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Synthesis of Water Soluble Camptothecin-Polyoxetane Conjugates via Click Chemistry

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Abstract

Water soluble camptothecin (CPT)-polyoxetane conjugates were synthesized using a clickable polymeric platform P(EAMO) that was made by polymerization of acetylene-functionalized 3-ethyl-3-hydroxymethyl oxetane (*i.e.*, EAMO). CPT was first modified with a linker 6-azidohexanoic acid via an ester linkage to yield CPT-azide. CPT-azide was then click coupled to P(EAMO) in dichloromethane using bromotris(triphenylphosphine) copper(I)/N,N-diisopropylethylamine. For water solubility and cytocompatibility improvement, methoxypolyethylene glycol azide (mPEG-azide) was synthesized from mPEG 750 g mol⁻¹ and click grafted using copper(II) sulfate and sodium ascorbate to P(EAMO)-*g*-CPT. ¹H NMR spectroscopy confirmed synthesis of all intermediates and the final product P(EAMO)-*g*-CPT/PEG. CPT was found to retain its therapeutically active lactone form. The resulting P(EAMO)-*g*-CPT/PEG conjugates were water soluble and produced dose-dependent cytotoxicity to human glioma cells and increased γ -H2AX foci formation, indicating extensive cell cycle-dependent DNA damage. Altogether, we have synthesized CPT-polymer conjugates able to induce controlled toxicity to human cancer cells.

Keywords

glioma; nanomedicine; brush polymer; polymer-drug conjugates; anticancer drug delivery; polyoxetane

INTRODUCTION

Covalently coupling anticancer drugs to polymeric carrier represents a viable approach to improving drug pharmacokinetic and pharmacodynamics. Camptothecin (CPT) is a potent and selective DNA topoisomerase I (TOP I) inhibitor, which binds to the TOP I and DNA through hydrogen bonds and stabilizes the enzyme–DNA complex that leads to DNA damage and subsequent cell apoptosis. CPT and its water soluble derivatives (*e.g.*, irinotecan) can be used to treat many types of cancer such as brain cancer, colon cancer, and lung cancer.^{1–3} CPT possesses a planar pentacyclic ring structure and has low solubility in

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water. It exists in two forms—a therapeutically active lactone form and a therapeutically inactive carboxylate form.⁴ Since the lactone form rapidly transforms into the carboxylate form at physiological pH, it is important to maintain CPT in the lactone form in drug-polymer coupling reactions and extend its stability with the use of polymeric carrier. Additionally, any inadvertent structural changes of the drug should be avoided to achieve expected therapeutic outcomes. The hydroxyl group of CPT is a primary site in CPT-polymer coupling reactions because chemical modification of CPT through alkylation or acylation of the hydroxyl group does not disrupt the lactone ring and indeed enhances its stability.⁵

The hydroxyl group of CPT contributes to the stabilization of the TOP I and DNA complex via a hydrogen bond formed with the side chain on aspartic acid at position 533 (Asp533) of the enzyme. To fully recover the biological activity of the drug, a cleavable linkage, typically an ester bond,⁶ connecting the drug to the polymer via the hydroxyl group is commonly used. Interestingly, Tong and Cheng used CPT as an initiator to polymerize D, L-lactide and obtained CPT-terminated polylactide.⁷ CPT-polylactide conjugates were formulated into nanoparticles. However, the release of CPT from this system still relies on hydrolytic cleavage of ester bond. In some systems, CPT is coupled to the carrier through a heterobifunctional spacer to utilize highly efficient controlled synthesis such as click chemistry. Copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry yields high coupling efficiency and allows precise control of compositions and functionalization.^{8–10} Therefore CuAAC click chemistry has attracted considerable attention in drug delivery.^{10, 11} For example, Parrish and Emrick modified CPT with an azide-containing spacer and click coupled it to an acetylene functionalized copolymer made from α -propargyl- δ -valerolactone and ϵ -caprolactone monomers.¹² We recently synthesized water soluble cytocompatible polyethylene glycol (PEG)-grafted polyoxetane brush polymers, made through ring-opening polymerization of acetylene-functionalized 3-ethyl 3-hydroxymethyloxetane (EAMO) monomer followed by a click reaction with methoxypolyethylene glycol azide (mPEG-azide). The uniformly distributed alkyne pendant groups make this new platform well suited for delivery of therapeutic and diagnostic agents. In this work, we investigated the utility of this new carrier for CPT delivery. The chemistry used in CPT coupling reaction, structural characterization, and therapeutic effectiveness were studied.

