



Insertion of hydrophobic spacers on dodecalysines as potential transfection enhancers

Clothilde Le Guen, Candice Dussouillez, Antoine Kichler, Delphine Chan-Seng

► To cite this version:

Clothilde Le Guen, Candice Dussouillez, Antoine Kichler, Delphine Chan-Seng. Insertion of hydrophobic spacers on dodecalysines as potential transfection enhancers. *European Polymer Journal*, 2021, 157, pp.110654. 10.1016/j.eurpolymj.2021.110654 . hal-03308204

HAL Id: hal-03308204

<https://hal.science/hal-03308204>

Submitted on 29 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Insertion of Hydrophobic Spacers on Dodecalysines as Potential Transfection Enhancers

Clothilde Le Guen,^a Candice Dussouillez,^b Antoine Kichler,^b Delphine Chan-Seng^{a,}*

^a Université de Strasbourg, CNRS, Institut Charles Sadron UPR 22, F-67000 Strasbourg, France

^b Université de Strasbourg, CNRS, Laboratoire de Conception et Application des Molécules Bioactives UMR 7199, ITI InnoVec, F-67000 Strasbourg, France

* corresponding author: delphine.chan-seng@ics-cnrs.unistra.fr (D. Chan-Seng)

ABSTRACT

The use of cationic polymers in gene therapy aims at improving the circulation, protection and delivery of nucleic acids. The modification of poly(L-lysine) (PLL), including the insertion of hydrophobic moieties, is an efficient strategy to enhance the transfection efficiency of this synthetic delivery system. PLL is usually modified through either the extremities of the polymer chains or the amino groups present on the side chains. Herein the synthesis of dodecalysines as model of PLL with hexyl spacing units regularly distributed along the oligomer backbone is reported. Oligolysines terminated with azidohexyl groups were prepared by solid-phase synthesis and ligated by copper-assisted alkyne-azide cycloaddition using propargylamine. The iterative ligation of α -azidohexyloligolysines was more efficient when performed on resin as compared to the ligation in solution. The plasmid DNA transfection efficiency of the hydrophobically modified dodecalysines was then compared to that of unmodified dodecalysine. The results show that when formulated with the helper lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) the hydrophobized dodecalysines exhibited a higher efficiency than the unmodified one. Interestingly, the efficiency could also be increased by the addition of a fusogenic peptide, which paves the way for modifications of the oligolysines that could lead to improved activities.

KEYWORDS: Oligolysine, hydrophobic spacer, solid-phase synthesis, ligation, DNA delivery

1. INTRODUCTION

Solid-phase synthesis (SPS) is a well-established method to prepare peptides,[1, 2] oligonucleotides,[3, 4] and oligosaccharides[5, 6] and has been a source of inspiration in the field of polymer synthesis to reach sequence-defined oligomers and polymers[7-9] especially to tune the properties of biomaterials.[10, 11] SPS has been in particular used to generate non-viral vectors for the delivery of nucleic acids by designing oligomers from novel building blocks or by including for example lysine residues on oligomers. In this field lysine residues are often used because poly(L-lysine) has been shown to be efficient for complexing DNA.[12] Solid-phase peptide synthesis has been exploited to enhance the transfection of oligolysines by inserting at precise location other α -amino acids such as cysteine residues to promote formation of reducible disulfide bridges[13, 14] and histidine residues to induce endosomal escape.[15] The addition of specific peptide sequences have been also investigated such as RGD for enhanced nuclear entry[16] and GGFLGF for endosomal cleavage.[17]

Among the features considered in the design of non-viral polymer-based vectors for nucleic acid delivery, the introduction of hydrophobic moieties improves mainly the stability of the complex formed by condensation of the nucleic acid with the cationic polymer, and the cellular uptake. Post-polymerization modification of the most commonly used cationic polymers in this field (*e.g.* polyethylenimine, poly(L-lysine), chitosan) allows the introduction of aliphatic chains, fatty acids, polymers such as Pluronic® and poly(γ -benzyl L-glutamate), and cholesterol derivatives.[18] Hydrophobic modification has been also performed by design of the polymers for example, by copolymerizing amine-containing and hydrophobic comonomers[19, 20] or distributing oligolysines pendent to a polymer backbone.[21, 22] Solid-phase synthesis is an approach providing easy access to insert hydrophobic moieties precisely located along the backbone of sequence-defined oligomers. We have previously reported the solid-phase synthesis of oligolysine end-capped with an alkyl azide and sequence-defined oligomers containing amino acid residues and aliphatic spacing units.[23-26] In the former case, solid-phase peptide synthesis using Fmoc-Lys(Boc)-OH has been performed to prepare a Boc-protected tetralysine followed by on resin coupling of 6-azidohexanoic acid before cleavage from the resin and removal of the Boc protecting group. In the latter case, the oligomers have been synthesized in a similar manner by on resin iterative addition of protected amino acids, azidoalkanoic acids, and propargylamine through amidification and copper-assisted alkyne-azide cycloaddition reactions. For the synthesis of long sequences, the fully iterative approach as previously described could be time-consuming and source of deleted sequences. These limitations are usually overcome using ligation approaches.[27, 28]

Herein a ligation strategy of oligolysine end-capped with an hexyl azide is proposed to introduce regularly spaced hydrophobic spacing units on a dodecalysine. Dodecalysine has been selected as model as the minimal number of lysine residues to induce complexation of DNA is eight.[29] Preliminary results on the transfection performance of two synthesized sequence-defined oligomers with hydrophobic hexyl spacing units every four and six lysine residues respectively and dodecalysine as reference in the presence of dioleoylphosphatidylethanolamine (DOPE) is presented to highlight the effect of the presence of hydrophobic spacers on DNA delivery.

