# NOVEL LIPID:

## ACHIEVING WORLD-CLASS NUCLEIC ACID DELIVERY EFFICIENCY AND SAFETY

**CL4H6** HIGH-PERFORMANCE IONIZABLE LIPID

JSR Life Sciences

Novel lipid: Achieving world-class nucleic acid delivery efficiency and safety

# HIGH-PERFORMANCE IONIZABLE LIPID CL4H6

JSR Corporation has developed a manufacturing technology for the ionizable lipid CL4H6, which was developed in collaboration with Dr. Hideyoshi Harashima and Dr. Yusuke Sato from Hokkaido University. Under the license for testing and research use, we manufacture and sell CL4H6, Lipid nanoparticle (LNPs) formulations using CL4H6 are expected to be used for applications such as cancer treatment and gene editing.

※ Patented by Hokkaido University.

※ A signed Material Transfer Agreement (MTA) with JSR Corporation is required for purchase.

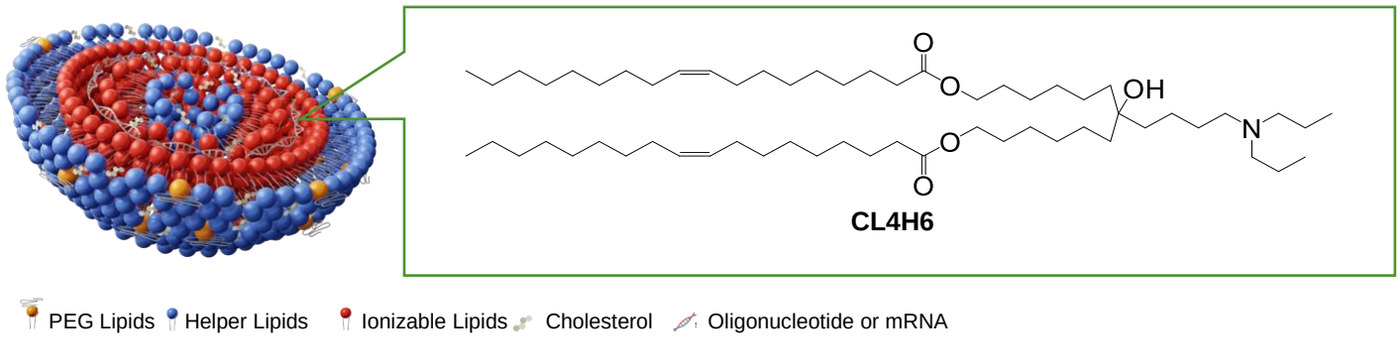
※ For use in pharmaceutical research and development only.

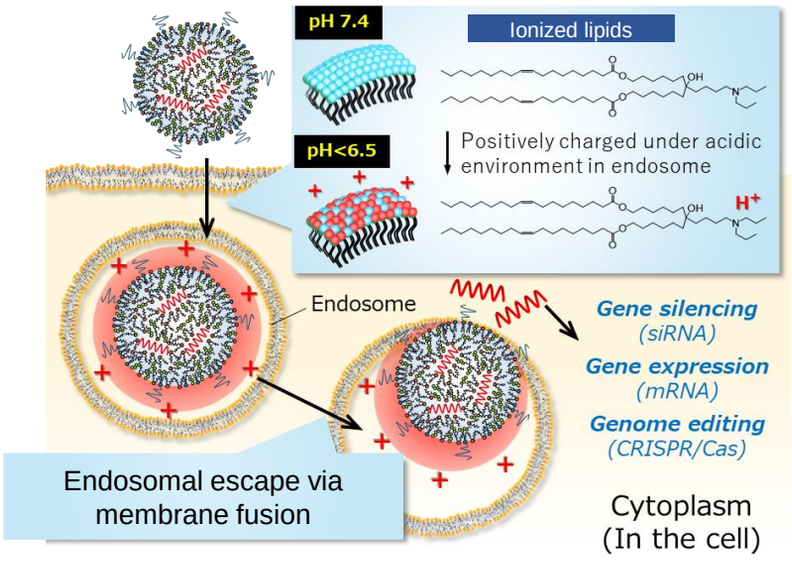
※ A license agreement with Hokkaido University is required for use in pharmaceutical applications.

