# **Supporting Information**

for

Polyphosphoester-based conjugates as a platform for ultra-high paclitaxel-loaded multifunctional nanoparticles

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#### **Experimental section:**

**Materials**. N,N-dimethylformamide (DMF), ethyl acetate, acetone, diethyl ether, copper(I) bromide, acetone, diethyl ether, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), acetic acid, 3-butyn-1-ol, triethylamine (TEA), *N*,*N*/dicyclohexylcarbodiimide (DCC), 6-bromohexanoic acid, 4-(dimethylamino)pyridine (DMAP), sodium azide, copper(II) acetate monohydrate, sodium ascorbate, *N*,*N*,*N'*,*N''*,*P*-pentamethyldiethylenetriamine (PMDETA), poly(ethylene glycol) methyl ether (average  $M_n \sim 2,000$  Da, PEO), methanol and 5-(iodoacetamido)fluorescein were used as received from Sigma-Aldrich Company (St. Louis, MO). 2-chloro-2-oxo-1,3,2-dioxaphospholane (95%) was used as received from Thermo Fisher Scientific Inc (Pittsburgh, PA). Paclitaxel (PTX) was used as received from Cedarburg Hauser Pharmaceuticals (Denver, CO). Chelex 100 Resin was used as received from Bio-Rad Laboratories (Hercules, CA). Tetrahydrofuran (THF) and dichloromethane (DCM) were dried through columns (J. C. Meyer Solvent Systems, Inc., Laguna Beach, CA). Nanopure water (18 MΩ·cm) was acquired by means of a Milli-Q water filtration system, Millipore Corp. (St. Charles, MO).

**Instrumentation**. <sup>1</sup>H NMR, <sup>31</sup>P NMR and <sup>13</sup>C NMR spectra were recorded on an Inova 300 MHz or Mercury 300 MHz spectrometer interfaced to a UNIX computer using VnmrJ software. Chemical shifts were referenced to the solvent resonance signals.

The DMF gel permeation chromatography (GPC) was conducted on a Waters Chromatography, Inc. (Milford, MA) system equipped with an isocratic pump model 1515, a differential refractometer model 2414, and a four-column set of 5  $\mu$ m Guard (50 × 7.5 mm), Styragel HR 4 5  $\mu$ m DMF (300 × 7.5 mm), Styragel HR 4 5  $\mu$ m DMF (300 × 7.5 mm), and Styragel HR 2 5  $\mu$ m DMF (300 × 7.5 mm). The system was equilibrated at 70°C in pre-filtered DMF containing 0.05 M LiBr, which served as polymer solvent and eluent (flow rate set to 1.00 mL/min). Polymer solutions were prepared at a concentration of *ca*. 3 mg/mL and an injection volume of 200  $\mu$ L was used. Data collection and analysis were performed with Empower 2 v. 6.10.01.00 software (Waters,

Inc.). The system was calibrated with polystyrene standards (Polymer Laboratories, Amherst, MA) ranging from 615 to 442,800 Da.

IR spectra were recorded on an IR Prestige 21 system (Shimadzu Corp.) and analyzed using IRsolution v. 1.40 software.

Ultraviolet-visible spectroscopy (UV-vis) absorption measurements were made using a UV-2550 system (Shimadzu Corp.) equipped with a TMSPC-8 thermoelectric temperature controlling system using quartz cuvettes. Spectra were analyzed by using Tm analysis software module 1,2,1,0 and UV-Probe v. 2.33 software.

Glass transition temperatures ( $T_g$ ) were measured by differential scanning calorimetry on a Mettler-Toledo DSC822<sup>®</sup> (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 10 °C /min. Measurements were analyzed using Mettler-Toledo STARe v. 7.01 software. The  $T_g$  was taken as the midpoint of the inflection tangent, upon the third heating scan. Thermogravimetric analysis was performed under N<sub>2</sub> atmosphere using a Mettler-Toledo model TGA/SDTA851<sup>e</sup>, with a heating rate of 5 °C /min. Measurements were analyzed by using Mettler-Toledo STARe v. 7.01 software.

