Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Ring-Opening Metathesis Polymerization-Induced Self-Assembly (ROMPISA) of a Cisplatin Analogue for High Drug-Loaded Nanoparticles

Daniel B. Wright,[a,‡] Maria Proetto,[a,‡] Mollie A. Touve,[a] and Nathan C. Gianneschi*[a]

[a]Department of Chemistry, Department of Materials Science and Engineering, and Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113, United States of America

Contents

Methods and Materials

Materials

(Oligo(ethylene glycol)) norbornene dicarboximide, quaternary amine phenyl norbornene dicarboximide, cisplatin analogue norbornene dicarboximide were synthesised according to adjusted previous literature reports.[1-4] All other materials were used as received from Aldrich, Fluka, Apeiron catalyst or Acros.

Synthesis

General procedure for dispersion ROMP

To a stirred solution of (Oligo(ethylene glycol)), (OEG), norbornene dicarboximide (eqv. dependent on block length) in degassed 18 mΩ water, mixture was added Hoveyda-Grubbs 2nd generation catalyst (1 Eqv.). The reaction was left to stir under nitrogen at room temperature for 30 min. An aliquot of the reaction was removed and quenched with ethyl vinyl ether. To the remaining solution was added cisplatin analogue norbornene dicarboximide and quaternary amine phenyl norbornene dicarboximide or 2-(diisopropylamino)ethyl norbornene dicarboximide, (Eqv. Dependent on block length) and the reaction was stirred under nitrogen at room temperature for 1 h, after which the solution was quenched by adding ethyl vinyl ether and were characterized by SEC-MALS and ^{1}H NMR spectroscopy.

Characterization methods

^{1}H Nuclear magnetic resonance

^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance III Au 400 (400 MHz) in *N,N*-dimethyl-d_6-formamide or DMSOd_7. Chemical shifts are given in ppm downfield from tetramethylsilane (TMS).

Size exclusion chromatography Multi-Angle Light Scattering

Size exclusion chromatography multi-angle light scattering (SEC-MALS) measurements were performed on a set of Phenomenex Phenogel 5u, 1K-75K, 300 x 7.80 mm in series with a Phenomex Phenogel 5u, 10K-1000K, 300 x 7.80 mm columns with HPLC grade solvents as eluents: dimethylformamide (DMF) with 0.05M of LiBr at 40 ^{o}C with a Wyatt Dawn Heleos II and a Optilab T-rEX. The molecular weights of the synthesized polymers were calculated using Astra software after normalizing the detectors with a 30K PS standard.

Dynamic light scattering (DLS)

Measurements were performed at an angle of 90 ° with a Wyatt Dynapro NanoStar operating at λ_0 = 658 nm and at 25 °C ± 1 °C. Data were collected in triplicate with 100 s run times. The concentration dependence of D is given by $D = D_0(1+k_DC)$ where k_D is the dynamic second virial coefficient and D_0 the diffusion coefficient used for computing the hydrodynamic radius (R_h) of the scatterers according to the Stokes-Einstein equation. Here, measurements were performed only at a concentration where interactions could be neglected here at least for solutions so that this concentration is ~ D_0.

Scanning transmission electron microscopy (STEM) and energy dispersive X-Ray spectroscopy (EDX) analysis

Small aliquots (4 μL) of sample were applied onto 400 mesh carbon grids (Ted Pella, INC.) that had been glow discharged using a PELCO easiGlow glow discharge unit for 90 s. Excess sample solution was wicked away with filter paper, rinsed with water, and allowed to dry. TEM imaging was conducted on a HD-2300A STEM (Hitachi High Technologies America, Inc., Schaumber, IL USA) with micrographs recorded on a Digiscan II System (Gatan Inc., Pleasanton, CA, USA). Samples were then analyzed by EDX (NSS Spectral Analysis System, Thermo Fischer Scientific).

Zeta Potential

Zeta-potentials were obtained on a Zetasizer Nano (Malvern Instruments Ltd.) with nanoparticles suspended in Milli-Q water at room temperature.

***In-vitro* cytotoxicity**

The human cancer cell lines HeLa and CAOV3 were stored in DMEM (Dulbecco's modified Eagle's Medium) containing 1% glutamine, with 1% non essential amino acids, 1% penicillin-streptomycin, 1% sodium pyruvate, and 10% FBS (fetal bovine serum), from now on referred as complete media, in a humidified atmosphere (5% CO_2) at 37 ^oC.

