Supporting Information for:

Y Shaped mPEG-PLA Cabazitaxel Conjugates: Well-Controlled Synthesis by Organocatalytic Approach and Self-Assembly into Interface Drug-Loaded Core-Corona Nanoparticles

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Materials and Methods

Nuclear Magnetic Resonance (NMR): NMR spectra were recorded at room temperature on Bruker Avance 300 MHz, Bruker Avance 400 MHz and Bruker Avance 500 MHz devices equipped with a cryoprobe. The chemical shifts δ in ^{1}H and ^{13}C are reported in ppm with respect to the residual solvent and the chemical shifts δ in ^{29}Si are reported in ppm with respect to Me_4Si as external standard. The coupling constants *J* are given in hertz. The following abbreviations have been employed to describe the signals: s (singlet), br (broad), d (doublet), t (triplet), q (quartet) and m (multiplet).

Steric exclusion chromatography (SEC): the number-average molar masses M_n, the weight-average molar masses M_w and the polydispersity index (M_w/M_n) were measured by steric exclusion chromatography (SEC) at 35 °C with a triple detection line composed of an Alliance Waters e2695, of a MALS miniDAWN (Wyatt) light scattering detector, of a Viscostar-II (Wyatt) viscometer and of a Waters 2414 refractometer. THF is used as eluent at a flow rate of 1.0 mL/min. A Styragel (WAT054405) precolumn and two Shodex (KF-802.5 and KF-804) columns are used. The calibrations are carried out with polystyrene standards (400-100 000 g/mol). The samples are prepared in the following way: the product to be analyzed (10 to 20 mg) is dissolved in 1 mL of THF containing toluene as marker. The solution is subsequently filtered using a 0.45 μm filter.

Mass spectrometry: chemical ionization (DCI) mass spectra were recorded on a Thermo Fisher Scientific DSQ spectrometer.

MALDI-TOF MS analyses: MALDI-TOF-MS analyses were performed on a MALDI MicroMX from Waters equipped with a 337 nm nitrogen laser. An accelerating voltage of 20 kV was applied. Mass spectra of 1000 shots were accumulated. The polymer sample was dissolved in CH_2Cl_2 at a concentration of 1 mg/mL. The cationization agent used was NaI dissolved in MeOH at a concentration of 10 mg/mL. The matrix used was dithranol and was dissolved in CH_2Cl_2 at a concentration of 10 mg/mL. Solutions of matrix, salt, and polymer were mixed in a volume ratio of 3:1:1 respectively. The mixed solution was hand-spotted on a stainless steel MALDI target and left to dry. The spectrum was recorded in the reflectron mode. Baseline corrections and data analyses were performed using MassLynx version 4.1 and Polymerix Software, Sierra Analytics, Version 2.0.0.

UPLC conditions: UPLC analyses were performed on a UPLC chain equipped with a pump, an automatic injector and an UV PDA detector (Acquity UPLC, Waters). The conditions employed were as follows:

Column: Acquity UPLC BEH C18 1.7 µm 2.1x50 mm

Flux: 0.3 mL/min; column temperature was 35 °C

Ultraviolet (UV) dual detection mode at 227 nm and 275 nm

Gradient mobile phase: 0.1% Trifluoroacetic acid (TFA)/Water and 0.1% TFA/CH_3CN

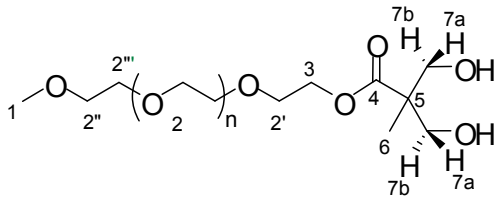
Retention time for cabazitaxel = 0.97 min

Experience duration: 5 min

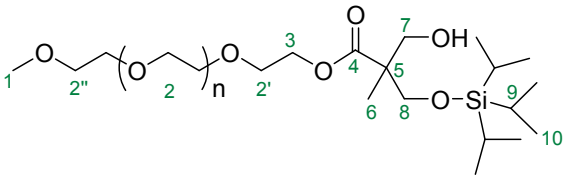
Time(min)	Flux (mL/min)	%A (0.1% TFA/Water)	%B (0.1% TFA/CH_3CN)
0	0.3	30	70
1	0.3	30	70
2	0.3	0	100
4	0.3	0	100
4.2	03	30	70

Elemental Analyses: Microanalyses (elementary and traces) C, H, N, Pd, S and F were performed by the Central Analytic Center (SCA, CNRS-Solaize).