Transmission electron microscopy (TEM) was conducted on a Hitachi H-7500 microscope, operating at 100 kV. Samples for TEM measurements were prepared as follows: 4 µL of the dilute solution (with a polymer concentration of 0.1 mg/mL) was deposited onto a carbon-coated copper grid, and after 2 min, the excess of the solution was quickly wicked away by a piece of filter paper. The samples were then negatively stained with 1 *wt*% phosphotungstic acid (PTA) aqueous solution. After 1 min, the excess staining solution was quickly wicked away by a piece of filter paper of filter paper and the samples were left to dry under ambient conditions overnight. The average diameter of nanoparticles on TEM grid was obtained by measuring the core domain of 200 sphere particles at different area of TEM specimen and the standard deviation was presented as error.

DLS measurements were conducted using a Delsa Nano C from Beckman Coulter, Inc. (Fullerton, CA) equipped with a laser diode operating at 658 nm. Scattered light was detected at 165° angle and analyzed using a log correlator over 70 accumulations for a

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0.5 mL of sample in a glass size cell (0.9 mL capacity). The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of ca. 10 kcps. The calculation of the particle size distribution and distribution averages was performed using CONTIN particle size distribution analysis routines using Delsa Nano 2.31 software. The peak averages of histograms from intensity, volume and number distributions out of 70 accumulations were reported as the average diameter of the particles. All determinations were repeated 10 times.

The zeta potential values of the nanoparticles were determined by Delsa Nano C particle analyzer (Beckman Coulter. Fullerton, CA) equipped with a 30 mW dual laser diode (658 nm). The zeta potential of the particles in suspension was obtained by measuring the electrophoretic movement of charged particles under an applied electric field. Scattered light was detected at a 30° angle at 25 °C. In each measurement, NaCl solution was added to adjust the sample to 10 mM. The zeta potential was measured at five regions in the flow cell and a weighted mean was calculated. These five measurements were used to correct for electroosmotic flow that was induced in the cell due to the surface charge of the cell wall. All determinations were repeated 5 times.

Inductively coupled plasma-mass spectrometry (ICP-MS) was performed on a 7500ce Agilent, quadrupole mass spectrometer, equipped with an octapole reaction cell for removal of polyatomic interferences, and using 2% HNO<sub>3</sub> as the matrix and TI (III) as internal standard.

#### Synthesis of 6-azidohexanoic acid

In a 100 mL round-bottom flask equipped with a magnetic stirring bar, 6-bromohexanoic acid (3.88 g, 20 mmol) and sodium azide (2.6 g, 40 mmol) were added and dissolved in DMF (20 mL). After being stirred under room temperature for 36 h, the reaction mixture was added 30 mL DCM and then extracted with water (30 mL), brine (30 mL) and saturated NaHCO<sub>3</sub> aqueous solution (30 mL) respectively. The combined organic layers were dried over MgSO<sub>4</sub> and filtered. The filtrate was concentrated in *vacuo*, and the resulting mixture was purified by column chromatography on silica gel using

hexane/EtOAc gradient as eluent and gave 6-azidohexanoic acid as a pale yellow liquid (2.48 g, yield: 79%) <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  1.42 (m, 2H, N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.64 (m, 4H, N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.36 (t, 2H, *J* = 7 Hz, CH<sub>2</sub>CH<sub>2</sub>COOH), 3.27 (t, 2H, *J* = 7 Hz, N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 9.70 (br, 1H, COO*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm):  $\delta$  24.2, 26.1, 28.5, 33.8, 51.2, 178.9. FT-IR (cm<sup>-1</sup>): 3600-3100, 2931, 2092, 1700, 1242, 941. HRMS: calculated [M-H]<sup>-</sup> for C<sub>6</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub>: 156.0773, found: 156.0777.

