium was then replaced with 0.1 mg mL^{-1} PNTC-*g*-BODIPY-*g*-PEG$_{550}$. At 2, 4, and 6 h time points, the nanoparticle solution was removed and cells washed with PBS (1 mL × 3). Cells were fixed using 4% paraformaldehyde, and cell membranes were permeabilized using 0.5% Triton X-100

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in cytoskeleton stabilization (CS) buffer (0.1 M PIPES, 1 mM EGTA, and 4% (w/v) 8000 MW polyethylene glycol) at 37 °C on a dry block for 10 min, rinsed thrice for 5 min each in CS buffer, and incubated in 0.1% sodium borohydride in PBS at ambient temperature for 10 min. Samples were then blocked in 5% donkey serum for 20 min at 37 °C, washed with 1% donkey serum and incubated for 1 h with rhodamine phalloidin (1:200), followed by DAPI (1:10) for 10 min to stain the cell nuclei. Cells were imaged using fluorescence DAPI ($\lambda_{ex}/\lambda_{em}$ = 350/470 nm), FITC ($\lambda_{ex}/\lambda_{em}$ = 490/525 nm) and TRITC ($\lambda_{ex}/\lambda_{em}$ = 556/563 nm) channels. At least 10 pictures from each glass slide (19 mm in diameter) were taken. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

9-Norbornene-2,4,8,10-tetraoxaspiro[5,5]undecan-3-one (NTC) was synthesized through the acid-catalyzed acetal formation of pentaerythritol with 5-norbornene-2-carboxyaldehyde and the subsequent ring closure reaction with ethyl chloroformate, following previously reported procedures for the preparation of spirocyclic carbonate monomers (Scheme 1, Figure S1).[53, 54, 56, 57] The monomer was obtained after a two-step synthesis in 60% yield and purified using filtration and recrystallization. The organocatalyzed ring opening polymerization (ROP) of NTC was initiated from benzyl alcohol in $CHCl_3$ at ambient temperature, using 5 mol% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst (Scheme 1, Figures S2-S3). This procedure can be undertaken in air without extensive drying of monomers and solvents, thus presenting a simple and scalable method for polymer preparation.[58] The polymerization exhibited a first order kinetic and the number-average molecular mass, M_n, displayed a linear increase with increasing monomer conversion (Figures 1 and S4, and Table S1). The resultant polymers exhibited predictable molecular mass and narrow molecular mass distributions ($Đ_M$ = 1.09-1.20) at a range

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of degrees of polymerization (DP) (10, 20, 50, 100, 250), which indicate the controlled nature of this process with minimal adverse side reactions (*i.e.* transesterification) occurring during ROP (Figure 1). Importantly, ^{1}H NMR spectroscopy and MALDI-ToF-MS confirmed complete retention of the norbornene functionality (Figures S2 and S3).

Scheme 1. Synthesis of NTC from pentaerythritol and NTC ROP initiated from benzyl alcohol. a) 5-norbornene-2-carboxyaldehyde, HCl, deionised H_2O, 80 ˚C to 25 ˚C; b) Ethyl chloroformate, Et_3N, THF, 0 ˚C to 25 ˚C; c) Benzyl alcohol, 5 mol% DBU, $CHCl_3$, 25 ˚C.