2. RESULTS AND DISCUSSION

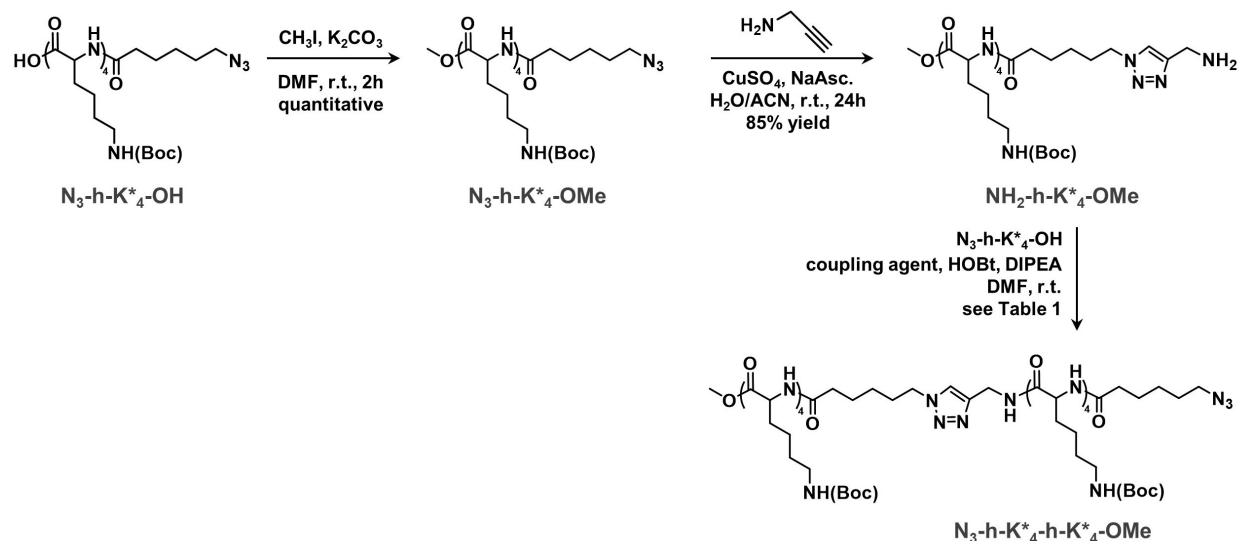
2.1. Synthesis of Boc-protected α -azidohexyl-oligolysines

Boc-protected α -azidohexyl-oligolysines were synthesized as previously reported for N_3 -h-K*₄-OH[23] by solid-phase synthesis (Scheme S1 and Figure S1). Briefly, a 2-chlorotrityl chloride resin was functionalized with Fmoc-Lys(Boc)-OH in the presence of diisopropylethylamine (DIPEA) followed by removal of the Fmoc protecting group using a solution of piperidine in DMF. After determining the loading density by gravimetry, the other lysine residues were added iteratively in two steps: i) addition of Fmoc-Lys(Boc)-OH using *N,N,N',N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) as coupling agent in the presence of 1-hydroxybenzotriazole hydrate (HOBt) and DIPEA, and ii) treatment with piperidine to remove the Fmoc group. The oligolysine grafted on the resin was end-capped with 6-azidohexanoic acid using the same reaction conditions as for the addition of Fmoc-Lys(Boc)-OH affording N_3 -h-K*_x-O-resin where * represents the Boc protecting group and x is the number of lysine residues (x = 4 or 6). N_3 -h-K*_x-OH were recovered after cleavage from the resin using a solution of 2,2,2-trifluoroethanol (TFE) in CH₂Cl₂ and precipitation in diethyl ether/*n*-hexanes in 70-85 % yield.

2.2. Ligation of Boc-protected α -azidohexyl-oligolysines

To avoid the addition of multiple Boc-protected α -azidohexyl-oligolysine blocks simultaneously, the ligation of two N_3 -h-K*₄-OH was performed in three steps (Scheme 1): i) protection of the ω extremity of one of the two N_3 -h-K*₄-OH (N_3 -h-K*₄-OMe), ii) addition of propargylamine by copper-assisted alkyne-azide cycloaddition reaction to prepare the corresponding tetralysine block terminated by a primary amine (NH₂-h-K*₄-OMe), and iii) ligation with an unmodified N_3 -h-K*₄-OH to obtain N_3 -h-K*₄-h-K*₄-OMe. The first step consisted in the protection of the carboxylic acid group by methylation using iodomethane in the presence of potassium carbonate in DMF affording N_3 -h-K*₄-OMe quantitatively. For the second step, various catalyst/ligand systems (*e.g.* CuBr with *N,N,N',N'',N''*-pentamethyldiethylenetriamine, CuSO₄ with sodium ascorbate) under different reaction conditions (*e.g.* reaction times, molar ratios of reagents,

