Supporting Information

Design, Synthesis and Biological Evaluation of a Robust and Biodegradable Dendrimer Drug Carrier

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PE-G_1-$(AspBoc)_8$ (11). Compound **6** (434 mg) was added to a 20 mL reaction vial and dissolved in MeOH (10 mL). Activated Pd/C (10 wt%, 44 mg) was added and the reaction put under hydrogen atmosphere. After 1 h, the reaction appeared complete by MALDI. Filtration and removal of the solvent via rotary evaporation gave **11** (315 mg) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 12H), 1.45 (s, 72H), 2.81-3.04 (br d, 16H), 4.16-4.41 (m, 24H), 4.63 (br s, 8H), 5.72 (br s, 6H), 6.52 (br s, 2H); ^{13}C NMR (100 MHz, MeOD) δ 18.3, 28.8, 37.1, 47.7, 51.4, 63.3, 67.0, 80.8, 82.0, 157.5, 172.4, 173.3, 174.0; MS (MALDI-TOF) Calc $[M]^+$ ($C_{97}H_{148}N_8O_{56}$) *m/z* = 2320.9. Found $[M+Na]^+$ *m/z* = 2344.0.

PE-G_1-$(Asp(Glu(NNBoc)_2)PEO)_8$ (12). Compound **9** (150 mg, 30 μmol COOH), compound **10** (232 mg, 620 μmol), and pentachlorophenol (164 mg, 620 μmol) were added to a 20 mL reaction vial. Under a nitrogen atmosphere, DMF (600 μL) was added, followed by triethylamine (86 μL, 620 μmol); upon dissolution, EDC (118 mg, 620 μmol) was added and the reaction stirred at room temperature overnight. The reaction was dialyzed against MeOH in 12-14 kDa MWCO dialysis with 3 solvent changes over 24 h. Concentration of the bag contents *in vacuo* gave **12** (140 mg) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 24H), 1.45 (br s, 110 H), 2.7-3.0 (br m, 66 H), 3.38 (s, 24 H), 3.5-3.9 (br m, 4070 H), 4.1-4.5 (br m, 50H).

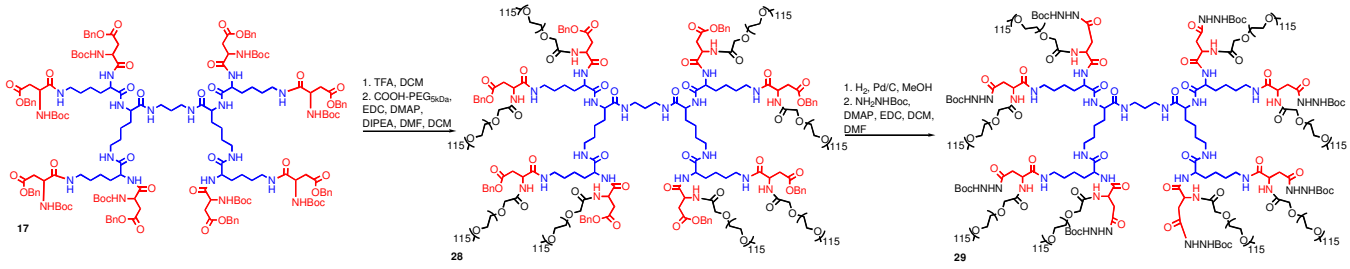
PE-G_1-$(Asp(Glu(NNDox)_2)PEO)_8$ (14). Compound **12** (15.2 mg, 6.1 μmol NNBoc) was dissolved in 1:1 TFA:DCM for 1 h. The solvent was removed by rotary evaporation. The solid was redissolved in DCM and evaporated twice to remove residual TFA. The solid was dissolved in MeOH (1 mL), pyridine (50 μL), and acetic acid (50 μL), and doxorubicin (10 mg, 17 μmol) was added. The reaction was purged with nitrogen and stirred at 60 °C in the dark for 16 h. The reaction mixture was loaded directly onto a Sephadex LH-20 column and eluted with methanol. The first dark red band was collected and the solvent removed by rotary evaporation. The solid material was further purified using a Biorad PD-10 column with water as the eluent. After lyophilization, 17 mg of red powder remained. The Dox loading was quantified using the absorbance at 486 nm (ε = 11,500) (*1*) to be 14.8% wt %, or 68% of the maximum theoretical loading.