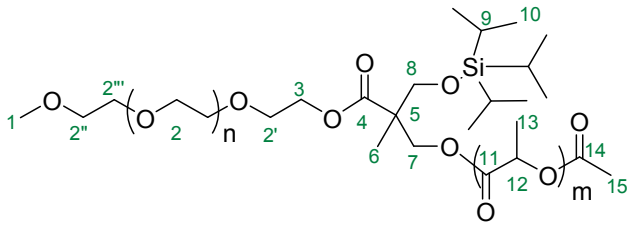
Atom labeling for NMR assignments:



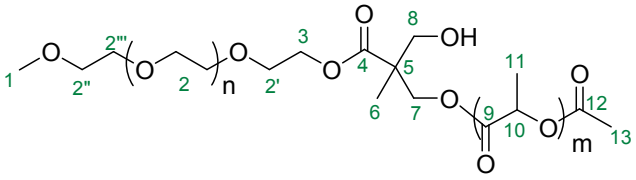
mPEG diol 1



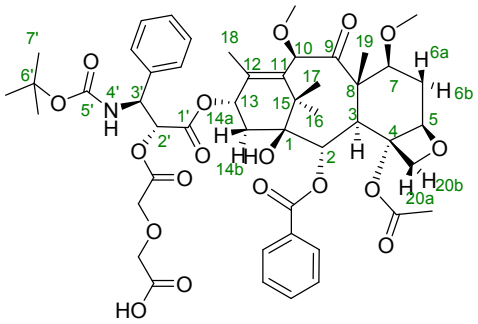
Monoprotected mPEG diol 2



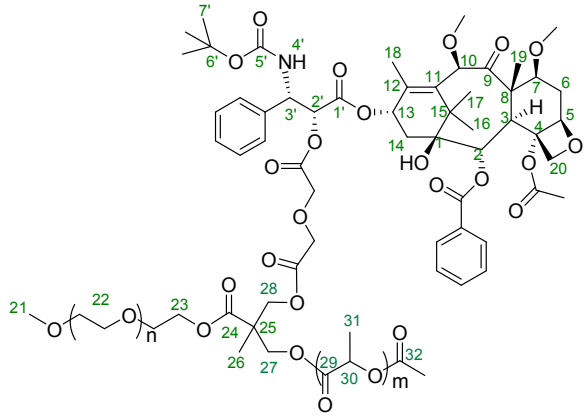
Protected Y-shaped mPEG-PLA copolymer 3.



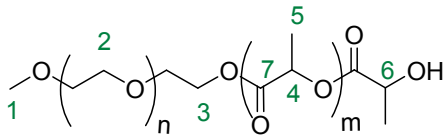
Deprotected Y-shaped mPEG-PLA copolymer 4.



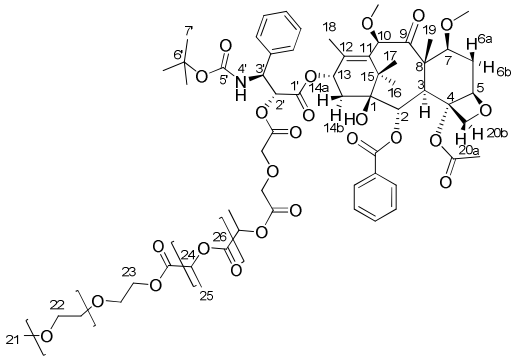
Diglycolyl-Cabazitaxel.



Y-Shaped mPEG-PLA / Cabazitaxel conjugate 5



Linear copolymer mPEG-PLA-OH 6.



Linear mPEG-PLA-diglycolyl / Cabazitaxel conjugate 7.

