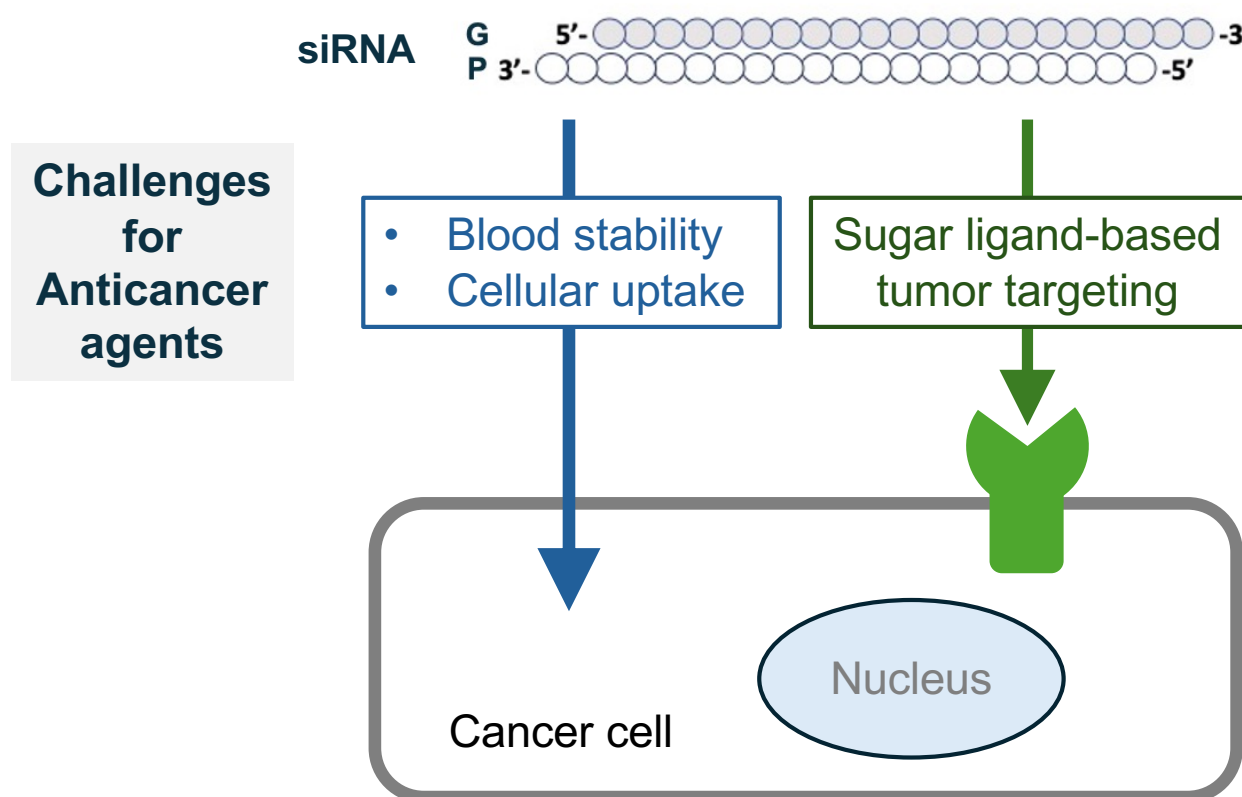


Chemical modifications for siRNA-based anticancer agents

Institute of Microbial Chemistry (Collaboration with Gifu University)



Challenges of siRNA-based anticancer agents

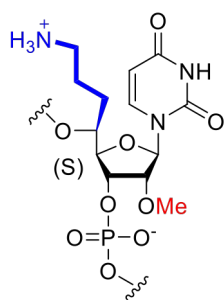
1. Blood stability
2. Intracellular delivery
3. Selective tumor targeting

Our approaches

- 1 **5'-AP-2'-OMe-rN** enhances the serum stability of siRNA
- 2 Carrier free siRNA design with **a novel cholesterol analog**
- 3 CI-M6PR-Mediated Targeted Delivery of **Mannose-6-Phosphate-Conjugated** siRNA

