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Synthesis and Evaluation of Polymeric Gold Glyco-Conjugates as Anti-Cancer Agents

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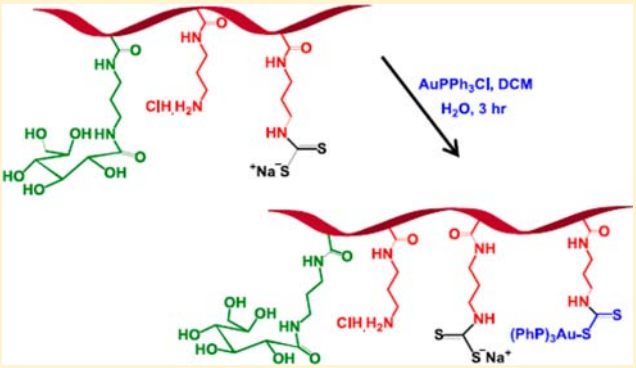
Supporting Information

ABSTRACT: The antitumor activity of organo-gold compounds is a focus of research from the past two decades. A variety of gold stabilizing ligands such as vitamins and xanthanes have been prepared and explored for their 'chelating effect' as well as for their antitumor activity. Dithiocarbamates (DTC) compounds and their metallic conjugates have been well explored for their antiproliferative activities. In this study, glycopolymer based DTC-conjugates are prepared by reversible addition−fragmentation chain transfer polymerization (RAFT) and subsequently modified with gold(I) phosphine. These polymer-DTC derivatives and their gold compounds are tested for their *in vitro* toxicity in both normal and cancer cell lines. The Au(I) phosphine conjugated cationic glycopolymers of 10 kDa and 30 kDa are evaluated for their cytotoxicity profiles using MTT assay. Au(I) compounds are well-known for their mitochondrial toxicity, hence hypoxic cell lines bearing unusually enlarged mitochondria are subjected to these anticancer compounds. It is concluded that these polymeric DTC derivatives and their gold conjugates indeed show higher accumulation as well as cytotoxicity to cancer cells under hypoxic conditions in comparison to the normoxic ones. Hypoxic MCF-7 cells showed significant sensitivity toward the low molecular weight (10 kDa) glycopolymer-Au(I) complexes.



INTRODUCTION

Therapeutic resistance in cancer diseases presents a formidable challenge to the researchers, therefore an innovative approach to their effective management is required. The discovery of cisplatin and other platinum-based anticancer compounds demonstrates some promise in treating the proliferative tumors.[1−4] However, severe toxic and undesirable side-effects in this class of compounds has encouraged researchers to look for alternative metal-based molecules.[1−5] The use of gold for medicinal purposes, 'chrysotherapy', dates back two thousand years. Gold(I) complexes are well-known for their anti-arthritic and anti-inflammatory properties.[1,2] However, the limited efficacy of Auranofin as an anticancer drug has led to the synthesis of a variety of gold(I) complexes, and their structure activity relationships have been studied for cancer treatment.[1−3,6−12] The modification of ligands was considered one of the approaches to overcome the toxic side effects of gold(I) complexes. Vitamin K3, pyridine, phosphane, and naphtalamide-based gold(I) complexes have been investigated, due to their electronic similarity with phosphines, and have been tested for their antiproliferative properties, but were less selective.[2,8−11,13−17]

One possible approach to enhance the selectivity, and to overcome the passive targeting to the tumor site, is the conjugation of metal complexes with nontoxic polymers.[18−21] Well-defined copolymers have been produced *via* living radical polymerization (LRP), are conjugated to anticancer compounds, and are studied for their cellular uptake, toxicity, and biodistribution.[19−21,43] This study focuses on the conjugation of Au(I) triphenylphosphine complexes with well-defined hydrophilic copolymers using dithiocarbamate (DTC) as a stabilizing ligand. DTCs are versatile ligands with antiproliferative properties that demonstrate strong covalent interactions between Au and sulfur, and can form complexes with a variety of transition metals in a number of oxidation states due to their 'chelate effect'. The combinations of antitumor features of metal ions with chemoprotective properties of DTCs may provide an innovative and effective strategy to cancer management. DTC is expected to coordinate with metal center, hence preventing the interactions of gold with other SH containing biomolecules, which is associated with general nephrotoxicity.[5] A number of DTC-based metal-conjugated complexes such as gold(III), copper(II), zinc(II), and ruthenium(III) complexes have been developed.[23−25]

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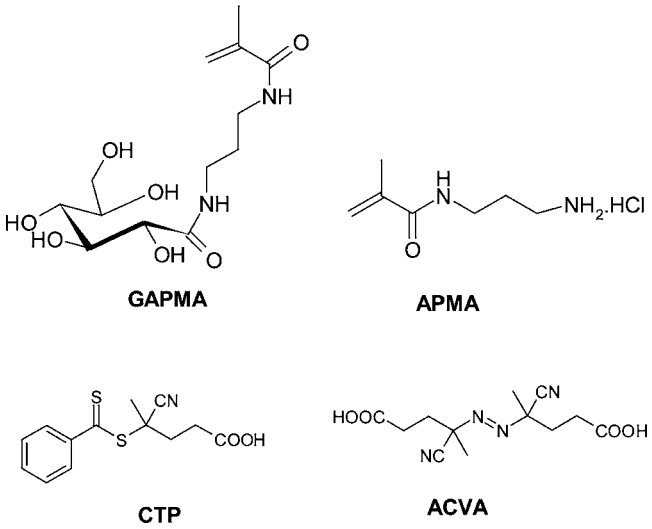
Interestingly, gold(I) and (III)-based DTC derivatives exhibit higher anticancer activity in comparison to cisplatin,[1,9] which indicates that the backbone of DTC can offer biocompatibility and water solubility leading to potential anticancer compounds.[25,26]

In the current study, DTC compounds are prepared in polymeric form to incorporate the desired backbone chemistry. The role of carbohydrates as delivery vectors, targeting moieties, and in cell−cell communication is well-established.[27−35] Therefore, conjugating polymeric carbohydrate chain to DTC compounds can provide an effective strategy for the development of cancer drugs. For this purpose cationic glycopolymers having varying degree of polymerization are synthesized in random architecture *via* reversible addition−fragmentation chain transfer polymerization (RAFT). The copolymers are modified by Makovnikov addition reaction to yield DTC-derived polymeric chains.[36−38] The structure of monomers, polymers, polymer synthesis, and DTC-modification of copolymers is depicted in Schemes 1 and 2, respectively.

