

# A Scalable Method For Squalenoylation And Assembly Of Multifunctional $^{64}\text{Cu}$ -Labeled Squalenoylated Gemcitabine Nanoparticles

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## Supplemental Materials

**Figure S1.** NMR of TBDMS-Gem (7).

**Figure S2.** ESI-MS of TBDMS-Gem (7) squalenoylated TBDMS-protected gemcitabine, and SqGem (8)

**Figure S3.** NMR of SqGem (8).

**Figure S4.** SqGem-CholPEG and SqGem-CholPEG-Chol NPs size and morphology.

**Figure S5.** Morphology of SqGem-CholPEG and SqGem-CholPEG-Chol NPs after cooling.

**Figure S6.** Efficacy of SqGem-CholPEG NPs compared to gemcitabine after 24h incubation.

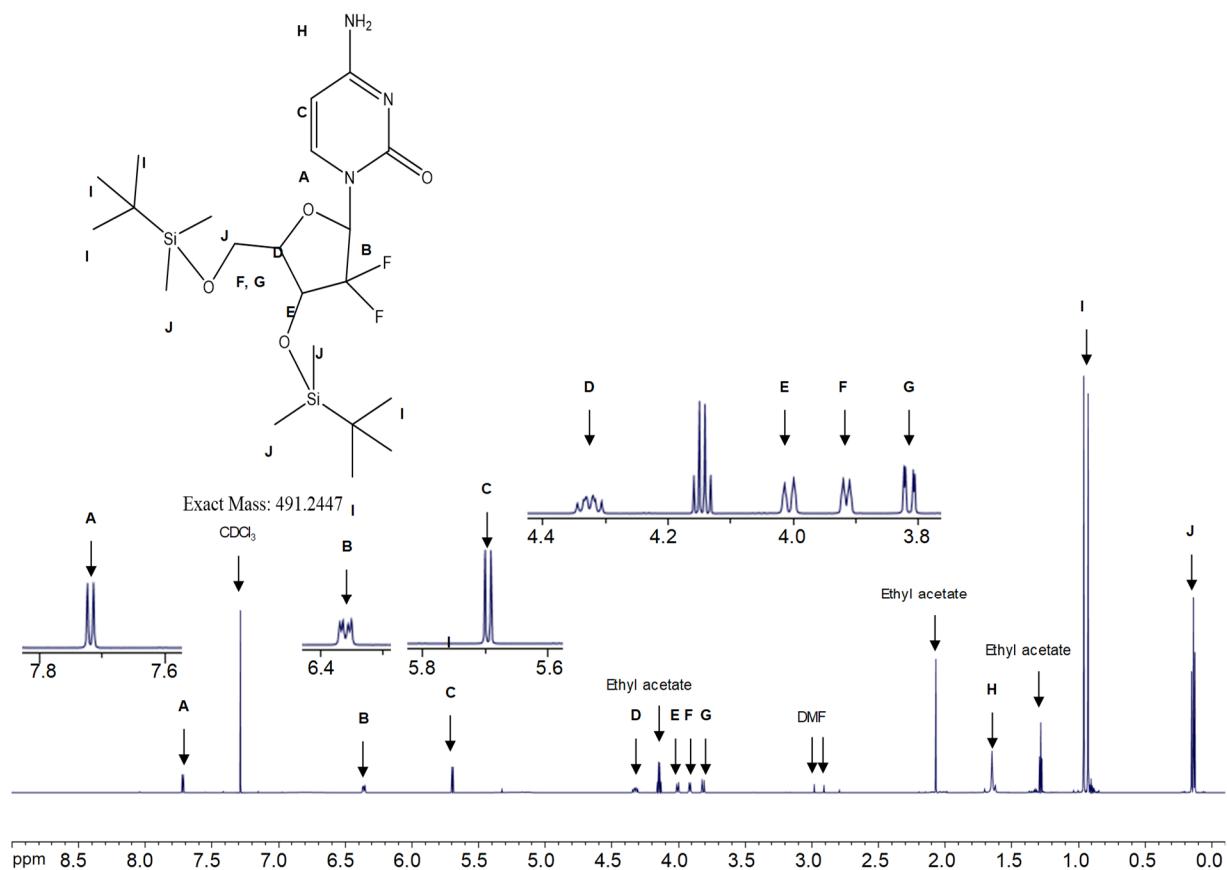
**Figure S7.** NMR of SqNHS (8).