solvent) were investigated. The optimal conditions were obtained using 2 equivalents of propargylamine in the presence of 0.2 equivalent of copper sulfate and 1.5 equivalents of sodium ascorbate in a mixture of water and acetonitrile at room temperature for 24 h. $\text{NH}_2\text{-h-K}^*_4\text{-OMe}$ was purified by precipitation into water and obtained in 85% yield. Finally, the ligation of $\text{N}_3\text{-h-K}^*_4\text{-OH}$ with $\text{NH}_2\text{-h-K}^*_4\text{-OMe}$ was conducted through the formation of an amide linkage (Table 1). The first attempt consisted in using the conditions employed for the addition of Fmoc-Lys(Boc)-OH when synthesizing $\text{N}_3\text{-h-K}^*_4\text{-OH}$, *i.e.* 3 equivalents of HBTU as coupling agent in the presence of HOBT (3 equivalents) and DIPEA (6 equivalents) in DMF at room temperature for 1 h. However, no formation of $\text{N}_3\text{-h-K}^*_4\text{-h-K}^*_4\text{-OMe}$ was observed. Increasing the reaction time to 24 h did not show any improvement on the reaction. As the proper activation of the carboxylic acid group is important to efficiently form amide linkages,[30] different coupling agents based on aminium salts (*i.e.* HBTU and 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HATU)), carbodiimide (*i.e.* 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC)), and phosphonium salts (*i.e.* bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP), and 1H-benzo-triazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)) were considered. All the reactions were conducted for 16 h at room temperature. After purification by extraction to remove the excess of coupling agents and urea side products, the residue was characterized by ^1H NMR spectroscopy and mass spectrometry to determine the extent of the reaction. The results showed that HATU and PyBroP did not allow the formation of the desired product ($\text{N}_3\text{-h-K}^*_4\text{-h-K}^*_4\text{-OMe}$), EDC led to the formation of traces of $\text{N}_3\text{-h-K}^*_4\text{-h-K}^*_4\text{-OMe}$ while PyBOP was the most efficient coupling reagent (Table 1). However, the purification of the reaction was arduous due to the presence of the large excess of $\text{N}_3\text{-h-K}^*_4\text{-OH}$ used (3 equivalents relative to $\text{NH}_2\text{-h-K}^*_4\text{-OMe}$). The reaction was thus attempted with a lower amount of $\text{N}_3\text{-h-K}^*_4\text{-OH}$ (1.5 equivalents), but the purification remained difficult.



Scheme 1. Ligation of two $\text{N}_3\text{-h-K}^*_4\text{-OH}$ in solution through i) methylation of the terminal carboxylic acid of one of the two $\text{N}_3\text{-h-K}^*_4\text{-OH}$, ii) addition of propargylamine by copper-assisted alkyne-azide cycloaddition reaction, and iii) coupling with an unmodified $\text{N}_3\text{-h-K}^*_4\text{-OH}$.

Table 1. Reaction conditions for the ligation of $\text{N}_3\text{-h-K}^*_4\text{-OH}$ on $\text{NH}_2\text{-h-K}^*_4\text{-OMe}$.

| coupling agent | reaction time (h) | formation of $\text{N}_3\text{-h-K}^*_4\text{-h-K}^*_4\text{-OMe}$ |
|----------------|-------------------|--|
| HBTU | 1 | no |
| HBTU | 24 | no |
| HATU | 16 | no |
| EDC | 16 | traces |
| PyBroP | 16 | no |
| PyBOP | 16 | yes, but isolation difficult |

To minimize the difficulties encountered for the purification of $\text{N}_3\text{-h-K}^*_4\text{-h-K}^*_4\text{-OMe}$, the ligation was performed on the resin previously used to prepare the Boc-protected α -azidohexyl-oligolysines (Scheme 2). This approach permits to rinse the growing oligomers grafted on the resin after each step to remove easily the unreacted molecules. Instead of cleaving $\text{N}_3\text{-h-K}^*_4\text{-OH}$ from the resin, $\text{N}_3\text{-h-K}^*_4\text{-O-resin}$ was modified with propargylamine by copper-assisted alkyne-azide cycloaddition reaction. As the resin is based on polystyrene, the previous conditions used in solution would induce deswelling of the resin due to the presence of water and thus limit the accessibility of the active sites on the resin for the reaction to occur. The addition of propargylamine was conducted with CuBr in the presence of N,N,N',N'',N'' -pentamethyldiethylenetriamine (PMDETA) in CH_2Cl_2 . The reaction was monitored by FT-IR

N₃-h-K^{*}₄-O-resin

NH₂-h-K^{*}₄-O-resin

N₃-h-K^{*}₄-h-K^{*}₄-O-resin

N₃-h-K^{*}₄-h-K^{*}₄-h-K^{*}₄-OH

1) propargylamine, CuBr, PMDETA, CH₂Cl₂, r.t.
 2) N₃-h-K^{*}₄-OH, HBTU, HOBT, DIPEA, DMF, r.t.
 3) TFE, CH₂Cl₂

Scheme 2. On resin ligation of three N₃-h-K*₄-OH through i) addition of propargylamine by copper-assisted alkyne-azide cycloaddition reaction, and ii) coupling with an unmodified N₃-h-K*₄-OH.

2.3. Evaluation of the lysine-based oligomers as non-viral vectors

Before evaluating the transfection activity of the oligomers, the Boc protecting groups of N_3 -h-K*₄-h-K*₄-h-K*₄-OH and N_3 -h-K*₆-h-K*₆-OH were removed following the previously reported protocol[23] affording **K4x3** and **K6x2** respectively. **K12** (*i.e.* dodecalysine without any spacing unit) was used for comparison purposes. Each oligomer was tested for its ability to promote the delivery of a reporter plasmid in the Human colorectal carcinoma cell line HCT116. Therefore, increasing amounts of oligomer were mixed with a constant amount of a luciferase expression plasmid and incubated with the cells. As control, complexes generated with a poly(L-lysine) with a degree of polymerization of 215 (**K215**) were included. The results showed that all three oligomers were poorly efficient as their activity remained more than 10-fold below that of **K215** (Figure S5) - which in the absence of helper compounds is by itself also rather a poor transfection vector[31] (see also Figure 2 **K215** in comparison with DOTAP). This result is not unexpected since it was previously shown that shorter poly(L-lysine)s are less efficient than longer ones.[32] Next, we tested whether addition of helper agents could improve the activity of our oligomers. Therefore, the transfection was performed by adding the lysosomotropic agent chloroquine that was previously shown to increase the transfection efficiency of vectors such as lactosylated poly(L-lysine)s.[33] However, the addition of this weak base did not increase the efficiency (data not shown). The naturally-occurring phospholipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was then investigated by addition to the complexes. The helper activity of the zwitterionic lipid DOPE relies on its capacity to increase endosomal escape of the complexes through its fusogenic activity.[34, 35] Many commercially available lipidic formulations (Lipofectamine, Lipofectine, SAINT-red, DC-Chol/DOPE, etc.) contain a mixture of a cationic lipid and the helper lipid DOPE. But DOPE proved also able to increase the DNA and siRNA transfection activity of non-lipidic formulations, in particular of oligoureas, a class of peptidomimetic foldamers composed of ethylene diamine type units connected by urea bonds.[36, 37]

