Supporting Information

A neutral lipid envelope-type nanoparticle composed of a pH-activated and vitamin E-scaffold lipid-like material as a platform for a gene carrier targeting renal cell carcinoma

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S1 Synthesis of Palms

S1.1. General Procedures

All reagents were obtained from commercial sources and were used without further purification. Thin layer chromatography was performed on Merck TLC plates silica gel 60. $^1$H-NMR spectrum was recorded on JEOL ECP400 ($^1$H 400MHz) or ECA600 ($^1$H 600MHz) spectrometer.

S1.2. Synthesis of ssPalm$_M$

ssPalm$_M$ was synthesized according to the method outlined in Scheme S1.2.

$$\text{HO-SS-SS-OH} \xrightarrow{\text{MsCl, TEA, acetonitrile}} \text{MsO-SS-SS-O} \xrightarrow{\text{K$_2$CO$_3$, acetonitrile}} \text{OH}$$

1 \text{ 97%}

$$\text{HO-SS-SS-NH-OH} \xrightarrow{\text{R:C$_{15}$H$_{37}$, DIC, DMAP, TEA, dichloromethane}} \text{OH}$$

2 \text{ 91%}

$$\text{R-O-SS-SS-NH-O} \xrightarrow{} \text{R-O-SS-SS-NH-O}$$

3 \text{ 15%}

Scheme S1.2 Synthesis of ssPalm$_M$ (compound 3)

S1.2. Synthesis of Palm$_M$

<mesylation>

2,2'-Dithiodiethanol (15 g, 97.2 mmol) was dissolved in acetonitrile (143 ml) at 20-25°C.
Triethylamine (33.8 g, 328 mmol) was added to the solution, which was then stirred at 20-25°C for 5 minutes. Methanesulfonyl chloride (34.5 g, 300 mmol) was then added at 0-5 °C, and stirring was continued at 20-25°C for 3 hours. After confirming the complete consumption of 2,2’-dithiodiethanol by thin layer chromatography (TLC), the reaction was quenched by the addition of ethanol (29 mL) and insoluble matter was removed by filtration. Dichloromethane (150 ml) and 10 wt% sodium hydrogen carbonate aqueous solution (150 g) were added to the filtrate, and it was stirred for 5 minutes. After standing for 10 minutes, the aqueous layer was removed. The organic layer was washed with water (4×150 g), dried over sodium sulfate, filtered and evaporated under vacuum to yield the crude dimesyl compound (29.4 g).

<amination>

The crude dimesyl compound (5.0 g, 16 mmol) was dissolved in acetonitrile (127 ml) at 40 °C. Potassium carbonate (5.5 g, 39.8 mmol) was added to the solution and it was stirred at 25 °C for 5 minutes. A solution of 3-(methylamino)-1-propanol (7.2 g, 80.8 mmol) was prepared in acetonitrile (9.2 ml) at 25 °C. This solution was dropped in the acetonitrile solution of the dimesyl compound over a period of 1.5 hours. After confirming the complete consumption of the dimesyl compound by TLC, the reaction solution was filtered and evaporated under vacuum. The residue (13.2 g) was dissolved in chloroform (132 ml), and it was washed with 10 wt% sodium chloride aqueous solution (5×132 ml). The organic layer was evaporated under vacuum to yield the diamino compound (4.3 g).

<acylation>

Myristic acid (1.5 g, 6.7 mmol) was dissolved in dichloromethane (5.8 ml). 4-Dimethylamino pyridine (0.082 g, 0.67 mmol) and triethylamine (0.68 g, 6.7 mmol) were added to the solution at 0-5 °C. The diamino compound (1 g, 3.4 mmol) and diisopropylcarbodiimide (1.7 g, 13.5 mmol) were then dissolved in dichloromethane (5.8 ml) at 25 °C. This solution was dropped in the dichloromethane solution of myristic acid over a 1 hour period. After confirming the complete
consumption of the diamino compound by TLC, the reaction solution was filtered and evaporated under vacuum. The residue (7.2 g) was dissolved in acetonitrile (38 ml), and the solution was cooled (10 °C) to crystallize the desired compound. It was stirred for 1 hour at 10 °C, and filtered. After repeating the same process of crystallization two additional times, the crystal was dissolved in hexane (45 ml). The hexane solution was washed with acetonitrile (6×38 ml), and evaporated under vacuum to yield SSPalmM compound (0.7 g). 1H-NMR (600 MHz, CDCl3); δ0.85-0.90 (t, CH3-CH2-, 6H); δ1.22-1.35 (m, CH3-(CH2)10-, 40H); δ2.67-2.70 (q, -N(CH3)-CH2-CH2-S-, 4H); δ2.78-2.82 (q, -N(CH3)-CH2-CH2-S-, 4H); δ4.10-4.13 (t, -CH2-C(O)-O-CH2-, 4H)
S1.3 Synthesis of \( \text{SSPalm}_A \)