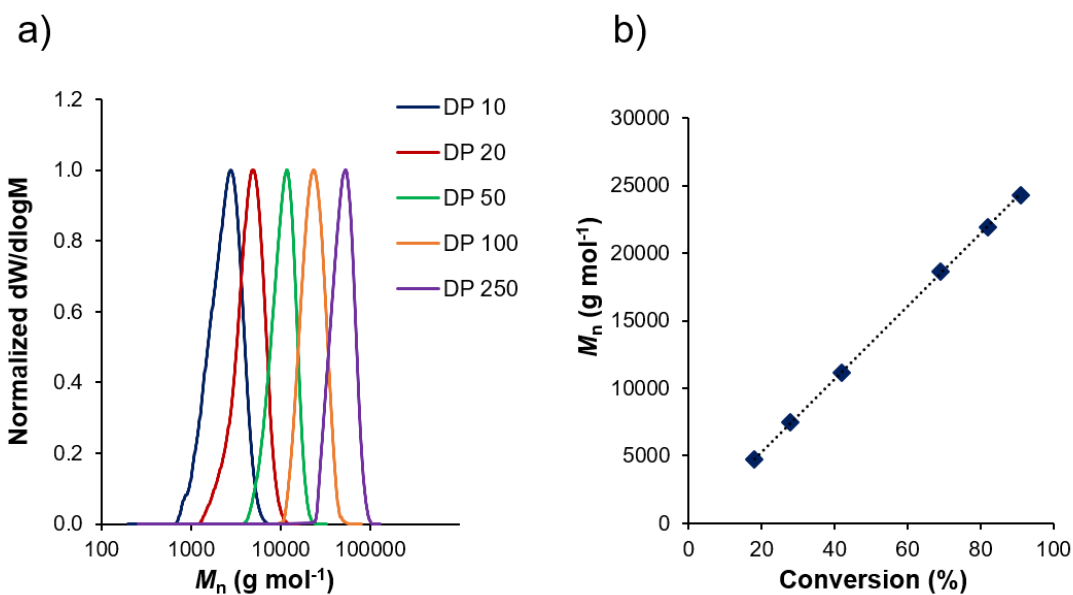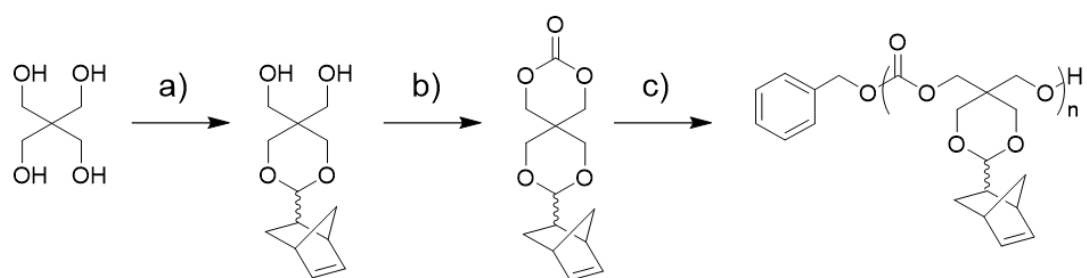**Figure 1.** a) Size exclusion chromatograms (SEC) of NTC polymerizations with increasing $[M]_0/[I]$; b) Plot of M_n (obtained *via* SEC) against monomer conversion (obtained *via* ^{1}H NMR spectroscopy) throughout the ROP of NTC (5 mol% DBU, $CHCl_3$, 25 ˚C), where dotted line represents linear fit to the data.