To evaluate the effect of this additive, DNA complexes were generated using different oligomer/DOPE ratios and their activity was measured on HCT116 cells. The results showed that when associated with DOPE, the efficiency of oligomers **K4x3** and **K6x2** was significantly increased (Figure 1) reaching the level of **K215**, while this was not the case with **K12**. To note, when DOPE was mixed with plasmid DNA without the presence of a cationic vector no detectable levels of luciferase were found with 5 μ g but measurable levels of luciferase were obtained when using 10 μ g of DOPE (Figure S6). When the **K4x3**/DOPE/DNA complexes were generated in a sequential manner (*i.e.* instead of mixing oligomer **K4x3** with DOPE before addition of DNA, we first generated the oligomer/DNA complexes before adding DOPE) the resulting complexes performed slightly less well (Figure S7). A similar light decrease was also observed when the

ternary **K4x3**/DOPE/DNA complexes were generated in water in place of 150 mM of sodium chloride suggesting an effect of salts on structuration of the complexes (Figure S7). How the different components of the complexes exactly get organized remains to be studied.

Lastly, the ability to increase the activity by adding a fusogenic peptide to the **K4x3**/DNA complexes was investigated. Fusogenic peptides, mostly derived from the amino-terminal sequence of hemagglutinin of the influenza virus, have been widely used in order to increase the escape from the endosomes of DNA complexed with polymers [38-41] or lipids [42, 43]. These anionic peptides have a disordered conformation at pH 7 but as the pH decreases, a situation which occurs in late endosomes, the carboxyl groups of aspartate and glutamate side chains become protonated and the disappearance of the anionic repulsion allows the transition into an amphipathic α -helical conformation. As a consequence, the peptides can interact with membranes to form pores or induce membrane fusion/lysis.[44] In 1996, Gottschalk and coworkers designed such a peptide, JTS-1 (GLFEALLELLESLWELLLEA), that showed a pH-dependent lytic activity on erythrocytes and the authors found that addition of this amphipathic peptide to plasmid DNA complexed by a cationic peptide strongly increased the transfection efficiency.[45] The results obtained with oligomer **K4x3** showed that addition of JTS-1 highly increased the activity since levels approx. 17-fold higher than that of **K215** were observed (Figure 2), even though the activity remained below that of the commercialized cationic lipid DOTAP (*N*-(1-(2,3-dioleoyloxy)propyl)-*N,N,N*-trimethylammonium chloride).[46]

To check whether the negatively charged fusogenic peptide adsorbs electrostatically to the oligomer/DNA complexes the surface charge was measured before and after addition of JTS-1. While the zeta potential of 20 μ g **K4x3**/1.5 μ g DNA was of +27.3 mV, it decreased to +20.7mV when 10 μ g JTS-1 were added. These results suggest that at least some of the JTS-1 peptides bind to the positively charged DNA complexes.

Importantly, the cell toxicity of the oligomer/DNA +/- DOPE or JTS-1 was estimated by measuring the amounts of proteins present in each well. The DNA complexes displayed no cytotoxicity when compared to untreated cells (Figures 1, 2, and S5-S7).