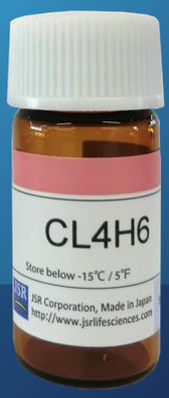
## Ionizable Lipid CL4H6

Ionizable lipids are used in lipid nanoparticle (LNPs) formulations to protect and deliver nucleic acids such as DNA, mRNA, and siRNA to target sites. Encapsulated nucleic acids within LNPs are efficiently delivered to target cells, protected from enzymatic degradation. The delivered payloads are released inside the cells and exert their functions. LNPs have attracted significant attention due to their immense potential in innovative therapies based on nucleic acids.

Lipid nanoparticles using functional lipid CL4H6, developed by Dr. Hideyoshi Harashima and Dr. Yusuke Sato from Hokkaido University, have been reported to demonstrate excellent performance in various applications such as mRNA vaccines, nucleic acid therapeutics, and genome editing in scientific papers and publications.




**Molecular Formula** : $C_{59}H_{113}O_5N$
**Molecular weight** : 916.55
**Physical State** : Liquid (Oil)
**Color** : Colorless or light yellow
**Size** : 50 mg, 100 mg, 1 g

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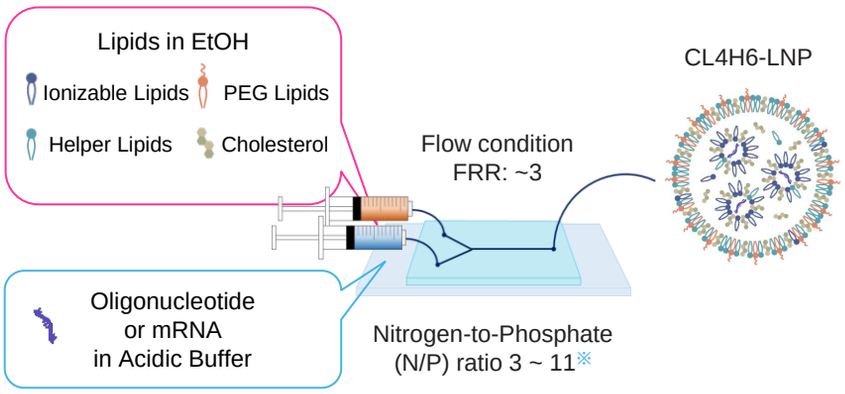
## Typical Conditions for the Preparation of LNPs Formulations Using CL4H6

Prepare ethanol solutions of each lipid (CL4H6, phospholipid, PEG lipid, cholesterol) and an aqueous solution of nucleic acid. Mix the lipid solution and nucleic acid solution using the appropriate equipment (microfluidic device, mixer, etc.). Perform dialysis to remove ethanol. If necessary, perform size selection using filters or other methods.




※ It may vary depending on the type of nucleic acid used.

### Typical buffer for nucleic acid solution

| Buffer | Conc. | pH |
|---|---|---|
| Acetate | 25 mM | 4 |
| Citrate | 10 mM | 3 |

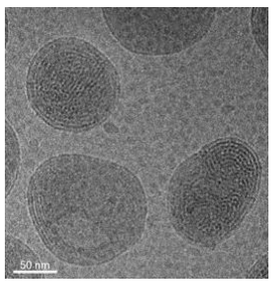
References： Sato Y. Harashima H. et al., *J. Control. Release,* **325**, 235-248 (2020)

## Basic Performance of LNPs Formulation Using CL4H6

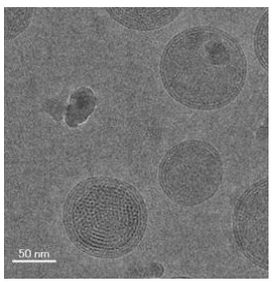
We prepared LNPs encapsulating various types of nucleic acids and evaluated their morphology by Cryo-TEM, applicability to mRNA with various length by encapsulation efficiency, and long-term stability by particle size change over time. From the Cryo-TEM images, we confirmed the formation of LNPs encapsulating siRNA and mRNA. Moreover, high nucleic acid encapsulation efficiency was achieved even for mRNAs with different chain lengths. Furthermore, the particle size of LNPs encapsulating siRNA was confirmed to be stable for a long period of time.