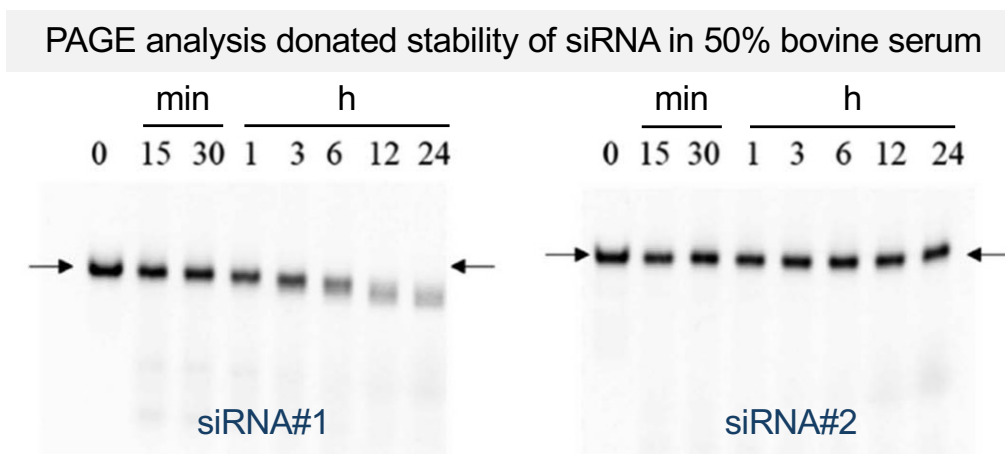
1 Introduction of 5'-(S)-AP-2'-OMe-rN Enhances the Serum Stability of siRNA

PCT/JP2023/018672, WO/2023/224108



5'-(S)-AP-2'-OMe
(S)-5'-C-aminopropyl-2'-O-methylnucleosides

siRNA#1	5'- GGCCU UUCACUACUCC UACUU -3'	Blue: 2'-O-methylnucleosides Red: 5'-(S)-AP-2'-OMe
	3'-U [^] U [^] C [^] C [^] GGAAAGUGAUGAGG AUG -5'-F	
siRNA#2	5'- GGCCU UUCACUACUCC UACUU -3'	
	3'-U [^] U [^] C [^] C [^] GGAAAGUGAUGAGG AUG -5'-F	

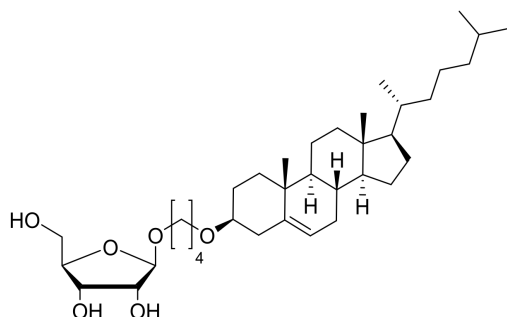


(Figures were reproduced from Ref.1)

(S)-5'-C-aminopropyl-2'-O-methylnucleosides improves the serum stability of therapeutic siRNAs without compromising their RNAi activities.

Ref.1 Kajino, Sakamoto, Ueno. *RSC Adv.*, 2022. **12**. 11454
doi: 10.1039/d2ra00705c

2 Carrier free siRNA design with a novel cholesterol analog

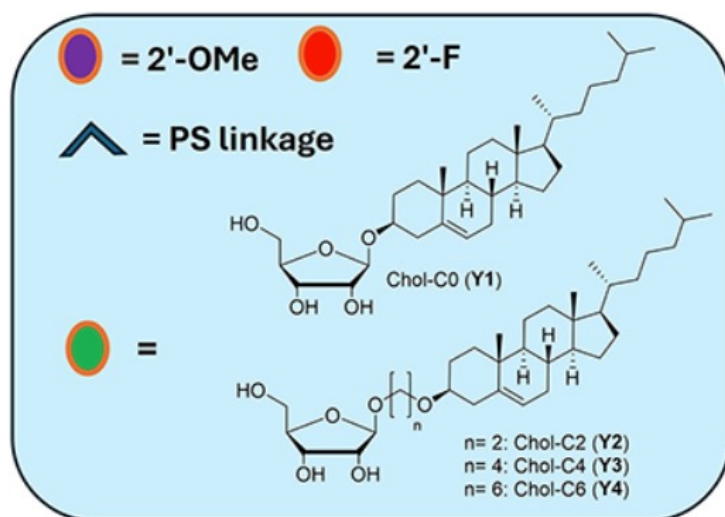
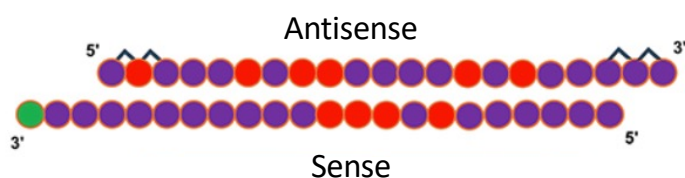


Shimada et al.