## Synthesis of azido-PTX, 4

In a 25-mL round flask equipped with a magnetic stirring bar, 6-azidohexanoic acid (204 mg; 1.3 mmol) and PTX (920 mg; 1.08 mmol) were added and dissolved in dichloromethane (10 mL). After stirring at r.t. for 1 h, DCC (268 mg; 1.30 mmol) and DMAP (27 mg; 0.23 mmol) were added. The mixture was heated to reflux for 3 days, filtrated, concentrated, and then separated by flash chromatography using silica gel with hexane and ethyl acetate as eluent in gradient (until hexane/ethyl acetate = 50/50, v/v) and gave the targeted compound as a pale yellow solid (840 mg, Yield: 78.3 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm): δ 1.13 (s, 3H, (C-16)-CH<sub>3</sub>), 1.25-1.40 (m, 5H, (C-17)-CH<sub>3</sub> and N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.50-1.70 (m, 7H, (C-19)-CH<sub>3</sub> and N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.82-1.95 (m, 5H, (C-6)-CH, 1-OH and (C-18)-CH<sub>3</sub>), 2.15 (m, 1H, (C-14)-CH), 2.23 (s, 3H, 10-OAc), 2.34-2.62 (m, 8H, 4-OAc, (C-6)-CH, (C-14)-CH, 7-OH and CH<sub>2</sub>CH<sub>2</sub>COO(PTX)), 3.21 (t, 2H, J = 7 Hz, N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.81 (d, 1H, J = 7 Hz, (C-3)-CH), 4.20 (d, 1H, J = 8 Hz, (C-20)-CH), 4.31 (d, 1H, J = 8 Hz, (C-20)-CH), 4.46 (m, 1H, (C-7)-CH), 4.94 (dd, 1H, J = 9 Hz, J = 2 Hz, (C-5)-CH), 5.51 (d, 1H, J = 3 Hz, (C-2')-CH), 5.68 (d, 1H, J = 7 Hz, (C-2)-CH), 5.95 (dd, 1H, J = 9 Hz, J = 3 Hz, (C-3')-CH), 6.23-6.29 (m, 2H, (C-10)-CH and (C-13)-CH), 6.85 (d, 1H, J = 9 Hz, 3'-NH), 7.34-7.64 (m, 11H, PhH), 7.73 (d, 2H, J = 8 Hz, PhH), 8.14 (d, 2H, J = 8 Hz, PhH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm):  $\delta$  9.6, 14.9, 20.9, 22.2, 22.7, 24.2, 26.0, 28.4, 33.5, 35.6, 43.2, 45.6, 51.1, 52.8, 58.5, 71.8, 72.2, 73.9, 75.1, 75.6, 76.5, 79.2, 81.1, 84.5, 126.5, 127.1, 128.5, 128.8, 129.1, 129.2, 130.3, 132.1, 132.8, 133.7, 137.0, 142.8, 167.1, 168.1, 169.8, 171.3, 172.5, 203.9. FT-IR (cm<sup>-1</sup>): 2939, 2098, 1782, 1659, 1528, 1450, 1365, 1234, 1065, 980, 902, 794. HRMS: calculated [M+Li]<sup>+</sup> for C<sub>53</sub>H<sub>60</sub>N<sub>4</sub>O<sub>15</sub>Li:

999.4215, found: 999.4187.

## Synthesis of butynyl phospholane (BYP, 2) Monomer

To a stirred solution of 3-butyn-1-ol (7.40 g, 106 mmol) and triethylamine (11.7 g, 116 mmol) in 200 mL of anhydrous THF at 0 °C were dropwisely added a solution of COP (15.1 g, 106 mmol) in 50 mL of anhydrous THF, and the reaction mixture was allowed to stir for 12 h. After complete conversion of COP, as confirmed by TLC, the reaction mixture was filtered and the filtrate was concentrated. The concentrated filtrate was distilled under reduced pressure to obtain a faint yellow and viscous liquid (121-124 °C, 0.4 mmHg, 12.1 g, Yield: 65 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  2.05 (s, 2H, POCH<sub>2</sub>CH<sub>2</sub>C=C*H*), 2.62 (t, *J* = 6.0 Hz, 2H, POCH<sub>2</sub>CH<sub>2</sub>C), 4.27-4.20 (m, 2H, POCH<sub>2</sub>CH<sub>2</sub>C), 4.49-4.37 (m, 4H, POCH<sub>2</sub>CH<sub>2</sub>OP). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm):  $\delta$  20.7, 66.2, 70.6, 79.1. <sup>31</sup>P NMR (CDCl<sub>3</sub>, ppm):  $\delta$  17.32. HRMS: calculated [M+H]<sup>+</sup> for C<sub>6</sub>H<sub>10</sub>O<sub>4</sub>P: 177.0317, found: 177.0308. IR (cm<sup>-1</sup>): 3350 - 3175, 3050-2850, 1474, 1280, 1011, 926, 841, 748.