**Figure S8.** MALDI of SqNHS (8).

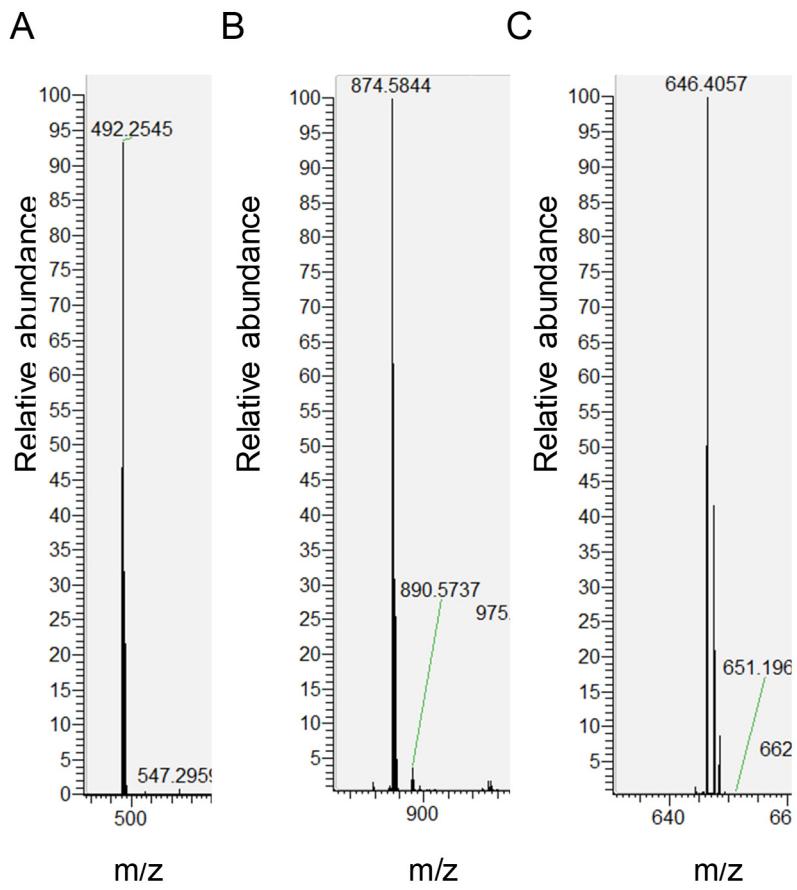
**Figure S9.** MALDI of SqNOTA (10).

