Poly(2-oxazoline)-based vectors for gene delivery applications

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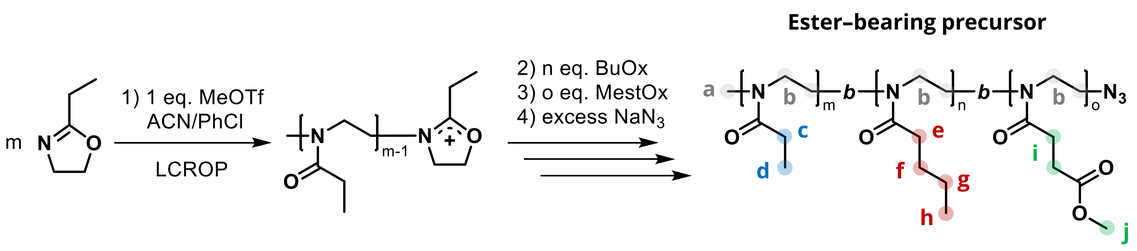
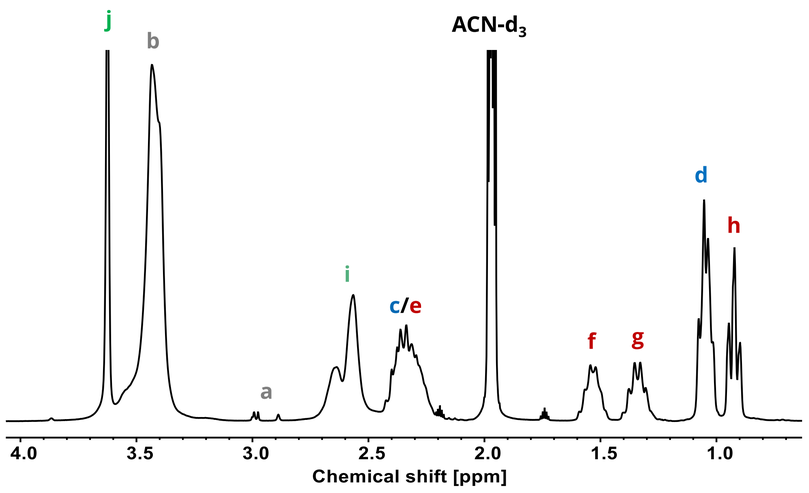
Motivation

Cationic polymers (CPs) have emerged as promising vectors for gene delivery purposes, owing to their ability to facilitate lysosomal escape. However, most highly transfecting CPs, such as Poly(ethylene imine), suffer from poor biocompatibility and cytotoxicity.[1] Furthermore, modifications to reduce cytotoxicity can lead to decreased transfection efficiency and complicated synthesis protocols.[2] In this respect Poly(2-oxazoline)s (POx) offer a versatile platform to circumvent these issues.[3] Not only do POx benefit from increased biocompatibility, but they also exhibit “stealth-behavior”, leading to prolonged blood retention times.[4] POx can be synthesized from a library of functional monomers and facile strategies for post-polymerization modification are available. Herein, we present the synthesis of an easily tunable gene delivery platform based on amine-functionalized POx.

Polymer Synthesis



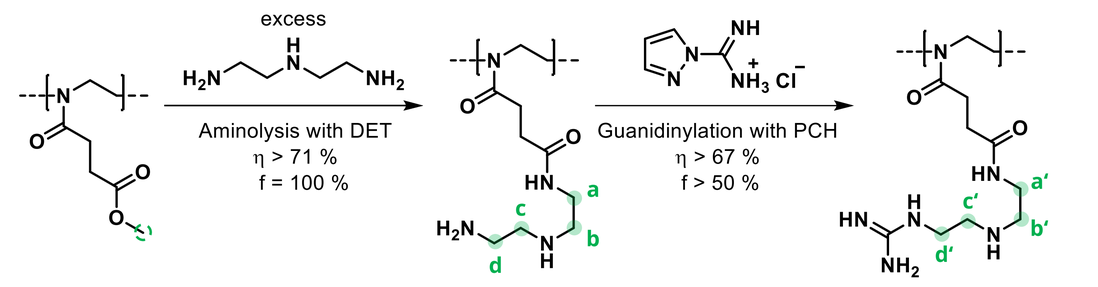


Structure	$M_{n,NMR}$[a] [kg/mol]	$M_{n,SEC}$[b] [kg/mol]	$Đ$[b]	$f_{term.}$[c] [%]	η [%]
$(EtOx)_{35}$–b–$(MestOx)_{24}$–N_3	7.3	5.5	1.10	100	91
$(EtOx)_{34}$–ran–$(MestOx)_{23}$–N_3	7.0	5.8	1.13	100	87
$(EtOx)_{34}$–b–$(BuOx)_{20}$–b–$(MestOx)_{29}$–N_3	10.5	7.5	1.15	81	91

[a]determined via SEC in DMAc, [b]determined via ^{1}H-NMR , [c]determined via ^{1}H-NMR after click reaction.