#### Synthesis of PEO-*b*-PBYP, 3

A solution of BYP (0.528 g, 3.0 mmol) and PEO (0.200 g, 0.1 mmol) in anhydrous dichloromethane (0.7 mL) was transferred into a flame-dried 5-mL shell vial equipped with a rubber septum and a stir bar. At 25 °C, a solution of DBU (0.023 g, 0.15 mmol) in anhydrous dichloromethane (0.1 mL) was injected into the vial *via* syringe, while being maintained under a nitrogen gas atmosphere. After being stirred for 4 min, the reaction vial was unstoppered and a solution of acetic acid (excess) in dichloromethane was added *via* pipet into the reaction mixture to quench the reaction. After the reaction was quenched, the conversion was monitored by <sup>31</sup>P NMR and reached 99%. The PEO-*b*-PBYP, **3** was purified by precipitation from dichloromethane into diethyl ether (3x), and was then dried under vacuum, to give an average yield of 80%. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  2.18-2.02 (br, POCH<sub>2</sub>CH<sub>2</sub>C=CH), 2.66-2.54 (br, POCH<sub>2</sub>CH<sub>2</sub>C=CH), 3.36 (s, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.87-3.49 (br, CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>), 4.43-3.87 (br, POCH<sub>2</sub>CH<sub>2</sub>OP, POCH<sub>2</sub>CH<sub>2</sub>C). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm):  $\delta$  20.5, 65.8-66.3, 69.9, 70.8, 79.5. <sup>31</sup>P NMR

(CDCl<sub>3</sub>, ppm):  $\delta$  -1.73. GPC:  $M_n$  = 12200 g/mol, PDI = 1.17. DSC:  $T_g$  = - 34.2 °C,  $T_m$  = - 37.1 °C. TGA in N<sub>2</sub>: 50–270 °C, 37% mass loss; 270–340 °C, 33% mass loss, 30 % mass remaining above 600 °C. IR (cm<sup>-1</sup>): 3700-3100, 3100-2750, 1643, 1428, 1353, 966, 810.

# Synthesis of PEO-b-(PBPY-g-PTX), 5

In a 10-mL Schlenk flask equipped with a magnetic stirring bar, PEO<sub>2k</sub>-b-PBYP<sub>30</sub>, 3 (73 mg; 0.01 mmol), azido PTX, 4 (150 mg; 0.15 mmol), CuBr (21.6 mg; 0.15 mmol) and PMDETA (26.7 mg; 0.15 mmol) were added and dissolved in DMF (1 mL). The reaction mixture was deoxygenated by freeze-pump-thaw (4x) and then placed in a preheated reaction bath at 40 °C. After 24 h, the resulting mixture was precipitated from acetone into ethyl ether (3x) to remove unreacted azide-functionalized PTX. The crude product was collected and dissolved in 10 mL acetone formed clear solution. The acetone solution was transferred to dialysis tubing (MWCO: 8 kDa) and dialyzed against nanopure water with the existence of Chelex 100 resin (100-200 mesh) for 2 days, to remove copper ion and trigger self-assembly. A bluish-colored micelle solution was obtained and then passed through a 450 nm polypropylene filter to get rid of dust and large aggregates. The micelle solution was lyophilized to give a faint yellow powder with a yield of 90%. The amount of residual copper, analyzed by ICP-MS, was about 60 ppm. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  1.14-2.80 (broad multiple peaks, protons from PTX and CH<sub>2</sub>C=CH), 2.91 (br, CH<sub>2</sub>C≡C*H*), 3.05 (br, N<sub>3</sub>C*H*<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.37 (s, 3H, PEO-OC*H*<sub>3</sub>), 3.64 (br, C*H*<sub>2</sub> from PEO), 3.75 (br, CH from PTX(C-3)-CH), 3.82-4.60 (br, POCH<sub>2</sub>CH<sub>2</sub> from PPE backbone and POCH<sub>2</sub>CH<sub>2</sub>CCH from side chain), 4.90 (br, CH from PTX(C-5)-CH), 5.50 (br, CH from PTX(C-2')-CH), 5.62 (br, CH from PTX(C-2)-CH), 5.92 (br, CH from PTX(C-3')-CH), 6.19 (br, CH from PTX(C-13)-CH), 6.32 (br, CH from PTX(C-10)-CH), 7.28-7.68 (br, PhH from PTX), 7.77 (br, PhH from PTX), 8.13 (br, PhH from PTX). <sup>31</sup>P NMR (CDCl<sub>3</sub>, ppm): δ -1.72. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, 25 °C, ppm): δ 9.7, 14.8, 20.9, 22.8, 23.9, 25.7, 26.7, 29.8, 33.3, 35.3-35.9 (multiple overlapping br), 43.2, 45.8, 49.9, 53.4, 58.4, 66.3-67.1 (multiple overlapping br), 70.5, 71.9, 74.0, 75.0, 78.9, 81.0, 84.4,