**Table S1.** Biodistribution of SqNOTA and SqNOTA-SqGem NPs after 4h of circulation.

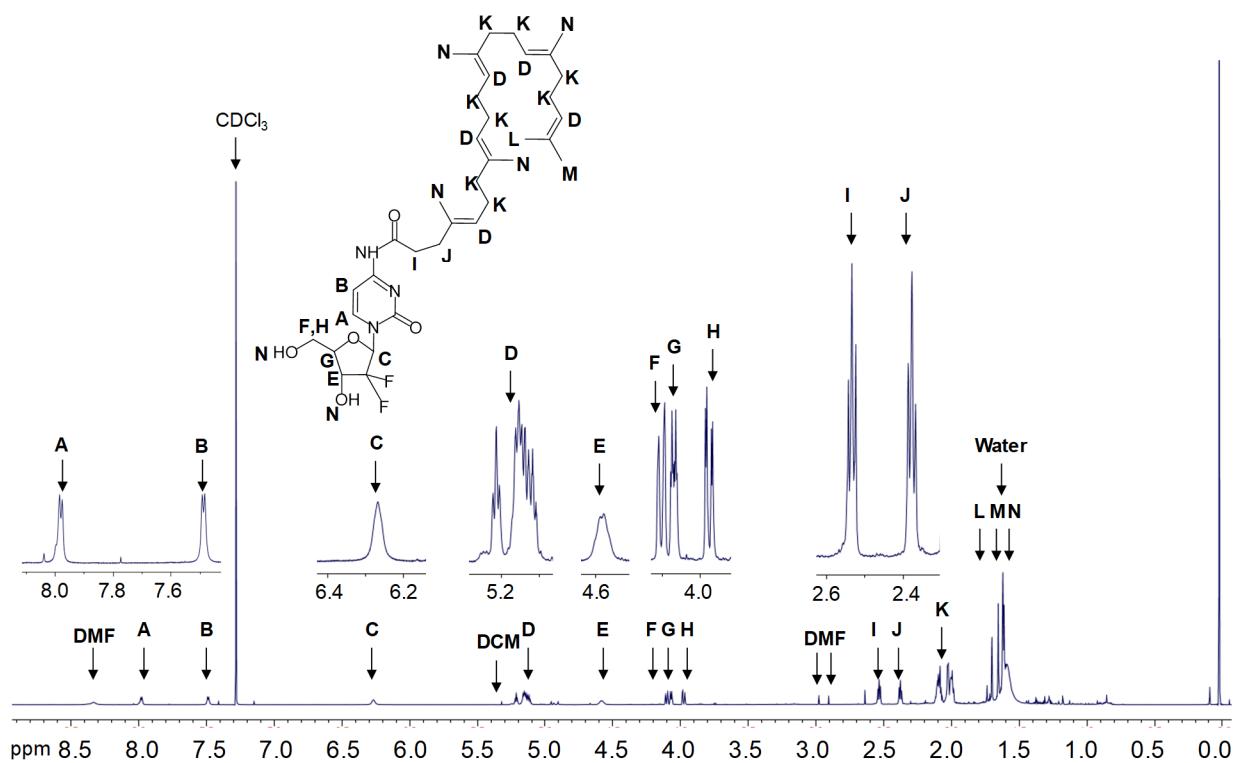
**Table S2.** Pharmacokinetics of SqNOTA and SqNOTA-SqGem NPs after 4h of circulation.