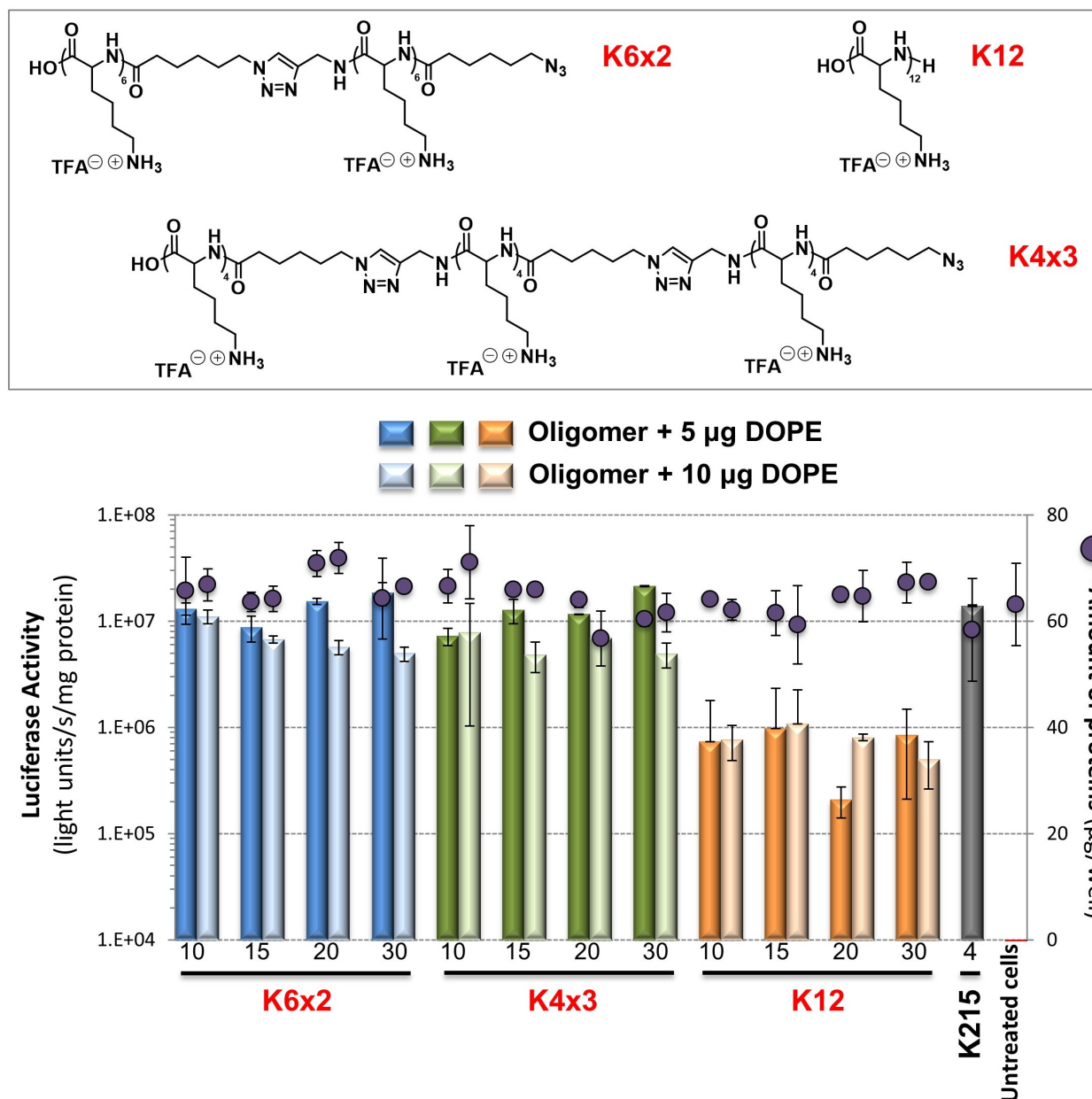


Figure 1. Evaluation of the DNA transfection activity of oligomer/DOPE mixtures on human HCT116 cells. Transfection was performed in 48 well-plates using 0.75 μg/well of the plasmid encoding for luciferase. Increasing amounts of polymer were mixed with either 5 μg (dark bars) or 10 μg of DOPE (light bars) in 150 mM NaCl before addition of a constant amount of plasmid. The indicated amounts of polymer in μg are given for 1.5 μg of DNA. The complexes were incubated with cells for 2h30 in serum-free medium. Thereafter, the medium was removed and replaced with serum-containing medium. Luciferase activity was measured 24 h after the beginning of the transfection and it is expressed as total light units/1 s/mg protein, and the average of duplicates is shown. Error bars represent the standard deviation of the mean. Cell toxicity was estimated by total protein quantification and compared to untreated cells (purple dots; right axis).

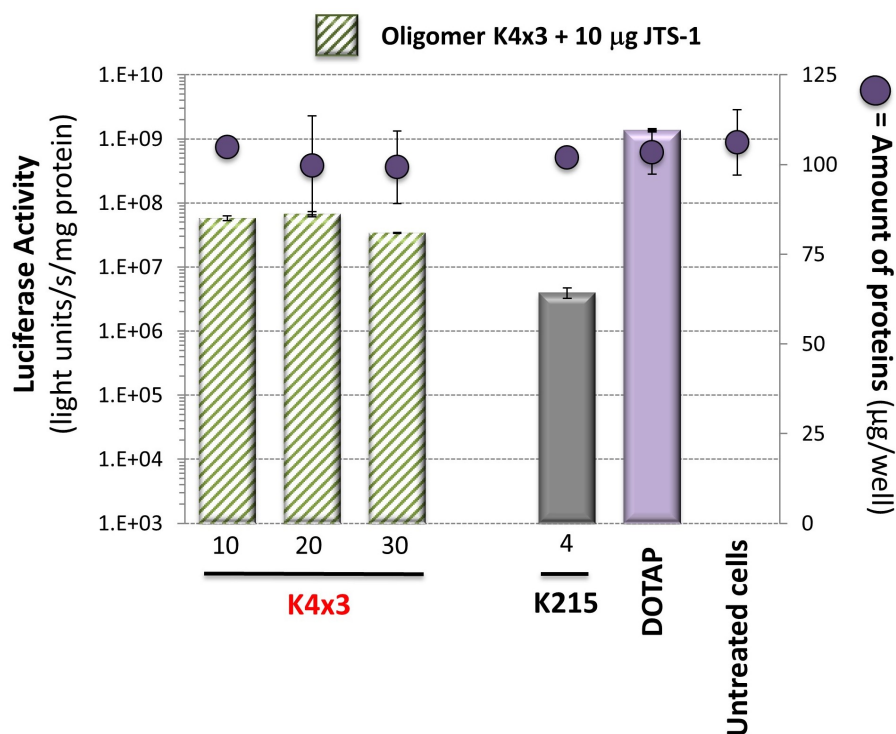


Figure 2. Addition of the fusogenic peptide JTS-1 to oligomer/DNA mixtures on human HCT116 cells. Transfection was performed in 48 well-plates using 0.75 µg per well of DNA. Increasing amounts of oligomer were mixed with a constant amount of plasmid. Then 10 µg of the peptide JTS-1 was added to the complexes. The indicated amounts of vector in µg are given for 1.5 µg of DNA. DOTAP was used at an amine to phosphate (N/P) ratio of 3. The complexes were incubated with cells for 2h30 in serum-free medium. Thereafter, the medium was removed and replaced with serum-containing medium. Luciferase activity was measured 26 h after the beginning of the transfection and it is expressed as total light units/1 s/mg protein, and the average of duplicates is shown. Error bars represent the standard deviation of the mean. Cell toxicity was estimated by total protein quantification and compared to untreated cells (purple dots; right axis).

