

Electronic Supplementary Information for Dalton Transactions
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Hyperbranched Polymers for Controlled Release of Cisplatin

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Supplementary Information

Materials:

Polymers: Boltorn H40 was obtained from Perstorp, PG2 from Hyperpolymers. Succinic anhydride, *cis*-diamminedichloroplatinum(II), anhydrous pyridine, 4-(dimethylamino)pyridine and 1,2-phenylenediamine were obtained from Sigma-Aldrich Canada. N,N-dimethylformamide – Fisher, Spectranalysed grade, Spectra/Por 7 pretreated dialysis membranes, molecular weight cut off (MWCO) 1000, and 1cm³/cm width, from Spectrum Labs.

NMR spectra were run on a Bruker Avance 400dir Spectrometer. UV-Vis

Methods:

Modification of H40

Boltorn H40 (10 g, 0.002 moles, 0.128 moles OH based on molecular weight of 5.1 kDa and 64 terminal OH groups) was added to pyridine (50 cm³) and stirred rapidly to facilitate dissolution. Dimethylaminopyridine (0.2 g, 0.0016 moles) was added followed by the slow addition of succinic anhydride (14 g, 0.14 moles). An additional volume of pyridine (20 cm³) was added to ensure the resulting solution was not too viscous for efficient stirring. The reaction was stirred overnight at room temperature (approx 22°C). Water was added (50 cm³) and the mixture stirred for 30 minutes. Solvents were removed by rotary evaporation with the periodic addition of small portions of water to enable better evaporation of pyridine by azeotropic distillation. The residue was dissolved in methanol and dialyzed against distilled water for 16 hours. The dialysis buffer was changed four times, each time with a greater methanol concentration. The final buffer was 70 % methanol in distilled water. The solvent was removed by rotary evaporation and the polymer dried in a vacuum oven over night.

δ_{H} (400 MHz, MeOD-d₄, MeOH): 1.15-1.4 (br m, initiator core, CH₃ on polymer); 2.6 (br,s, CH₂CH₂COOH); 3.3 (br, m, CH₃OH, solvent); 3.7 (br, m, CH₂OH); 4.2-4.4 (br m, CH₂OR); 4.9 (br, s, OH)

δ_C (100 MHz, MeOD-d₄, MeOH): 17.9, 18.4 (CH₃ on polymer); 29-30.2(C(O)CH₂CH₂COOH); 47.9-50.0 (quarternary carbons in polymer backbone); 65.6-67.2 (CH₂O); 173.5-174.8 (C(O)CH₂); 176.0 (COOH)

Modification of PG2

Pyridine (50 cm³) was added to PG2 (10 g, 0.005 moles, 0.100 moles OH based on molecular weight of 2 kDa, and 20 terminal OH groups) and stirred rapidly to facilitate dissolution. Dimethylaminopyridine (0.2 g, 0.0016 moles) was added followed by the slow addition of succinic anhydride (12 g, 0.12 moles). The reaction was stirred overnight at room temperature (approx 22°C). Water was added (50 cm³) and the mixture stirred for 30 minutes. Solvents were removed by rotary evaporation with the periodic addition of small portions of water to enable better evaporation of pyridine by azeotropic distillation. The residue was dissolved in methanol and dialyzed against distilled water for 16 hours. The dialysis buffer was changed four times, each time with a greater methanol concentration. The final buffer was 70 % methanol in distilled water. The solvent was removed by rotary evaporation and the polymer dried in a vacuum oven over night.

δ_H (400 MHz, MeOD-d₄, MeOH): 0.84 (3H, br s, CH₃, Polymer Initiator,); 2.55 – 2.70 (br s, OCOCH₂CH₂COOH), 3.26 (br s, 1H); 3.30 – 3.32 (13H, m, CH₃OH), 3.35 (20H, br s, CH₃CH₂C(CH₂O)₃); 3.45 – 3.81 (br m, CH, CH₂, polymer backbone); 4.08 – 4.40 (br m, CH₂OCO); 5.04 (s, CH₂OCO); 5.04 – 5.24 (br m, CHOCO)
 δ_C (100 MHz, MeOD-d₄, MeOH): 30.0 (C(O)CH₂CH₂COOH); 64.0-65.0 (CH₂OH, unreacted primary alcohol groups in polymer); 67.0 (CH₂ in polymer); 70.0-74.0(CH₂O and CHO groups in polymer); 78.5, 80.5 (CH in polymer); 174.0 (C(O)CH₂CH₂COOH); 176.0 (C(O)CH₂CH₂COOH)

Formation of platinum-hyperbranched polymer complexes

H40 Solution

A solution of modified H40 polymer in water was prepared by dissolving polymer in dilute sodium hydroxide solution (0.05M NaOH) to give a 10 mg/cm³ polymer solution.