Figure S1. ^{1}H NMR spectrum of monoprotected mPEG diol **2** ($CDCl_3$, 500 MHz, 298K)

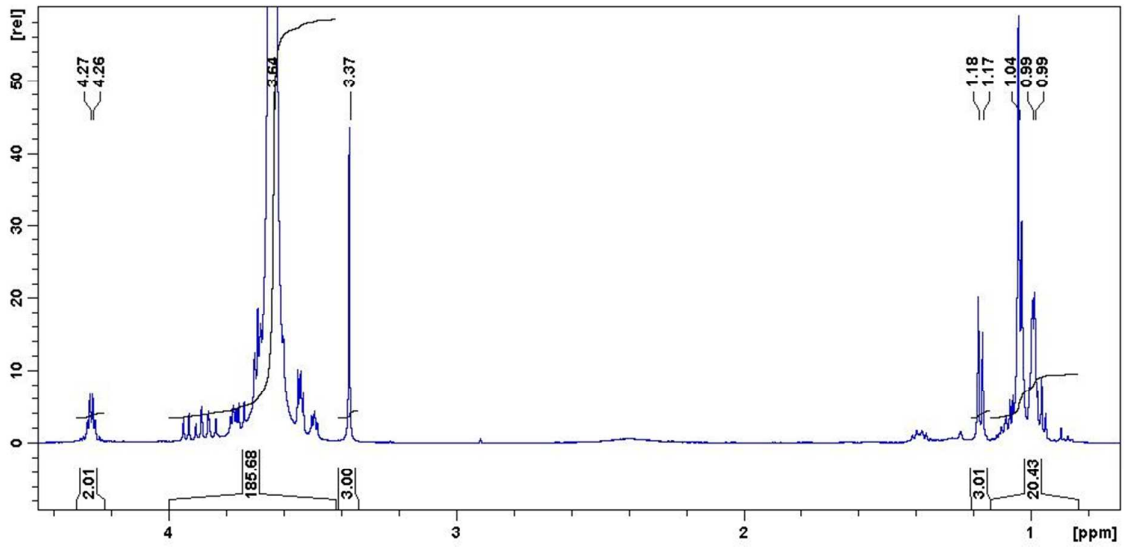


Figure S2. ^{13}C NMR spectrum of monoprotected mPEG diol **2** ($CDCl_3$, 125.7 MHz, 298K):

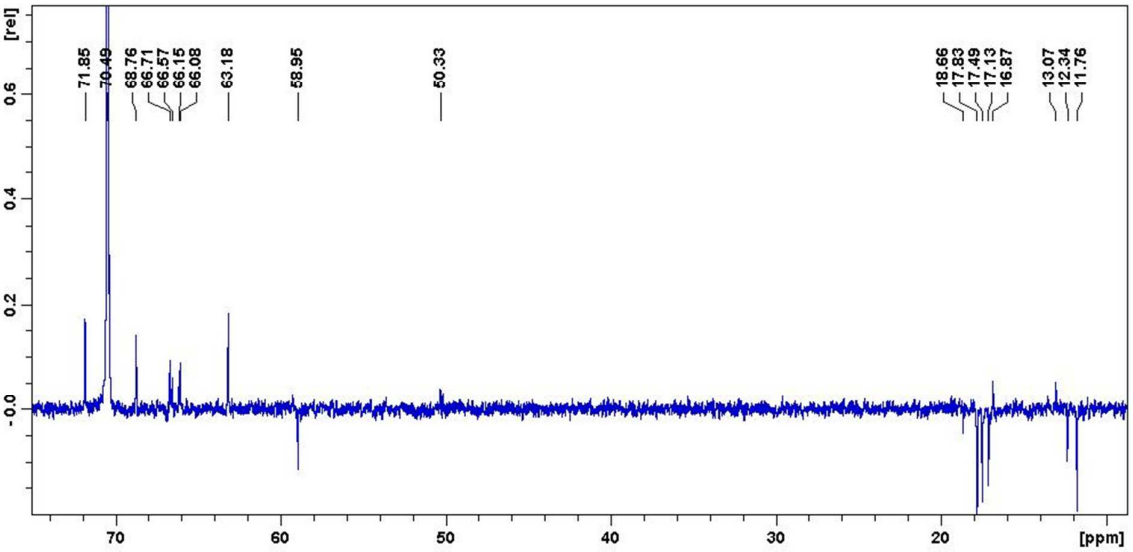


Figure S3. ^{1}H NMR spectrum of mPEG-PLA copolymer **3** ($CDCl_3$, 500 MHz, 298 K)