3. CONCLUSION

In the present work two new lysine-rich oligomers with hydrophobic spacers regularly distributed have been synthesized using SPS and on resin ligation. The strategy proposed for the synthesis of the oligomers should offer the ability to prepare blocks having each a function (*e.g.* oligolysine for complexation with nucleic acids as described in this work) and attach them covalently to each other by ligation in a specific order paving the way to enhance the transfection of lysine-based oligomers. Although only low DNA transfection activity by themselves was observed, addition of helper agents could importantly increase their efficiency as compared to the corresponding unmodified oligolysine (*i.e.* same number of lysine residues and without hydrophobic spacers). Given the reported results endosomal release of the complexes has some limitation since addition of fusogenic compounds led to high activity. These encouraging results suggest that the introduction of moieties such as histidines [47, 48] known to enhance

endosomal escape on one of the blocks of the oligomers could reach the development of lysine-based oligomers with interesting nucleic acid delivery capabilities.

4. EXPERIMENTAL

4.1. Materials

2-Chlorotriptyl chloride (CTC) resin (100-200 mesh, 1.6 mmol g⁻¹), Fmoc-L-Lys(Boc)-OH (>99%), *N,N,N',N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 99%), and 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 99%) were purchased from Iris Biotech. 1-Hydroxybenzotriazole hydrate (HOBt, ≥97%), copper(II) bromide (CuBr, 98%), *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 99%), anhydrous *N,N*-dimethylformamide (anhydrous DMF, 99.8%), anhydrous dichloromethane (anhydrous CH₂Cl₂, ≥99.8%), and *N,N*-dimethylformamide (DMF, >99%) were purchased from Sigma-Aldrich. 2,2,2-*N,N*-Diisopropylethylamine (DIPEA, >99%), propargylamine (>95%), trifluoroethanol (TFE, >99%), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC, >98%), and bromotripyrrolidinophosphonium hexafluoro-phosphate (PyBroP, >98%) were purchased from TCI. Iodomethane (CH₃I, 99%, stabilized with copper) and L-ascorbic acid sodium salt (99%) were purchased from Alfa Aesar. 1H-Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 98%) was purchased from ABCR. Copper sulfate(II) pentahydrate (>98%), methanol (MeOH, 99.8%), ethanol (EtOH, absolute, 99.99%), dichloromethane (CH₂Cl₂, pure, stabilized with amylene), *n*-hexane (pure), and diethyl ether (Et₂O, 99%) were purchased from Carlo Erba. Hydrochloric acid (HCl, 37% in water) and acetic acid (99.7%) were purchased from Fisher Chemical. Potassium carbonate (K₂CO₃, 99%) was purchased from VWR. Acetonitrile (ACN, Chromasolv, >99.9%) was purchased from Honeywell. Docecalysine, TFA salt (K₁₂, ≥98%) was purchased from GenScript. All chemicals were used as received, except if noted otherwise. Dialysis membrane in regenerated cellulose with molecular cutoff of 2000 Da (SpectrumLab) were purchased from Roth. CuBr was washed with acetic acid, subsequently with absolute EtOH, filtered and stored under inert atmosphere prior to use. 6-Azidohexanoic acid was synthesized as previously reported.[23] The Kaiser test was conducted as described in the literature.[49] All the syntheses on solid support were performed in solid phase extraction (SPE) tubes (60 mL polypropylene SPE tubes with polyethylene frits, 20 μm porosity purchased from SUPELCO®).

4.2. Characterization

^1H and ^{13}C NMR spectra were recorded in $\text{DMSO}-d_6$ or MeOD on a 400 MHz Bruker Avance III HD spectrometers equipped with a BBO type probe at 25 °C.

Fourier transform infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer using the attenuated total reflectance (ATR) technique for wavenumber between 600 and 4000 cm^{-1} .

Mass measurements were carried out on a Bruker Daltonics microTOF spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with an orthogonal electrospray (ESI) interface. Calibration was performed using Tuning mix (Agilent Technologies). Sample solutions were introduced into the spectrometer source with a syringe pump (Harvard type 55 1111: Harvard Apparatus Inc., South Natick, MA, USA) with a flow rate of 5 $\mu\text{L min}^{-1}$.

4.3. Synthesis of Boc-protected α -azidohexyl- ω -carboxylic acid-oligolysines

$\text{N}_3\text{-h-K}^*_4\text{-OH}$ was synthesized as previously reported.[23] $\text{N}_3\text{-h-K}^*_6\text{-OH}$ was prepared using the same protocol that is briefly described below.

Loading of the resin. CTC resin (1.00 g, 1.60 mmol, 1 eq.) was weighed in a SPE tube, swollen in CH_2Cl_2 , and washed three times with CH_2Cl_2 . Fmoc-Lys(Boc)-OH (1.50 g, 3.20 mmol, 2 eq.) was added to the resin. The tube was degassed by performing three vacuum/argon cycles. Under argon atmosphere, 6 mL of anhydrous CH_2Cl_2 was added to the tube, followed by 1.1 mL of DIPEA (6.40 mmol, 4 eq.). The tube was agitated using an orbital stirrer for 1 h at room temperature. The solution was filtered and the resin was washed six times with DMF (addition of DMF, agitation for 2 min, and filtration). 30 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (80/15/5) was added to the tube, agitated for 10 min at room temperature, and filtered (twice). The resin was then washed six times with DMF. The deprotection of the Fmoc group was performed by adding a 25 v% piperidine solution in DMF to the tube that was agitated for 3 min. After filtration, this step was repeated with an agitation of 20 min. The resin was washed six times with DMF, three times with CH_2Cl_2 , three times with MeOH, and six times with CH_2Cl_2 . The resin was dried under vacuum for 48 h. The loading density of the resin was determined by gravimetry as 1.23 mmol Boc-protected lysine per gram of resin (77%).