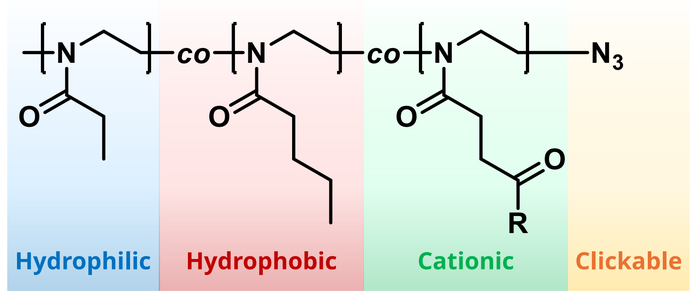
Defined **Easily tuneable** **Azide-terminated**

Functionalization

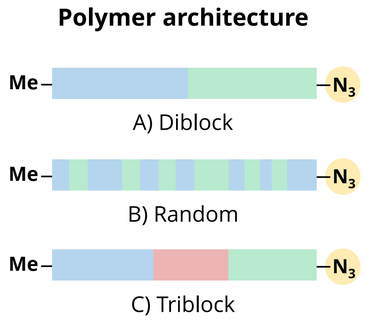


Simple **High yielding** **Efficient**

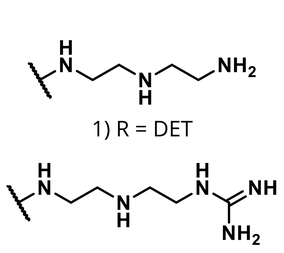
Gene Delivery Platform



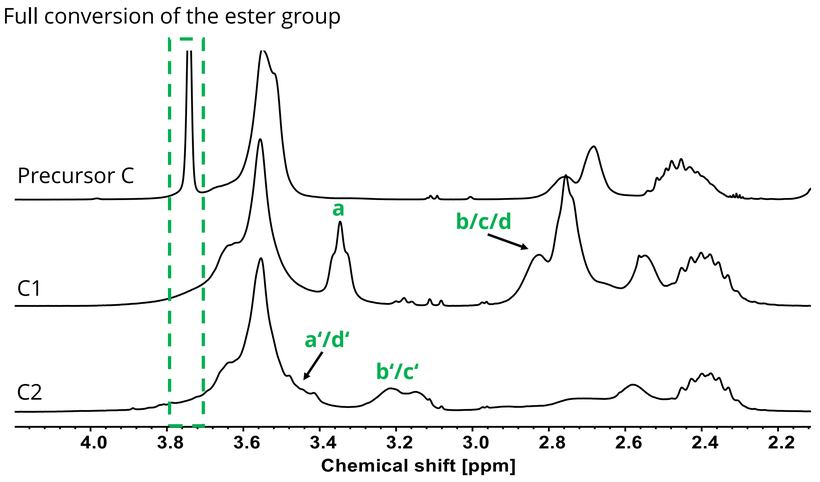
Polymer architecture



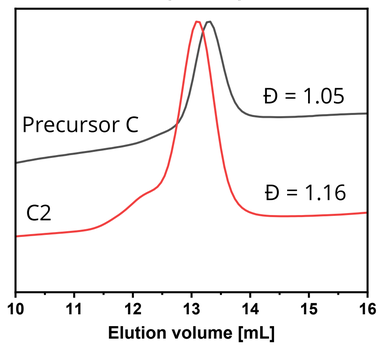
Cationic motifs



Full conversion of the ester group



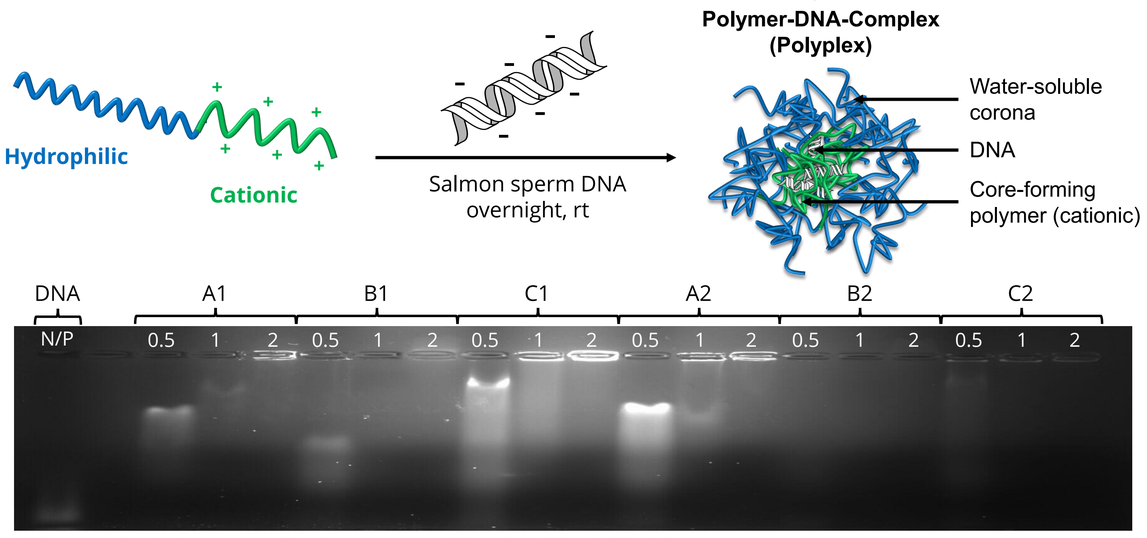
SEC (DMSO)



Sakaguchi test

Red color indicates presence of monosubstituted guanidines.

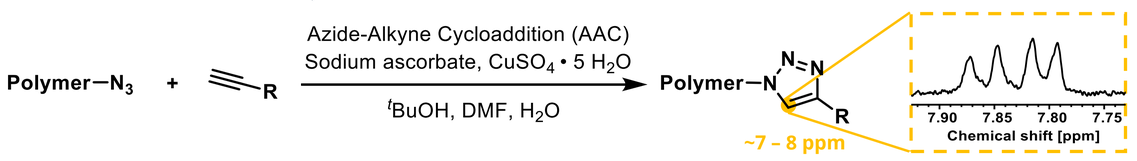
Polyplex-Formation

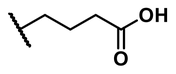
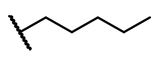
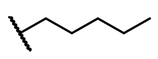


AGE results of cationic polymers. [1 wt% agarose gel, 120 V, 15 W, 400 mA, 50 min].

DNA-Complexation **Best architecture: B) Random** **Best cationic motif: 2) DET-Guanidine**

Click Chemistry



Polymer	R	Conditions	$\delta_{triazole}$ [ppm]	f[a] [%]
Precursor A		Microwave 50 W, 100 °C, 3 h	7.83 (D_2O)	100
Precursor B			7.83 (D_2O)	100
Precursor C			7.60 (ACN-d_3)	81
A1		Microwave 50 W, 100 °C, 3 h	8.08 (D_2O)	72
B1			8.06 (D_2O)	92
C1			8.08 (D_2O)	84