EXPERIMENTAL SECTION

Materials

Bromotris(triphenylphosphine) copper(I) CuBr(PPh₃)₃, N,N-diisopropylethylamine (DIPEA), 4-dimethylaminopyridine (DMAP), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), copper(II) sulfate (CuSO₄), (+)-sodium L-ascorbate, magnesium sulfate MgSO₄, deuterated solvents, dichloromethane (DCM), and other solvents were purchased from Acros (Morris Plains, NJ). 20(S)-Camptothecin (95%) (CPT) and silica gel 60 (40–63 μ m, 230–400 mesh) were purchased from Sigma-Aldrich (St. Louis, MO). Cy5.5 azide was purchased from Lumiprobe (Hallandale Beach, FL). 6-Azidohexanoic acid (AHA) was synthesized by us following a reported method¹³ with slight modifications. Methoxypolyethylene glycol azide (mPEG-azide) was synthesized from mPEG 750 g mol⁻¹.¹⁴ Dialysis tubing, snake skin MWCO 3500, was obtained from Thermo Fisher Scientific (Pittsburg, PA).

Instrumentation

¹H NMR spectra were recorded on a Bruker AVANCEIII 600 MHz spectrometer. UV/Vis spectra were measured on an Agilent 8453 spectrophotometer. Fluorescence spectra were

recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Luciferase assay was measured on an EnVision 2103 multilabel Reader (PerkinElmer).

Synthesis of azide-functionalized camptothecin (CPT-azide)

Azide was introduced to CPT via a hydrophobic spacer AHA.¹⁵ CPT (190mg, 0.55mmol), AHA (170 mg, 1.1mmol), DMAP (66 mg, 0.55 mmol), and EDC (0.2 g, 1.1 mmol) were dissolved in 50 ml of DCM and stirred for 36h (Scheme 1). Afterwards, the reaction solution was poured into water and extracted with DCM. The combined organic fractions were dried over MgSO_4 . Upon removal of DCM by rotary evaporation, the remaining residue was purified by column chromatography on silica gel using DCM/methanol (90/2.5: v/v). Yield: 60%. ^1H NMR (CDCl_3 , 600 MHz): δ (ppm) 8.41 (s, 1H), 8.23 (d, $J=8.4$ Hz, 1H), 7.95 (d, $J=8.4$ Hz, 1H), 7.84 (t, $J=6.8$ Hz, 1H), 7.68 (t, $J=7.6$ Hz, 1H), 7.21 (s, 1H), 5.68 (d, $J=17.04$ Hz, 1H), 5.42 (d, $J=17.04$ Hz, 1H), 5.29 (s, 2H), 3.22 (t, $J=6.8$ Hz, 2H), 2.51 (m, 2H), 2.28 (m, 1H), 2.16 (m, 1H), 1.68 (m, 2H), 1.59 (m, 2H), 1.44 (m, 2H), 0.98 (t, $J=7.2$ Hz, 3H).

Synthesis of P(EAMO)-g-CPT conjugates

Ring-opening polymerization of acetylene-functionalized 3-ethyl 3-hydroxymethyloxetane (EAMO) to make P(EAMO) ($M_n = 4640 \text{ g mol}^{-1}$, $\text{PDI}=2.45$) was described in detail previously.¹⁴ P(EAMO) (32 mg, 0.2 mmol acetylene equivalent) and CPT- N_3 (25 mg, 0.05 mmol) were dissolved in 3 ml of DCM. To the solution DIPEA (0.2 ml, 1 mmol) was added followed by addition of $\text{CuBr}(\text{PPh}_3)_3$ (9.6 mg, 0.01 mmol). The reaction mixture was heated at reflux with stirring for 24 h under nitrogen and then dialyzed against DCM for 48 h. After DCM was removed by rotary evaporation, P(EAMO)-g-CPT conjugates were precipitated in cold ether, filtered, and dried. Yield: 51%. ^1H NMR (CDCl_3 , 600 MHz): δ (ppm) 8.40 (br.s, 1H), 8.18 (br.s, 1H), 7.94 (br.s, 1H), 7.82 (br.s, 1H), 7.65 (br.s, 1H), 7.40 (br.s, 1H, triazole), 7.20 (s, 1H), 5.66 (br.d, 1H), 5.40 (br.d, 1H), 5.29 (br.s, 2H), 4.56 (br.s, 2H), 4.30 (br.s, 2H), 4.10 (s, 2H), 3.38 (s, 2H), 3.21 (s, 4H), 2.48 (m, 2H), 2.42 (s, 1H, alkyne), 2.27 (br.s, 1H), 2.14 (br.s, 1H), 1.91 (br.s, 2H), 1.68 (br.s, 2H), 1.38 (br s, 2H (b, b') and 2H (13)), 0.98 (br.s, 3H), and 0.84 (br.s, 3H).

