SUPPORTING INFORMATION
for

# Synthesis of polyethylene glycol-poly(glycerol carbonate) block copolymeric micelles as surfactant-free drug delivery systems

Danielle M. Fitzgerald[1], Heng Zhang[1], Catalina Bordeianu[1], Yolonda L. Colson[2], and Mark W. Grinstaff[1]*

[1]Departments of Chemistry and Biomedical Engineering, Boston University, Boston, MA 02215
[2]Division of Thoracic Surgery, Department of Surgery, Massachusetts General Hospital, Boston MA 02114
*Corresponding author E-mail: mgrin@bu.edu

## 1. General Information

All manipulations involving air- and/or water-sensitive compounds were carried out in a glovebox under argon environment. Benzyl glycidyl ether was refluxed over $CaH_2$ and fractionally distilled under argon atmosphere prior to use. Excess water was removed from poly(ethylene glycol) via azeotropic distillation with toluene and lyophilized prior to use. Carbon dioxide (99.995%, bone dry) was purchased from Airgas and used as received. The cobalt salen catalyst was synthesized according to literature.[1] Pd/C (10%) was purchased from Strem and used as received.

*NMR experiments.* $^1$H and $^{13}$C NMR spectra were recorded on a Varian 500 MHz type ($^1$H, 500 MHz; $^{13}$C, 125 MHz) spectrometer. Their peak frequencies were referenced against the solvent, chloroform-$d_6$ at δ 7.26 ppm for $^1$H NMR and δ 77.16 ppm for $^{13}$C NMR.

*Gel Permeation Chromatography.* All polymer molecular weights were determined by gel permeation chromatography versus polystyrene standards using THF as the eluent at a flow rate of 1.0 mL/min with a refractive index detector. The exception is the analysis of mPEG-*b*-PGC-OBn synthesized from mPEG 10 kDa, which was analyzed in the same manner but using aqueous buffer solution containing 0.10 M sodium nitrate, 0.010 M sodium phosphate, and 0.02 wt% sodium azide (pH 7.4) as the eluent and polyethylene glycol as the standards.

*Particle Size Analyzer.* Nanoparticle diameter, polydispersity, and zeta potential measurements were acquired on a Brookhaven NanoBrook Particle Size Analyzer at 640 nm. Dynamic light scattering was performed with a 90˚ scattering angle.

*Scanning Electron Microscopy.* Scanning electron micrographs were obtained at 3 kV with a 6.0 mm working distance and 30 μm aperture (Zeiss Supra 55). Prior to imaging, nanoparticles were sputter coated with a gold-palladium target at 20 mA, with a target-to-mesh distance of 3 cm.

*UV-Vis Absorption Spectroscopy.* Paclitaxel was quantified using UV-Vis absorption spectroscopy on a SpectraMax iD3 microplate reader (Molecular Devices, LLC). Samples were diluted into the range of the calibration curve (2.5 mg/mL – 6.4 ng/mL) and 200 μL of each were placed in the well of a 96-well plate. Absorption was read at 230 nm and the paclitaxel concentration was determined from the calibration curve.

*Cell Culture.* NIH 3T3 murine fibroblasts were selected for use as a representative cell line of healthy tissue. A549 human lung carcinoma, SKOV3 human ovarian adenocarcinoma, and MDA-

MB-231 human breast adenocarcinoma cell lines were selected as they are diseases commonly treated with paclitaxel. All cell lines were purchased from ATCC.

a. *NIH 3T3*: Cells were cultured in Dulbecco's Modified Eagle's Medium (Corning®; Cat. No. 10-013-CV) media supplemented with 10% bovine bovine serum and 100 U/100 µg/mL penicillin/streptomycin at 37˚C and 5% $CO_2$.
b. *A549*: Cells were cultured in F-12K Medium (Gibco™; Cat. No. 21127-022) supplemented with 10% fetal bovine serum and 100 U/100 µg/mL penicillin/streptomycin at 37˚C and 5% $CO_2$.
c. *SKOV3*: Cells were cultured in McCoy 5a Medium Modified (Gibco™; Cat. No. 16600-082) supplemented with 10% fetal bovine serum and 100 U/100 µg/mL penicillin/streptomycin at 37˚C and 5% $CO_2$.
d. *MDA-MB-231*: Cells were cultured in RPMI Medium (Corning®; Cat. No. 10-0040-CV) supplemented with 10% fetal bovine serum and 100 U/100 µg/mL penicillin/streptomycin at 37˚C and 5% $CO_2$.