Scheme 1. Chemical Structure of Monomers, 3-Gluconamidopropyl Methacrylamide (GAPMA), 3-Aminopropyl Methacrylamide (APMA), Chain Transfer Agent (4-cyanopentanoic acid dithiobenzoate, CTP), and Initiator (4′-azobis(4-cyanovaleric acid, ACVA)



DTC-functionalized glycopolymers were then reacted with gold salt to yield linear gold(I) phosphine derivatives, as shown in Scheme 3.

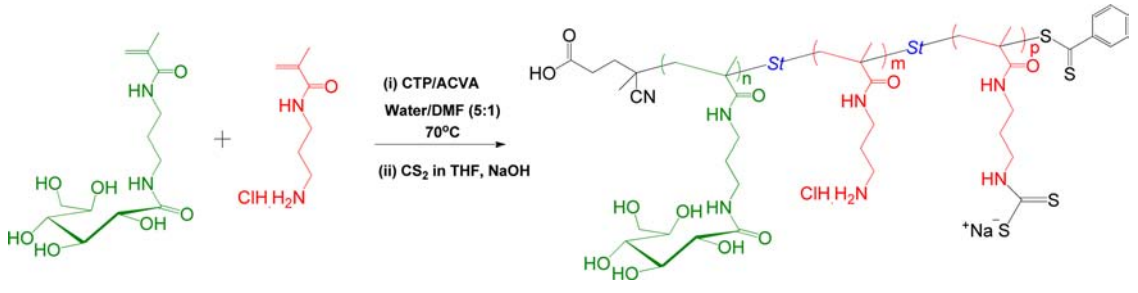
These novel glycopolymer-DTC derivatives and their metal complexes were evaluated for their cytotoxicity profiles in a number of cell lines. Toxicity studies of these materials were also investigated in hypoxic atmosphere in selected cancer cell lines, in order to determine their activity under mimicked *in vivo* cancer conditions. The uptake of DTC compounds was studied by modifying DTC-glycopolymers, with fluorescent probes, and by detecting the amount of fluorescence in treated cells. Finally the uptake of gold-complexes in cancer cells was studied by inductive coupling plasmon mass spectrometer (ICP-MS).

EXPERIMENTAL SECTION

Materials and Methods. ^{1}H NMR spectra of polymers were recorded using a Varian spectrometer (500 MHz) to confirm and determine the chemical structures of the synthesized polymers. The molecular weights and polydispersities of the synthesized polymers were determined by gel permeation chromatography (GPC) with two Waters Ultrahydrogel linear WAT011545 columns and Viscotek model 270 dual detector. Sodium acetate (0.50 M)/acetic acid (0.50 M) buffer or DMF at a flow rate of 1.0 mL/min was used as eluent. Dynamic light scattering (DLS) was performed with a ZetaPlus-Zeta Potential Analyzer (Brookhaven Instruments Corporation) at a scattering angle θ = 90°. Fourier transform infrared (FT-IR) spectral analyses using KBr pellets of the synthesized samples were carried out on a Nicolet8700 (Thermo) instrument and the diffuse reflectance spectra were scanned over a range of 4000−400 cm^{-1} wavenumbers. Monomers, 3-aminopropylmethacrylamide (APMA) and 3-glucoamidopropyl methacrylamide (GAPMA), were prepared in-house according to previously reported procedures.[39,40] Fluorescein isothiocyanate (FITC), carbon disulfide, cytarabine, cisplatin, and MTT dye were purchased from Sigma Aldrich, Canada. All solvents were purchased from Caledon Laboratories and used without any further purification. All cell culture products, including DMEM, FBS, antibiotics, and Trypsin with EDTA were obtained from Invitrogen.

Preparation of *p*(GAPMA$_m$-*st*-APMA$_n$). The statistical copolymer of GAPMA and APMA was prepared according to previously reported procedures.[40] Typically, GAPMA (1.00 g, 3.13 × 10^{-3} mol) was dissolved in doubly distilled water (4.5 mL) in a small test tube followed by the addition of an aqueous solution (0.5 mL) of APMA (0.139 g, 7.81 × 10^{-4} mol). A solution of ACVA (2.43 mg, 0.087 × 10^{-4} mmol) and 4-cyanopentanoic acid dithiobenzoate (CTP) (4.86 mg, 0.174 × 10^{-4} mmol) in *N*,*N*′-dimethylformamide (DMF) (1 mL) was then added. The mixture was degassed for 30 min. The flask was then transferred to a preheated oil bath at 70 °C for 20 h. The polymer was precipitated out of acetone, washed with methanol to remove unreacted monomer, and dried in air. Polymers were characterized by ^{1}H NMR spectroscopy and GPC.

Scheme 2. Synthesis of the Cationic Glycopolymer *via* RAFT Process and Subsequent Modification of the Amine Groups to Generate the Dithiocarbamate Polymers



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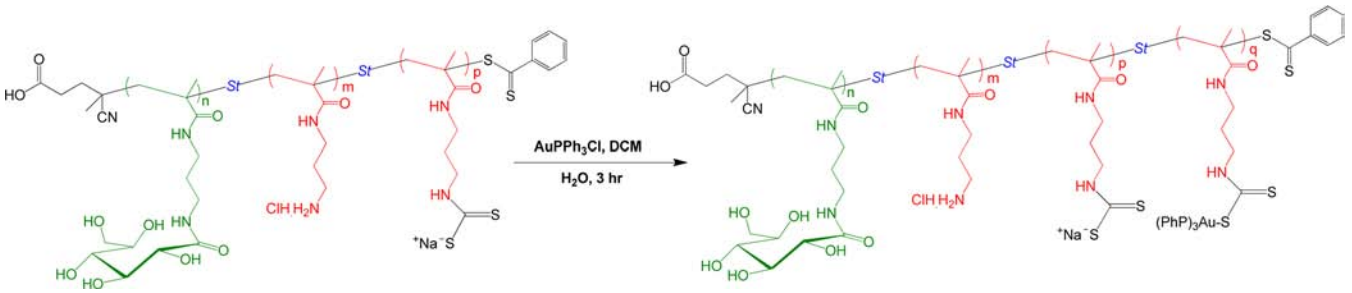
Scheme 3. Synthesis of Glycopolymer-DTC Gold Conjugates



Synthesis of DTC Compounds. The DTCs were synthesized with slight modifications to previously reported method.[36] All values are based on the amount of the APMA monomer in the polymer. In a typical synthesis, the polymer (200 mg) was added to carbon disulfide in THF in the presence of sodium hydroxide at pH ≤ 10. The reaction was stirred at room temperature for 24 h and the solvent was removed with a rotary evaporator. The solid products were washed with methanol and THF to remove unreacted starting materials. The products were characterized by ^{1}H NMR and IR spectroscopies.

