SUPPORTING INFORMATION

A new 3D Organotypic Model of Ovarian Cancer to help evaluate the Antimetastatic Activity of RAPTA-C Conjugated Micelles

Mingxia Lu, Claire Henry, Haiwang Lai, Yee Yee Khine, Caroline Ford, Martina H. Stenzel

Table S1. Synthesis conditions and characterization of polymers.

Polymer(NMR)	Reactants concentration (mol L^{-1})			Conv. (%) of Monomer		$M_{n,nmr}$ (g mol^{-1})	$M_{n,sec}$ (g mol^{-1})	$Đ$
	AFru	CEA	PLA RAFT agent	AFru	CEA			
PLA_{247}-*b*-P[1-*O*-$AFru_{46}$-*s*-CEA_{12}]	9.8×10^{-1}	2.4×10^{-1}	8.2×10^{-3}	38	40	30200	24960	1.34
PLA_{247}-*b*-P[1-*O*-$AFru_{90}$-*s*-CEA_{24}]	9.8×10^{-1}	2.4×10^{-1}	8.2×10^{-3}	75	80	42100	23070	1.39
PLA_{247}-*b*-P[1-*O*-AFru]$_{39}$	9.8×10^{-1}	-	8.2×10^{-3}	33	-	26900	20540	1.37
PLA_{247}-*b*-P[1-*O*-AFru]$_{89}$	9.8×10^{-1}	-	8.2×10^{-3}	74	-	38600	14980	1.46

Table S2. Characterizations of micelles size via DLS and ruthenium concentration via ICPOSE.

Micelle	Polymer	Size (nm)	PDI	[Ru] (mmol L^{-1})[a]
M1	PLA_{247}-*b*-P[1-*O*-$AFru_{46}$-*s*-(CEA-RAPTA-C)$_{12}$]	185	0.266	3.14×10^{-2}
M2	PLA_{247}-*b*-P[1-*O*-$AFru_{90}$-*s*-(CEA-RAPTA-C)$_{24}$]	125	0.141	3.14×10^{-2}
M3	PLA_{247}-*b*-P[1-*O*-AFru]$_{39}$	163	0.224	-
M4	PLA_{247}-*b*-P[1-*O*-AFru]$_{89}$	133	0.218	-

[a]ICPOES results in mg L^{-1} were converted to mmol L^{-1} using the molecular weight of Ru = 101.07 g mol^{-1}

MATERIALS AND METHODS

All chemicals were reagent grade and used as received, unless otherwise specified. 1,3,5-triaza-7-phosphaadamantane (PTA; 97%, Aldrich), methanol (HPLC grade, APS), dichlororuthenium(II) (p-cymene) dimer (RuCl2(p-cymene) dimer; 97%, Aldrich), dichloromethane (DCM; >99.8%, Aldrich), n-hexane (95%, Ajax Finechem), 2-chloroethanol (>99%, Aldrich), sodium iodide (NaI; >99.5%, Aldrich), tin(II) 2-ethyl hexanoate (SnOct2; 95%, Aldrich), and 3,6-dimethyl-1,4-dioxane-2,5-dione (lactide; Aldrich), toluene (99%, Ajax), acryloyl chloride (97%, Lancaster), 2-chloroethanol (>99%, Aldrich), triethylamine (Et3N; >99%, Aldrich), *N*,*N*-dimethylacetamide (DMAc; 99.9%, Aldrich), N,N-dimethylformamide (DMF; 99%, Aldrich), D-fructose (99%, Aldrich), Sulfuric acid (95%-98%, Ajax Finechem), silica gel (60 Å , 70–230mesh, Aldrich), Nile Red (Aldrich), acetone (HPLC grade, Aldrich), sodium hydroxide (98%, Aldrich), deuterated dimethylsulfoxide-d6 (DMSO-d_6; Cambridge Isotope Laboratories), ethyl acetate (EtOAc; 95%, Ajax Finechem), diethyl ether (99%, Ajax Finechem), anhydrous acetone (0.0075% H_2O, Merck Millipore), were used without any further purification. 2-Hydroxyethyl acrylate (HEA; 96%, Aldrich) was destabilized by passing it over a column of basic alumina. 2,2-Azobis(isobutyronitrile) (AIBN; 98%, Fluka) was purified by recrystallization from methanol. The RAFT agent benzyl (2-hydroxyethyl) carbonotrithioate (BHCT) was synthesized according to literature procedures.[24]

Synthesis of dichlororuthenium (II) (p-cymene)(1,3,5-triaza-7-phosphaadamantane)(RAPTA-C). RAPTA-C was synthesized according to the previous report.[16] $RuCl_2$ (p-cymene) dimer (50 mg, 82 μmol) and PTA (26 mg, 160 μmol) were dissolved in 35 mL of anhydrous methanol. The reaction was refluxed under nitrogen for 5 hours. Then the solution was filtered, and methanol was removed under reduced pressure. Red-orange crystals were formed using a DCM-hexane mixture and the compound was confirmed by NMR.

Synthesis of PLA MacroRAFT agent. The synthesis of the PLA MacroRAFT agent was described in detail previously.[25] 3,6-Dimethyl-1,4-dioxane-2,5-dione (3 g, 21 μmol) and

BHCT (0.05 g, 0.21 μmol) were mixed and stirred under vacuum at 120 °C for 12 h. After purging with nitrogen, $SnOct_2$ (0.01 g, 0.027 μmol, solution in 1mL toluene) was added. The temperature was increased to 140 °C and the mixture was left to react for 5 h. After cooling, dichloromethane was added to dissolve the cooled polymer mixture. Under stirring, the polymer solution was added dropwise to an excess of chilled methanol. The precipitated product was dried under vacuum.