Synthesis of P(EAMO)-g-CPT/PEG conjugates

P(EAMO)-g-CPT (10 mg, 27 μmol acetylene equivalent) and mPEG-azide (25 mg, 32 μmol) were dissolved in a mixture of THF/water (3/1: v/v). CuSO_4 (8 mg, 32 μmol) and sodium ascorbate (12 mg, 60 μmol) were added to the solution. The reaction mixture was stirred at 70 °C under N_2 for 24 h. Upon removal THF by rotary evaporation, the remaining solution was dialyzed against water for 20 h and freeze-dried to obtain P(EAMO)-g-CPT/PEG conjugates. Yield: 81%. According to ^1H NMR spectroscopy, the remaining alkynes reacted completely with mPEG-azide. That is to say, the resulting P(EAMO)-g-CPT/PEG conjugates had an average of 22.5 PEG chains per polymer. ^1H NMR (CDCl_3 , 600 MHz): δ (ppm) 8.41 (br.s, 1H), 8.18 (br.s, 1H), 7.94 (br.s, 1H), 7.81 (br.s, 1H), 7.67 (br.s, 1H of CPT and 1H of triazole ring), 7.56 (br.s, 1H of triazole ring), 7.20 (s, 1H), 5.67 (br.s, 1H), 5.41 (br.s, 1H), 5.28 (br.s, 2H), 4.52 (br.s, 6H), 4.28 (br.s, 2H), 3.86 (s, 2H), 3.64 (br.s, protons in repeat units of PEG), 3.38 (s, 2H(c,c') and 3H (j')), 3.17 (s, 4H), 2.48 (s, 2H), 2.27 (br.s, 1H), 2.15 (br.s, 1H), 1.91 (br.s, 2H), 1.68 (br.s, 2H), 1.38 (br.s, 2H (b, b') and 2H (13)), 0.96 (br.s, 3H), 0.76 (br.s, 3H).

In addition, P(EAMO)-g-PEG without CPT attachment was also synthesized with the same degree of PEGylation as P(EAMO)-g-CPT/PEG. A portion of P(EAMO)-g-PEG was further labeled with Cy5.5 azide. The click reaction was performed in a mixture of DMSO/water (1/1: v/v) using the same conditions as above. The obtained polymer was purified on Sephadex G-50 using chloroform as eluent. An average of 1 Cy-5.5 molecule was attached to the polymer chain according to fluorometry.

Cell toxicity assays

Human glioma U1242/luc-GFP cells stably expressing the reporter luciferase¹⁶ were exposed to various CPT equivalent concentrations of polymer-CPT conjugates or polymer control samples (n=4) in a 96-well microtiter plate for 48 h followed by luciferase assay to assess survival of cells. Repair foci formation was carried out using anti- γ -H2AX antibody (Upstate/Millipore, MA) and secondary anti-mouse-Alexa Fluor-488 antibody (Molecular Probes-Invitrogen).^{17, 18} Cells were imaged using a Zeiss LSM 710 Meta imaging system and analyzed using PerkinElmer's Volocity software.