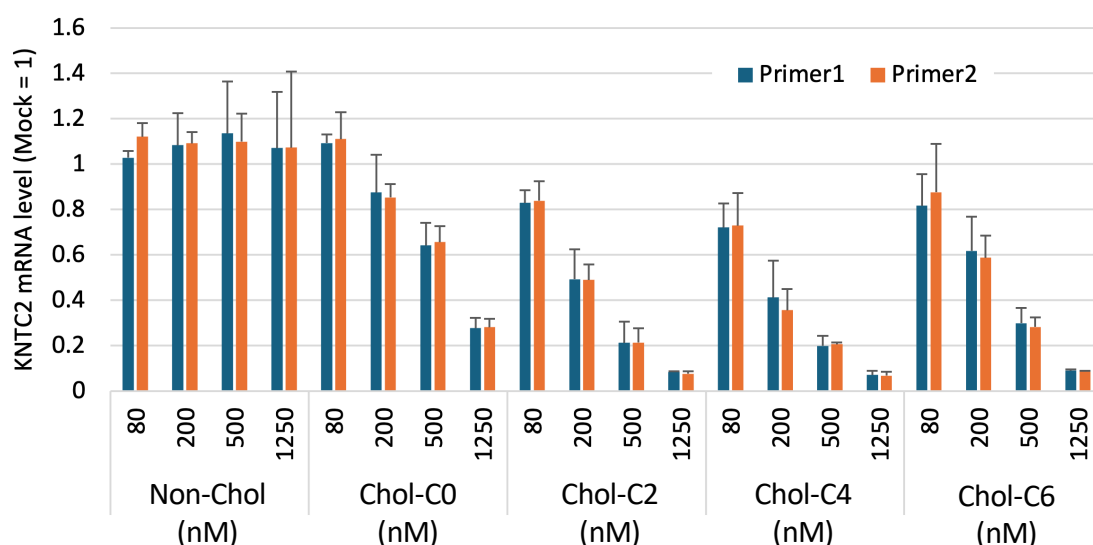
ASC Omega, 2026. **11**. 40426-40440

- Novel 1' –sugar-tethered cholesterol analogs
- Enhanced siRNA duplex stability
- Efficient carrier-free intracellular delivery
- Chol-C2 and Chol-C4 exhibit maximal gene silencing

