SYNTHESIS OF LIPID-POLY(N-METHYL N-VINYLACETAMIDE) CONJUGATES AS ALTERNATIVES TO PEG FOR THE STABILIZATION OF LIPIDIC VECTORS

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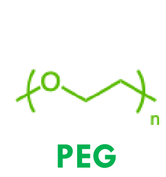
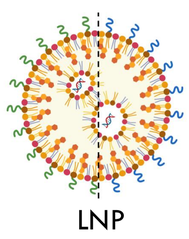
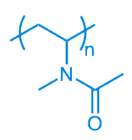
1 Introduction

Efficient gene delivery is essential for many therapeutic strategies, particularly those involving nucleic acids. The latter being unstable in biological environments, they require the use of nanocarriers like **lipid nanoparticles (LNPs)**. To enhance their stability and prolong their circulation time in the bloodstream, LNPs are commonly PEGylated but **PEG presents limitations,** including accelerated blood clearance (ABC) triggered by anti-PEG antibodies, immunogenicity, as well as hindered cellular uptake and endosomal escape. Preliminary studies within the LIPEGALT consortium highlighted the potential of DSPE-**poly(N-methyl N-vinylacetamide)** (DSPE-PNMVA$_{24}$) as valuable **alternative to PEG** in terms of safety, reduced ABC effects, and enhanced efficacy in siRNA delivery.[1-3]

+ Low cytotoxicity
+ Stealth properties
- Hypersensitivity
- ABC effect
- PEG dilemma







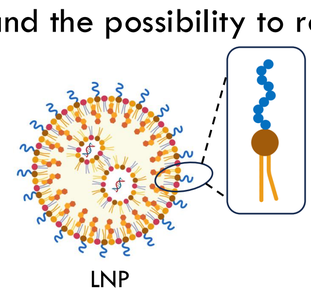
DSPE-PNMVA$_{24}$ (LIPEGALT project)

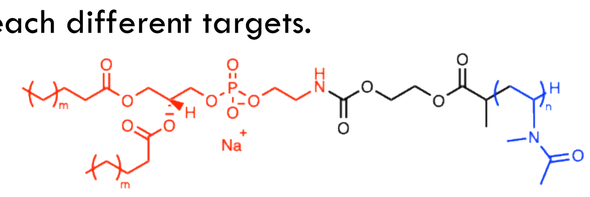
+ Low cytotoxicity
+ Stealth properties
+ No/low hypersensitivity
+ Limited ABC effect
+ Higher transfection efficiency

Cholesterol · Helper lipid · Ionizable lipid · Lipid-polymer · siRNA/tRNA

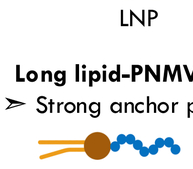
2 Objectives

Optimize the structure of the lipid-PNMVA conjugates dedicated to the formulation of LNPs, in particular the **degree of polymerization** of the PNMVA and the **nature of the lipid anchor,** to further enhance the safety, the transfection efficiency and the possibility to reach different targets.

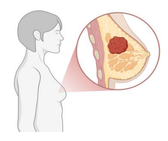




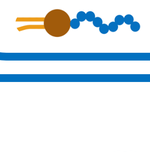
Long lipid-PNMVA (C18)
➢ Strong anchor properties



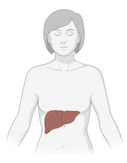
Prolonged systemic circulation → Tumor accumulation