PG2 Solution

A solution of modified PG2 polymer in water was prepared by dissolving polymer in dilute sodium hydroxide solution (0.05M NaOH) to give a 20 mg/cm³ polymer solution.

Cisplatin Solution

Cisplatin solution was prepared to 1.25 mg/cm³ in distilled water or saline.

Complex Formation

Cisplatin and polymer were mixed according to table with each sample prepared in triplicate, having a total volume of 5 cm³ with 5 mg total mass of cisplatin and 0.01 M in sodium hydroxide. The samples were incubated at 37°C for 72 hours under static conditions if required.

Cisplatin	Polymer	Reaction Buffer	Incubation	Release Buffer
5 mg	None	Water	N/A	Water
		Saline	N/A	Saline
5 mg	H40 10 mg	0.01M sodium hydroxide solution	0	Water
				Saline
5 mg	H40 10 mg	0.01M sodium hydroxide solution	72 hours	Water
				Saline
5 mg	PG2 20 mg	0.01M sodium hydroxide solution	0	Water
				Saline
5 mg	PG2 20 mg	0.01M sodium hydroxide solution	72 hours	Water
				Saline

Platinum Complex Release Studies

Release studies of cisplatin and related compounds were conducted by dialysis. 12 cm lengths of dialysis membrane were soaked in water to remove preservative and clamped using weighted dialysis clamps. Polymer-drug solution was pipetted into the bags and a clip applied to seal the bags. In some cases sodium chloride was added to the polymer-drug solutions prior to filling dialysis bags, ensuring the concentration was approximately 0.9 % w/w saline. The dialysis bags were trimmed to allow approx 0.5 cm³ of membrane either end. Release was carried out in saline (500 cm³, 0.9 %) in a media bottle (500 cm³), at 37°C and 75 rpm agitation. Samples of release buffer (0.5 cm³) were taken at each time point (0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 hours).

Cisplatin Assay

Cisplatin concentrations were determined by the o-phenylenediamine (OPDA) colorimetric assay developed by Golla *et al.*^{15,16}. A solution of ortho-phenylenediamine (OPDA) in N,N-dimethylformamide (1.6 mg/cm³) was prepared. Samples from dialysis experiments were mixed with an equal volume of OPDA solution, and the resulting mixture boiled in a water bath for 30 minutes, ensuring no water entered the sample tube. Samples were analysed by UV-Visible spectroscopy, reading at 704 nm. Standard curves were generated using standard samples of cisplatin in each release medium (fig. S3), as both media and sodium hydroxide concentration were found to have an effect on the assay. The linear range for this assay was found to be 0.5 – 16 µg/cm³.

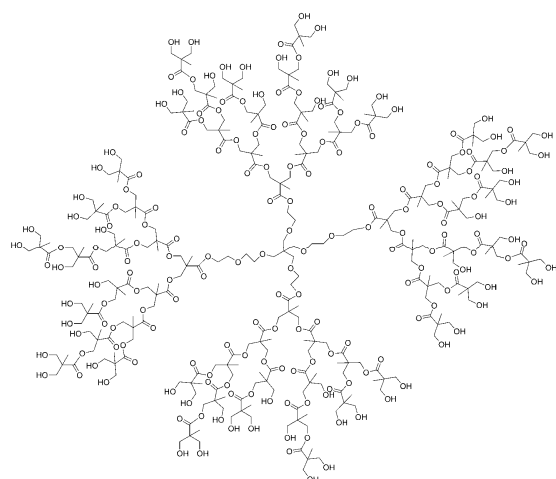


Fig. S1 Hyperbranched polyester, boltorn H40 modified with carboxylic acid groups