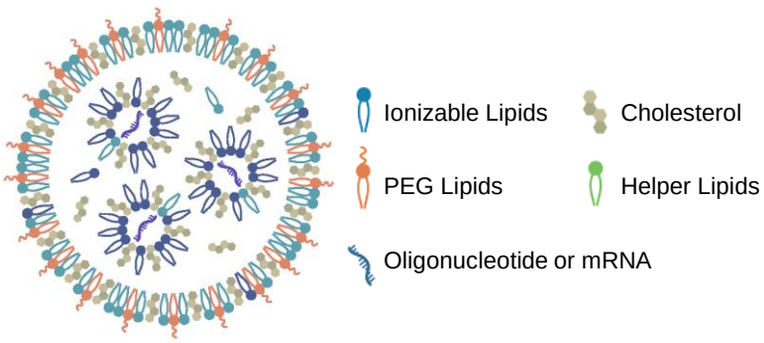
### Cryo-TEM Image of CL4H6-LNPs

FLuc mRNA-LNP (1929nt)




FⅦ siRNA-LNP

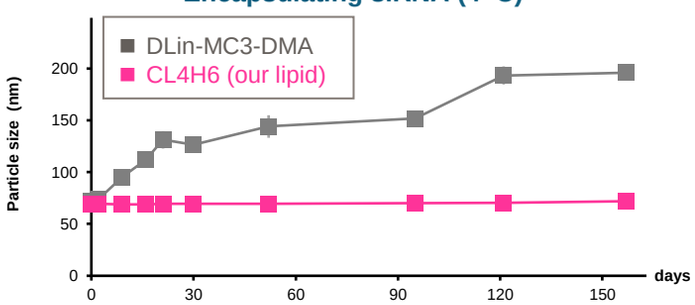



### Encapsulation Efficiency of mRNA with Different Chain Lengths in CL4H6-LNPs

| mRNA | EGFP (996nt) | FLuc (1929nt) | Cas9 (4521nt) |
|---|---|---|---|
| Encapsulation efficiency rate | 93% | 90% | 86% |
| Particle size | 84 nm | 85 nm | 80 nm |

### Time Course Variation of Particle Size in LNPs Encapsulating siRNA (4°C)

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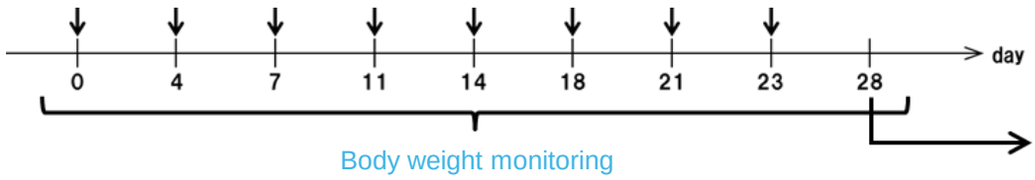
## Toxicity Evaluation of Lipid Nanoparticle (LNPs) Formulations Using CL4H6

CL4H6-LNPs were administered to mice, and body weight monitoring and measurement of hematological parameters were conducted. We confirmed that there were no abnormalities observed in terms of systemic toxicity and hepatotoxicity.

References： Sato Y. Harashima H. et al., *J. Control. Release*, **295**, 140-152 (2019)

### CL4H6-LNP i.v. injections (0.3 or 1.0 mg siRNA/kg/dose)

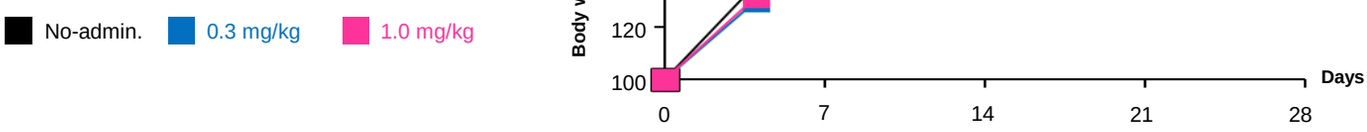



※ 120-400 times greater ED50 compared to CL4H6-LNP

Body weight monitoring

Measurement of hematological parameters

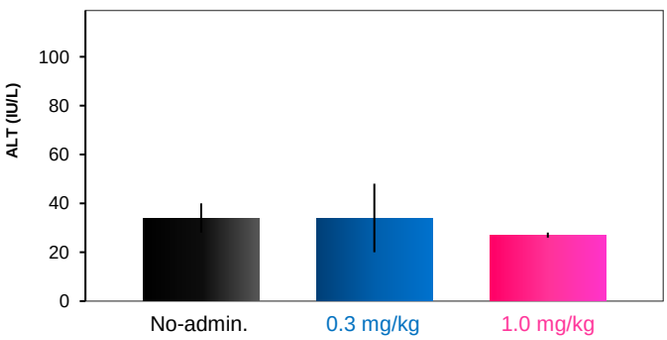
### Systemic Toxicity (Body Weight Monitoring)