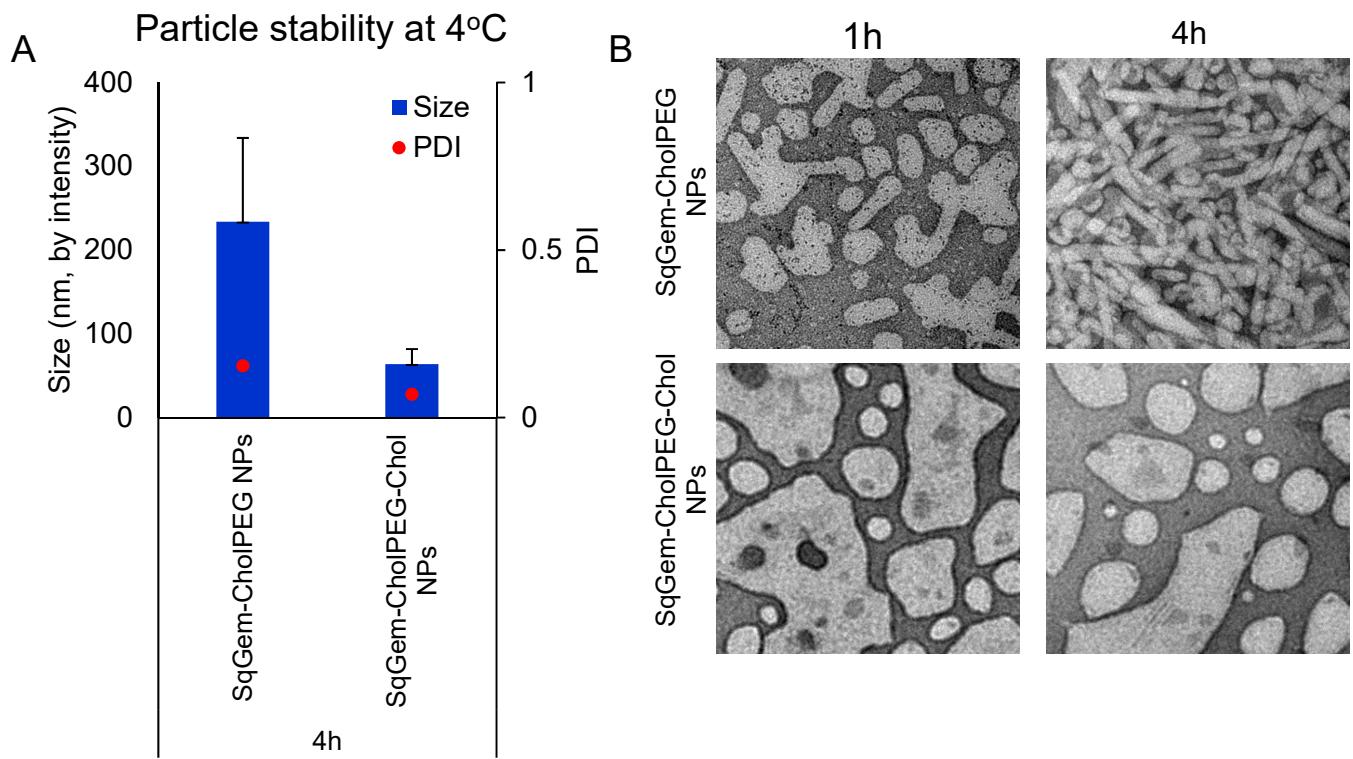
**Figure S1.** NMR of TBDMS-Gem (**7**) in  $\text{CDCl}_3$  at 800 MHz.



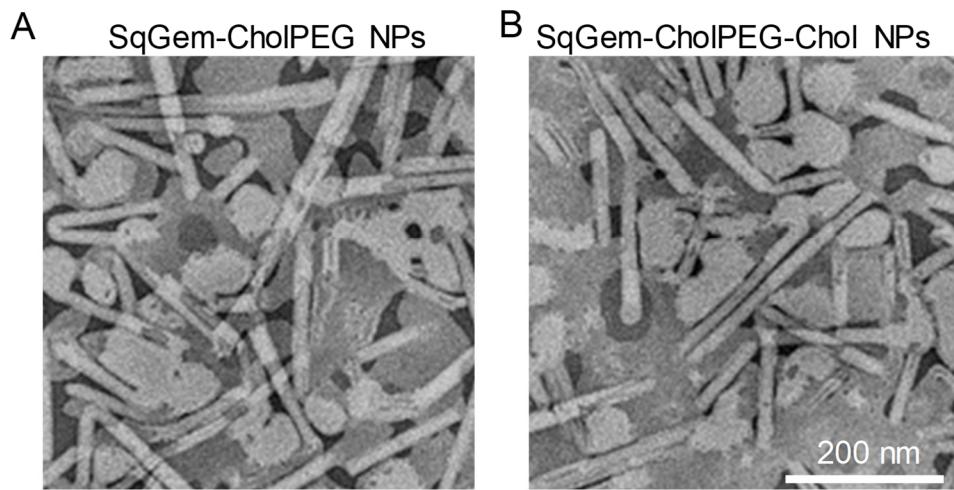
**Figure S2.** ESI-MS of TBDMS-protected gemcitabine (**7**) with an exact mass of 491.24 (A), squalenoylated TBDMS-protected gemcitabine (**8**) with an exact mass of 873.57 (B), and of SqGem (**9**) with an exact mass of 645.41 (C).