Short lipid-PNMVA (C14)
➢ Weak anchor properties

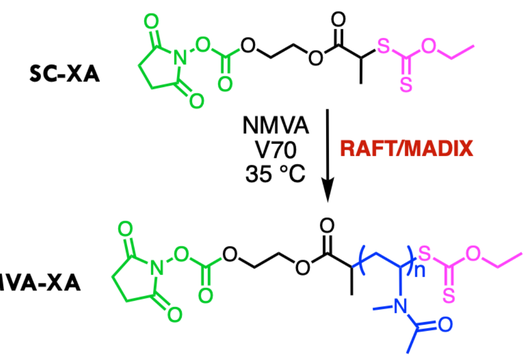


Reduced systemic circulation → Liver accumulation



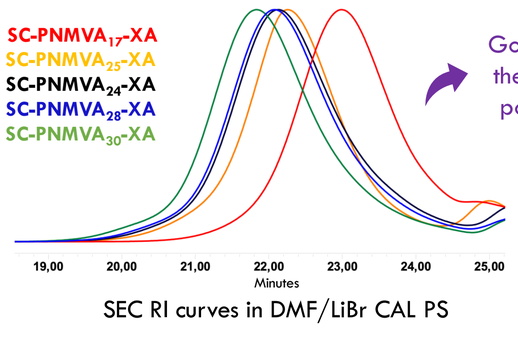
3 Results

A RAFT polymerization



Time (min)	Conv (%)[a]	$M_{n\ th}$ [b]	$M_{n\ SEC}$ [c]	$M_{p\ SEC}$ [c]	$Đ_{SEC}$ [c]	$M_{n\ MALLS}$ [d]	$Đ_{MALLS}$ [d]	DP_{MALLS} [d]
150	21	1040	1410	1510	1.11	1670	1.39	17
165	37	1830	1790	1980	1.15	2500	1.18	25
180	49	2430	2070	2450	1.19	2470	1.27	24
210	52	2580	2130	2520	1.19	2780	1.19	28
240	60	2970	2390	2870	1.21	2970	1.21	30

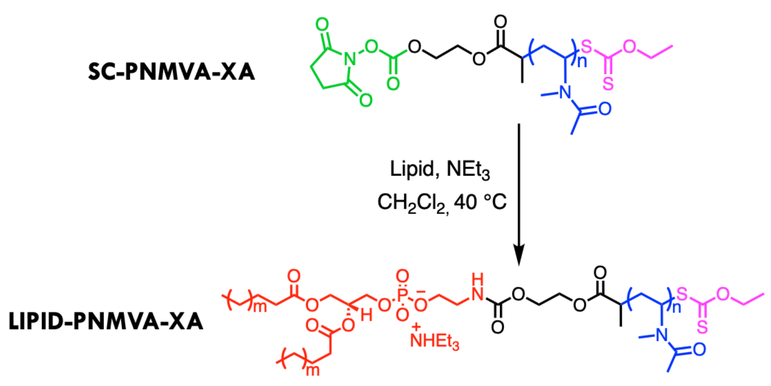
Table1. Synthesis of SC-PNMVA-XA by RAFT. Conditions: bulk polymerization, 35 °C, $[NMVA]_0/[SCXA]_0)] = 50$, $[V70]_0/[SCXA]_0)] = 1$. [a] monomer conversion measured by gravimetry. [b] $M_{n\ th} = [([NMVA]_0/[SCXA]_0)]^* conv^* MM_{NMVA}$, $M_{n\ th\ 100\%} = 4960$ g/mol. [c] Determined by SEC calibration PS. [d] Determined by SEC MALLS.