Norbornene moieties are versatile and able to participate in a number of conjugation reactions, including halide addition, cycloaddition, thiol-ene reactions, and (hetero) Diels-Alder reactions.[59-62] As such, in order to highlight its versatility, poly-NTC (PNTC) (DP 10) was modified with benzyl azide *via* a 1,3-dipolar cycloaddition, with 2,5-dioxopyrrolidin-1-yl 6-(6-(pyrimidin-2-yl)-1,2,4,5-te trazin-3-yl) benzoate *via* an inverse electron demand Diels-Alder (DA$_{inv}$) reaction, and with 1-dodecanethiol *via* a photoinduced radical thiol-ene addition (Figure 2a). Analysis by FT-IR spectroscopy (Figure 2b) and ^{1}H NMR spectroscopy (Figure S5) confirmed quantitative functionalization, with complete disappearance of the alkene norbornene peaks. Furthermore, SEC analysis revealed that the number average molecular mass of each modified polymer increased with no significant change in dispersity ($Đ_M$ = 1.14-1.12) (Figure 2c). This is indicative of the controlled nature of the post-polymerization modifications, with minimal adverse side reactions *i.e.* backbone degradation or chain-chain coupling, and demonstrates the versatility of this approach to enable the conjugation of additives through routes that are the most synthetically accessible.
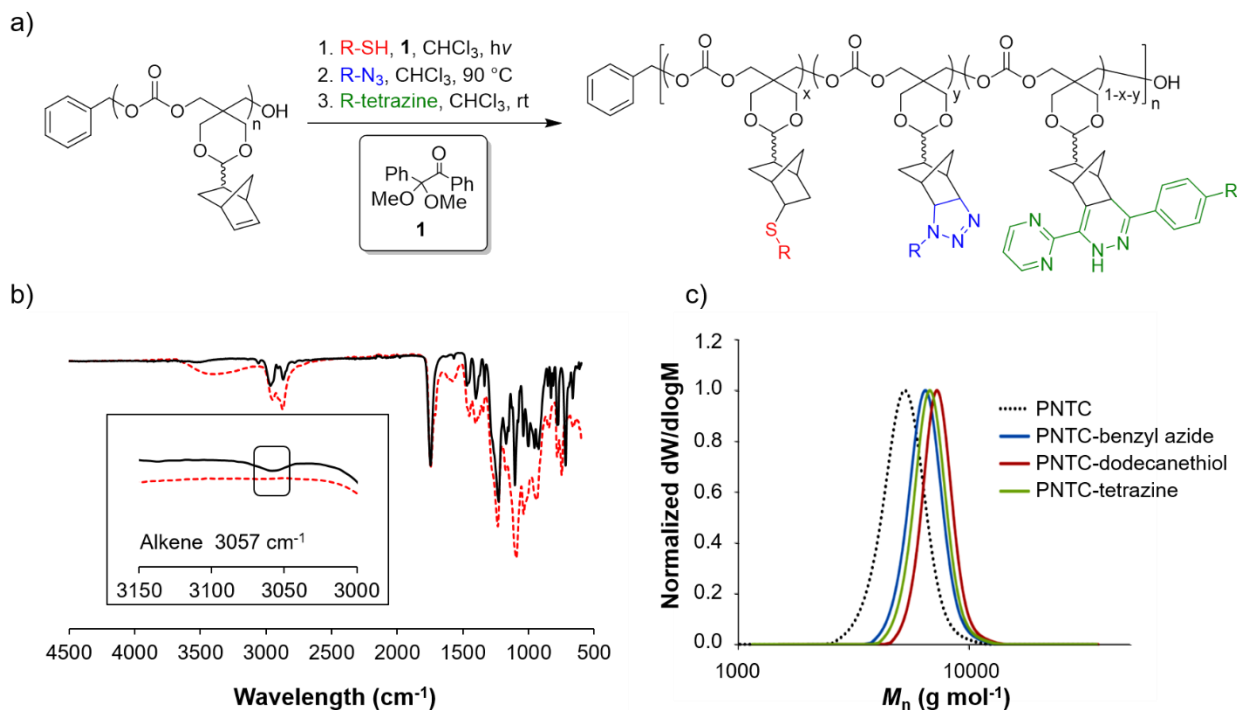
Figure 2. Post-polymerization functionalization of PNTC. a) Scheme of post polymerization functionalization reactions *via* photoinduced radical thiol-ene addition, 1,3-dipolar cycloaddition, and inverse electron demand Diels-Alder (DA_{inv}) reaction; b) FT-IR spectra of PNTC before (solid black line) and after (dashed red line) functionalization with dodecanethiol, showing the disappearance of the norbornene alkene peak at 3057 cm^{-1}; c) SEC of PNTC before (dashed line) and after (solid lines) functionalization with benzyl azide (blue), dodecanethiol (red) and tetrazine (green).

Beyond the versatility in the conjugation approach, a second advantage is represented by the pH responsive acetal bond.[36, 37] To demonstrate the cleavability of this bond, modified PNTCs were subjected to acidic conditions (pH 5) in order to determine the capability of these functional polycarbonates to release the grafted fragments. As expected, SEC analysis after 16 h (Figure 3 and Table S2) revealed a significant decrease in molar mass, with an increase in dispersity ($Đ_M$ = 1.23-1.27). Interestingly, however, it was noted that each of the cleaved polymers exhibited