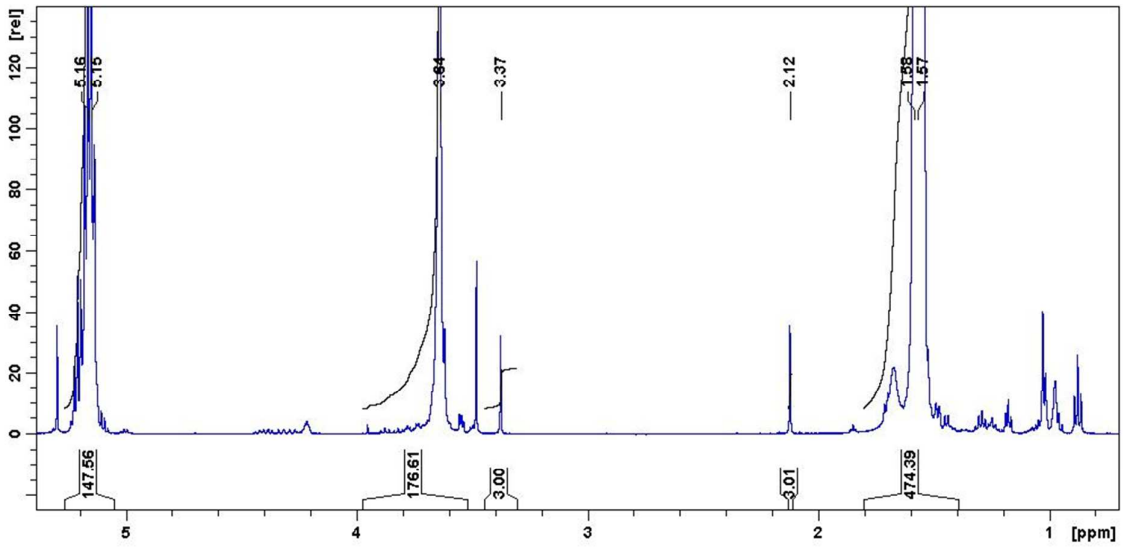


Figure S4. ^{1}H NMR spectrum of the Y-shaped mPEG-PLA / cabazitaxel conjugate **5** ($CDCl_3$, 500 MHz, 298 K)

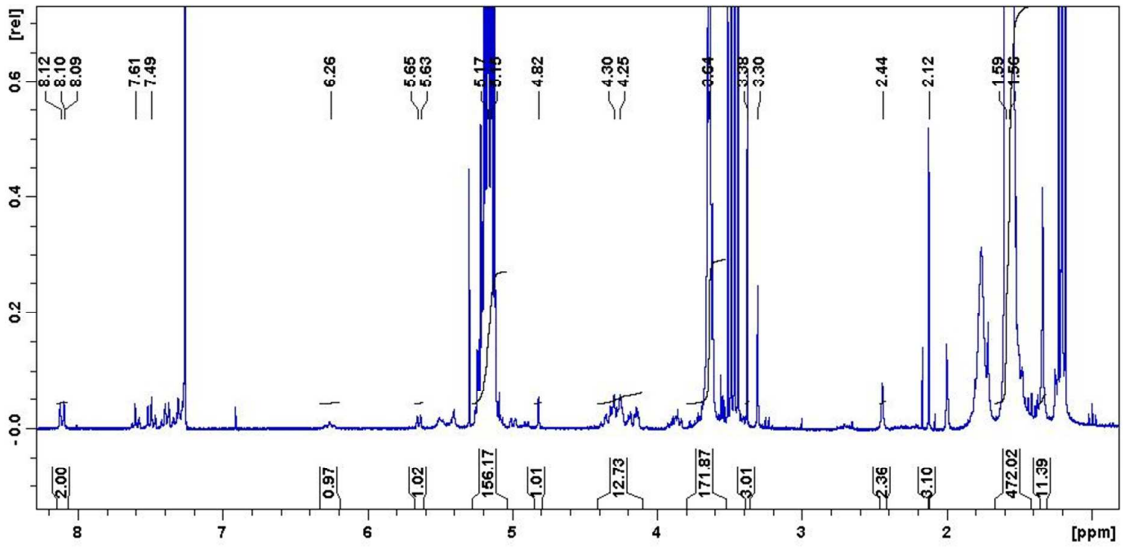


Figure S5. ^{1}H NMR of the linear-shaped copolymer mPEG-PLA-OH **6** ($CDCl_3$, 500 MHz, 298K)

