

Gene-Directed Enzyme Prodrug Therapy by Dendrimer-Like Mesoporous Silica Nanoparticles against Tumor Cells

Supplementary Material

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Synthesis of DOXO-Gal

Synthesis of 4-formylphenyl-(2, 3, 4, 6-tetra-O-acetyl-B-D-galactopyranoside) 3:

4-Hydroxybenzaldehyde **1** (244 mg, 2.0 mmol) was dissolved in 7 mL of a 0.286 M solution of aqueous NaOH (80 mg, 2.0 mmol) and cooled down in an ice bath. Acetobromo- α -D-galactose **2** (1033 mg, 2.5 mmol) was dissolved in acetone (10 mL) and added dropwise under stirring. The mixture was allowed to stand for 10 min in the ice bath and then stirred overnight at room temperature. The mixture was then placed into water (50 mL) and extracted with ethyl acetate (3 \times 30 mL). The organic phase was dried with MgSO₄, filtered and the solvent was evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (silica, 2 \times 30 cm) from hexane:ethyl acetate (7:3) to hexane:ethyl acetate (1:1), to get 4-formylphenyl-(2, 3, 4, 6-tetra-O-acetyl-B-D-galactopyranoside) **3** (400 mg, 44%) as a white solid. ¹H-NMR, ¹³C-NMR and MS spectrometry were in accordance with the literature [1].

Compounds **4**, **5** and **6** were prepared according to literature procedures [1]. In the case of compound **5**, 3 equivalents of triethylamine were used as base instead of pyridine.

Synthesis of N-(α -D-galactopyranosylbenzyloxycarbonyl)-doxorubicin (DOXO-Gal):

Compound **6** (680 mg, 0.66 mmol) was placed in a 100 mL round bottom flask and solved in a mixture MeOH:dichloromethane (32:6 mL). A suspension of K₂CO₃ (92 mg, 0.66 mmol) in methanol was added dropwise (10 mL) and the colour changed from deep red to deep purple. The suspension was stirred for 1 h at rt. The reaction mixture was neutralized by addition of HCl in 2M in Et₂O (1 mL) and the colour turned to orange. The mixture was evaporated under reduced pressure and purified by flash chromatography (silica, 2 × 20 cm) in dichloromethane:methanol (4:1), to get N-(α ,D-galactopyranosylbenzyloxycarbonyl)-doxorubicin (**DOXO-Gal**) (410 mg, 72%) as a red solid. ¹H-NMR, ¹³C-NMR and MS spectrometry in accordance with literature [1].