*In Vitro Cytotoxicity Assays*. Subconfluent cells were seeded at 1600, 1600, 2500, and 3750 cells per well in 96-well culture plates for NIH 3T3, A549, SKOV3, and MDA-MB-231 cells, respectively. Cells were adhered for 24 hours, after which the media was replaced with either mPEG-*b*-PGC-OBn (control) or mPEG-*b*-PGC-*g*-PTX nanoparticles (treatment) dissolved in the appropriate media for each cell line. After 36 hours of incubation, cell viability was measured using a colorimetric MTS assay (Abcam, ab197010) and calculated as a percentage of absorbance from untreated cells at each time point. The half maximal inhibitory concentration ($IC_{50}$) of paclitaxel against each cell line was determined used four-parameter logistic fit analysis.

## 2. Synthetic Procedures

a. *Representative synthesis of mPEG-b-PGC using mono-methoxy terminated PEG as chain transfer agent*.

Benzyl glycidyl ether (BGE) (1.53 mL, 10 mmol, 1.0 eq.), colbalt salen catalyst (*S,S*-SalcyCo$^{III}$TBDDNP) (10.4 mg, 0.005 mmol, 0.0005 eq.), and monomethyl PEG (MW = 1900, 380 mg, 0.2 mmol, 0.02 eq.) were added to a high-pressure autoclave. The autoclave was transferred out of the glovebox and immersed into a 50˚C oil bath for 30 min to dissolve mPEG in BGE. Then, the autoclave was charged with 220 psi of $CO_2$. The reaction was allowed to run at 50˚C for 2 hr. After that, the $CO_2$ pressure was released and an aliquot was taken for $^1$H NMR analysis to determine percent conversion. The reaction mixture was then diluted with minimal DCM and precipitated dropwise into cyclohexane 2-3 times. The precipitated polymer was dried under vacuum and 1.34 g (88%) of yellow semi-solid polymer was collected. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.20-7.50 (br, 5H), 5.00-5.30 (br, 3H), 4.30-4.60 (br, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 116.0, 153.6, 134.8, 128.8, 128.4, 73.5, 68.0, 66.2, 53.7.

b. *Debenzylation of mPEG-b-PGC*

mPEG-*b*-PGC (540 mg) was added into a high-pressure autoclave and dissolved completely in 7 mL 5:1 ethyl acetate:methanol. Pd/C (10% dry basis, 50% wet with water) (160 mg, 30 wt% of polymer) was added. The autoclave was charged with 400 psi $H_2$. After 12 hours stirring at room temperature, the pressure was released and the reaction mixture was filtered through celite. The solvent was removed under reduced pressure and the polymer was dried under vacuum to afford 328 mg (91%) of mPEG-*b*-PGC. $^1$H NMR

(500 MHz, DMSO-$d_6$): δ 4.75-4.85 (br, 1H), 4.12-4.35 (br, 2H), 3.52-3.58 (br, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 168.0, 153.7, 73.6, 67.0.

c. *Synthesis of mPEG-b-PGC-g-SA*

mPEG-*b*-PGC (300 mg, 0.034 mmol, 1.0 eq.), succinic anhydride (3.8 mg, 0.038 mmol, 1.1 eq.), and 4-dimethylaminopyridine (2.3 mg, 0.019 mmol, 0.05 eq.) were added into a 5 mL round bottom flask. 1 mL of DMF was added to dissolve the solid and the reaction was stirred at room temperature overnight. Then, the reaction mixture was added dropwise into diethyl ether, centrifuged, and the upper layer was decanted off and discarded. The solid was re-dissolved in 5:1 ethyl acetate:methanol and added dropwise into diethyl ether to precipitate again and completely remove DMF. The mixture was centrifuged and the polymer was isolated as a rubbery, weakly elastic solid. $^1$H NMR (500 MHz, $CDCl_3$): δ 4.95-5.13 (br, 1H), 4.12-4.43 (br, 4H), 3.40-3.60 (br, 5H), 2.23-2.62 (br, 4H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.0, 153.6, 134.8, 128.8, 128.4, 73.5, 68.0, 66.2, 53.7.

d. *Synthesis of mPEG-b-PGC-g-PTX Conjugate*

mPEG-*b*-PGC-*g*-SA (100 mg, 0.011 mmol, 1.0 eq.), PTX (4.9 mg, 0.006 mmol, 0.5 eq.), and DCC (1.2 mg, 0.006 mmol, 0.55 eq.) were added into a round bottom flask. 1 mL DMF was added to dissolve the solid and the reaction was stirred at room temperature overnight. The reaction mixture was filtered to remove the precipitate. The filtrate was added dropwise into diethyl ether, centrifuged, and the upper layer was decanted off and discarded. The solid was re-dissolved in DCM and added dropwise into diethyl ether to precipitate again and to completely remove DMF. The solution was centrifuged and the conjugate was isolated as a white powder. $^1$H NMR (500 MHz, $CDCl_3$): δ 5.75-5.85 (br, 0.32H), 5.50-5.60 (br, 0.31H), 5.30-5.41 (dd, 0.56H), 5.00-5.30 (br, 1H).