Synthesis of GP-10. Carbon disulfide (11.2 μL, 0.19 mmol) in THF (5 mL) was added dropwise to *p*(GAPMA$_{27}$-*st*-APMA$_8$) (27.60 mg, 0.16 mmol) in sodium hydroxide (0.01 M) solution. The product was redissolved in distilled water and freeze−dried to yield a flaky pale pink solid.

^{1}H NMR (D_2O), ppm: 4.09 (s, 1H, Ar), 4.32 (s, 1H, Ar), 3.83 (s, 1H, Ar), 3.76 (s, 2H, CH_2−Ar), 3.66 (s, 1H, Ar), 3.28 (s, br, 4H), 3.14 (s, br, 4H), 2.65 (s, br, CH_2−NCS_2), 1.70 (s, br, 4H, $2CH_2$), 1.10 (s, br, 3H, CH_3), 0.93 (s, br, 3H, CH_3). IR (diamond, ATR): ν(C−S) = 988 cm^{-1}, ν(N−C) = 1478 cm^{-1}.

Synthesis of GP-30. Carbon disulfide (12 μL, 0.20 mmol) in THF (5 mL) was added dropwise to *p*(GAPMA$_{82}$-*st*-APMA$_{26}$) (29.80 mg, 0.17 mmol) in sodium hydroxide (0.01 M) solution. The product was redissolved in distilled water and freeze−dried to yield a flaky pale pink solid. ^{1}H NMR (D_2O), ppm: 4.11 (s, 1H, Ar), 4.02 (s, 1H, Ar), 3.80 (s, 1H, Ar), 3.75 (s, 2H, CH_2−Ar), 3.65 (s, 1H, Ar), 3.28 (s, br, 4H), 3.14 (s, br, 4H), 2.65 (s, br, CH_2−NCS_2), 1.70 (s, br, 4H, $2CH_2$), 1.10 (s, br, 3H, CH_3), 0.93 (s, br, 3H, CH_3). IR (diamond, ATR): ν(C−S) = 988 cm^{-1}, ν(N−C) = 1443 cm^{-1}.

Synthesis of Gold(I) Complex. The gold(I) complex was synthesized according to literature procedure with slight modification.[1] The dithiocarbamate polymers (GP-10 or GP-30) (50 mg) were dissolved in distilled water and were added dropwise to a stirring dichloromethane solution of triphenylphosphine gold(I) chloride (30 mg, 0.06 mmol) under an inert atmosphere for 3 h at 20 °C. The phases were separated and organic phase was washed 3 times with distilled water. The combined aqueous phase was removed *in vacuo* using a rotary evaporator and the white solid product was obtained. The solid obtained was dissolved in water and was extensively dialyzed to remove unreacted gold and reaction impurities. The solution was freeze−dried to obtain white precipitates. The gold content in the polymer was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and was 63.94 mg/g of the polymer. ^{31}P{^{1}H} NMR (D_2O): 23.4 ppm.

Size and Charge of Polymer Samples. GP-10, GP-30, GP-10($AuPPh_3$), and GP-30($AuPPh_3$) were added in OMEM supplemented with 10% FBS at a final concentration of 0.03 mg/mL. The sizes of samples were studied using Brookhaven dynamic light scattering instrument over a period of 4 h. The net charges of samples were determined in deionized water at a concentration of 2 mg/mL using Brookhaven zeta potential instrument.

Fluorescent Labeling of DTC-Polymers. The samples GP-10 and GP-30 were dissolved in 4% $NaHCO_3$ solution. FITC-DMSO solution (1 mg/mL) was added in small aliquots in aqueous solution of samples. The mixture was incubated in the dark for 3 days, followed by its dialysis in deionized water, and then freeze−dried to obtain yellow powder.

Cell Culture. Hep G2, HEK 293T, Human dermal fibroblasts, and MCF-7 cells were grown in low-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin/streptomycin) in a humidified atmosphere at 37 °C and 5% CO_2. At about 80% confluency, the cells were trypsinized with 0.25% trypsin with EDTA and were cultured twice a week. For performing hypoxia-selective studies, EMT-6 (cultured in complete DMEM medium supplemented with 10% fetal bovine serum) and MCF-7 cells (cultured in complete RPMI 1640 medium supplemented with 10% fetal bovine serum) were incubated under 5% CO_2 and 95% N_2, according to previously established procedure.[42]

Cellular Uptake of Fluorescent DTC-Compounds. The cells were cultured as mentioned above and were seeded in 24 well tissue culture plates. Hep G2 (60 000 cells/well), HEK 293T (80 000 cells/well), MCF-7 (12 000 cells/well), and fibroblasts (10 000 cells/well) were seeded in 24 well plate in duplicates, and were allowed to adhere overnight. The media was then changed and supplemented with serum containing fresh media, followed by the addition of fluorescent-polymer at final concentration of 0.2 mg/mL. The cells were incubated for an additional 4 h, followed by a wash (DPBS), and then typsinized using clear trypsin. The cells were fixed using 3.7% formalin in DPBS. The treated cells were analyzed using TECAN microplate reader while untreated cells were used as negative control. The cells were excited at 485 nm in 96 well black plates and emission spectra were recorded at 530 nm.

All cytotoxicity studies to evaluate the compounds against EMT-6 and MCF-7 cells under hypoxic conditions were performed in triplicate. Upon 80% confluence, the target cells were exposed to 0.06 mg/mL of fluorescent polymers at 37 °C. The medium was removed after 2 h of incubation; the cells were washed twice with the PBS buffer and then trypsinized, centrifuged, and fixed with 3.7 wt % formalin in PBS. The cells were excited at 485 nm in 96 well black plates and emission spectra were recorded at 530 nm.