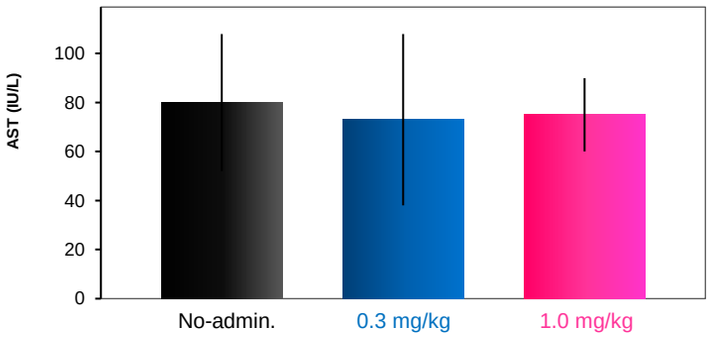






### Hepatotoxicity (Measurement of Hematological Parameters)



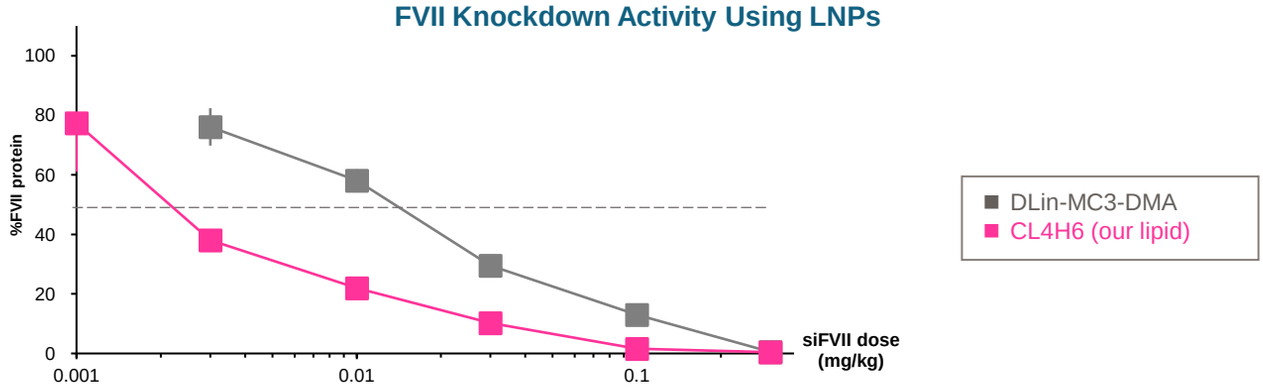




## Expected Applications

We compared the FVII knockdown activity of MC3-LNPs (Dlin-MC3-DMA (Cas RN® 1224606-06-7) containing LNPs) and CL4H6-LNPs containing siRNA for FVII. We confirmed superior knockdown activity of CL4H6-LNPs compared to that of MC3-LNPs. (ED50 0.0025 mg/kg). Furthermore, a series of studies have shown potential applications of CL4H6 in cancer immunotherapy and gene editing.

References： Sato Y. Harashima H. et al., *J. Control. Release*, **295**, 140-152 (2019)

### FVII Knockdown Activity Using LNPs

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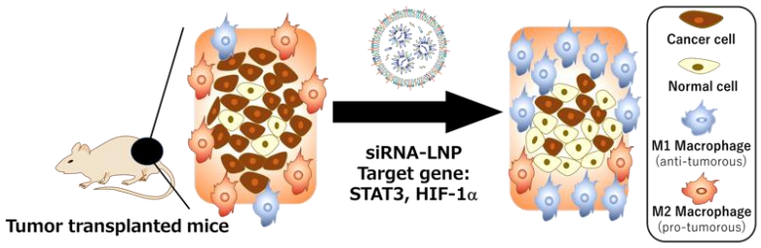
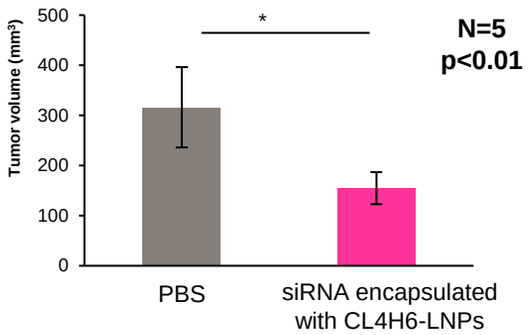
## Cancer Immunotherapy Using CL4H6-encapsulated siRNA LNPs Formulation

Reduction of tumor issue was confirmed.