e. *Formation of mPEG-b-PGC-g-PTX Nanoparticles*

Nanoparticles were formed using an oil-in-water mini-emulsion sonication method. An aqueous phase was formed by dissolving mPEG-*b*-PGC-*g*-PTX in 10 mM sodium phosphate buffer (pH 7.4). An organic phase was formed by dissolving PGC-OBn, synthesized according to literature procedures[2], in methylene chloride. The aqueous and organic phases were combined and pulse-sonicated for 30 min. The nanoparticle solution was then stirred open to air for 3 hours to evaporate off residual methylene chloride and dialyzed (10 kDa molecular weight cutoff Snakeskin tubing) in 5 mM sodium phosphate buffer for 24 hours.

## 3. Supplemental Figures and Tables

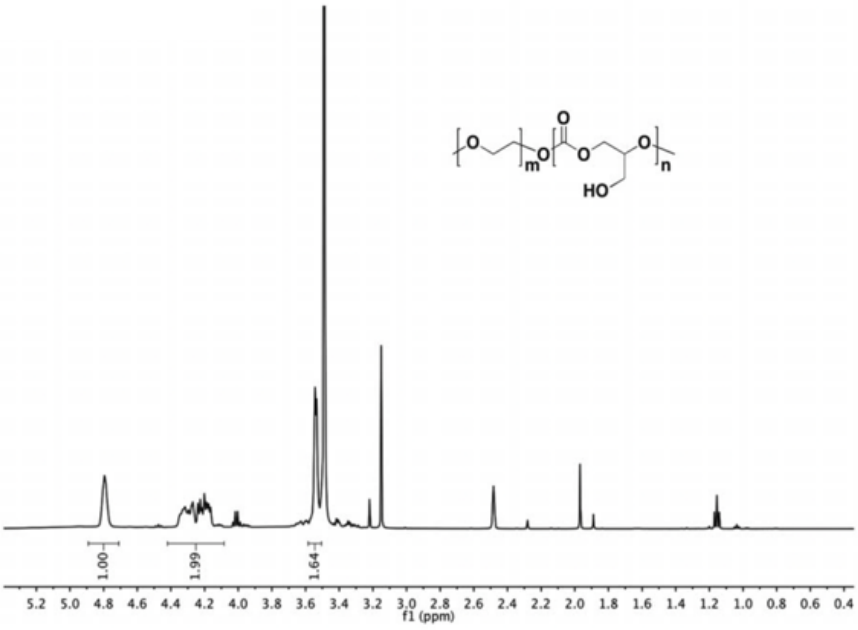


Figure S1. $^{1}$H NMR of mPEG-*b*-PGC in $CDCl_3$.

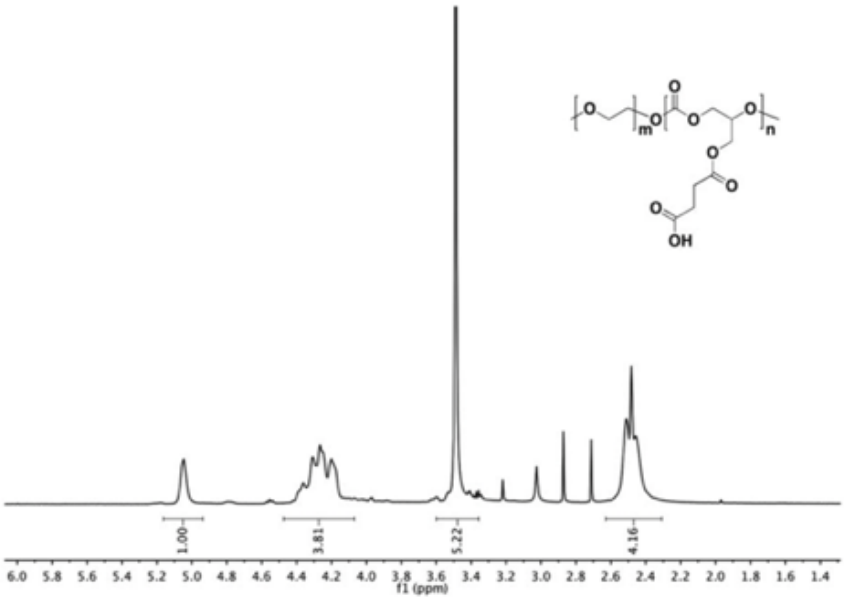


Figure S2. $^{1}$H NMR of mPEG-*b*-PGC-*g*-SA in $CDCl_3$.