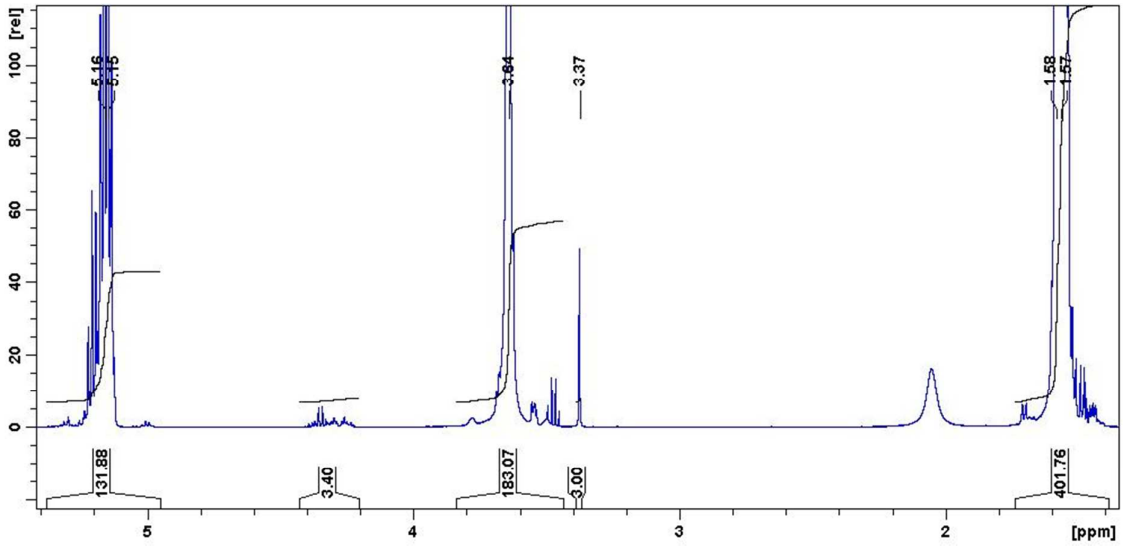


Figure S6. ^{1}H NMR of linear-shaped mPEG-PLA / cabazitaxel conjugate **7** ($CDCl_3$, 500 MHz, 298 K)