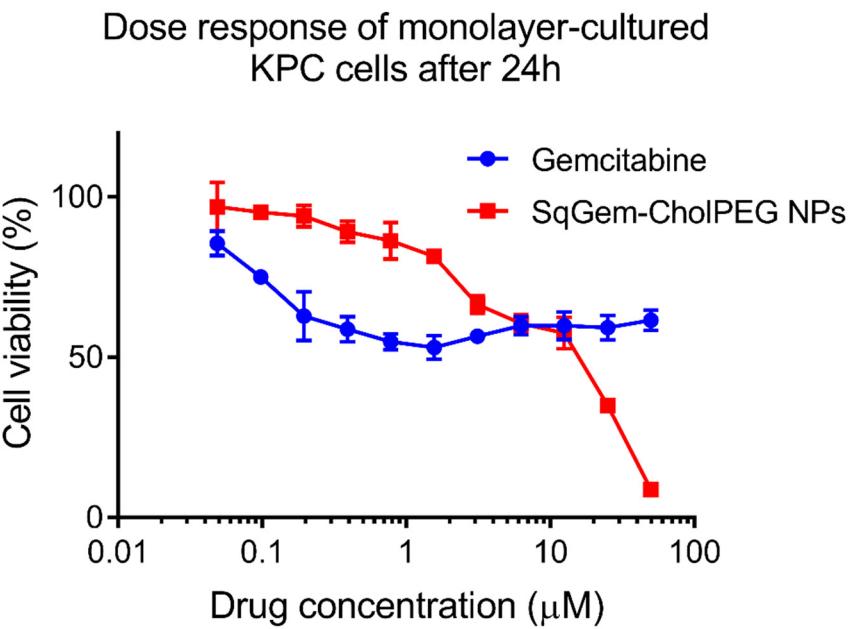
**Figure S3.** NMR of SqGem (**8**) in  $\text{CDCl}_3$  at 800 MHz.



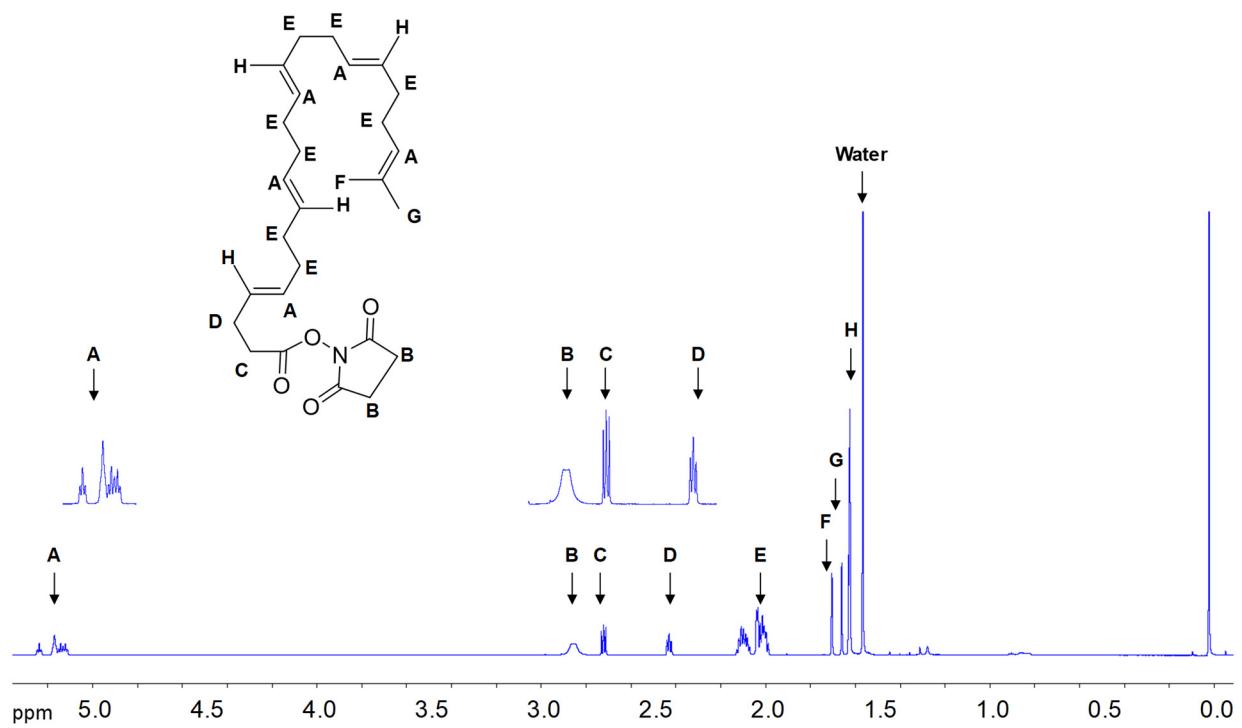
**Figure S4.** SqGem-CholPEG and SqGem-CholPEG-Chol NPs size and morphology. SqGem-CholPEG NPs with and without cholesterol (Chol) were evaluated at 4°C using dynamic light scatter to measure size and polydispersity index (PDI) (A) and transmission electron microscopy (B).