RESULTS AND DISCUSSION

We developed a new family of water soluble cytocompatible PEG-grafted polyoxetane brush polymers via ring-opening polymerization of acetylene-functionalized 3-ethyl-3-hydroxymethyl oxetane (EAMO) and subsequent click coupling of P(EAMO) with mPEG azide.¹⁴ The presence of alkyne group in each repeat unit resulted in a polymeric backbone with a high density of alkyne groups (DP = 30) available for drug coupling or attachment of solubilizing side chains and/or other biologically active molecules using CuAAC click chemistry. Due to the high efficiency of click chemistry, PEG grafting density in P(EAMO)-*g*-PEG can be precisely controlled by changing the feed molar ratio of mPEG-azide to alkyne of P(EAMO). Importantly, P(EAMO)-*g*-PEG brush polymers with a wide range of PEG grafting density are water soluble and cytocompatible.¹⁴

Since the lactone ring of CPT is responsible for drug binding to TOP I enzyme, it is critical to maintain CPT in the lactone form during coupling reactions. Acylation of the hydroxyl group of CPT has been commonly employed based on the observation that modifications at this site do not change the structure of the lactone ring. As shown in Scheme 1, CPT was acylated with a bifunctional hydrophobic spacer AHA carrying both azide and carboxyl groups using EDC/DMPA coupling reaction.¹⁵ In this reaction, CPT was coupled to AHA via an ester linkage. The reaction was performed in DCM at room temperature. The resulting CPT-azide was purified using silica gel column chromatography. A double doublet for methylene in the CPT lactone ring is clearly seen in the ¹H NMR spectrum of CPT-azide (Fig. 1), indicating that the lactone ring remained intact. Furthermore, proton signals between 1.3 and 1.75 ppm corresponding to methylene protons in the aliphatic chain of AHA were identified. The proton signal of methylene next to azide appears at 3.25 ppm.

The choice of reaction conditions for click coupling CPT-azide is also crucial since it can result in structural change of the drug and loss of activity. A general catalyst/ligand/solvent reaction condition for CuAAC click chemistry is CuI/alkyl amine/DMF. However, such a condition results in transformation of CPT into the corresponding hydroxy derivative in the presence of oxygen.¹⁹ To avoid this problem, CPT-azide was click grafted to P(EAMO) using CuBr(PPh₃)₃/DIPEA/DCM. CuBr/2,2-bipyridine/DMSO click reaction condition was also found to be efficient in click coupling CPT-azide to P(EAMO).

¹H-NMR spectrum of the resultant P(EAMO)-*g*-CPT (Fig. 2) confirms click coupling of CPT to P(EAMO). A singlet at 7.40 ppm assigned to proton (*e*) in the triazole linkage indicates the success of the click reaction. Furthermore, the chemical shift of methylene next to azide in CPT-azide moved downfield to 4.3 ppm due to triazole formation and appears as a broad singlet. Similarly, the formation of triazole resulted in a downfield chemical shift of methylene (*d*) to 4.56 ppm, which became distinct from the chemical shift of methylene (*d'*) adjacent to the unreacted alkyne. All the proton chemical shifts of CPT in the drug-polymer conjugates were identical to those of CPT-azide, indicating that no structural change of CPT occurred during the click reaction. A singlet at 2.42 ppm is assigned to acetylene proton (*e'*), indicating the presence of unreacted alkynes. The ratio of proton integral of methylene (*d*)

adjacent to the triazole linkage resulting from the CPT coupling to that of methylene (δ') adjacent to the unreacted alkyne revealed that an average of 7.5 CPT molecules were coupled to the polymer.

The P(EAMO)-*g*-CPT conjugates are water insoluble. To make them water soluble, P(EAMO)-*g*-CPT conjugates were further grafted with mPEG-azide via click chemistry catalyzed by CuSO₄ in the presence of sodium ascorbate. The complete disappearance of proton signals at 4.10 ppm and 2.42 ppm associated with alkyne as well as the appearance of a broad methylene peak of PEG (δ') at 3.64 ppm and a singlet at 7.67 ppm assigned to methine (ϵ') in the newly formed triazole linkage (Fig. 3) confirmed the incorporation of PEG and complete substitution of remaining alkynes. Importantly, the resulting P(EAMO)-*g*-CPT/PEG conjugates were water soluble.