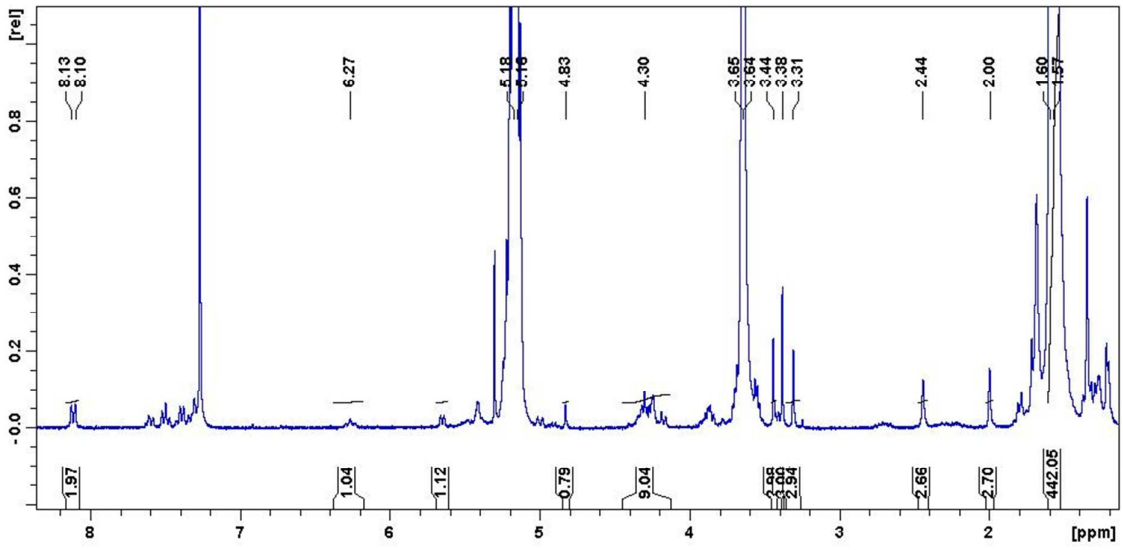
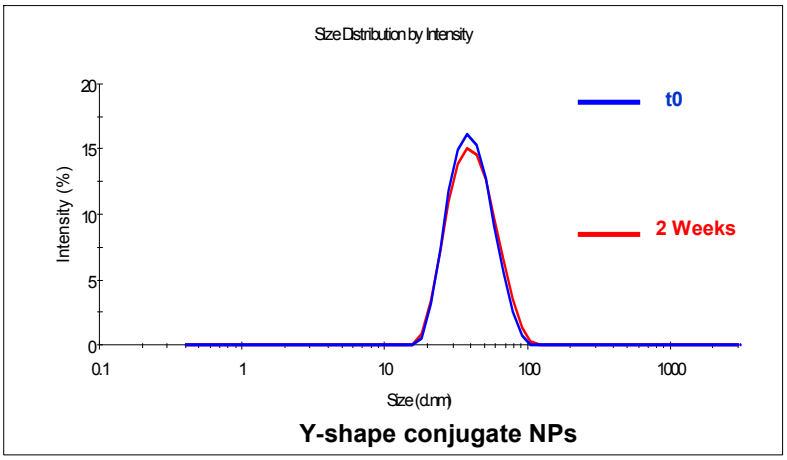
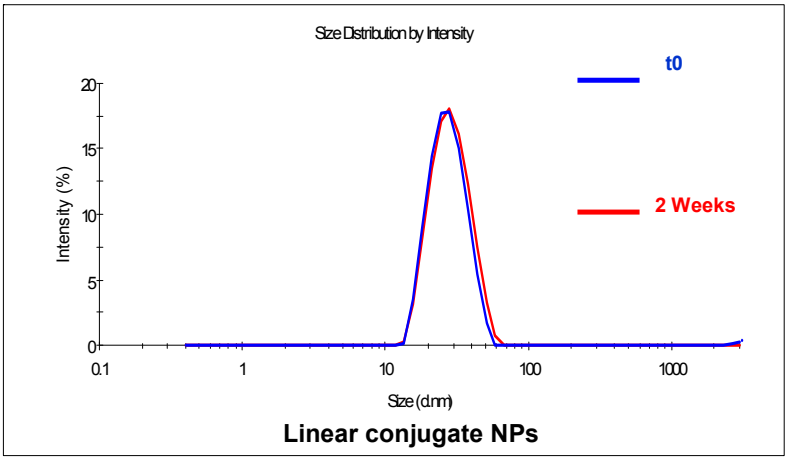


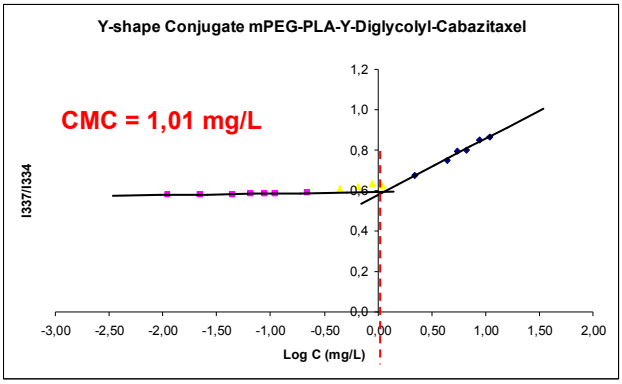
Figure S7. DLS traces of Y shaped and linear mPEG-PLA Cabazitaxel nanoparticles at t= 0 and after 2 weeks.