NMR characterization of DOXO-Gal

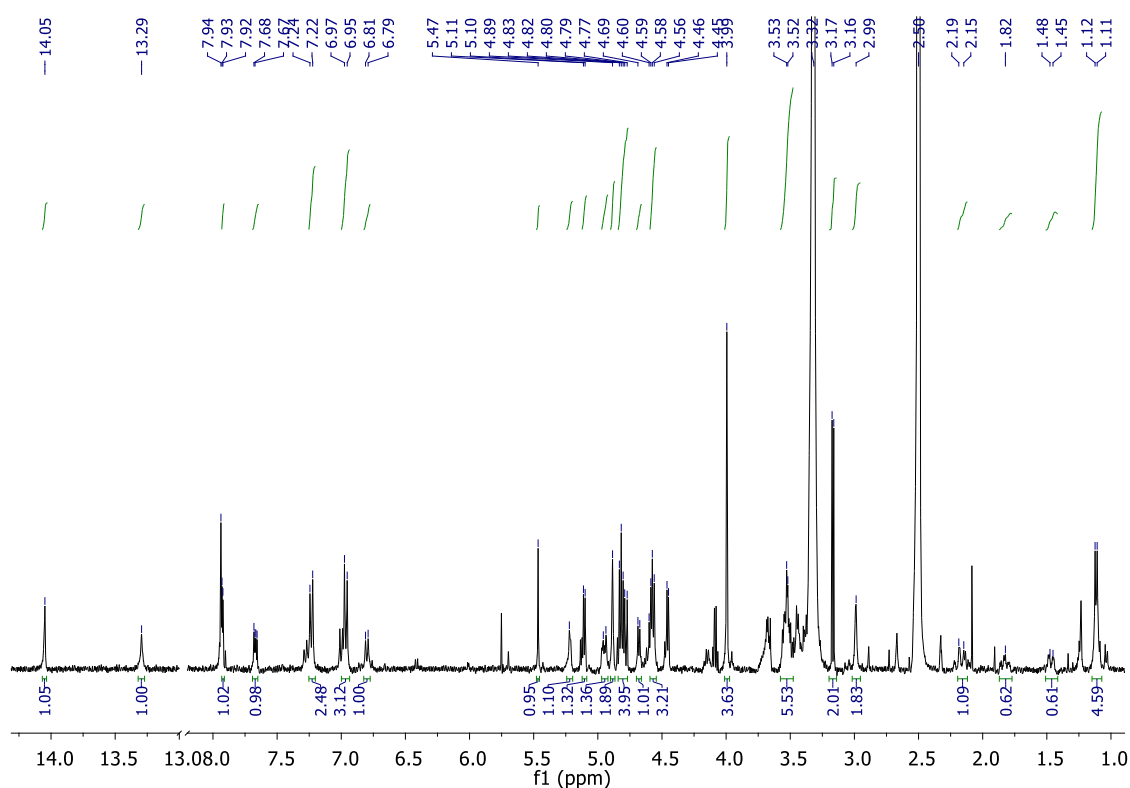


Figure S1. ¹H-NMR spectrum of DOXO-Gal in DMSO-d₆.

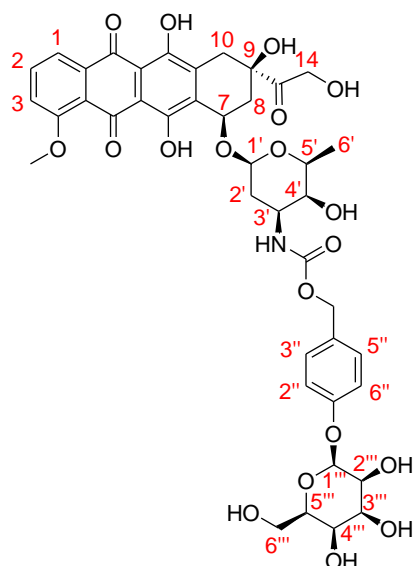


Table S1. ^1H -NMR data spectrum of **DOXO-Gal** in DMSO-d_6

(ppm)	multiplicity	Number of protons	Asignation
1.11	d	3	6'
1.43	d	1	2'
1.82	m	1	2
2.15	d	1	8
2.21	d	1	8
2.99	d	2	10
3.17-3.50	m	5	4',5',2''',3''',4'''
3.53	m	1	3'
3.99	s	3	Ar-O-CH ₃
4.10	m	1	5'''
4.56-4.60	m	2	14
4.70-4.80	m	2	Ar-CH ₂ -O
4.82	d	1	6'''
4.90	m	1	7
5.25	d	1	1'''
5.45	d	1	1'
6.79	d	1	1
6.95	d	2	3'',5''
7.22	d	2	2'',6''
7.68	d	1	3
7.93	m	1	2