In a 96 well plate, a 100 μL of a cell suspension of culture medium at 2,000 cells/mL (HeLa and CAOV3) was plated into each well and incubated for 24 h. After 24 h, the media was removed from the wells and 100 μL aliquots of different concentrations of the compounds were added so that the DMF was 0.1%. The plates were incubated for 4 days under cell culture conditions. 20 μL

of Cell Titer Blue was added to each well. It was incubated for 4 h and then the fluorescence (560/590nm) was recorded using a Perkin Elmer EnSpire Multimode Plate Reader.

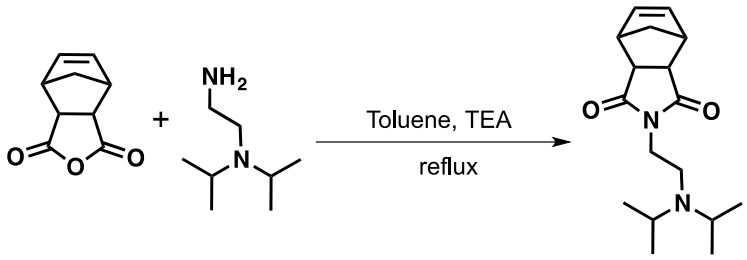
Cellular uptake studies

$2.1x10^6$ CAOV-3 cells were seeded in a T-75 flask and incubated for 48 h. The media was removed and cells were incubated for 6 h with a 1 μM nanoparticle solution in respect to Pt in complete media previously dissolved in DMF at 1 mM stock solutions. The compound-containing media was removed, cells were washed twice with DPBS, trypsinized and resuspended in 10 mL of complete media. Cells were centrifuged down (500 rcf, 5 min) and the supernatant was removed. Pellets were stored at -20 ^oC until further analysis. For protein and Pt quantification, pellets were resuspended in 1 mL milliQ water. Samples were ultrasonicated while keeping them chilled with ice at a 40% amplitude, 3 cycles of 20 sec each and 10 sec rest (Fisherbrand™ Model 505 Sonic Dismembrator). For protein quantification, a 1/20 dilution of the sample was prepared.in milliQ-water. 20 μL of each sample was placed in a 96-well plate and 200 μL of the Bradford reagent was added to each well. The plate was gently mixed and the absorbance at 595 was measured using a Perkin Elmer EnSpire Multimode Plate Reader. A calibration curve was plotted for each experiment using HSA as a protein standard (concentrations: 0, 0.025, 0.05, 0.1, 0.2, 0.25 and 0.3 mg/ml). Three wells were measured for each condition. For Au quantification, a 0.5 ml aliquot was removed and added to 8 ml milliQ water, 0.5 mL concentrated HCl, 0.5 mL HNO_3 and 0.5 mL H_2O_2, digested in a microwave (Milestone EthosEZ Microwave Digestion System) and analyzed by ICP-MS (Thermo iCAP Q ICP-MS). The cellular uptake is expressed as ng Pt/mg Protein and it is expressed as an average of at least 3 independent experiments.

Additional data for cisplatin-monomer synthesis and characterization

Exo-5-norborneneamidomalonic acid diethyl ester was synthesized as described[5] and the precursor cis-[$PtI_2(NH_3)_2$] was synthesized according to previous literature.[6] *Cis*-[$PtI_2(NH_3)_2$] (172 mg, 0.35 mmol) was added to a solution of Ag_2SO_4 (0.109 g, 0.35 mmol) in H_2O (~4 mL), and the mixture was stirred at 40 °C overnight in the dark. It was then filtered to remove AgI, and barium *exo*-5-norborneneamidomalonate (obtained from reaction between $Ba(OH)_2 \cdot 8H_2O$ (0.110 g, 0.35 mmol) and the *exo*-5-norborneneamidomalonic acid diethyl ester (0.103 g, 0.35 mmol)) was added to the filtrate. The mixture was stirred at 40 °C overnight in the dark, and $BaSO_4$ precipitated. The precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was washed with MeOH and Et_2O and dried under vacuum. Yield: 0.135 g, 83%; ^{1}H NMR (DMSOd_7): δ=7.46 (d, *J*=8.2 Hz, 1H, NH), 6.1 (m, 2H, C*H*=C*H*), 5.61 (d, *J*=8.1 Hz, 1H, NHC*H*), 4.24 (s, 6H, 2 NH_3), 2.80 (m, 2H, C*H*CH_2), 2.25 (m, 1*H*, C*H*C(O)), 1.76 (m, 1H, CH_2CHC(O)), 1.58 (m, 1H, CH_2CH), 1.13 (m, 2H, CH_2CHC(O), CH_2CH).