comparable number average molar mass (M_n = 4.1-4.7 kg mol^{-1}), which is indicative of complete cleavage.
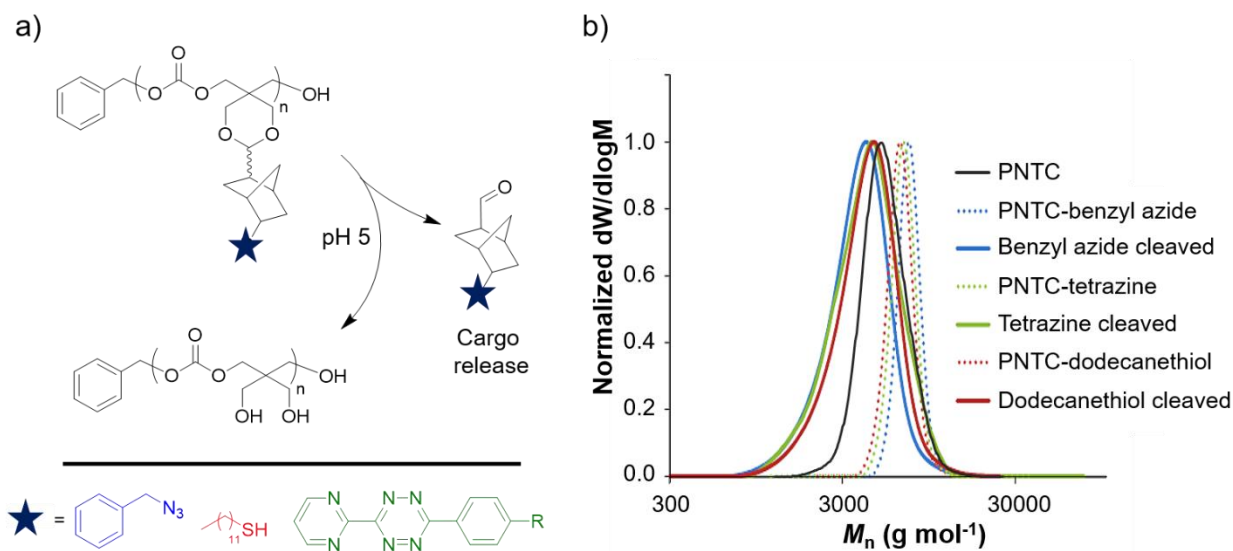

Figure 3. (a) Scheme and (b) and size exclusion chromatograms of PNTC (solid black line) functionalized with benzyl azide (blue), tetrazine (green) and dodecanethiol (red), before (dashed lines) and after (solid lines) cleavage with 0.01 M HCl at pH 5.0 for 16 h.

In order to efficiently serve as a delivery carrier, the polymer conjugate should be able to self-assemble into nanostructures that isolate the cargo from the external environment and deliver it inside a cell. For this reason, an amphiphilic copolymer was synthesized by the introduction of poly(ethylene glycol) (PEG) to the polymer backbone *via* the norbornene functionality. 3-Mercaptopropionate-functionalized PEG methyl ether (M_n = 550 g mol^{-1}) (PEG$_{550}$-SH) was grafted to PNTC (DP20) using a radical thiol-ene addition. As with the 1-dodecanethiol addition, ^{1}H NMR spectroscopy and SEC analysis show that full grafting was achieved (Figure S6 and S7). The amphiphilic graft copolymer PNTC-*g*-PEG$_{550}$ subsequently self-assembled *via* a solvent exchange method in water to obtain micelles ~12 nm in diameter, as confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses (Figure S8). Particle

disassembly at pH 5.0 was evaluated using static light scattering (SLS). After 16 h of incubation at acidic pH, the micelles' molecular weight decreases, and the calculated aggregation number ($N_{agg.}$) switches from 27 polymer chains per micelle to 3, indicating disassembly of the nanostructures (Table S3). The biocompatibility of the resultant micelles was assessed by incubating A549 and IMR-90 cells with an increasing concentration of nanoparticles, from 0 to 2 mg mL^{-1} up to 72 h. Viability was found to be higher than 95% for both cell lines used (Figure S9), which suggests that this graft-copolymer-based nanoparticle system has a great potential for drug delivery.