Good control on the DP and low polydispersity

SEC RI curves in DMF/LiBr CAL PS

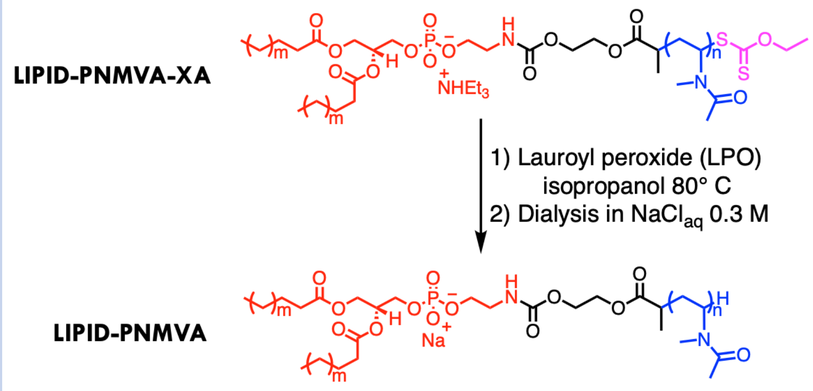
B Lipid conjugation



Starting compound	Final compound	$M_{n\ SEC}$	$Đ_{SEC}$	$M_{n\ NMR}$
SC-PNMVA$_{20}$-XA	DSPE-PNMVA$_{18}$-XA	1800	1.14	1800
SC-PNMVA$_{17}$-XA	DSPE-PNMVA$_{20}$-XA	1750	1.16	2000
SC-PNMVA$_{24}$-XA	DSPE-PNMVA$_{29}$-XA	2850	1.19	2900
SC-PNMVA$_{25}$-XA	DSPE-PNMVA$_{33}$-XA	2150	1.20	3300
SC-PNMVA$_{28}$-XA	DSPE-PNMVA$_{39}$-XA	2850	1.21	3900
SC-PNMVA$_{25}$-XA	DPPE-PNMVA$_{19}$-XA	2300	1.16	1900
SC-PNMVA$_{24}$-XA	DPPE-PNMVA$_{23}$-XA	2800	1.21	2300
SC-PNMVA$_{28}$-XA	DPPE-PNMVA$_{30}$-XA	2700	1.17	3000
SC-PNMVA$_{17}$-XA	DMPE-PNMVA$_{13}$-XA	1800	1.12	1300
SC-PNMVA$_{24}$-XA	DMPE-PNMVA$_{20}$-XA	2350	1.27	2000
SC-PNMVA$_{25}$-XA	DMPE-PNMVA$_{22}$-XA	2200	1.15	2200
SC-PNMVA$_{28}$-XA	DMPE-PNMVA$_{23}$-XA	2700	1.18	2300

Table 2. Synthesis of LIPID-PNMVA-XA. Conditions : $[SC\text{-}PNMVA\text{-}XA]_0/[LIPID]_0/[NEt_3]_0 = 1/1/4.2$, 40 °C, 1 h.