Carrier free siRNA design
for efficient *KNTC2* gene silencing



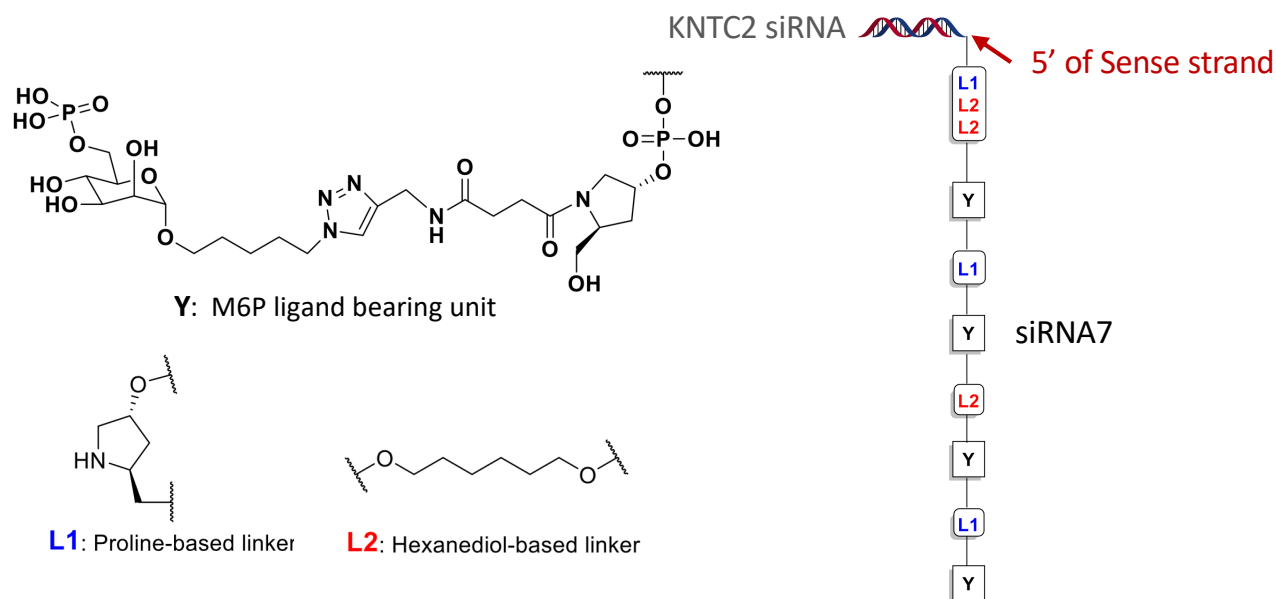
LFA free in
HCT116 cells



(Figures were reproduced from Ref.2)

3 CI-M6PR-Mediated Targeted Delivery of Mannose-6-Phosphate-Conjugated siRNA

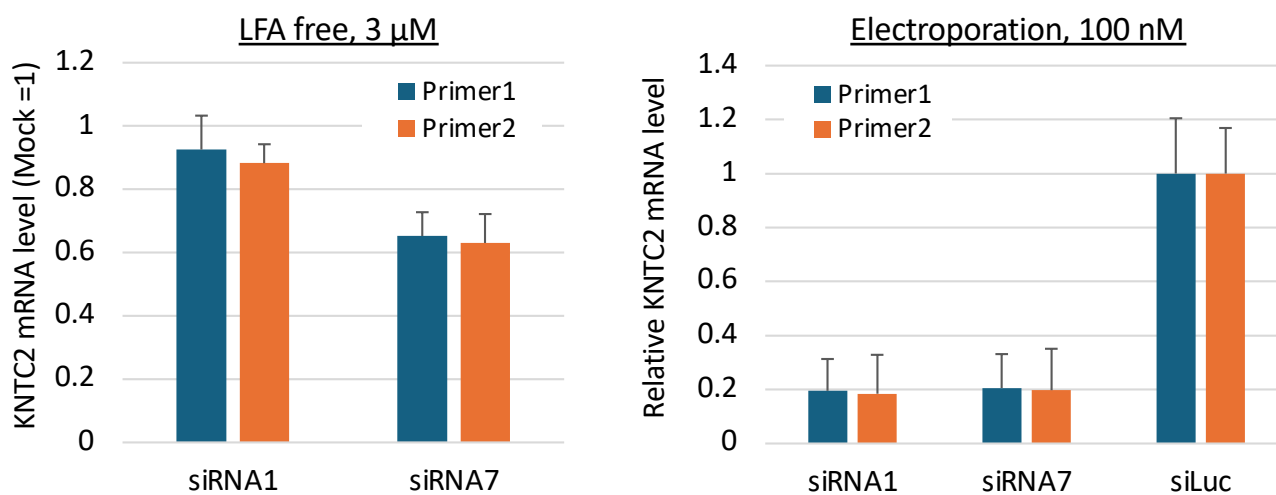
Sato, Mahmoud, Sakamoto, Ueno. *ASC Omega*, 2025. **10**. 62813-62827



	Sequence ^a	T_m^b (°C)
siRNA1	5'-uag uca AcU UGg uau auu uuu-3'	76.4
	3'-u•u•a uCA gUu gaa CCa Uau a•A•a-5'	(±0.2)
siRNA7	5'-Y L1 Y L2 Y L1 Y L2 L2 L1 uag uca AcU UGg uau auu uuu-3'	74.7
	3'-u•u•a uCA gUu gaa CCa Uau a•A•a-5'	(±0.2)

Small letter: 2'-OMe Capital letter: 2'-F • : phosphorothioate

Gene silencing activity in K562 cells



(Figures were reproduced from Ref.3)