Inductive Coupling Plasmon-Mass Spectrometer (ICP-MS). Hep G2, HEK 293T, MCF-7, and fibroblasts cells were maintained in humidified atmosphere at 37 °C and 5% CO_2 as described above. Upon 80% confluency the cells were exposed to 0.01 mg/mL GP-10($AuPPh_3$) and GP-30($AuPPh_3$) samples in DMEM media containing 10% FBS and 1% antibiotic for 4 h.

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The cells were washed twice with PBS and digested with 50% concentrated HNO_3 in water (5 mL). Hypoxic cells, EMT-6 and MCF-7, were cultured as described above. Upon 80% confluency, the target cells were exposed to the 0.0015 mg/mL GP-10($AuPPh_3$) at 37 °C. The medium was removed after 2 h of incubation and the cells were washed twice with the PBS buffer. The cells were then trypsinized and centrifuged. 50% HNO_3 (5 mL) was added to each sample to lyse the cells. The gold content in each solution was measured using inductively coupled plasma mass spectrometry (ICP-MS).

Determination of IC_{50} Values. Hep G2 (20 000 cells/well), HEK 293T (8000 cells/well), MCF-7 (4000 cells/well), and human dermal fibroblasts (4000 cells/well) were seeded in 96 well tissue culture plates in duplicates and were allowed to adhere overnight. The medium was then removed and fresh serum containing medium having varying concentrations (0.002 mg/mL to 0.4 mg/mL) of GP-10, GP-30, GP-10($AuPPh_3$), and GP-30($AuPPh_3$), cytarabine or cisplatin were then added. The cells were incubated for 18 h with the drugs under evaluation. The cell viability was determined using MTT assay. Briefly, 25 μL of MTT dye was added per well and cells were incubated for two hours, followed by the addition of MTT lysis buffer. The cells were incubated overnight and plate was read at 570 nm using TECAN microplate reader.

To determine hypoxia-selective toxicity of the study agents, exponentially growing cells were trypsinized and seeded into 96-well plates at the density of 800 cells/well for EMT-6 and 2000 cells/well for MCF-7 cells. GP-10 and GP-10($AuPPh_3$) were diluted with either DMEM medium or RPMI 1640 medium and exposed to the cells in 96 well plates at a volume of 100 μL to produce a final concentration from 0.0039 to 0.25 mg/mL. The plates were incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% N_2. The cell viability was evaluated, as described above using MTT assay.

RESULTS AND DISCUSSION

The importance of polymeric vectors in gene and drug delivery is well-recognized.[18−21,28−35,40] In addition to providing water solubility that facilitates nonspecific clearance, thereby reducing the toxicities of the system, these carbohydrate based polymeric vectors also offer multivalency and targeting properties to the tumor site. The synthesis of carbohydrates based anticancer agents is under extensive exploration to enhance the efficacy and decrease the side effects of anticancer compounds.[19−21,40] Due to the growing concern of the side effect of Pt-based drugs for chemotherapy, focus is shifted on the synthesis and careful design of gold complexes for cancer treatments.[1,2] Gold-based therapeutics are studied for their anti-inflammatory and anticancer activities from decades.[1,2,15] The anticancer activity of gold-complexes is associated with their mitochondrial toxicity, hence causing autophagy and apoptosis in targeted cells. The mechanism of toxicities of gold species in living cells has been reviewed.[4] The complex chemical approaches to vary the hydrophilicity of the resultant complexes are used to produce multidentate gold compounds bearing different types of phosphine ligands.[9,11−17] Moreover, cationic complexes bearing tetrahedral geometries are prepared to enhance the targeting efficacies to the mitochondria of cells. Gold(I) phosphine compounds bearing DTC moieties, such as auranofin analogues, have been reported and are found to be more potent than cisplatin against several cancer cell lines.[1] The exploration of backbone chemistry of DTC compounds has been proposed, but has not yet materialized for gold-based complexes.[25,26] We herein propose a novel approach to incorporate multivalency, hydrophilicity, and targeting features to traditional gold(I) phosphine complexes. To the best of our knowledge, there are no reports to date where gold(I) phosphine DTC complexes attached to a glycopolymer backbone have been synthesized and their toxicity profiles have been studied.

Extensive synthetic and structure−activity correlation studies by our group on carbohydrate-based copolymers[28−35] have helped us in identifying the molecules, sizes, and compositions of copolymers for their applications in optimized gene delivery efficacies.[28−31] Copolymers with statistical architectures are ideal for the desired biological features.[28−31,19−21,40] We therefore synthesized amine-based glycopolymers of statistical architecture using 3-aminopropyl methacrylamide (APMA) and glycomonomer 3-gluconamidopropyl methacrylamide (GAPMA) of varying molecular weights as monomers. The small amount of APMA was added to the glycopolymer in random fashion for the facile modification of free amine to afford dithiocarbamate (DTC) moiety using the Markovnikov addition reaction.[36] The selected copolymers were of low (10 kDa) as well as of high molecular weights (30 kDa), in order to incorporate optimum hydrophilic properties to the resulting gold(I) phosphine complexes. DTC-modified low molecular weight cationic glycopolymers were labeled as GP-10; similarly DTC-modified high molecular weight copolymers were labeled as GP-30. The structures of modified polymers were confirmed by ^{1}H NMR, mass spectroscopy, and FTIR-spectra (Supporting Information Figures S1−S5).

The DTC-modified copolymers were highly water-soluble. ^{1}H NMR spectrum was quantified to determine the final amount of DTC, free amine, and GAPMA in the polymers, as shown in Table 1. The polymeric chains showed 67% (for GP-10) and 86% (for GP-30) conversion of free amine into DTC moieties. All polymeric chains were slightly cationic, due to the presence of small number of free amines on the chain. FTIR spectra of polymer alone were compared with DTC-modified polymer and the presence of a C−S functional group was apparent at 988 cm^{-1} (Figure S5).