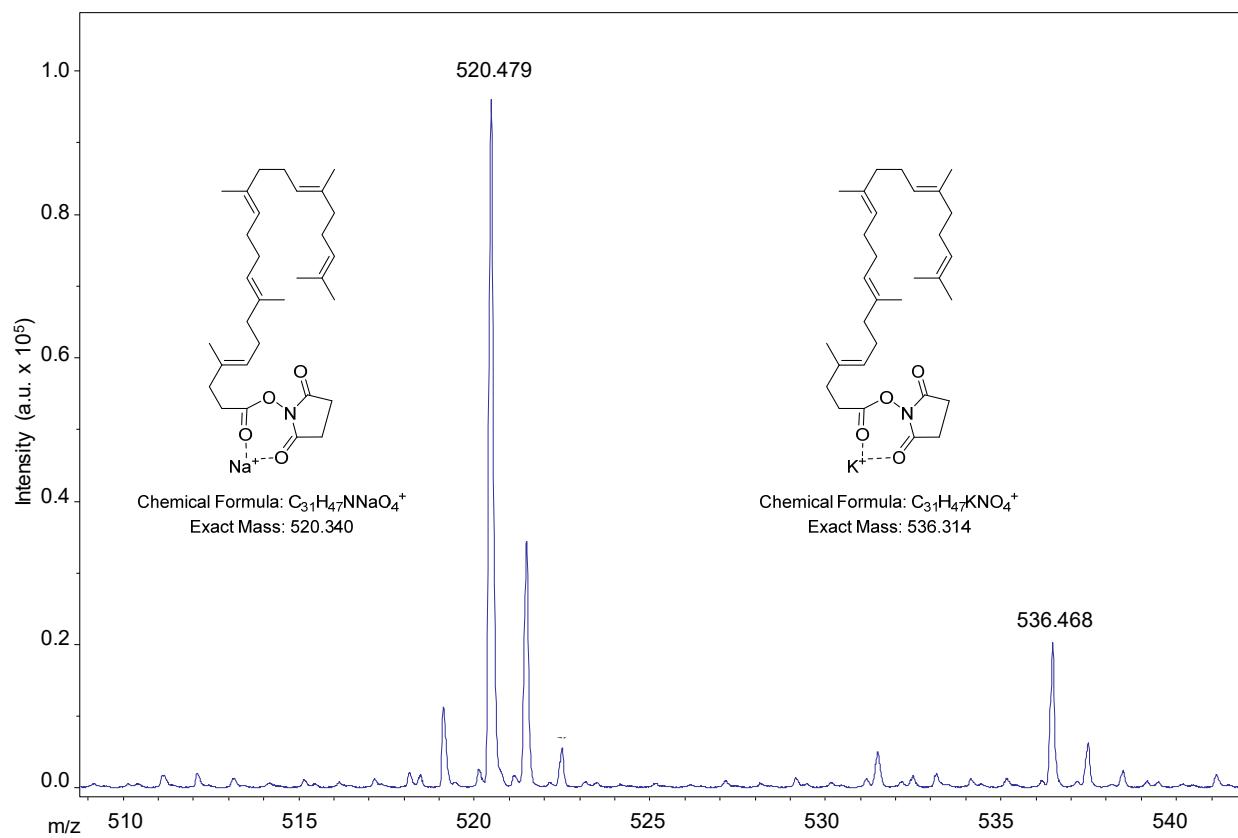
**Figure S5.** Morphology of SqGem-CholPEG and SqGem-CholPEG-Chol NPs after cooling.  
Transmission electron microscopy images of SqGem-CholPEG NPs with (A) and without 20% Chol (B)  
were kept at 4°C for 4h and then warmed to room temperature.