PLL-G_2-$(Asp(NNBoc)PEO)_8$ (20). Compound **19** (110 mg, 22 μmol COOH) and *t*-butyl carbazate (29.1 mg, 220 μmol) were added to a 20 mL reaction vial. Under a nitrogen atmosphere, DMF (500 μL) was added; upon complete dissolution, HBTU (83.5 mg, 220 μmol) and *N,N*-diisopropylethylamine (DIPEA) (80 μL, 440 μmol) were added and the reaction stirred at room

temperature overnight. The reaction was dialyzed against MeOH in 3,500 MWCO dialysis with 3 solvent changes over 18 h. SEC analysis of the isolated solid indicated a high degree of polymer degradation.

To circumvent the degradation of dendrimer **19**, PEGylation of dendrimer **17** with carboxymethyl-PEG afforded dendrimer **28**, which had PEG attached through the more stable amide linkage. The benzyl ester protecting groups were removed via hydrogenolysis and the free acids were functionalized with *t*-butyl carbazate to give dendrimer **29**..

Scheme SI 1. PEGylation of lysine dendrimer through an amide bond.



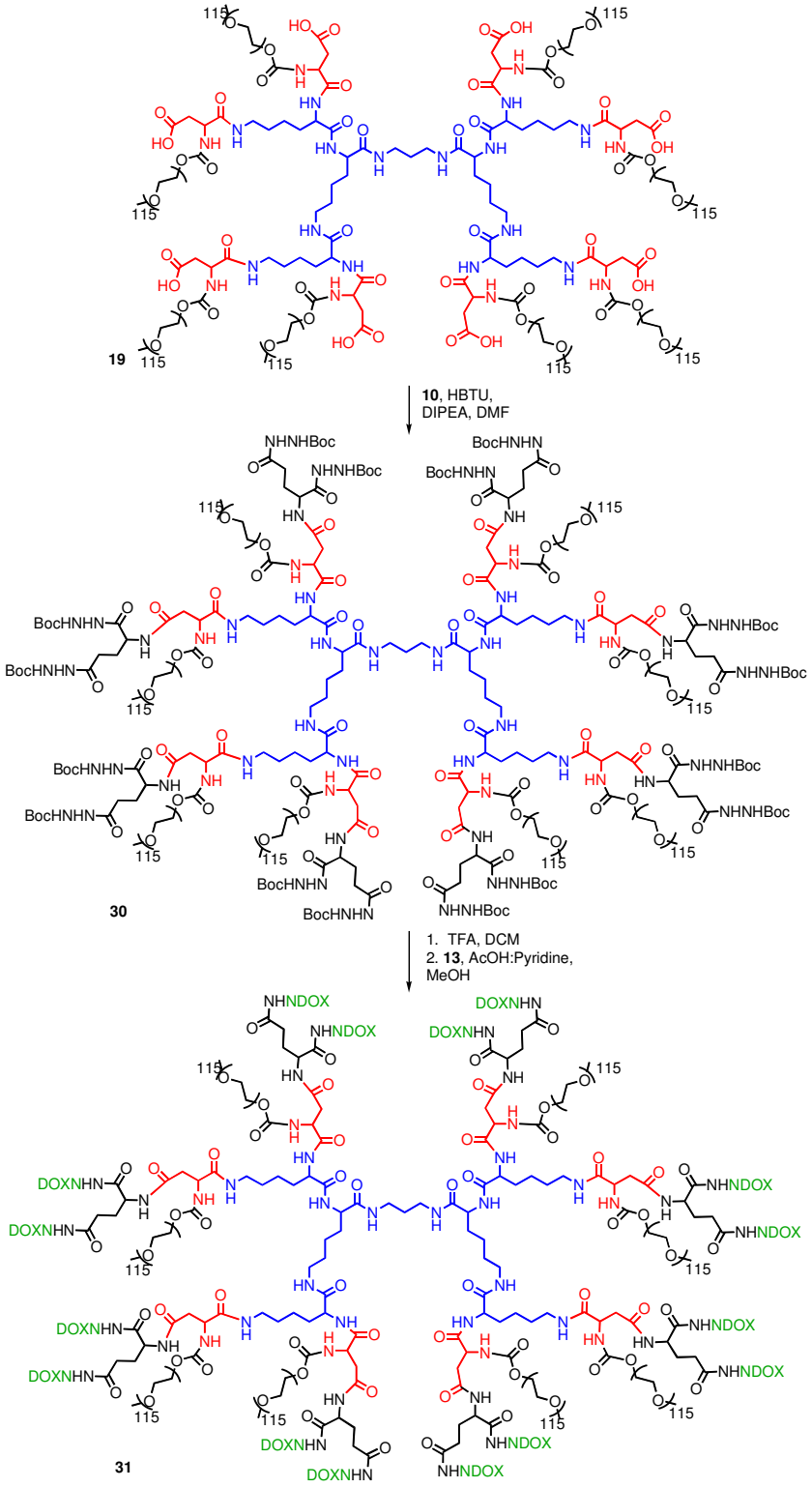
PLL-G$_2$-(Asp(Bn)-Amide-PEO)$_8$ (28). Deprotected compound **17** (158.4 mg, 0.373 mmol NH_3) and carboxymethyl-PEG (2.14g, 0.428 mmol) were added to a 20 mL reaction vial. Under a nitrogen atmosphere, DMF (4 mL) and DCM (0.6 mL) were added. After using a warm water bath to dissolve the starting material, DMAP (112 mg, 0.92 mmol), EDC (480 mg, 2.50 mmol), and DIPEA (210 µL, 1.21 mmol) were added. After stirring overnight, the reaction was considered complete and acetic anhydride (10 µL, 0.106 mmol) was added to acylate any remaining amines. After 4 h, n-butylamine (200 µL, 2.02 mmol) was added to deactivate any activated PEG chains. The reaction mixture was then precipitated into cold ether (120 mL), dissolved in water and dialyzed against water in 100 kDa MWCO dialysis with one solvent change over 24 h. The retained water was lyophilized to yield a white powder (1.82 g). ^{1}H NMR (D_2O, 500 MHz): δ 1.18 (br s, 15), 1.30 (br s, 13), 1.54 (br s, 8), 1.62 (br s, 6), 2.7-2.9 (br m, 18), 3.01 (br s, 19), 3.32 (s, 24), 3.4-3.8 (br m, ~4200), 4.24 (br m, 25), 4.4-4.6 (br m, 15), 5.1 (br s, 16), 7.3 br (m, 40). DMF SEC: Mn: 33,700 Da, Mw: 37,000 Da PDI: 1.06.