Table 1. Molecular Weights of the Cationic Glycopolymers As Determined from GPC and Chemical Compositions of Copolymers and Their DTC Derivatives As Determined from ^{1}H NMR

				polymer after modification		
code	molecular weight (kDa)	M_w/M_n	polymer before modification	GAPMA	APMA	DTC
GP-10	10	1.2	$p(GAPMA_{27}$-*st*-$APMA_8)$	27	3	5
GP-30	30	1.25	$p(GAPMA_{82}$-*st*-$APMA_{26})$	82	4	22

DTC is an attractive ligand bearing antioxidant properties, which can complex with a variety of metals in different oxidation states. DTC-derived compounds are reported to show various biological activities including anticancer activities.[25,26] Moreover, it is shown that the conjugation of DTC with cisplatin does not negatively impact its antiproliferative properties.[24] To combine the antiproliferative properties with potential antitumor and anti-inflammatory properties of gold compounds, polymer−DTC conjugates synthesized above were reacted with gold(I) phosphine. This yielded polymeric-DTC-gold complexes, GP-10($AuPPh_3$) and GP-30($AuPPh_3$), that separated as white precipitates from the

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corresponding reaction solutions. The precipitates were filtered, collected, purified, and freeze−dried to yield fine white powder. The powder was dispersible in water at the concentration at 2 mg/mL and formed stable colloids without any precipitation, which were stored at 4 °C for more than 6 months. The presence and amount of gold content in the two samples was confirmed by ICP-MS and ^{31}P NMR. As shown in ^{31}P NMR, the phosphorus peak shifted from the 33.18 ppm (gold-phosphine complex) to 23 ppm confirming the formation of gold−polymer conjugate (Supporting Information Figure S6 and S7). ICP-MS of GP-10($AuPPh_3$) and GP-30($AuPPh_3$) samples showed that although the percent conversion of amine into DTC moieties was higher for 30 kDa polymers in comparison to 10 kDa polymers, the gold content was higher for GP-10($AuPPh_3$) sample (0.696 ppm/0.06 mg/mL), in comparison to GP-30($AuPPh_3$) sample (0.438 ppm/0.06 mg/mL). The lower degree of modification of GP-30 with gold(I) phosphine may be associated to the bulkiness of GP-30 polymeric chains, as compared to GP-10 polymer. DTC-modified copolymers and their gold complexes were analyzed for their net sizes and charges using DLS and zeta potential instrument, respectively (Figure 1).

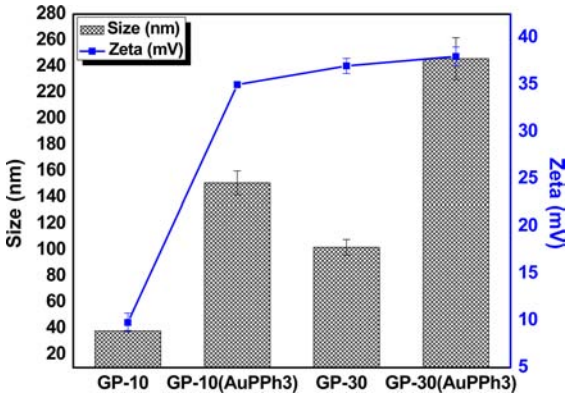

Figure 1. Aggregation of the polymers and polymer-Au conjugates in serum containing media. The net charge of the aggregates is determined in deionized water using zeta potential instrument.

It was shown that complexes GP-10 and GP-30 are smaller in size in comparison to the corresponding gold complexes. The increase in size of GP-10($AuPPh_3$) and GP-30($AuPPh_3$) reflected the hydrophobicity of the compounds upon modification. As shown in Figure 1, all samples contained strong net positive charge in deionized water. The stability of these samples in serum containing media was then studied as a function of time (Figure 2). It was found that all samples showed high stability in the presence of serum containing media over a period of 4 h and no aggregation of samples was observed due to the precipitation of samples in media.

These samples were then studied for their cellular uptake and toxicity profiles in different cell lines. Cytotoxicity evaluations of Au(I) phosphine-based complexes using DTC ligands in selected cancer cell lines has been reported. However, these complexes were not polymeric and were not very soluble in aqueous solution.[36] To evaluate the toxicities of these compounds, cytarabine was used as a standard for GP-10 and GP-30 and cisplatin was used as a standard for GP-10($AuPPh_3$) and GP-30($AuPPh_3$) samples. Cell lines included in the evaluation of toxicity profiles were malignant MCF-7 and Hep G2, nonmalignant HEK 293T (transformed), and human dermal fibroblasts (primary) and hypoxic MCF-7 and EMT-6 cells (Table 2, Supporting Information Figures S8−S33). IC_{50} values of the drugs were calculated using OriginPro Software and data was fitted using gmoidal fit.

In general, it was found that cisplatin (IC_{50} = 66.7 μM) was 30-fold more effective in inhibiting cell growth, in comparison to cytarabine (IC_{50} = 2.05 mM). GP-10 and GP-30 samples showed higher activity in comparison to cytarabine. The gold complexes of these copolymers showed a significantly higher degree of inhibition of proliferation, and their IC_{50} values were comparable to those of cisplatin. A correlation of copolymers' molecular weights to the toxicity of these compounds indicated that GP-10 complexes showed higher toxicity than GP-30 complexes. As discussed above, the DTC content is slightly higher for GP-30 sample. This decrease in toxicity can be due to the masking of DTC by the higher densities of the carbohydrate residues, in comparison to GP-10 sample. Another possible explanation to the suboptimal function of DTC moieties in GP-30 sample can be due to the decreased lipophilicity of the complexes upon conjugation with high molecular weight sugar chains. This trend can also be explained by DLS measurements of the samples (Figure 2). DLS analysis showed that modification of GP-10 with gold complexes caused 4-fold increase in the size of complexes, possibly due to the high hydrophobicity of the resulting samples. In contrast, modification of GP-30 with gold complexes only increased the size of complexes by 2-fold. We further studied the uptake of these gold complexes in MCF-7 and Hep G2, nonmalignant HEK 293T (transformed), and human dermal fibroblasts (primary) and hypoxic MCF-7 and EMT-6 cells lines. For this purpose, GP-10 and GP-30 samples were labeled with FITC. The labeling density was higher for GP-30 in comparison to GP-10, possibly due to the presence of a larger number of free amine groups in GP-30 sample, as determined by ^{1}H NMR. The labeling density of two polymers was compared. It was found that uptake of these complexes was not directly

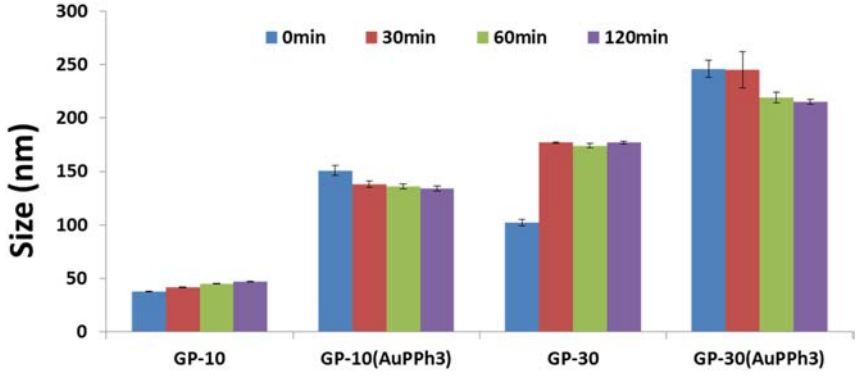

Figure 2. Stability of glycopolymer-DTC and gold conjugates in serum containing media over a period of 2 hr as studied by dynamic light scattering.