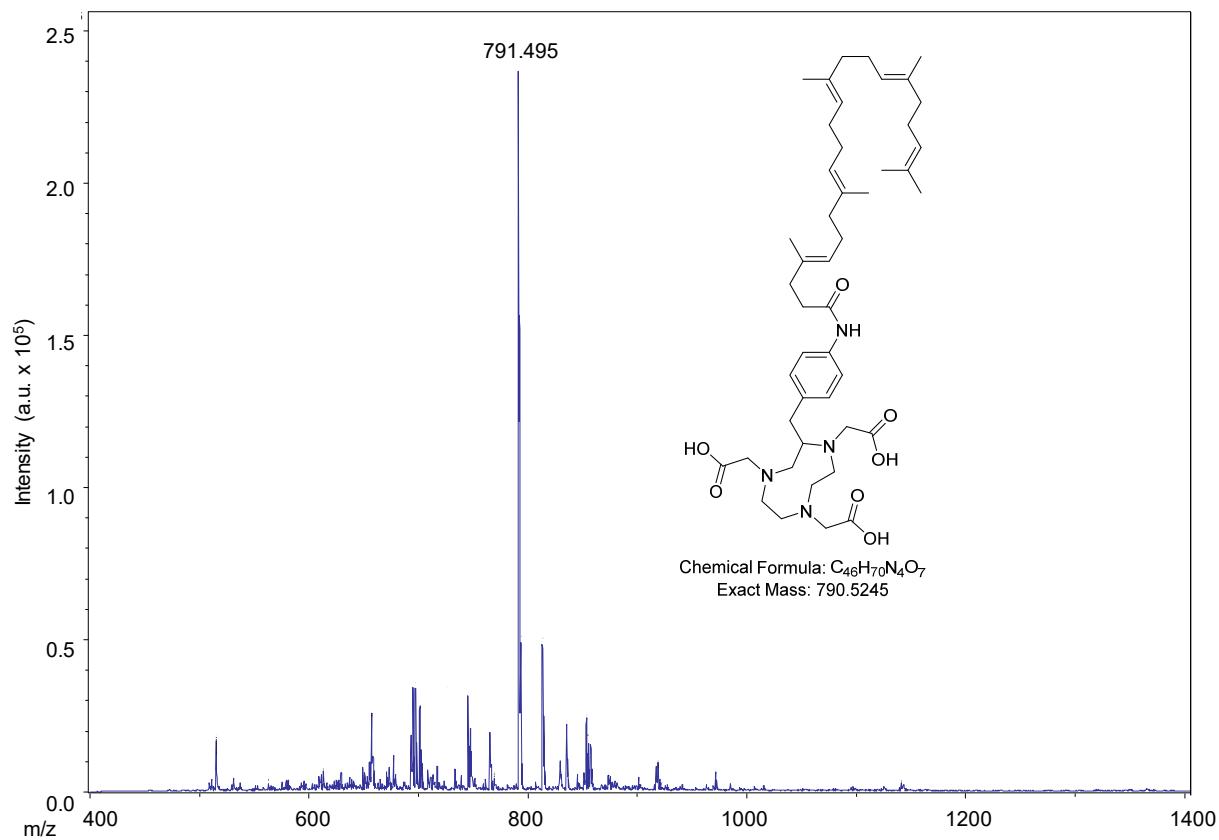
**Figure S6.** Efficacy of SqGem-CholPEG NPs compared to gemcitabine. Dose response curves of SqGem-CholPEG NPs and gemcitabine in monolayer cultured KPC cells after 24h continuous drug incubation.