A2		rt, 3d	-	-
B2			-	-
C2			-	-

[a]determined from ^{1}H-NMR via integration of the triazole proton.

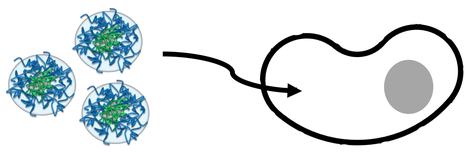
Successful

Successful
However: Metal-free approach desired due to complexation of Cu with DET side chains

Unsuccessful
Reaction with the guanidine group → no triazole formed

Outlook

Transfection studies



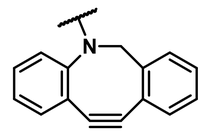
In vitro and *In vivo*

Further adjustments

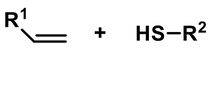
Block { Lengths, Sequences, Structures

Alternative click-approaches

Strain promoted AAC



Thiol-ene coupling



References
[1] Casper, J.; Schenk, S. H.; Parhizkar, E.; Detampel, P.; Dehshahri, A.; Huwyler, J., *JCR* **2023**, *362*, 667-691.
[2] Fernandes, J. C.; Qiu, X.; Winnik, F. M.; Benderdour, M.; Zhang, X.; Dai, K.; Shi, Q., *Int J Nanomedicine* **2013**, *8*, 4091–4102.
[3] Yamaleyeva, D. N.; Makita, N.; Hwang, D.; Haney, M. J.; Jordan, R.; Kabanov, A. V., *Macromol Biosci* **2023**, *23*, 2300177.
[4] Han, Y.; He, Z.; Schulz, A.; Bronich, T. K.; Jordan, R.; Luxenhofer, R.; Kabanov, A. V., *Mol Pharm* **2012**, *9*, 2302–2313.

GFF TUD GESELLSCHAFT VON FREUNDEN UND FÖRDERERN DER TU DRESDEN E. V.