Addition of protected lysine residues. Fmoc-Lys(Boc)-OH (1.63 g, 3.48 mmol, 3 eq.), HOBt (0.47 g, 3.48 mmol, 3 eq.), and HBTU (1.31 g, 3.48 mmol, 3 eq.) were dissolved in anhydrous DMF (7 mL) followed by the addition of DIPEA (1.2 mL, 6.96 mmol, 6 eq.). The solution was added to the SPE tube containing the resin and the reaction was agitated for 1 h at room temperature with an orbital stirrer. After filtration, the resin was washed six times with DMF and six times with CH_2Cl_2 . The Kaiser test was performed: i) if the solution was blue, the coupling reaction was repeated or ii) if the solution was yellow, the Fmoc removal reaction was performed as previously using a solution of piperidine in DMF.

Insertion of 6-azidohexanoic acid. 6-Azidohexanoic acid (0.55 g, 3.48 mmol, 3 eq.), HOBT (0.47 g, 3.48 mmol, 3 eq.), and HBTU (1.31 g, 3.48 mmol, 3 eq.) were dissolved in anhydrous DMF (7 mL) followed by the addition of DIPEA (1.2 mL, 6.96 mmol, 6 eq.). The solution was added to the SPE tube and the reaction was agitated for 1 h at room temperature with an orbital shaker. After filtering the solution, the resin was rinsed six times with DMF, three times with CH₂Cl₂, three times with MeOH, and six times with CH₂Cl₂. The Kaiser test was performed: i) if blue, the coupling reaction was repeated or ii) if yellow, FTIR spectroscopy was performed to confirm the presence of the azide (2100 cm⁻¹).

Cleavage from the resin. The resin was transferred to a glass vial and a solution of CH₂Cl₂/TFE (4/1) was added. The solution was agitated at room temperature for 45 min and then the filtrate was collected. This step was performed four times and then the resin was washed three times with CH₂Cl₂. The filtrates were combined and concentrated by rotary evaporation. The product was precipitated in Et₂O/*n*-hexane (2:1) and the suspension was centrifuged. The supernatant was removed to isolate the product as a white solid that was dried under vacuum overnight.

N₃-h-K*₆-OH. 78 % yield. ¹H NMR (DMSO-*d*₆, 2.50 ppm): δ 7.94-7.85 (m, 6H, -NH-C(=O)-), 6.71-6.38 (m, 6H, NH(Boc)), 4.26-4.09 (m, 6H, -C(=O)-CH-NH-), 3.30 (t, ³J_{HH} = 7.0 Hz, 2H + H₂O, -CH₂-N₃), 2.90-2.84 (m, 12H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 2.14-2.13 (m, 2H, -NH-C(=O)-CH₂-), 1.70-1.20 (m, 97H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc), -C(=O)-CH₂-CH₂-CH₂-CH₂-N₃, -CH₃ from Boc). ¹³C NMR (DMSO-*d*₆, 39.52 ppm): δ 173.5 (-C=O), 172.3 (-C=O), 172.0 (-C=O), 171.5 (-C=O), 171.4 (-C=O), 171.3 (-C=O), 155.6 (-C=O), 77.4 (-C(CH₃)₃ from Boc), 52.7 (-C(=O)-CH-NH-), 52.5 (-C(=O)-CH-NH-), 52.3 (-C(=O)-CH-NH-), 51.9 (-C(=O)-CH-NH-), 50.6 (-CH₂-N₃), 35.0 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 31.8 (-CH₂-NH(Boc)), 31.6 (-CH₂-NH(Boc)), 31.0 (-CH₂-NH(Boc)), 30.8 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 29.3 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 29.2 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 28.3 (-CH₃ from Boc), 28.0 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 25.8 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 24.8 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 22.8 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.7 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.5 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.1 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)). FTIR: 2098 cm⁻¹. HR ESI (m/z): [M+Na]⁺ calculated from C₇₂H₁₃₁N₁₅O₂₀Na, 1548.9587; found, 1548.9583.

4.4. Chemical ligation of two N₃-h-K*₄-OH in solution

Synthesis of N₃-h-K*₄-OMe. N₃-h-K*₄-OH (300 mg, 0.28 mmol, 1.0 eq.) and K₂CO₃ (51 mg, 0.37 mmol, 1.3 eq.) were placed in a round bottom flask. Three vacuum/argon cycles were performed. Anhydrous DMF (6 mL) and CH₃I (18 μL, 42 mmol, 1.5 eq.) were added. The reaction mixture was stirred at room temperature for 2 h and concentrated on a rotary evaporator. The residue was purified by precipitation in a solution of Et₂O/*n*-hexane (2:1). The suspension was centrifuged for 20 min. Filtrate was removed and

the product was dried overnight under vacuum. N₃-h-K*₄-OMe was obtained quantitatively as a white solid.

¹H NMR (DMSO-*d*₆, 2.50 ppm): δ 8.17-7.76 (m, 4H, -NH-C(=O)-), 6.75-6.39 (m, 4H, -NH(Boc)), 4.25-4.15 (m, 4H, -C(=O)-CH-NH-), 3.60 (s, 3H, CH₃-C(=O)-), 3.29 (t, ³J_{HH} = 7.00 Hz, 2H, -CH₂-N₃), 2.88-2.86 (m, 8H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 2.14-2.10 (m, 2H, -NH-C(=O)-CH₂-), 1.70-1.20 (m, 66H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc), -C(=O)-CH₂-CH₂-CH₂-CH₂-N₃, -CH₃ from Boc). ¹³C NMR (DMSO-*d*₆, 39.52 ppm): δ 172.3 (-C=O), 172.1 (-C=O), 171.8 (-C=O), 171.6 (-C=O), 171.2 (-C=O), 155.5 (-C=O), 77.3 (-C-(CH₃)₃ from Boc), 52.5 (-C(=O)-CH-NH-), 52.3 (-C(=O)-CH-NH-), 52.1 (-C(=O)-CH-NH-), 51.9 (-C(=O)-CH-NH-), 51.7 (CH₃O-C(=O)), 50.5 (-CH₂-N₃), 34.9 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 31.8 (-CH₂-NH(Boc)), 31.5 (-CH₂-NH(Boc)), 30.9 (-CH₂-NH(Boc)), 30.5 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 29.2 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 29.1 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 29.0 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 28.2 (-CH₃ from Boc), 27.9 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 25.7 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 24.7 (-C(=O)-CH₂-CH₂-CH₂-CH₂-N₃), 22.7 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.6 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.4 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.0 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)). ESI (m/z): [M+Na]⁺ calculated from C₅₁H₉₃N₁₁O₁₄Na, 1106.6796; found, 1106.6812.