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related to their toxicities (Supporting Information Figure S34). For example, the uptake of GP-10-FITC was slightly higher in fibroblasts in comparison to other cell lines (Figure 3). However,

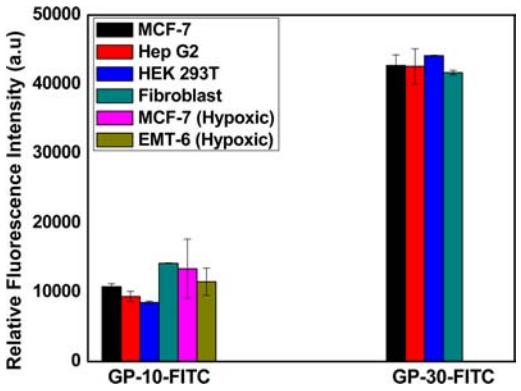

Figure 3. Cellular uptake of FITC-labeled GP-10 and GP-30 samples in different cell lines.

a high accumulation of GP-10-FITC polymers in fibroblasts did not translate into their higher cytotoxicity (IC_{50} 37 μM). Although accumulation of GP-10-FITC in HEK-293T cells was low, they showed high sensitivity with low IC_{50} values. Similarly, GP-30-FITC showed higher accumulation in HEK293T cells, but they were less cytotoxic. The studies with Hep G2 and MCF-7 indicated that there was no significant difference in uptake of both GP-10-FITC and GP-30-FITC, but Hep G2 cells showed less sensitivity to these polymers.

Gold content in treated cells was analyzed by ICP-MS, as shown in Figure 4. MCF-7 cells showed significantly higher accumulation of GP-10($AuPPh_3$), as compared to Hep G2 cells. In contrast, there was no significant difference in uptake of GP-30($AuPPh_3$) sample in all cell lines studied. However, all cell lines showed high sensitivity toward GP-10($AuPPh_3$). Interestingly, despite high accumulation of GP-10($AuPPh_3$) in MCF-7 cells, their IC_{50} values were higher than other cell lines studied.

Cytotoxicity studies with GP-10 and GP-10($AuPPh_3$) were then studied under hypoxic conditions in EMT-6 and MCF-7 cancer cells. Hypoxic cancer cells are characterized by the presence of exceptionally large mitochondria, which are responsible for their resistance to apoptosis. GP-10 and its gold analogue were chosen due to their high potency and accumulation in different cell lines. Au(I) phosphine complexes are interesting due to their implications in mitochondrial toxicity, leading to cell death.[2,4,12] MCF-7 and EMT-6 hypoxic cells were treated with these compounds. It was found that although EMT-6 cells showed significantly higher accumulation of gold complexes, MCF-7 showed enhanced toxicity in the presence of GP-10($AuPPh_3$) with IC_{50} of 0.4 μM. To the best of our knowledge this is the first approach where polymeric Au(I) DTC conjugates are prepared and their cytotoxicity in hypoxic cells are tested. High potency of GP-10($AuPPh_3$) conjugates in hypoxic breast cancer cells and their high accumulation in EMT-6 cells shows a proof-of-principle promise in the desired direction for the cancer treatment.

CONCLUSIONS

The study provides a comprehensive account of the synthesis of glycopolymer-DTC compounds and their gold(I) phosphine complexes in a facile manner. The composition of DTC/DTC-Au and glycopolymer was critical in maintaining the activity of these anticancer agents. The lower molecular weight version of the polymer showed higher activity toward different cell lines, especially toward MCF-7 cells. However, human embryonic kidney cells behaved differently and showed significant toxicity upon exposure to high molecular weight version of the polymer conjugates. This indicates that GP-10 and GP-10($AuPPh_3$)

Table 2. Inhibitory Concentration 50 (IC_{50}) of DTC and DTC Derived Gold Compounds in Different Cell Lines

	IC_{50} (μM)					
drugs	MCF-7	HepG2	HEK 293T	fibroblasts	MCF-7 (Hypoxic)	EMT-6 (Hypoxic)
Cisplatin	66.7	100	33.3	100	-	-
Cytarabine	2098	1316	1152	1440	-	-
GP-30	3.7	26	6.3	10.3	-	-
GP-30($AuPPh_3$)	1.7	5	0.67	1.33	-	-
GP-10	26	41	3	37	8.4	14
GP-10($AuPPh_3$)	4	2	4	2	0.4	1.3

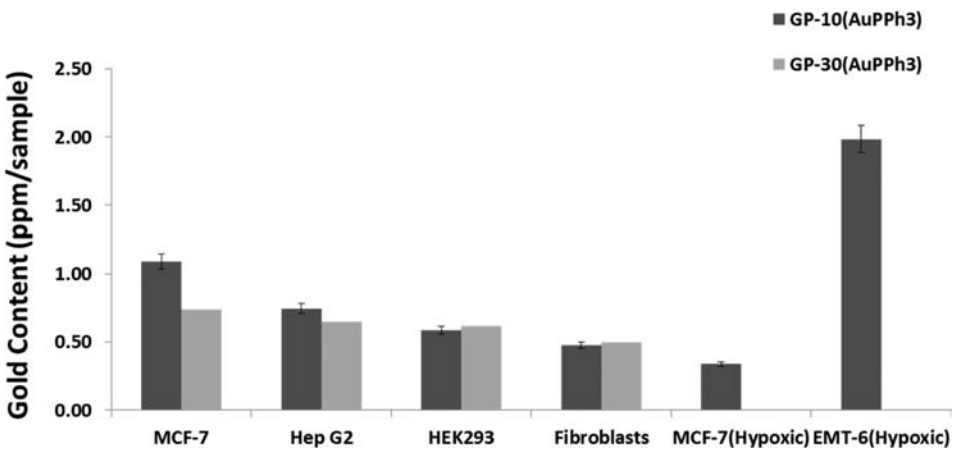

Figure 4. Gold content in different cell lines as determined by inductively coupled plasma mass spectrometer (ICP-MS).