PLL-G$_2$-(Asp-Amide-PEO)$_8$ (28a). Compound **28** (991mg, 1.98 mmol) was added to a 20 mL reaction vial and dissolved in MeOH (6 mL). Activated Pd/C (10 wt%, 210 mg) was added and the reaction put under hydrogen atmosphere. The reaction stirred overnight and then the Pd/C was filtered off. The solution was then precipitated into ether to give **28a** (550 mg) as an off-white solid. ^{1}H NMR (D_2O, 500 MHz) δ 1.18 (br d, 15), 1.36 (br s, 14), 1.57 (br s, 8), 1.66 (br s, 7), 2.7-2.9 (br m, 18), 3.05 (br s, 19), 3.25 (s, 24), 3.4-3.8 (br m, ~4000), 4.0-4.3 (br m, 23).

PLL-G$_2$-(Asp(NNBoc)-Amide-PEO)$_8$ (29). Compound **28a** (691 mg, 0.138 mmol COOH), *t*-butyl carbazate (192 mg, 1.45 mmol), and DMAP (180 mg, 1.47 mmol) were added to a 20 mL reaction vial. Under a nitrogen atmosphere, DMF (5.5 mL) and DCM (1 mL) were added. After using a warm water bath to dissolve the starting material, the solution was cooled to 20 ^{o}C and EDC (268 mg, 1.40 mmol) was added and the reaction was stirred overnight. The reaction was precipitated into cold ether (200 mL), dissolved in water and dialyzed against water in 3500 MWCO dialysis, changing the water after 2 and 8 hours. The retained water was lyophilized to yield a white powder (460 mg). ^{1}H NMR (D_2O, 500 MHz) δ 1.1-1.6 (br m, 94), 1.64 (br s, 8), 1.73 (br s, 7), 2.7-2.9 (br m, 17), 3.12 (br s, 19), 3.32 (s, 24), 3.4-3.8 (br m, ~4200), 4.0-4.3 (br m, 23).