\( \text{SSPalm}_A \) was synthesized according to the method outlined in Scheme S1.3.

\[
\begin{align*}
\text{HO} & \quad \text{S} & \quad \text{S} & \quad \text{HO} \\
\rightarrow & \quad \text{MsCl} & \quad \text{TEA, acetonitrile} & \quad \text{MsO} & \quad \text{S} & \quad \text{S} & \quad \text{O} & \quad \text{Ms} \\
& & & \quad 1 & \quad 97\% \\
\text{HO} & \quad \text{N} & \quad \text{S} & \quad \text{S} & \quad \text{N} & \quad \text{HO} \\
\rightarrow & \quad \text{K}_2\text{CO}_3, \text{acetonitrile} & \quad \text{EDC, DMAP} & \quad \text{chloroform} & \quad \text{N} & \quad \text{H} & \quad \text{O} & \quad \text{H} & \quad \text{O} & \quad \text{N} & \quad \text{S} & \quad \text{O} & \quad \text{N} & \quad \text{S} & \quad \text{O} \\
& & & \quad 2 & \quad 91\% \\
\text{all trans-retinoic acid} & \quad \text{Compound 2 (0.40 g, 1.3 mmol)} & \quad \text{all trans-retinoic acid} & \quad (0.97 g, 3.2 mmol) & \quad \text{were dissolved in} & \quad \text{hydrogen chloride (6.0 g).} & \quad 4-\text{dimethylamino pyridine (0.070 g, 0.54 mmol) and} & \quad \text{TEA, acetonitrile} \\
& & & & \quad \text{acetonitrile} & \quad \text{K}_2\text{CO}_3, \text{acetonitrile} & \quad \text{EDC, DMAP} & \quad \text{chloroform} & \quad \text{hydrogen chloride (6.0 g).} & \quad 4-\text{dimethylamino pyridine (0.070 g, 0.54 mmol) and}
\end{align*}
\]

Scheme S1.3 Synthesis of \( \text{SSPalm}_A \) (compound 4)

S1.3 Synthesis of \( \text{SSPalm}_A \)

Mesylation and amination are performed as mentioned above.