In order to investigate the internalization of the nanoparticles into the cells, fluorescent dyes were conjugated to PNTC *via* either thiol-ene addition, with BODIPYC$_{10}$-SH, or 1,3-dipolar cycloaddition with 4-(11-azido-undecanamide)-*N*-methyl phthalimide (Figures S10-S13). As expected, loading was predictable based on the amount of dye added (10% for BODIPYC$_{10}$-SH and 25% for 4-(11-azido-undecanamide)-*N*-methyl phthalimide)), demonstrating the high efficiency of the post-polymerization reactions. In both cases, fluorescent nanoparticles of 15 nm in diameter were obtained upon grafting of PEG$_{550}$-SH to the remaining norbornene units, as characterized by DLS and TEM (Figures 4 and S14) which showed that the addition of these hydrophobic components did not have a significant effect on the size of the nanoparticles.
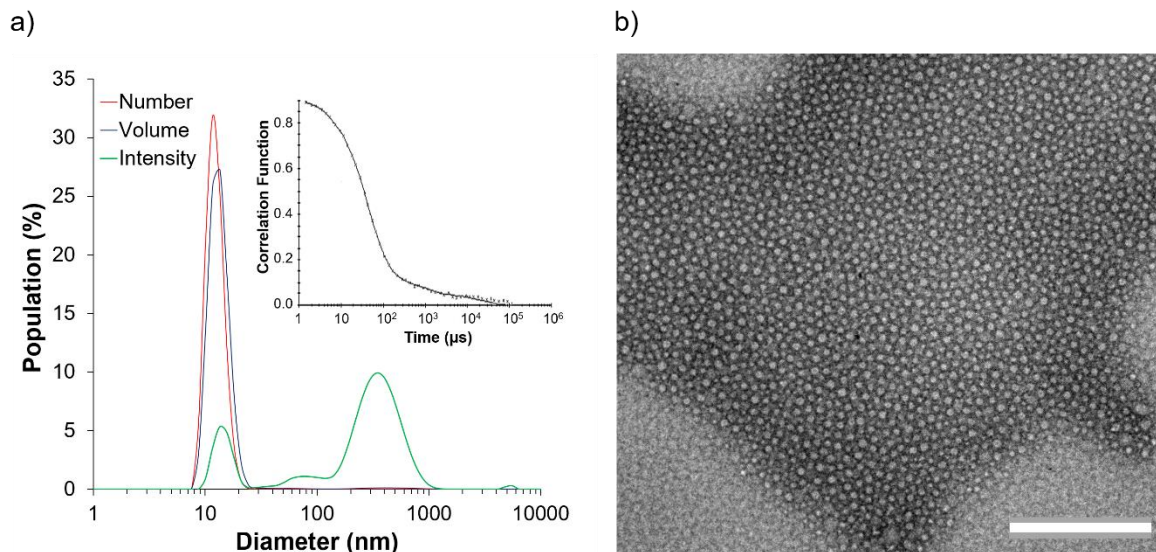
Figure 4. (a) DLS (173˚) distribution by intensity, volume and number, with correlation function (inset) showing a single particles distribution. The peak at 370 nm shown in the intensity distribution is likely to be an artifact due to the absorbance and fluorescence of the nanoparticles. (b) TEM micrograph of PNTC-*g*-BODIPY-*g*-PEG$_{550}$ nanoparticles stained with 1% uranyl acetate. Scale bar = 500 nm. Particle size was measured as 15 nm by DLS (PD = 0.2), and 14 ± 0.3 nm by TEM.