An alternate approach involved attachment of a glutamic acid spacer (**10**) to dendrimer **18**. Subsequent deprotection of the hydrazides allowed for up to 16 doxorubicin molecules to be attached.

Scheme SI 2. Drug attachment through bifunctional hydrazide drug linker.



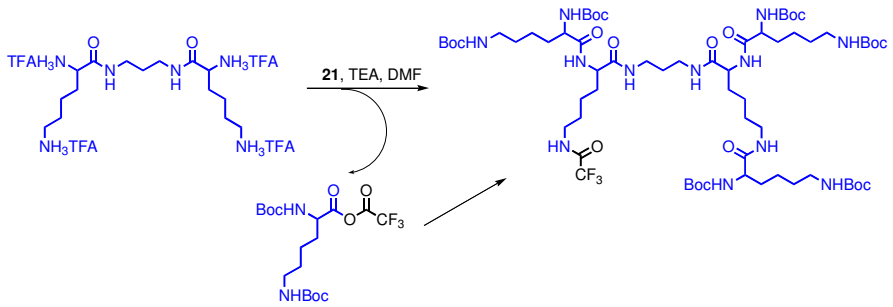
PLL-G$_2$-Asp(GluNNBoc$_2$)PEG$_8$ (30). Compound **18** (1.0 g, 0.22 mmol COOH) and DMF (4 mL) were added to a 20 mL vial. Under a nitrogen atmosphere, **10** (810 mg, 2.2 mmol) and HBTU

(830 mg, 2.2 mmol) were added. The mixture was stirred 5 min, and then DIPEA (760 μL, 4.4 mmol) was added. After 24 h, water was added and the solution was dialyzed against water in 3500 MWCO dialysis for 24 h. The retained water was lyophilized to yield a white powder (1.1 g, quant). ^{1}H NMR (500 MHz, D_2O): δ 1.47 (br s, 180), 1.6-1.9 (br m, 18), 2.2 (br m, 8), 2.4 (br m, 16), 2.7-2.9 (br m, 18), 3.21 (br s, 19), 3.39 (s, 24), 3.5-4.0 (br m, ~4300), 4.24 (br m, 25), 4.4-4.6 (br m, 15).

PLL-G_2-Asp(GluNNDox$_2$)PEG$_8$ (31). Compound **30** (166 mg, 66 μmol NNBoc) was dissolved in 1:1 TFA:DCM for 2 h. The solvent was removed by rotary evaporation, then again by azeotropic distillation twice with toluene under vacuum. The solid was dissolved in MeOH and evaporated twice to remove residual TFA. The solid was dissolved in MeOH (3 mL), pyridine (100 μL), and acetic acid (100 μL), and doxorubicin (100 mg, 170 μmol) was added. The reaction was purged with nitrogen and stirred at 60 °C in the dark for 16 h. The reaction mixture was loaded directly onto a Sephadex LH-20 column and eluted with methanol. The first dark red band was collected and the solvent removed by rotary evaporation. The solid material was further purified using a Biorad PD-10 column with water as the eluent. After lyophilization 163 mg of red powder remained. The Dox loading was quantified using the absorbance at 486 nm (ε = 11,500) (*1*) to be 16% wt %, or 73% of the maximum theoretical loading.

During the synthesis of polylysine dendrimers, we observed an additional side reaction that may be of interest to other polymer and dendrimer chemists. Complete removal of trifluoroacetic acid (TFA) after Boc deprotection steps was found to be critical; otherwise, TFA was found to add into the activated ester to form a mixed anhydride, which can cap the peripheral amines as the trifluoroacetamide. This side reaction was identified by MALDI-TOF analysis, and it was determined that the TFA counter ions on the dendrimer starting material do not cause this to occur.

Scheme SI 3. Side reaction caused by residual TFA.



References:

(1) Gabbay, E. J., Grier, D., Fingerle, R. E., Reimer, R., Levy, R., Pearce, S. W., and Wilson, W. D. (1976) Interaction Specificity of Anthracyclines with Deoxyribonucleic-Acid. *Biochemistry 15*, 2062-2070.