126.5-129.3 (multiple overlapping br), 130.2, 132.3, 132.8, 133.7, 137.0, 142.3, 166.9, 167.3, 170.0, 171.0, 172.4, 203.7. GPC:  $M_n = 18900$  g/mol, PDI = 1.12. FT-IR (cm<sup>-1</sup>): 3550-3100, 2940, 1728, 1643, 1450, 1366, 1242, 1072, 1026, 980, 802. TGA in N<sub>2</sub>: 200–420 °C, 60% mass loss; 40 % mass remaining above 420 °C.

## Solubility test of PEO-b-(PBPY-g-PTX), 5

Certain amount of powder like product PEO-*b*-(PBPY-*g*-PTX) was resuspended in certain amount of nanopure water and sonicated for 3min to obtain micelles. The insoluble drug conjugates were collected by ultracentrifuge at 7000 rpm for 5 min, and weighted after dried by the vacuum to calculate the highest concentration of the formulation.

## Synthesis of 5-(azidoacetamido)-fluorescein, 6

In a 10 mL round-bottom flask equipped with a magnetic stirring bar, 5-(iodoacetamido)fluorescein (100 mg, 0.19 mmol) and sodium azide (35.0 mg, 0.53 mmol) were added and suspended in DMF (5 mL). After being stirred under room temperature for 24 h, the DMF was removed by vacuum pump. The mixture was suspended into 30 mL 0.1 M HCl solution. The aqueous phase was extracted with 30 mL ethyl acetate for 4 times. The combined organic layers were dried over MgSO<sub>4</sub> and filtered. The filtrate was concentrated in *vacuo* and gave a yellow solid as the crude product (84 mg, yield: 98%). The product was dissolved in 8 mL DMF and stored at -20 °C. <sup>1</sup>H NMR (DMSO-d6, ppm):  $\delta$  4.28 (s, 2H, CH<sub>2</sub>N<sub>3</sub>), 6.70 (m, 4H, Ar-*H*), 6.82 (d, m, 2H, Ar-*H*), 7.33 (m, 2H, Ar-H), 8.05 (m, 1H, Ar-H), 8.52 (s, 1H, CON*H*), 10.67 (br, 1H, Ar-O*H*), 11.22 (s, 1H, COO*H*). HRMS: calculated [M+H]<sup>+</sup> for C<sub>22</sub>H<sub>15</sub>N<sub>4</sub>O<sub>6</sub>: 431.0991, found: 431.0620. UV-vis: (H<sub>2</sub>O)  $\lambda_{max}$  = 491.0 nm. Fluorescence: (H<sub>2</sub>O, pH=8.4)  $\lambda_{em}$  = 522.0 nm.