Synthesis of 2-chloroethyl acrylate. Acryoyl chloride (6 g, 660 μmol) was dissolved in 30 mL of DCM and added dropwise into a mixture of 2-chloroethanol (2.9 g, 730 μmol) and Et_3N (10.1 mL) combined in 70 mL of DCM, and reacted overnight in an ice-bath. The product was washed with Milli-Q water and extracted with DCM, and the solvent was then removed under reduced pressure, to give a pale yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 6.45 (d, 1H), 6.17 (dd, 1H), 5.84 (d, 1H), 4.38 (t, 1H), 3.69 (t, 1H).

Synthesis of 1-*O*-acryloyl-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (1-*O*-AiPrFru)**.** 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose was synthesized by the following procedure. D-fructose (24 g, 133 μmol) was dried under vacuum for 30 min, and was then added to a cooled solution of conc. sulphuric acid (23.33 mL) in acetone (466.67 mL) in a 2-liter round bottom flask. The mixture was stirred at room temperature for 1.5 hours. The mixture was then chilled before a cold solution of sodium hydroxide (73.33 g) in Milli-Q water (333.3 mL) was added under stirring. Then, acetone was removed under reduced pressure, and the resulting solution was extracted with DCM (3 × 200 mL). The organic phases were combined, subsequently washed with three portions of Milli-Q water (3 × 200 mL), dried with Na_2SO_4, and filtered, and the solvent was then removed under reduced pressure to obtain a crystalline solid. The crude product was recrystallized by dissolving it in boiling diethyl ether (5 mL g^{-1}), cooling and adding hexane (5 mL g^{-1}), to give 18.9 g (54.5%) of pure product. Synthesis of 1-*O*-AiPrFru followed this method. 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (5 g, 20 μmol) and Et_3N (5.5 mL, 40 μmol) were dissolved in 140 mL of anhydrous DCM and the solution was cooled to 0 °C under nitrogen atmosphere. Then a solution of acryloyl chloride (2.5 mL, 29.6 μmol) in 60 mL of anhydrous DCM was added dropwise. The resulting solution was stirred for two hours. The resulting mixture was added onto 300 mL of crushed ice, and extracted with ethyl acetate (3×100 mL). The combined organic phases were washed with saturated $NaHCO_3$ and water (3×100 mL); and dried with Na_2SO_4. The solvent was evaporated under a reduced pressure to give a crude viscous liquid. It was purified by flash chromatography, eluting with 2/1 hexane/ethyl acetate, and was obtained as a colourless

sticky liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 6.51 (d, 1H,), 6.19 (dd, 1H), 5.88 (d, 1H), 4.64 (dd, 1H), 4.53 (d, 1H), 4.38 (d, 1H), 4.27 (dd, 1H), 4.16 (m, 1H), 3.87 (m, 1H), 1.57, 1.51, 1.41, 1.37 (4s, 12H).

Polymerization of 1-*O*-AiPrFru and CEA with PLA MacroRAFT. 1-*O*-AiPrFru, CEA, PLA MacroRAFT, and AIBN as initiator were dissolved in DMAc, to give a ratio of [1-*O*-AiPrFru]/[CEA]/[MacroRAFT]/[AIBN] = 120:30:1:0.5. The solution was deoxygenated by purging with nitrogen for 45 min, and then polymerized at 70 °C. The polymer was precipitated into cold methanol, and dried under vacuum to give polylactide-b-(poly[1-*O*-acryloyl-2,3:4,5-di-*O*-isopropylidene-*β*-D-fructopyranose-co-2-chloroethyl acrylate]) (PLA-P[1-*O*-AiPrFru-CEA]) as a pale yellow solid.

Deprotection of the copolymer. The polymer PLA-P (1-*O*-AiPrFru-CEA) was mixed with 2 mL of TFA/H_2O (5:1 v/v) at room temperature and stirred for 40 min. The solution was dialyzed against Milli-Q water (MWCO = 3500 g mol^{-1}) for two days and freeze-dried to give a pale white powder (denoted as PLA-P [1-*O*-AFru-s-CEA]).

Conjugation of RAPTA-C to Polymer.

Finkelstein Reaction. The polymer and sodium iodide were dried under nitrogen for 30 min before dissolving in 10 mL of anhydrous acetone in a schlenk flask. The solution was refluxed at 70 °C for 7 days. The solution was dialyzed against Milli-Q water (MWCO = 3500 g mol^{-1}) for two days and freeze-dried to give a pale yellow solid.

Attachment of PTA to copolymer. The polymer and PTA were combined in a 10 mL schlenk flask, evacuated and filled with nitrogen before degassed DMSO-d_6 (1 mL) was added. The mixture was reacted for 7 days.

RAPTA-C Complexation. The ruthenium dimer was subsequently dissolved in the DMSO-PTA copolymer solution for 4 days to obtain an orange solution, which was analysed via NMR without further purification.

Synthesis of RAPTA-C. $RuCl_2$ (p-cymene) dimer (50 mg, 82 μmol) and PTA (26 mg, 160 μmol) were dissolved in 35 mL of anhydrous methanol. The reaction was refluxed under nitrogen for 5 hours. Then the solution was filtered, and methanol was removed under

reduced pressure. Red-orange crystals were formed using a DCM-hexane mixture and the compound was confirmed by NMR.

Micellisation of Drug-loaded copolymer. Typically, 0.25 mL of the RAPTA-C copolymer DMSO solution, where c(polymer) = 20 mg mL^{-1}, was diluted with 0.75 mL of DMF. Then the mixture was added to 1.75 mL of Milli-Q water by using a syringe pump, at a rate of 0.05 mL hr^{-1}. The deep red-orange solution was subsequently dialyzed against Milli-Q water (MWCO = 3500 g mol^{-1}) to give a pale orange solution, which was diluted to 7 mL.