Nanoparticle's characterisation

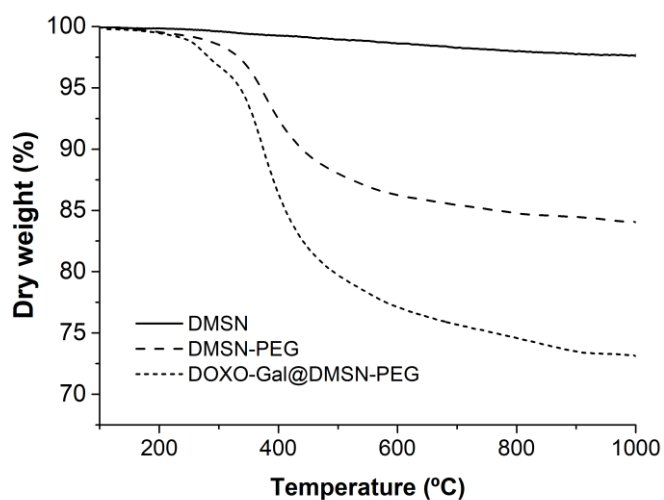


Figure S2. Thermogravimetric analysis of DMSN, DMSN-PEG and DOXO-Gal@DMSN-PEG. The percentage showed are normalised to the dry weight of each sample, which is taken at 100 °C.

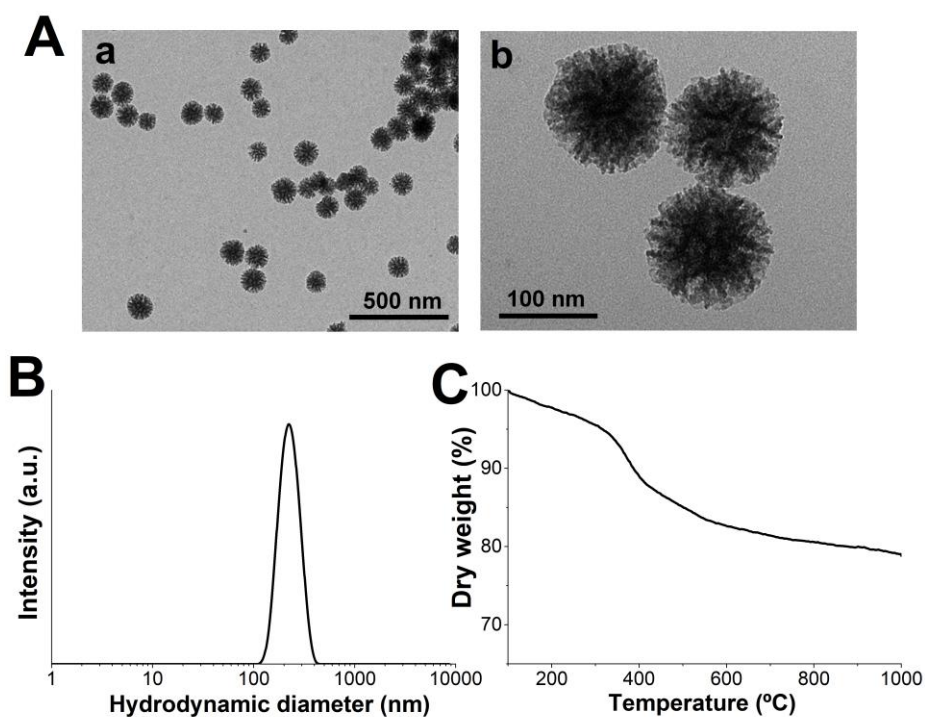


Figure S3. A) TEM images of Rubpy@DMSN-PEG (a and b). B) Intensity PSD DLS curve of Rubpy@DMSN-PEG. Sample measured was suspended in distilled water. Brief spin pulse was applied in order to remove large aggregates or sediments C) Thermogravimetric analysis of Rubpy@DMSN-PEG. The percentage showed are normalised to the dry weight of each sample, which is taken at 100 °C.

According to DMSN-PEG TG curve, the percentage of organic matter coming from $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ is 5.2% (52 μg / mg of nanoparticle).

Reference

1. Devalapally, H.K.; Navath, R.S.; Yenamandra, V.; Akkinapally, R.R.R.; Devarakonda, R.K. β -Galactoside prodrugs of doxorubicin for application in antibody directed enzyme prodrug therapy/prodrug monotherapy, *Arch. Pharm. Res.* **2007**, *30*, 723–732, doi: 10.1007/BF02977634.