<acylation>

Compound 2 (0.40 g, 1.3 mmol) and all trans-retinoic acid (0.97 g, 3.2 mmol) were dissolved in chloroform (6.0 g). 4-Dimethylamino pyridine (0.070 g, 0.54 mmol) and
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.78 g, 4.1 mmol) were added to the solution. The solution was stirred for 5 hours at 22-28 °C. After confirming the complete consumption of compound 2 by TLC, the reaction solution was filtered and evaporated under vacuum. The residue was purified by silica gel column chromatography with chloroform : methanol 100:0 - 95:5 (v:v) mixture as eluent, so that _SSPalm_A_ (0.42 g, 0.48 mmol) was obtained. ^1^H-NMR (600MHz, CDCl\textsubscript{3}); δ1.00-1.10 (s, -C(CH\textsubscript{3})\textsubscript{2}, 12H); δ1.45-1.50 (t, -C(CH\textsubscript{3})\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-, 4H); δ1.55-1.65 (m, -CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-, 4H); δ1.70-1.75 (s, -CH\textsubscript{2}-C(CH\textsubscript{3})=C-, 6H); δ1.80-1.90 (m, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-O-C(O)-, 4H); δ1.95-2.05 (m, -CH\textsubscript{2}-CH\textsubscript{2}-C(CH\textsubscript{3})=C-, =CH-C(CH\textsubscript{3})=CH-, 10H); δ2.20-2.30 (s, -N(CH\textsubscript{3})-, 6H); δ2.30-2.40 (s, -C(CH\textsubscript{3})=CH-C(O)-O-, 6H); δ2.45-2.55 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-O-C(O)-, 4H); δ2.65-2.75 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-S-, 4H); δ4.10-4.25 (d, -CH=CH-C(CH\textsubscript{3})=CH-CH=CH-C(CH\textsubscript{3})=, d, -CH=CH-C(CH\textsubscript{3})=CH-CH=CH-C(CH\textsubscript{3})=, 4H) ; δ6.10-6.20 (d, -CH=CH-C(CH\textsubscript{3})=CH-CH=CH-C(CH\textsubscript{3})=, 4H); δ6.20-6.35 (d, -CH=CH-C(CH\textsubscript{3})=CH-CH=CH-C(CH\textsubscript{3})=, 4H); δ6.95-7.05 (m, -C(CH\textsubscript{3})=CH-CH=CH-C(CH\textsubscript{3})=CH-, 2H)
**S1.4 Synthesis of ssPalmE**

ssPalmE was synthesized according to the method outlined in Scheme S1.4.

![Scheme S1.4 Synthesis of ssPalmE](image)

Scheme S1.4 Synthesis of ssPalmE (compound 5)
S1.4 Synthesis of \textsubscript{ssPalm}\textsubscript{E}

Mesylation and amination are performed as mentioned above.

\textless acylation\textgreater

Compound 2 (3.0 g, 10 mmol) and D-\textalpha-tocopherol succinate (12 g, 22 mmol) were dissolved in chloroform (45 g). 4-Dimethylamino pyridine (0.49 g, 4.0 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (5.4 g, 28 mmol) were added to the solution. The solution was stirred for 5 hours at 22-28 \degree C. After confirming the complete consumption of compound 2 by TLC, the reaction solution was evaporated under vacuum. The residue was purified by silica gel column chromatography with chloroform : methanol 100:0 - 95:5 (v:v) mixture as eluent, so that ssPalm\textsubscript{E} (6.0 g, 4.7 mmol) was obtained. \textsuperscript{1}H-NMR (600MHz, CDCl\textsubscript{3}); \delta 0.80-0.90 (d, (CH\textsubscript{3})\textsubscript{2}CH-, d, -CH\textsubscript{2}-CH(CH\textsubscript{3})\textsubscript{2}-CH\textsubscript{2} 24H); \delta 1.00-1.85 (m, -CH\textsubscript{2}-CH\textsubscript{2}-CH(CH\textsubscript{3})\textsubscript{2}-, -CH\textsubscript{2}-C\textsubscript{H}(CH\textsubscript{3})\textsubscript{2}-CH\textsubscript{2}-CH(\textsubscript{3})-CH\textsubscript{2}-, CH\textsubscript{3}-C(CH\textsubscript{2})\textsubscript{2}-O-, -CH\textsubscript{2}-CH\textsubscript{2}-C(CH\textsubscript{2})\textsubscript{2}-O-, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-O-C(O)-, 56H); \delta 1.95-2.10 (s, Ar-C\textsubscript{H}\textsubscript{3}, 18H); \delta 2.20-2.30 (s, -N(CH\textsubscript{3})\textsubscript{-}, 6H); \delta 2.40-2.50 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-O-C(O)-, 4H); \delta 2.55-2.60 (t, Ar-CH\textsubscript{2}-CH\textsubscript{2}-, 4H); \delta 2.60-2.70 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-S-, 4H); \delta 2.70-2.85 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-S-, t, Ar-O-C(O)-CH\textsubscript{2}-CH\textsubscript{2}-C(O)-, 8H); \delta 2.85-3.00 (t, Ar-O-C(O)-CH\textsubscript{2}-CH\textsubscript{2}-C(O)-, 4H); \delta 4.10-4.25 (t, -N(CH\textsubscript{3})-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-O-C(O)-, 4H)