P(EAMO)-*g*-CPT/PEG and control groups including free CPT, P(EAMO)-*g*-PEG, and P(EAMO)-*g*-PEG/Cy5.5 were tested on U1242/luc-GFP cells¹⁶ for cytotoxicity by luciferase assay. P(EAMO)-*g*-PEG and P(EAMO)-*g*-PEG/Cy5.5 were not toxic at concentrations up to 33 μ M. In contrast, P(EAMO)-*g*-CPT/PEG was found to be dose-dependent toxic, similar to free CPT (Fig. 4). As expected, P(EAMO)-*g*-CPT/PEG was less potent than free CPT at the same CPT equivalent concentration in causing toxicity, likely due to slow release. Chen *et al.* reported that the AHA linker connecting CPT to poly(methacryloyloxyethyl phosphorylcholine) was stable in aqueous solutions at different pHs.¹⁵ This observation prompted this group to employ more hydrophilic linkers for CPT coupling to accelerate CPT release. The lowest IC₅₀ of their polymer-drug conjugates possessing more hydrophilic linker was 2.3 μ M in colon (COLO205) adenocarcinoma cells.¹⁵ P(EAMO)-*g*-CPT/PEG having AHA linker described above was still potent in glioma cells. Cell viability dropped to 24% following 48 h incubation of 1 μ M of P(EAMO)-*g*-CPT/PEG. Given that toxicity of polymer-drug conjugates is also cell type-dependent, our preliminary work indicated that therapeutic application of P(EAMO)-*g*-CPT/PEG is promising. Indeed, in some applications, maintaining a stable linkage between the drug and the polymer before polymer-drug conjugates enter the target cell is a desirable strategy to avoid premature drug release.²⁰ Therefore, P(EAMO)-*g*-CPT/PEG is presumed to undergo ester linkage hydrolysis following cellular uptake to release CPT to recover its therapeutic activity, hence enabling sustained release and longer activity.

In addition, toxicity-induced repair foci formation in human U1242 glioma cells was examined. CPT interferes with DNA synthesis and is thus most toxic to cells in S phase¹. Therefore, DNA damage in the form of double-strand breaks are mostly seen in S/G2 cells which is detected by the accumulation of γ -H2AX foci. These cells treated with P(EAMO)-*g*-CPT/PEG produced extensive γ -H2AX foci after 24 h indicative of DNA damage (Fig. 5). It should be noted that the size of the foci in S-phase is relatively small compared to those formed in G1 by, for example, radiation. Note the absence of foci in a subset of cells treated with P(EAMO)-*g*-CPT/PEG, presumably those outside of S. Overall, P(EAMO)-*g*-CPT/PEG showed antitumor activity that supported release of CPT from the polymer and subsequent selective DNA damage in a subset of cells. Since the composition of CPT and PEG can be precisely modulated, a higher drug payload can be achieved without comprising water solubility of the polymer-drug conjugates. A systematic investigation is warranted to elucidate structure-activity relationships of P(EAMO)-*g*-CPT/PEG conjugates.

CONCLUSIONS

CPT in the lactone form has been successfully coupled to PEG-grafted polyoxetane brush polymers via CuAAC click chemistry. These polymer-drug conjugates were water soluble and demonstrated potent toxicity to glioma cells, while the carrier itself was not toxic.

Because of high efficiency and selectivity of the click chemistry, PEG-grafted polyoxetane brush polymers represent a modular platform for efficient delivery of anticancer drugs and functionalization.