Additional data for the pH sensitive monomer



To a stirred solution of 2-aminoethyldiisopropylamine (648 mg, 4.5 mmol) in dry toluene (50 mL) was added 5-norbornene-*exo*-2,3-dicarboxylic anhydride (492 mg, 3 mmol) and triethylamine (606 mg, 6 mmol). The reaction was heated to reflux overnight. The solvent was reduced to a minimum

under reduced pressure and was in a vacuum pump for further 5 h to yield a brown oil (860 mg, 99%). ^{1}H NMR (400 MHz, Cl_3CD-*d*) δ 6.27 (m, 2H), 3.47 (t, 2H), 3.26 (m, 2H), 3.01 (m, 2H), 2.66 (m, 2H), 2.57 (t, 2H), 1.50 (d, 1H), 1.34 (d, 1H), 0.97 (d, 12H).

Additional data for polymerization of (Oligo(ethylene glycol)), (OEG), norbornene dicarboximide

Polymer[a]	$M_{n\ Theo}$[b] (kDa)	$M_{n\ SEC}$[c] (kDa)	$Đ$[c]
20	7.0	8.1	1.08

[a] The block length of the block for P(OEG) [b]Determined by monomer conversion from 1H NMR spectroscopy. [c]alculated from SEC-MALS in DMF.

Additional data for copolymerization of cisplatin analogue norbornene dicarboximide and quaternary amine phenyl norbornene dicarboximide

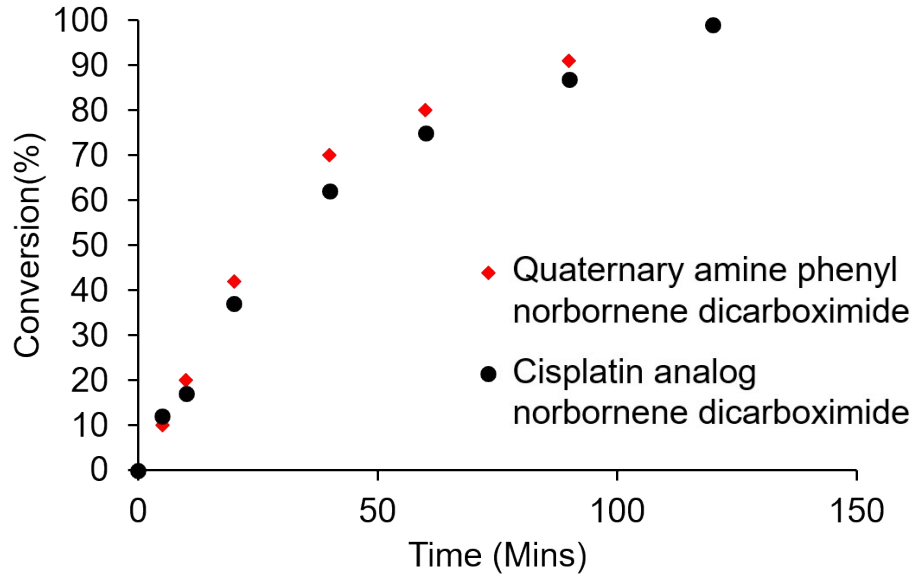


Figure S1. Conversion vs reaction time for cisplatin analogue norbornene dicarboximide and quaternary amine phenyl norbornene dicarboximide, example shown is polymer **20-20**.

Comparison of solvent switch assembly and ROMPISA formulation at 5 wt% solids

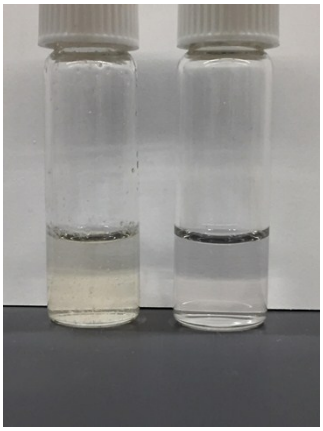


Figure S2. Picture of left) Solvent switch of **20-20** from DMF to water at 5 wt% solids, right) ROMPISA formulation of **20-20** at 5 wt% solids. Note the polymer precipitates in the left vial.

AUTHOR INFORMATION

| D.B.W. and M.T.P. contributed equally to this work

Corresponding Author

*E-mail: nathan.gianneschi@northwestern.edu (N.C.G.)

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