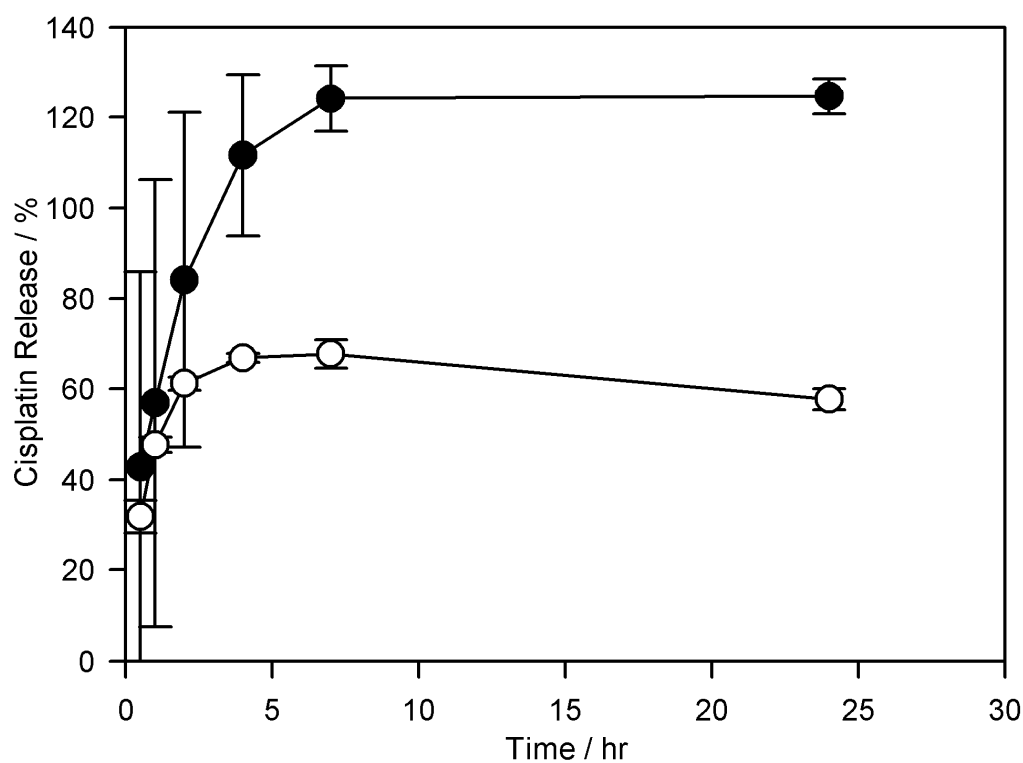


Fig. S2 Cumulative release of platinum complexes from H40 with no incubation time prior to dialysis. (n=3, ● – saline, ○ – water)

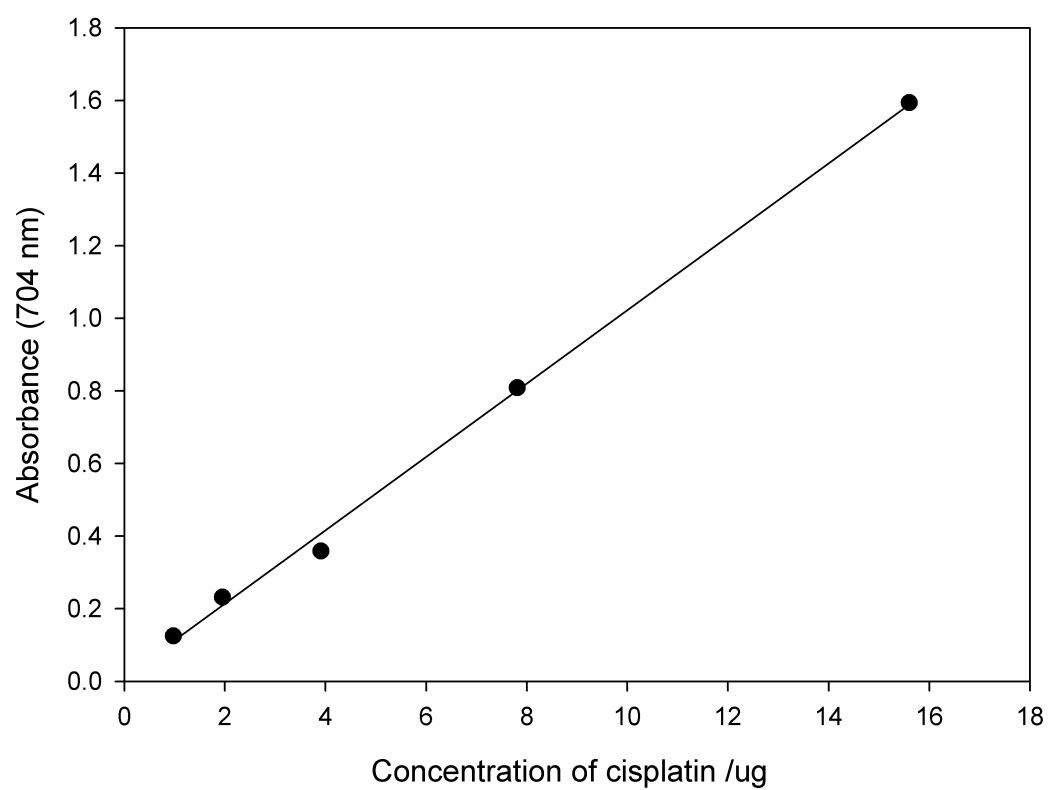


Fig. S3 OPDA Assay standard Curve, $R^2 = 0.9979$