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might be useful for further studies. Evaluation of GP-10 and GP-10($AuPPh_3$) in MCF-7 and EMT-6 under hypoxic conditions was done in order to mimic the therapy-resistant conditions in cancer diseases. It is logical to conclude that, regardless of uptake profile, both GP-10 and GP-10($AuPPh_3$) complexes showed significant toxicity toward MCF-7 cells under hypoxic conditions. In comparison to normoxic MCF-7 cells, hypoxic MCF-7 cells show significant sensitivity to GP-10($AuPPh_3$) compound. Hence, GP-10($AuPPh_3$) has the potential to serve as an anticancer compound for the treatment of breast cancer, without significantly affecting normal tissues.

■ ASSOCIATED CONTENT

Supporting Information

NMR data, comparison of labeling density of polymers, IC_{50} profiles of compounds in different cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Tiekink, E. R. T. (2008) Anti-cancer potential of gold complexes. *Inflammopharmacology 16*, 138−142.
(2) Ott, I. (2009) On the medicinal chemistry of gold complexes as anticancer drugs. *Coord. Chem. Rev. 253*, 1670−1681.
(3) Ronconi, L., Marzano, C., Zanello, P., Corsini, M., Miolo, G., Macca, C., Trevisan, A., and Fregona, D. (2006) Gold(III) dithiocarbamate derivatives for the treatment of cancer: solution chemistry, DNA binding, and hemolytic properties. *J. Med. Chem. 49*, 1648−1657.
(4) Wang, Y., and Chiu, J.-F. (2008) Proteomic approaches in understanding action mechanisms of metal-based anticancer drugs. *Met. Based Drugs*, 716329 DOI: 10.1155/2008/716329.
(5) Cattaruzza, L., Fregona, D., Mongiat, M., Ronconi, L., Fassina, A., Colombatti, A., and Aldinucci, D. (2011) Antitumor activity of gold(III)-dithiocarbamato derivatives on prostate cancer cells and xenografts. *Int. J. Cancer 128*, 206−215.
(6) Ronconi, L., Giovagnini, L., Marzano, C., Bettio, F., Graziani, R., Pilloni, G., and Fregona, D. (2005) Gold dithiocarbamate derivatives as potential antineoplastic agents: design, spectroscopic properties, and in vitro antitumor activity. *Inorg. Chem. 44*, 1867−1881.
(7) Coronnello, M., Mini, E., Caciagli, B., Cinellu, M. A., Bindoli, A., Gabbiani, C., and Messori, L. (2005) Mechanisms of cytotoxicity of selected organogold(III) compounds. *J. Med. Chem. 48*, 6761−6765.
(8) Suresh, D., Balakrishna, M. S., Rathinasamy, K., Panda, D., and Mobin, S. M. (2008) Water-soluble cyclodiphosphazanes: synthesis, gold(I) metal complexes and their in vitro antitumor studies. *Dalton Trans.*, 2812−2814.
(9) Gracia-Orad, A., Arizti, P., Sommer, F., Silvestro, L., Massiot, P., Chevallier, P., Gutierrez-Zorilla, J. M., Colacio, E., De Pancorbo, M. M., and Tapiero, H. (1993) The (S-thiotheophyllinate) (triphenylphosphine) gold (I), (tTAUP): a new gold complex as an anticancer agent. *Biomed. Pharmacother. 47*, 363−370.
(10) Tian, S., Siu, F.-M., Kui, S. C. F., Lok, C.-N., and Che, C.-M. (2011) Anticancer gold(I)-phosphine complexes as potent autophagy-inducing agents. *Chem. Commun. 47*, 9318−9320.
(11) Wetzel, C., Kunz, P. C., Kassack, M. U., Hamacher, A., Bohler, P., Watjen, W., Ott, I., Rubbiani, R., and Spingler, B. (2011) Gold(I) complexes of water-soluble diphos-type ligands: Synthesis, anticancer activity, apoptosis and thioredoxinreductase inhibition. *Dalton Trans.*, 9212−9220.
(12) Bindoli, A., Rigobello, M. P., Scutari, G., Gabbiani, C., Casini, A., and Messori, L. (2009) Thioredoxinreductase: A target for gold compounds acting as potential anticancer drugs. *Coord. Chem. Rev.*, 1692−1707.
(13) Barreiro, E., Casas, J. S., Couce, M. D., Sanchez, A., Sanchez-Gonzalez, A., Sordo, J., Varela, J. M., and Lopez, E. M. V. (2008) Synthesis, structure and cytotoxicity of triphenylphosphinegold(I) sulfanylpropenoates. *J. Inorg. Biochem. 102*, 184−192.
(14) Berners-Price, S. J., Bowen, R. J., Hambley, T. W., and Healy, P. C. (1999) NMR and structural studies of gold(I) chloride adducts with bidentate2,-3- and 4-pyridyl phosphines. *J. Chem. Soc., Dalton Trans.*, 1337−1346.
(15) Berners-Price, S. J., Bowen, R. J., Fernandes, M. A., Layh, M., Lesueur, W. J., Mahepal, S., Mtotywa, M. M., Sue, R. E., and Rensburg, C. E. J. (2005) Gold(I) and silver(I) complexes of 2,3-bis-(diphenylphosphino)maleic acid: Structural studies and antitumour activity. *Inorg. Chim. Acta 358*, 4237−4246.
(16) Caruso, F., Rossi, M., Tanski, J., Pettinari, C., and Marchetti, F. (2003) Antitumor activity of the mixed phosphine gold species chlorotriphenylphosphine- 1,3-bis(diphenylphosphino)propanegold-(I). *J. Med. Chem. 46*, 1737−1742.
(17) Pillarsetty, N., Katti, K. K., Hoffman, T. J., Volkert, W. A., Katti, K. V., Kamei, H., and Koide, T. (2003) In vitro and in vivo antitumor properties of tetrakis((trishydroxymethyl)phosphine)gold(I) chloride. *J. Med. Chem. 46*, 1130−1132.
(18) Allen, T. M. (2002) Ligand-targeted therapeutics in anticancer therapy. *Nat. Rev. Cancer 10*, 750−763.
(19) Huynh, V. T., Chen, G., De Souza, P., and Stenzel, M. H. (2011) Thiolyne and thiolene "click" chemistry as a tool for a variety of platinum drug delivery carriers, from statistical copolymers to cross-linked micelles. *Biomacromolecules 12*, 1738−1751.
(20) Huynh, V. T., De Souza, P., and Stenzel, M. H. (2011) Polymeric micelles with pendant dicarboxylato chelating ligands prepared via a michael addition for cis-platinum drug delivery. *Macromolecules 44*, 7888−7900.
(21) Huynh, V. T., Quek, J. Y., De Souza, P., and Stenzel, M. H. (2012) Block copolymer micelles with pendant bifunctional chelator for platinum drugs: effect of spacer length on the viability of tumour cells. *Biomacromolecules 13*, 1010−1023.
(22) Ronconi, L., and Fregona, D. (2009) The Midas touch in cancer chemotherapy: from platinum- to gold-dithiocarbamato complexes. *Dalton Trans.*, 10670−16080.
(23) Marzano, C., Trevisan, A., Giovagnini, L., and Fregona, D. (2002) Synthesis of a new platinum(II) complex: anticancer activity and nephrotoxicity in vitro. *Toxicol. In Vitro 16*, 413−419.
(24) Marzano, C., Bettio, F., Baccichetti, F., Trevisan, A., Giovagnini, L., and Fregona, D. (2004) Antitumor activity of a new platinum(II) complex with low nephrotoxicity and genotoxicity. *Chem. Biol. Interact. 148*, 37−48.
(25) Nabipour, H., Ghammamy, S., Ashuri, S., and Aghbolagh, Z. S. (2012) Synthesis of a new dithiocarbamate compound and study of its biological properties. *Org. Chem. J. 2*, 75−80.
(26) Nabipour, H., Ghammamy, S., and Rahmani, A. (2011) Synthesis of a new dithiocarbamate cobalt complex and its nanoparticles with the study of their biological properties. *Micro Nano Lett. 6*, 217−220.
(27) Spain, S. G., and Cameron, N. R. (2011) A spoonful of sugar: the application of glycopolymers in therapeutics. *Polym. Chem. 2*, 60−68.
(28) Ahmed, M., and Narain, R. (2011) The effect of polymer architecture, composition, and molecular weight on the properties of