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References

1. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer*. 2006; 6(10): 789–802. [PubMed: 16990856]
2. Mollica A, Stefanucci A, Feliciani F, Cacciatore I, Cornacchia C, Pinnen F. Delivery methods of camptothecin and its hydrosoluble analogue irinotecan for treatment of colorectal cancer. *Curr Drug Delivery*. 2012; 9(2):122–31.
3. Biddlestone-Thorpe L, Marchi N, Guo K, Ghosh C, Janigro D, Valerie K, Yang H. Nanomaterial-mediated CNS delivery of diagnostic and therapeutic agents. *Adv Drug Delivery Rev*. 2012; 64(7): 605–13.
4. Venditto VJ, Simanek EE. Cancer therapies utilizing the camptothecins: a review of the *in vivo* literature. *Mol Pharm*. 2010; 7(2):307–49. [PubMed: 20108971]
5. Zhao H, Lee C, Sai P, Choe YH, Boro M, Pendri A, Guan S, Greenwald RB. 20-O-acetylcampthothecin derivatives: evidence for lactone stabilization. *J Org Chem*. 2000; 65(15):4601–6. [PubMed: 10959865]
6. Cho JK, Chun C, Kuh HJ, Song SC. Injectable poly(organophosphazene)-camptothecin conjugate hydrogels: Synthesis, characterization, and antitumor activities. *Eur J Pharm Biopharm*. 2012; 81(3):582–90. [PubMed: 22554767]
7. Tong R, Cheng J. Controlled synthesis of camptothecin-poly(lactide) conjugates and nanoconjugates. *Bioconjugate Chem*. 2010; 21(1):111–21.
8. Kolb HC, Finn MG, Sharpless KB. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew Chem Int Ed Engl*. 2001; 40(11):2004–2021. [PubMed: 11433435]
9. Lallana E, Fernandez-Trillo F, Sousa-Herves A, Riguera R, Fernandez-Megia E. Click chemistry with polymers, dendrimers, and hydrogels for drug delivery. *Pharm Res*. 2012; 29(4):902–21. [PubMed: 22274559]
10. Lallana E, Sousa-Herves A, Fernandez-Trillo F, Riguera R, Fernandez-Megia E. Click chemistry for drug delivery nanosystems. *Pharm Res*. 2012; 29(1):1–34. [PubMed: 21913032]
11. Deshayes S, Maurizot V, Clochard MC, Baudin C, Berthelot T, Esnouf S, Lairez D, Moenner M, Deleris G. “Click” conjugation of peptide on the surface of polymeric nanoparticles for targeting tumor angiogenesis. *Pharm Res*. 2011; 28(7):1631–42. [PubMed: 21374102]
12. Parrish B, Emrick T. Soluble camptothecin derivatives prepared by click cycloaddition chemistry on functional aliphatic polyesters. *Bioconjugate Chem*. 2007; 18(1):263–7.
13. Lee JM, Kim J, Shin Y, Yeom CE, Lee JE, Hyeon T, Moon Kim B. Heterogeneous asymmetric Henry reaction using a chiral bis(oxazoline)-copper complex immobilized on magnetically separable mesocellular mesoporous silica support. *Tetrahedron: Asymmetry*. 2010; 21(3):285–291.
14. Zolotarskaya, OY.; Yuan, Q.; Wynne, KJ.; Yang, H. Synthesis and characterization of clickable polyethylene glycol-grafted polyoxetane brush polymers. unpublished results
15. Chen X, McRae S, Parelkar S, Emrick T. Polymeric Phosphorylcholine Camptothecin Conjugates Prepared by Controlled Free Radical Polymerization and Click Chemistry. *Bioconjugate Chem*. 2009; 20(12):2331–2341.
16. Golding SE, Rosenberg E, Valerie N, Hussaini I, Frigerio M, Cockcroft XF, Chong WY, Hummersone M, Rigoreau L, Menear KA, O'Connor MJ, Povirk LF, van Meter T, Valerie K. Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin,

- AKT and ERK prosurvival signaling, and inhibits migration and invasion. *Mol Cancer Ther.* 2009; 8(10):2894–902. [PubMed: 19808981]
17. Golding SE, Morgan RN, Adams BR, Hawkins AJ, Povirk LF, Valerie K. Pro-survival AKT and ERK signaling from EGFR and mutant EGFRvIII enhances DNA double-strand break repair in human glioma cells. *Cancer Biol Ther.* 2009; 8(8):730–8. [PubMed: 19252415]
 18. Golding SE, Rosenberg E, Neill S, Dent P, Povirk LF, Valerie K. Extracellular signal-related kinase positively regulates ataxia telangiectasia mutated, homologous recombination repair, and the DNA damage response. *Cancer Res.* 2007; 67(3):1046–53. [PubMed: 17283137]
 19. He X, Gao H, Lu W, Cai J. New Method for the Synthesis of 5-Hydroxycamptothecin Derivatives. *Synth Commun.* 2004; 34(23):4285–4291.
 20. Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer.* 2006; 6(9):688–701. [PubMed: 16900224]

Figure 1.
 ^1H NMR spectrum of CPT-azide in CDCl_3 .

Figure 2.
¹H NMR spectrum of P(EAMO)-g-CPT in CDCl₃.

Figure 3.
 ^1H NMR spectrum of P(EAMO)-g-CPT/PEG in CDCl_3 .

Figure 4.
In vitro cytotoxicity evaluation by luciferase assay.

Figure 5.
Effect of P(EAMO)-g-CPT/PEG on foci formation.

Scheme 1.

Synthesis of P(EAMO)-*g*-CPT/PEG conjugates via CuAAC click chemistry.