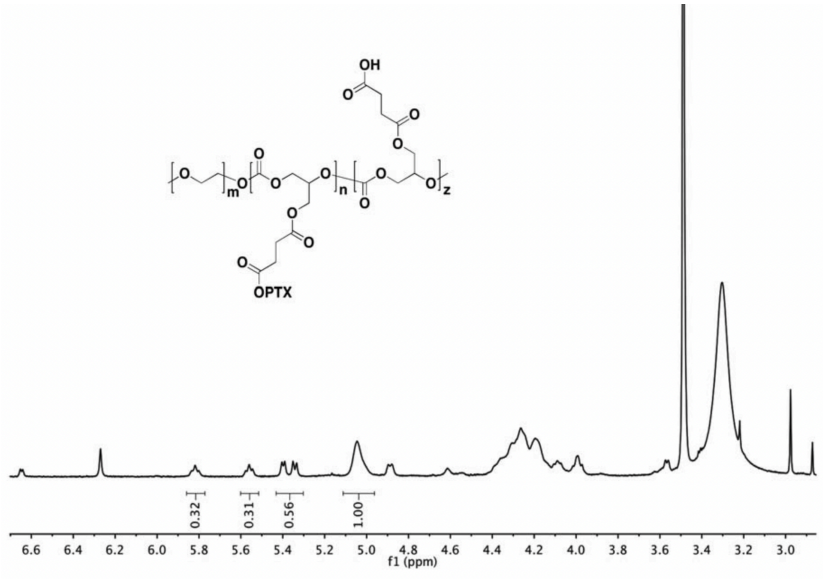


Figure S3. $^{1}$H NMR of mPEG-*b*-PGC-*g*-PTX in $CDCl_3$.

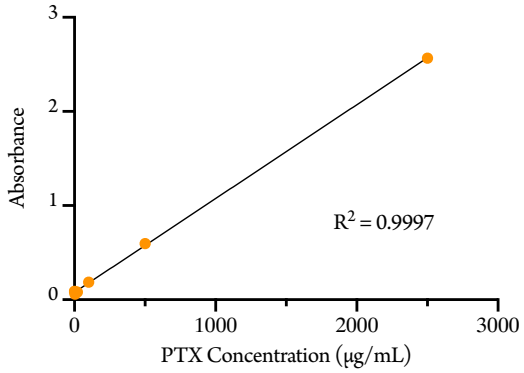


Figure S4. Standard curve for UV-Vis absorbance quantification of paclitaxel.

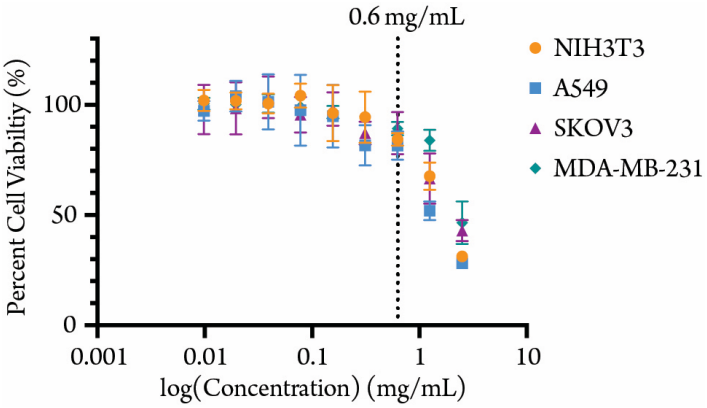


Figure S5. *In vitro* cytotoxicity of mPEG-*b*-PGC-OBn polymer against NIH 3T3, A549, SKOV3, and MDA-MB-231 cell lines.

Table S1. Monomer feed ratios, turnover frequency, and polydispersity for the tetrapolymerization of $CO_2$, mPEG, BGE, and PO.

| $f_{BGE}$ | $F_{BGE}$ | TOF ($h^{-1}$) | Time (min) | *Đ* |
|---|---|---|---|---|
| 0.2 | 0.22 | 288 | 20 | 1.04 |
| 0.3 | 0.32 | 458 | 20 | 1.04 |
| 0.5 | 0.49 | 497 | 30 | 1.03 |
| 0.7 | 0.67 | 539 | 40 | 1.04 |
| 0.8 | 0.81 | 342 | 40 | 1.05 |

## 4. References


1. Ren, W-M.; Liu, Z-W.; Wen, Y-Q.; Zjang, R., Lu, W-B., Mechanistic aspects of the copolymerization of $CO_2$ with epoxides using a thermally stable single-site cobalt(III) catalyst. *Journal of the American Chemical Society* **2009**, *131*(32), 11509-11518.
2. Zhang, H.; Grinstaff, M. W., Synthesis of Atactic and Isotactic Poly(1,2-glycerol carbonate)s: Degradable Polymers for Biomedical and Pharmaceutical Applications. *Journal of the American Chemical Society* **2013,** *135* (18), 6806-6809.