PNTC-*g*-BODIPY-*g*-PEG$_{550}$ nanoparticles were then incubated with A549 and IMR-90 cell lines. Nanoparticle internalization was investigated using a polymer concentration of 0.1 mg mL^{-1}, and time points were taken at 2, 4, and 6 h after incubation, at which point nanoparticles appear to homogeneously distribute within the cytosolic compartment for both cell types (Figures 5 and S15). This suggests that our novel polycarbonate can be used as a drug delivery vehicle, being highly biocompatible and able to enter the cell compartment.
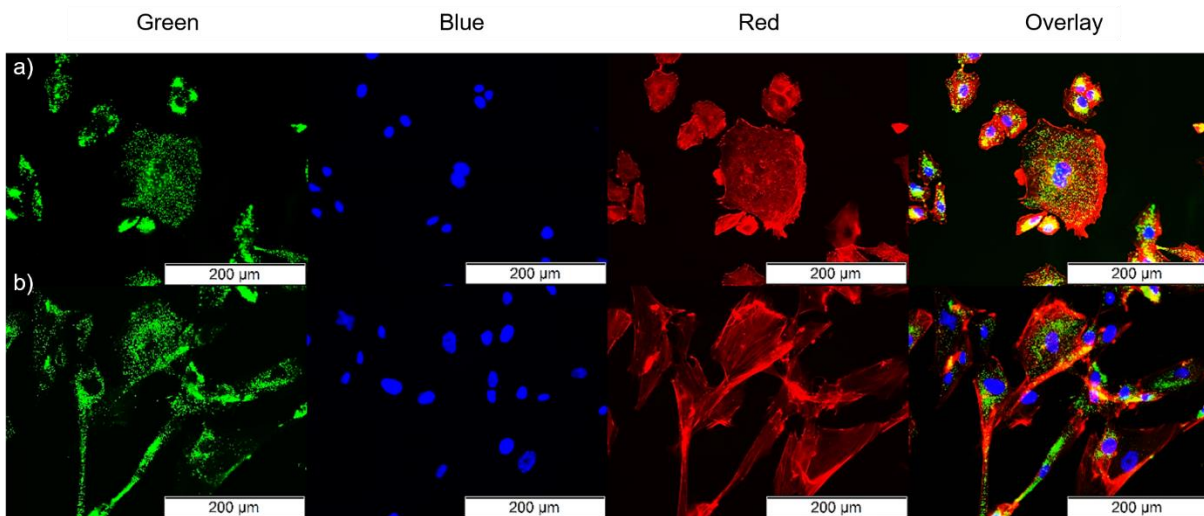
Figure 5. A549 (a), and IMR-90 (b) cell lines incubated for 6 h with 0.1 mg mL^{-1} of PNTC-*g*-BODIPY-*g*-PEG$_{550}$ nanoparticles. Nanoparticles fluoresce in green, cells cytoskeletons are stained with rhodamine phalloidin (red) and nuclei are stained with DAPI (blue).

CONCLUSIONS

Herein we report the monomer synthesis and polymerization of a novel, functional polycarbonate scaffold that contains both a versatile norbornene group for post-polymerization modification and a pH responsive acetal group for triggered release. Importantly, the scaffolds can be realized by simple and scalable chemistry. Taking advantage of these properties, the polycarbonate was conjugated to thiol functionalized BODIPYC$_{10}$, azide functionalized *N*-methyl phthalimide derivative, and PEG and self-assembled into polymeric micelles to explore their ability to be internalized into mammalian cells. Furthermore, the ability to precisely control the loading of small molecules on the polymer backbone offers great advantage for drug delivery purposes. Our polymer system was found to be highly biocompatible, with a viability higher than 95% after 72 h of incubation, and was internalized by the two human cell lines considered in this

study (A549 and IMR-90). The simplicity of this approach, in combination with the biodegradable and biocompatible polymers applied opens the possibility to further explore these polymeric systems for a range of nanomedicine therapies *in vivo*.

ASSOCIATED CONTENT

Experimental details, additional data, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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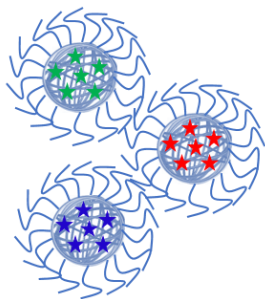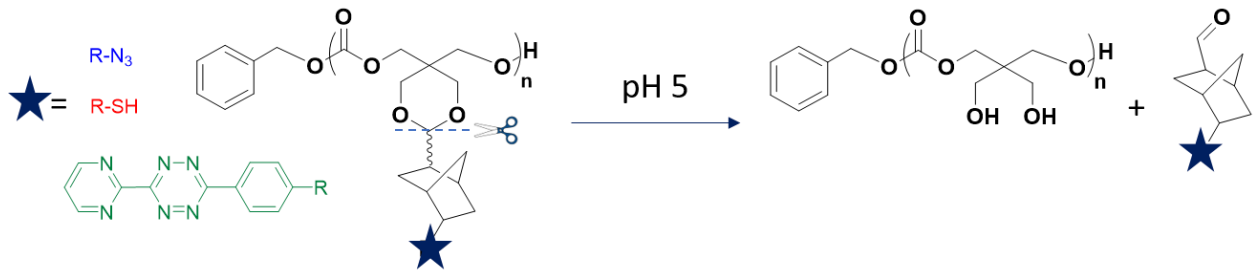
- ✓ Scalable
- ✓ Functionable
- ✓ pH responsive
- ✓ Cytocompatible
- ✓ Internalized by cells