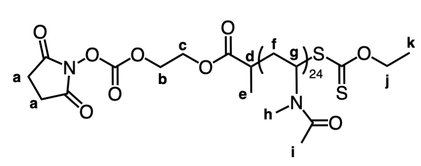
C Xanthate removal

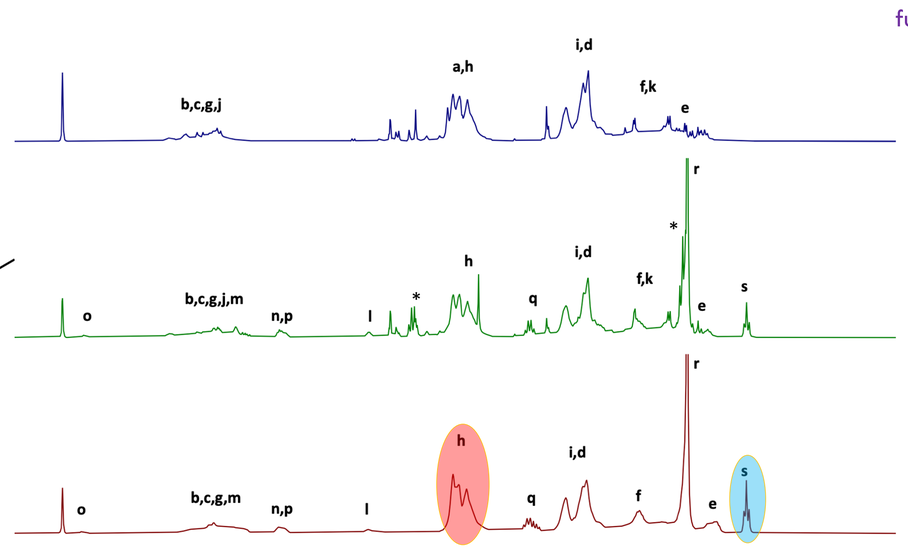


Starting compound	Final compound	$M_{n\ SEC}$	$Đ_{SEC}$	$M_{n\ NMR}$
DSPE-PNMVA$_{20}$-XA	DSPE-PNMVA$_{14}$	2000	1.12	1400
DSPE-PNMVA$_{18}$-XA	DSPE-PNMVA$_{17}$	2040	1.12	1700
DSPE-PNMVA$_{29}$-XA	DSPE-PNMVA$_{25}$	3150	1.16	2550
DSPE-PNMVA$_{33}$-XA	DSPE-PNMVA$_{26}$	2444	1.16	2600
DSPE-PNMVA$_{39}$-XA	DSPE-PNMVA$_{31}$	3000	1.17	3100
DPPE-PNMVA$_{19}$-XA	DPPE-PNMVA$_{14}$	2400	1.12	1400
DPPE-PNMVA$_{23}$-XA	DPPE-PNMVA$_{17}$	2950	1.15	1700
DPPE-PNMVA$_{30}$-XA	DPPE-PNMVA$_{24}$	3000	1.13	2400
DMPE-PNMVA$_{13}$-XA	DMPE-PNMVA$_{11}$	2050	1.08	1100
DMPE-PNMVA$_{20}$-XA	DMPE-PNMVA$_{19}$	2800	1.13	1900
DMPE-PNMVA$_{22}$-XA	DMPE-PNMVA$_{21}$	2500	1.09	2100
DMPE-PNMVA$_{23}$-XA	DMPE-PNMVA$_{24}$	2681	1.15	2400

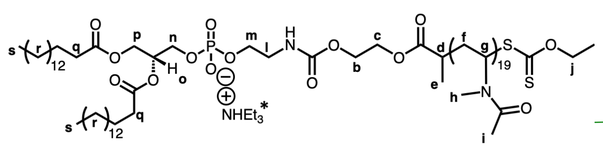
Table 3. Clivage of XA group. Conditions: 1) $[Lipid\text{-}PNMVA\text{-}XA]_0/[LPO]_0 = 1/1.4$, 80 °C, 24 h. 2) Dialysis in NaCl 0.3 M (24h)/water (24h).

SC-PNMVA$_{24}$-XA

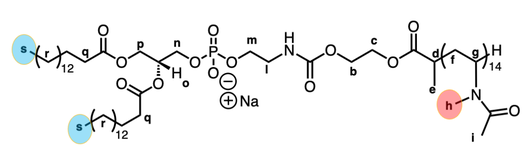




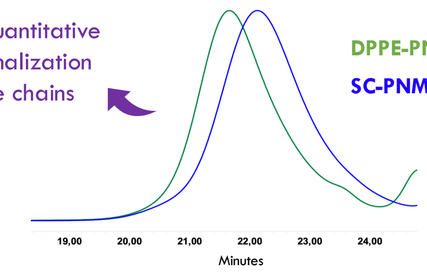
DPPE-PNMVA$_{19}$-XA



DPPE-PNMVA$_{14}$

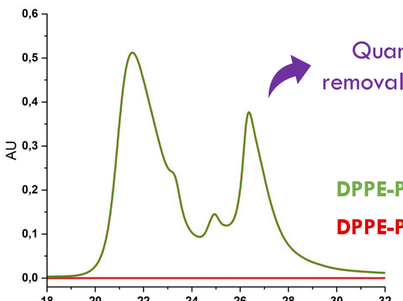


Near quantitative functionalization of the chains



SEC RI curves in DMF/LiBr CAL PS

Quantitative removal of the XA



SEC UV signals at 290 nm

4 Conclusion

A library of lipid-PNMVAs was developed by varying the lipid anchor and PNMVA chain length, providing new candidates for LNP surface modification. Future studies will evaluate their interaction with lipid membranes and hemocompatibility. The formulation of PNMVA-modified LNPs, initially with scramble siRNA, will be followed by in vitro and in vivo studies.

5 References and acknowledgments

[1] Berger M. et al. Journal of Controlled Release 2023, 361, 87–101. 2
[2] Berger M. et al. Advanced Healthcare Materials 2024, 13(8), 2302712.
[3] Debuigne A. et al. Patent 2024, WO2024227713A1 & EP4458870A1.

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