glycopolymer-based non-viral gene delivery systems. *Biomaterials 32*, 5279−5290.
(29) Ahmed, M., and Narain, R. (2012) The effect of molecular weight, compositions and lectin type on the properties of hyperbranchedglycopolymers as non-viral gene delivery systems. *Biomaterials 33*, 3990−4001.
(30) Ahmed, M., Jawanda, M., Ishihara, K., and Narain, R. (2012) Impact of the nature, size and chain topologies of carbohydrate-phosphorylcholine polymeric gene delivery systems. *Biomaterials 33*, 7858−7870.
(31) Ahmed, M., Jiang, X., Deng, Z., and Narain, R. (2009) Cationic glyco-functionalized single walled carbon nanotubes as efficient gene delivery vehicles. *Bioconjugate Chem. 20*, 2017−2022.
(32) Ahmed, M., Deng, Z., Liu, S., Lafrenie, R., Kumar, A., and Narain, R. (2009) Cationic glyconanoparticles: their complexation with DNA, cellular uptake, and transfection efficiencies. *Bioconjugate Chem. 20*, 2169−2176.
(33) Ahmed, M., Deng, Z., and Narain, R. (2009) Study of size dependent transfection efficiencies of gold nanoparticles in human cell line. *ACS Appl. Mater. Interface 1*, 1980−1987.
(34) Ahmed, M., and Narain, R. (2012) Intracellular delivery of DNA and enzyme in active form using degradable carbohydrate based nanogels. *Mol. Pharmaceutics 9*, 3160−3170.
(35) Sunasee, R., Wattanaarsakit, P., Ahmed, M., Lollmohamed, F., and Narain, R. (2012) Biodegradable, non-toxic cationic nanogels as non-viral gene delivery systems. *Bioconjugate Chem. 23*, 1925−1933.
(36) De Vos, D., Ho, S. Y., and Tiekink, R. T. (2004) Cytotoxicity profiles for a series of triorganophosphinegold(I) dithiocarbamates and triorganophosphinegold(I) xanthates. *Bioinorg. Chem. Appl. 2*, 141−154.
(37) Deng, Z., Bouchekif, H., Babooram, K., Housni, A., Choytun, N., and Narain, R. (2008) Facile synthesis of controlled-structure primary amine-based methacrylamide polymers via the reversible addition-fragmentation chain transfer process. *J. Poly. Sci. Part A: Polym. Chem. 46*, 4984−4996.
(38) Deng, Z., Ahmed, M., and Narain, R. (2009) Novel well-defined glycopolymers synthesized via the reversible addition fragmentation chain transfer process in aqueous media. *J. Polym. Sci., Part A: Polym. Chem. 47*, 614−627.
(39) Huynh, V. T., Binauld, S., De Souza, P. L., and Stenzel, M. H. (2012) Acid degradable cross-linked micelles for the delivery of cisplatin: A comparison with non-degradable crosslinker. *Chem. Mater. 24*, 3197−3211.
(40) Halimehjani, A. Z., Marjani, K., and Ashouri, A. (2010) Synthesis of dithiocarbamate by Markovnikov addition reaction in aqueous medium. *Green Chem. 12*, 1306−1310.
(41) Voegel, P. D., Quashnock, K. A., and Heil, K. M. (2005) Combined ^{31}P and ^{1}H NMR experiments in the structural elucidation of polynuclear thiolate complexes. *J. Chem. Educ. 82*, 630−633.
(42) Kumar, P., Shustov, G., Liang, H., Khlebnikov, V., Zheng, W., Yang, X.-H., Cheeseman, C., and Wiebe, L. I. (2012) Design, synthesis, and preliminary biological evaluation of 6-*O*-glucose-azomycin adducts for diagnosis and therapy of hypoxic tumors. *J. Med. Chem. 55*, 6033−6046.
(43) Peter, C. K., Nadine, E. B., and Bernhard, S. (2007) Towards polymer diagnostic agents - copolymers of *N*-(2-hydroxypropyl)-methacrylamide and bis(2-pyridylmethyl)-4-vinylbenzylamine: synthesis, characterisation and Re(CO)$_3$-labelling. *Eur. J. Inorg. Chem.*, 394−399.

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