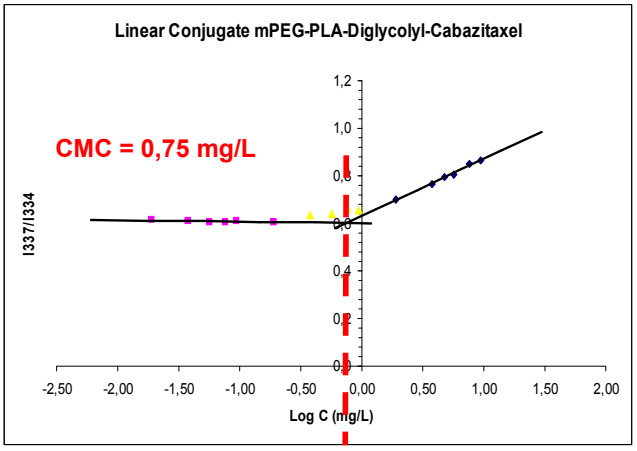




Determination of the critical micelle concentration (CMC) of Y shaped and linear mPEG-PLA Cabazitaxel conjugates. CMC of Y shaped and linear mPEG-PLA Cabazitaxel conjugates were determined using Pyrene as an extrinsic probe. Serial solutions with fixed Pyrene concentration of 6.15×10^{-7}M and various concentrations of polymer conjugates comprised between 1×10^{-2} and 10 mg/mL were prepared. Fluorescence spectra were obtained at 20°C. Fluorescence measurements were taken at an excitation wavelength of 400 nm and the emission monitored from 300 to 350 nm.

Figure S8. CMC Determination of Y shaped and linear mPEG-PLA Cabazitaxel conjugates. Intensity of Pyrene versus concentrations of conjugates





In vitro release studies of Cabazitaxel conjugates nanoparticles formulations. The *in vitro* release kinetics of free Cabazitaxel from nanoparticles of copolymer conjugates **5** and **7** have been assessed in plasma (buffered previously with a 500 mM phosphate buffer to a final concentration of 10 mM in plasma) obtained from Sprague Dawley rat, using a high performance liquid chromatography (HPLC) technique. To the vials containing plasma aliquots, standard cabazitaxel or cabazitaxel conjugate nanoparticles **5** and **7** (1 mg/mL) were added using a micropipette to achieve a final volume of 800 µL. The vials were then placed at 37 °C on an agitator (agitation speed 250 rpm). Sample analysis was carried out at 0 h, 1 h, 2 h, 4 h, 16 h and 24 hours. At each time interval, 100 µL of sample was collected into a vial containing 0.3 mL of acetonitrile:water 85:15 v/v, and agitated for 5 min to allow the precipitation of proteins and the extraction of free cabazitaxel. The contents were then subjected to centrifugation at 10,000 rpm for 10 min, and the clear supernatant was collected and analyzed by HPLC.

Free cabazitaxel recovery controls in spiked plasma were initially performed at 10µg/mL and 50µg/mL concentrations, and cabazitaxel was recovered with deviation of maximum 7% (3 distinct experiments carried out at 10µg/mL). Free diglycolyl-cabazitaxel controls in spiked plasma were also performed, at 10µg/mL and the total amount of cabazitaxel was recovered with a deviation of 5%, but it must be pointed out that partial hydrolysis of diglycolyl-cabazitaxel into cabazitaxel was always observed (about 15% to 30% after 1h sample incubation).

HPLC conditions employed were as follows:

Column : 150 mm Zorbax SB phenyl 3.5 µm

Flux : 1 mL/min ; column temperature was 30 °C

Ultraviolet (UV) dual detection mode at 230 nm (principal used for titration) and 210 nm

Isocratic mobile phase: Acetonitrile 60% / water 40% /Trifluoroacetic acid 0.006%

Retention time for Cabazitaxel = 4.1 min

Figure S9. Typical chromatogram of a recovery test sample containing 10µg/mL diglycolyl-cabazitaxel (t_{ret} 3.4 min) and 10µg/mL of cabazitaxel (t_{ret} 4min). A reference of plasma alone is superimposed.