# CATIONIC LIPIDS FOR LIPID NANOPARTICLE DELIVERY OF THERAPEUTICS TO HEPATIC STELLATE CELLS

## CROSS-REFERENCE TO RELATED APPLICATION(S)

This patent application claims the benefit of priority of U.S. application serial No. 62/975,116, filed February 11, 2020, which application is herein incorporated by reference.

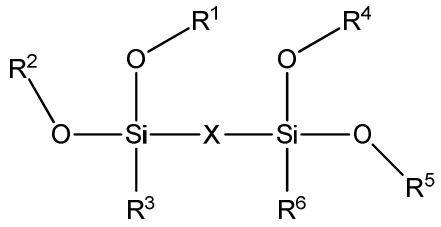
## BACKGROUND

Cationic lipid-containing nanoparticles have been used for the delivery of a variety of therapeutic agents. Currently, the difficulty and cost associated with the preparation of the cationic lipid component of these nanoparticles can limit their attractiveness for commercial development as delivery vehicles. Accordingly, there is a need for additional cationic lipids that can be incorporated into lipid nanoparticles. For example, there is a need for cationic lipids that can be prepared using less expensive and more efficient processes. There is also a need for cationic lipids that are relatively more biodegradable, such that the related lipid nanoparticles better degrade following delivery of an active agent or a therapeutic agent. There is also a need for lipid nanoparticle with characteristics for delivering therapeutic agents to hepatic stellate cells for the treatment, *e.g.*, of liver fibrosis.

## SUMMARY

The disclosure provides cationic lipids that are effective for delivering an active agent or therapeutic agent, such as a nucleic acid, when incorporated into lipid nanoparticles to hepatic stellate cells. The cationic lipids are also biodegradable, such that associated lipid nanoparticles better degrade following delivery of an active agent or a therapeutic agent.

Accordingly, in certain examples, provided herein a compound of formula (I):




(I)

wherein:

$R^1$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^2$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^3$ is $C_1$-$C_4$ alkyl or –O-$C_2$-$C_{30}$ hydrocarbyl;

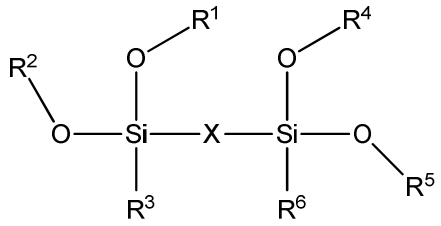
$R^4$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^5$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^6$ is $C_1$-$C_4$ alkyl or –O-$C_2$-$C_{30}$ hydrocarbyl; and

X is a divalent linking group.

In one aspect, the present disclosure refers to a compound of formula (I):




(I)

wherein:

$R^1$ is a $C_5$-$C_{30}$ hydrocarbyl;

$R^2$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^3$ is $C_1$-$C_4$ alkyl or –O-$C_2$-$C_{30}$ hydrocarbyl;

$R^4$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^5$ is a $C_2$-$C_{30}$ hydrocarbyl;

$R^6$ is $C_1$-$C_4$ alkyl or –O-$C_2$-$C_{30}$ hydrocarbyl; and

X is a divalent linking group.

In one aspect, the present disclosure refers to a composition comprising any one of the compounds disclosed herein.

Also provided are lipid particled comprising such compounds, and for methods of using the lipid particles, *e.g.*, to deliver therapeutics specifically to hepatic stellate cells to treat diseases.

In one aspect, the present disclosure refers to a lipid particle comprising any one of the compounds disclosed herein.

In one aspect, the present disclosure refers to a lipid particle disclosed herein for use in the *in vivo* delivery of a therapeutic agent to a mammalian subject.

In one aspect, the present disclosure refers to use of the lipid particle disclosed herein in the manufacture of a medicament for the *in vivo* delivery of a therapeutic agent to a mammalian subject.

In one aspect, the present disclosure refers to the lipid particle disclosed herein for use in treating a disease or disorder in a mammalian subject in need thereof.

In one aspect, the present disclosure refers to use of the lipid particle disclosed herein in the manufacture of a medicament for treating a disease or disorder in a mammalian subject in need thereof, wherein the disease or disorder is liver fibrosis or liver fibrosis associated non-alcoholic steatohepatitis (NASH) or alcoholic steatohepatitis (ASH).

In one aspect, the present disclosure refers to a method of delivering a therapeutic agent to a hepatic stellate cell (HSC), *in vitro*, comprising contacting the HSC with the lipid particle disclosed herein.

In one aspect, the present disclosure refers to a lipid particle disclosed herein for use in delivering a therapeutic agent to a hepatic stellate cell (HSC), *in vivo*, wherein the HSC is to be contacted with the lipid particle disclosed herein.

In one aspect, the present disclosure refers to use of the lipid particle disclosed herein in the manufacture of a medicament for delivering a therapeutic agent to a hepatic stellate cell (HSC), *in vivo*, wherein the HSC is to be contacted with the lipid particle disclosed herein.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Figure 1 depicts reelin mRNA knock-down 48 hours following IV administration of the LNP formulations in Balb/C Mice (n=4). LNP with a representative compound of the invention (presented in columns 3 and 4) has equivalent potency to a benchmark compound (**100**; presented in column 2), but at 4-times lower dose (0.025 mg/kg instead of 0.1 mg/kg).

Figure 2. Figure 2 depicts reelin mRNA knock-down 48 hours following IV administration of the LNP formulations in Balb/C Mice (n=4). An LNP with a representative compound of the invention (presented in column 3) has high specificity for HSCs, whereas an LNP with a benchmark compound (**101**; presented in column 2) designed for delivery to hepatocytes demonstrates no knock-down in HSCs. This comparative data demonstrates that lipids of the invention possess the desired quality of enabling LNP-delivery of therapeutic agents to HSCs.

Figure 3. Figure 3 depicts reelin mRNA knock-down 24 hours following IV administration of the LNP formulations in Balb/C Mice (n=4). LNP with a representative compound of the invention demonstrate that at about 0.03 mg/kg, maximum knock-down in HSCs is achieved.

Figure 4. Figure 4 depicts reelin mRNA knock-down 24 hours following IV administration of the LNP formulations in Balb/C Mice (n=4). A benchmark LNP (with compound **100**; presented in columns 2-4) requires higher doses (~1 mg/kg) to achieve equivalent knock-down to a representative lipid of the invention (presented in columns 5-7) (0.03 mg/kg).

Figure 5A-5B. Figures 5A-5B demonstrate that LNP formulated with a representative lipid of the invention (presented in columns 5-7) demonstrate tolerability as no significant increases in liver enzyme levels over a dose range, up to 3 mg/kg in this study, were evident. LNP with compound **100** are also included as the first set of data in Figure 5A (presented in columns 2-4).

Figure 6. Figure 6 demonstrates that LNP formulated with a representative lipid of the invention and with minimally modified siRNA show good duration of action (presented in the