**Figure S7.** NMR of SqNHS (**8**) in  $\text{CDCl}_3$  at 800 MHz.



**Figure S8.** MALDI of SqNHS (**8**).



**Figure S9.** MALDI of SqNOTA (**10**).

	SqNOTA		SqNOTA-SqGem NPs		
	Average	Standard Deviation	Average	Standard Deviation	P value
<b>Blood</b>	0.500	0.092	1.554	0.073	0.0001 ****
<b>Urine</b>	2.735	1.587	1.991	0.130	0.4185 n.s.
<b>Heart</b>	0.304	0.026	0.978	0.076	0.0001 ***
<b>Lungs</b>	0.904	0.209	2.187	0.565	0.0145 *
<b>Liver</b>	11.315	2.059	13.0641	1.046	0.1973 n.s.
<b>Spleen</b>	1.207	0.180	4.026	0.432	0.0003 ***
<b>Pancreas</b>	0.314	0.056	0.731	0.064	<0.0001 ****
<b>Kidneys</b>	1.791	0.440	2.647	0.186	0.0227 *
<b>Stomach</b>	0.928	0.410	1.293	0.592	0.2831 n.s.
<b>Intestine</b>	8.946	3.399	7.136	2.137	0.4083 n.s.
<b>Muscle</b>	0.122	0.020	0.206	0.022	0.0013 **
<b>Bone</b>	0.272	0.036	0.485	0.062	0.0022 *
<b>Brain</b>	0.296	0.045	0.337	0.026	0.1746 n.s.
<b>Whole body (%ID)</b>	52.84	1.418	72.92	3.444	0.0004 ***

\* p< 0.05, \*\* p < 0.01, \*\*\* p < 0.005, \*\*\*\* p<0.0001

**Table S1.** Biodistribution of SqNOTA and SqNOTA-SqGem NPs after 4h of circulation. Unit is percent injected dose per gram unless otherwise noted. Statistical analysis by multiple comparisons.

	SqNOTA		SqNOTA-SqGem NPs		
Time (minutes)	Average	Standard Deviation	Average	Standard Deviation	P value
<b>2.5</b>	35.851	1.843	44.809	6.310	0.0608 n.s.
<b>3.5</b>	33.699	4.423	39.543	4.171	0.1031 n.s.
<b>4.5</b>	33.344	4.374	35.972	3.731	0.3967 n.s.
<b>7.5</b>	28.870	3.046	33.600	3.689	0.0970 n.s.
<b>12.5</b>	24.557	3.123	29.072	2.212	0.0610 n.s.
<b>17.5</b>	21.372	4.081	26.377	2.222	0.0881 n.s.
<b>25</b>	17.264	3.446	22.310	1.495	0.0536 n.s.
<b>240</b>	0.572	0.278	1.839	0.260	0.0006 ***

\* p<0.05, \*\* p < 0.01, \*\*\* p < 0.005, \*\*\*\* p<0.0001

**Table S2.** Pharmacokinetics of SqNOTA and SqNOTA-SqGem NPs after 4h of circulation. Unit is percent injected dose per cubic centimeter. Statistical analysis by multiple comparisons.