# Synthesis of Fluorescein labeled PEO-b-( PBPY-g-PTX), 7

To an aqueous solution of the PEO-*b*-( PBPY-*g*-PTX) (1.1 mL, 4 mL) was added a solution of 5-(azidoacetamido)-fluorescein (10 mg/mL, 9  $\mu$ L, 1 dye per polymer) in DMF, a solution of copper(II) acetate monohydrate (21 mM, 10  $\mu$ L) and solution of sodium ascorbat (41  $_{S8}$ 

mM, 10 µL). The reaction mixture was allowed to stir for 1 days and was then transferred to presoaked dialysis tubing (MWCO *ca.* 6000-8000 Da) and extensively dialyzed against nanopure water with the existence of Chelex 100 resin (100-200 mesh) for 3 days to remove excess dye and copper catalyst.  $D_h$  (DLS, intensity) = 128 ± 93 nm;  $D_h$  (DLS, volume) = 40 ± 23 nm;  $D_h$  (DLS, number) = 28 ± 8 nm. UV-vis: (H<sub>2</sub>O)  $\lambda_{max}$  = 489.0 nm. Fluorescence: (H<sub>2</sub>O, pH=8.4)  $\lambda_{em}$  = 523.0 nm.

# Synthesis of the Control Polymer, 8

In a 10-mL Schlenk flask equipped with a magnetic stirring bar, PEO<sub>2k</sub>-b-PBYP<sub>30</sub>, **3** (100 mg; 0.013 mmol), 6-azidohexanoic acid (32.4 mg; 0.20 mmol), CuBr (28.4 mg; 0.20 mmol) and PMDETA (35.2 mg; 0.20 mmol) were added and dissolved in DMF (4 mL). The reaction mixture was deoxygenated by freeze-pump-thaw (4x) and then placed in a preheated reaction bath at 35 °C. After 24 h, the resulting mixture was transferred to dialysis tubing (MWCO: 3500 Da) and dialyzed against nanopure water with the existence of Chelex 100 resin (100-200 mesh) for 3 days, to remove copper ion and unreacted 6-azidohexanoic acid. The solution was lyophilized to give a faint yellow solid (102 mg, yield: 77%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  1.24-1.89 (br, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH2COOH), 2.24-2.02 (br, POCH<sub>2</sub>CH<sub>2</sub>C=CH), 2.40-2.52 (br, CH<sub>2</sub>CH<sub>2</sub>COOH), 2.69-2.54 (br, POCH<sub>2</sub>CH<sub>2</sub>C=CH), 3.09 (br, N<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.38 (s, CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.85-3.52 (br, CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>), 4.82-3.85 (br, POCH<sub>2</sub>CH<sub>2</sub>OP, POCH<sub>2</sub>CH<sub>2</sub>C), 7.76 (s, HC(=C)N). <sup>31</sup>P NMR (CDCl<sub>3</sub>, ppm): δ -1.71. <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm):  $\delta$  20.6, 24.3, 26.4, 28.9, 34.1, 59.1, 66.5-65.8, 70.6, 79.5, 165.0. FT-IR (cm<sup>-1</sup>): 3600-3200, 3150 – 2850, 1700, 1454, 1255, 842. DSC:  $T_{g}$  = - 37.5 °C,  $T_{m}$  = - 43.7 °C. TGA in N<sub>2</sub>: 100–250 °C, 9% mass loss; 250-420 °C, 46% mass loss, 45 % mass remaining above 420 °C.

# Cytotoxicity assays:

Human ovarian adenocarcinoma cells (OVCAR-3) (5x10<sup>3</sup> cells/well) and RAW 264.7 mouse macrophages (2x10<sup>4</sup> cells/well) were plated in 96-well plate in RPMI-1640 medium and Dulbecco's Modified Eagle's Medium (DMEM) (20% and 10% fetal bovine serum, for the OVCAR-3 and RAW 264.7, respectively and 1% penicillin/streptomycin). Cells were S9

incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 24h to adhere. Then, the medium was replaced with a fresh medium 1-h prior to the addition of the various formulations at concentrations ranged from  $1 \times 10^{-4}$  to 60  $\mu$ M of paclitaxel. The paclitaxel conjugate was prepared as described previously, and the Taxol<sup>®</sup>-mimicking formulation was prepared in similar composition to Taxol<sup>®</sup> (*i.e.* Cremophor-EL and ethanol, 1:1 v/v). For each well, 20  $\mu$ L of every formulation was added to 100  $\mu$ L of the medium. The cells were incubated with the formulations for 72h and washed once with phosphate-buffered saline (PBS) and 100 µL of the complete medium was added to the cells. 20 µL of the MTS combined reagent was added to each well (Cell Titer 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega Co., Madison, WI). The cells were incubated with the reagent for 3 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> protected from light. Absorbance was measured at 490 nm using SpectraMax M5 (Molecular Devices Co., Sunnyvale, CA). The cell viability was calculated based on the relative absorbance to the control untreated cells. The IC<sub>50</sub> values were calculated using GraphPad Prism four-parameter fit, considering the 0% and 100% viabilities correspond to the medium control (no cells) and cells-treated with PBS, respectively.

# Laser Scanning Confocal Microscopy (LSCM):

RAW 264.7 and OVCAR-3  $(1\times10^5 \text{ cells/well})$  cells were plated in six-well glass-bottom plates (MatTek Co., Ashland, MA) in DMEM and RPMI-1640 medium, respectively. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 24h to adhere. Then, the medium was replaced with a fresh medium 1-h prior to the addition of the fluorescein-labeled nanoparticles (final paclitaxel concentrations of 0.5  $\mu$ M for OVCAR-3 and 3 or 15  $\mu$ M for RAW 264.7). The cells were incubated with the formulation for 5h and washed extensively with PBS. Then, DRAQ-5 (Biostatus Ltd., Shepshed, Leicestershire, UK) was utilized to stain the nucleus (30-min incubation, followed by extensive washing with PBS). The cells were then fixed with 1% formaldehyde for 20 minutes, washed once with PBS. The cells were then stored in 1 mL PBS in the refrigerator. The cellular uptake of the nanoparticles was investigated by LSCM (LSM 510, Zeiss, Jena, Germany).

The images were collected under the same conditions (*e.g.* laser power and detector gain) for consistency, and  $\lambda_{\text{excitation}}$  and  $\lambda_{\text{emission}}$  of 488 and 633 nm were utilized for the fluorescein and DRAQ-5, respectively.



Figure S1. Optimization of click reaction with three different feed ratios of azido-PTX to alkyne group on PEO-*b*-PBYP. Three resulting polymers with feed ratios (20%, 50% and 100% respectively) were plotted in the figure as a function of conjugation efficiency (left), PTX loading capacity (left), polymer solubility in water (right) and PTX solubility in water (right).



Figure S2. Comparison of the HPLC spectra of **5** (top) and free PTX (bottom) confirmed the complete removal of free PTX by precipitation after the click reaction. The retention time of free PTX was about 9.15 min, while that of **5** was about 2.25 min.



Figure S3. DLS a) and GPC b) profiles of PEO-*b*-(PBYP-*g*-PTX) after kept 3 month in -20 °C.



Figure S4. <sup>31</sup>P NMR spectra of PEG-*b*-PBYP **3** as a function of time at different pH. (a). pH = 1; (b). pH = 7; (c). pH = 9.5; (d). pH = 12. (e). Polyphosphoester and oligo-phosphoester (-1.5 to -3.0 ppm) percentage in degradation mixture during the hydrolytic degradation.



Figure S5. The synthetic route of the control polymer, 8.



Figure S6. The synthetic route of fluorescein labeled PEO-*b*-(PBYP-*g*-PTX), **7**.



Figure S7. DLS results of **7**,  $D_h$  (intensity) = 128 ± 93 nm;  $D_h$  (volume) = 40 ± 23 nm;  $D_h$  (number) = 28 ± 8 nm.



Figure S8. Laser scanning confocal microscopy analysis of the mouse macrophages (A and B) and OVCAR-3 (C and D) that either untreated (A and C) or treated with fluorescein-labeled nanoparticles (0.5  $\mu$ M and 3  $\mu$ M for B and D, respectively). The nucleus were stained with DRAQ5 nuclear stain (blue panel), whereas the fluorescein appears in green (no uptake was observed at the tested concentrations). The transmitted light-images and merged images are also indicated. The changes in the nuclear morphology due to the treatment with the nanoparticles are demonstrated by the red arrows.