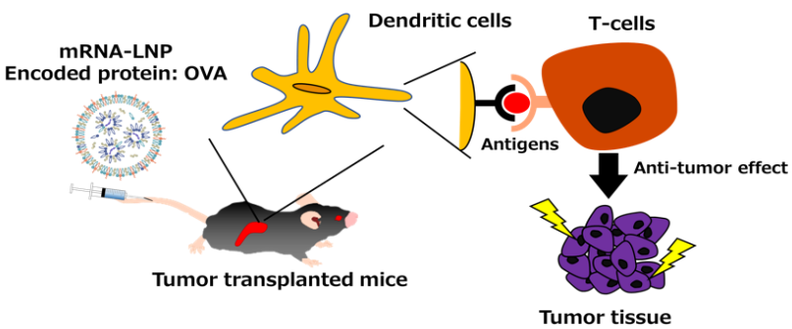

**Tumor tissue size after LNPs administration**



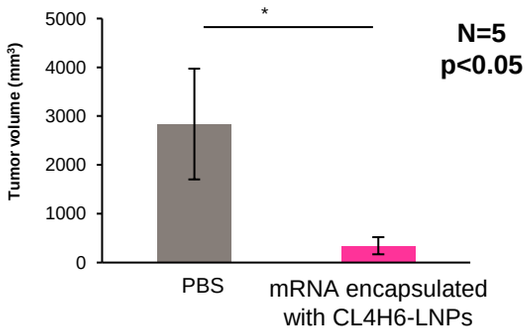

References： Sato Y. Harashima H. et al., *J. Control. Release*, **325**, 235-248 (2020)

## Cancer Immunotherapy Using CL4H6-encapsulated mRNA LNPs Formulation

Reduction of tumor cells was confirmed.



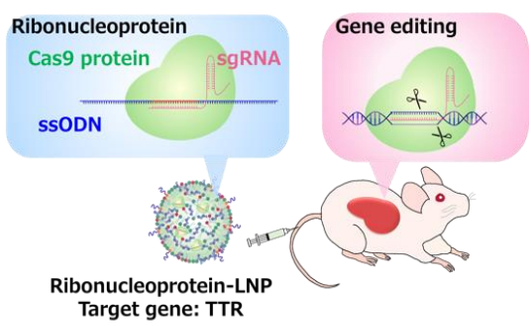

**Tumor tissue size after LNPs administration**



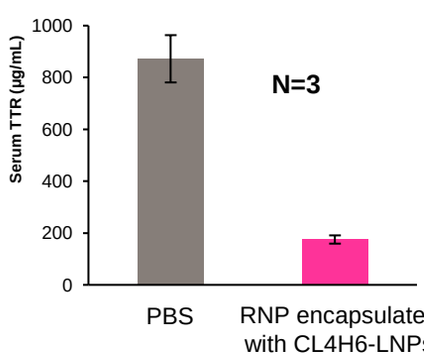

References： Sato Y. Harashima H. et al., *Pharmaceutics,* **14**, 1572 (2022)

## Gene Editing Using CL4H6-encapsulated Ribonucleoprotein (RNP) LNPs Formulation

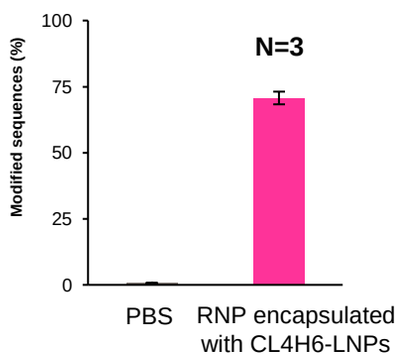
80% decrease in blood TTR concentration and high editing efficiency (70%) were confirmed.




**TTR concentration in blood after LNP administration**




**Editing efficiency of TTR gene in the liver**




References： Sato Y. Harashima H. et al., *J. Control. Release,* **355**, 406-416 (2023)

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## Disclaimer

The information in this brochure is based on publications made by Hokkaido University (*), which holds the patents for this lipid.


***** Sato Y. Harashima H. *et al.*, *J. Control. Release*, **325**, 235-248 (2020)
Sato Y. Harashima H. *et al. J. Control. Release*, **295**, 140-152 (2019)
Sato Y. Harashima H. *et al.*, *Pharmaceutics,* **14**, 1572 (2022)
Sato Y. Harashima H. *et al*, *J. Control. Release*, **355**, 406-416 (2023)


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