Synthesis of NH₂-h-K*₄-OMe. Sodium ascorbate (96 mg, 0.48 mmol, 1.5 eq.), copper sulfate (8 mg, 0.05 mmol, 0.1 eq.), and propargylamine (42 μL, 0.65 mmol, 2.0 eq.) were added to a solution of N₃-h-K*₄-OMe (350 mg, 0.32 mmol, 1.0 eq.) in H₂O/ACN (0.9/2.1 mL). The reaction mixture was stirred at room temperature for 24 h and concentrated on a rotary evaporator. The residue was purified by precipitation in water and the suspension was centrifuged. The product was obtained as brown solid in 85% yield.

¹H NMR (DMSO-*d*₆, 2.50 ppm): δ 8.17-7.77 (m, 5H, -NH-C(=O)- and -CH_{triazole}-), 6.70-6.36 (m, 4H, -NH(Boc)), 4.28-4.18 (m, 4H, -C(=O)-CH-NH-), 3.59 (s, 3H, CH₃-C(=O)-), 3.39 (br s, 2H + D₂O, -CH₂-NH₂), 2.86 (br s, 8H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 2.09 (m, 2H, -NH-C(=O)-CH₂-), 1.78-1.23 (m, 66H, -CH-CH₂-CH₂-CH₂-CH₂-NH(Boc), -C(=O)-CH₂-CH₂-CH₂-CH₂-triazole-, -CH₃ from Boc). ¹³C NMR (DMSO-*d*₆, 39.52 ppm): δ 172.3 (-C=O), 172.1 (-C=O), 171.8 (-C=O), 171.6 (-C=O), 171.2 (-C=O), 155.5 (-C=O), 77.3 (-C-(CH₃)₃ from Boc), 52.5 (-C(=O)-CH-NH-), 52.3 (-C(=O)-CH-NH-), 51.9 (-C(=O)-CH-NH-), 51.7 (CH₃O-C(=O)-), 34.8 (-C(=O)-CH₂-CH₂-CH₂-CH₂-triazole-), 31.8 (-CH₂-NH(Boc)), 31.5 (-CH₂-NH(Boc)), 30.9 (-CH₂-NH(Boc)), 30.6 (-C(=O)-CH₂-CH₂-CH₂-CH₂-triazole-), 30.4 (-triazole-CH₂-NH₂), 29.5 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 29.2 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 29.0 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 28.2 (-CH₃ from Boc), 27.9 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 25.4 (-C(=O)-CH₂-CH₂-CH₂-CH₂-triazole-), 24.6 (-C(=O)-CH₂-CH₂-CH₂-CH₂-triazole-), 22.7 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.5 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)), 22.4 (-CH-CH₂-CH₂-

CH₂-CH₂-NH(Boc)), 22.0 (-CH-CH₂-CH₂-CH₂-CH₂-NH(Boc)). ESI (m/z): [M+Na]⁺ calculated from C₅₄H₉₉N₁₂O₁₄, 1139.7398; found, 1139.7362.

General protocol for the ligation of N₃-h-K*₄-OH on NH₂-h-K*₄-OMe. N₃-h-K*₄-OH (141 mg, 0.13 mmol, 3 eq.), coupling agent (0.13 mmol, 3 eq.), HOBt (18 mg, 0.13 mmol, 3 eq.), and DIPEA (46 μL, 0.26 mmol, 6 eq.) were placed in a vial and dissolved in a mixture of DMF (1 mL) and CH₂Cl₂ (0.5 mL). The solution was added to a round bottom flask containing NH₂-h-K*₄-OMe (50 mg, 0.04 mmol, 1 eq.). The reaction mixture was stirred for the desired time (Table 1) at room temperature. CH₂Cl₂ and 10% NaOH_{aq} were added and the solution was extracted three times with CH₂Cl₂. The organic phases were combined, dried over Na₂SO₄, filtered, and concentrated by rotary evaporation. The residue was dissolved in MeOH and precipitated in a solution of Et₂O/*n*-hexane (2:1). The product was isolated by centrifugation and dried under vacuum overnight.

4.5. On resin chemical ligation

Addition of propargylamine by copper-assisted alkyne-azide cycloaddition reaction. Typically, CuBr (24 mg, 0.17 mmol, 1 eq.) was added to the SPE tube containing N₃-(h-K*_x)_y-O-resin (0.17 mmol of oligomer grafted on the resin) where x = 4 or 6 and y = 1 or 2. Three vacuum/argon cycles were performed. Anhydrous CH₂Cl₂ (4 mL) was added to tube followed by PMDETA (0.10 mL, 0.50 mmol, 3 eq.) and propargylamine (0.03 mL, 0.50 mmol, 3 eq.). The reaction mixture was agitated for 16 h under argon at room temperature. After filtering the solution, the resin was rinsed six times with DMF, three times with CH₂Cl₂, three times with MeOH, and six times with CH₂Cl₂. The extent of the reaction was monitored by the disappearance of the characteristic band of azides at 2100 cm⁻¹ by FT-IR spectroscopy; if still present the reaction was repeated.

Ligation of N₃-h-K*₄-OH on the oligomer grafted on the resin. N₃-h-K*₄-OH (0.50 mmol, 3 eq. relative to the loading of the resin), HBTU (186 mg, 0.50 mmol, 3 eq.), HOBt (67 mg, 0.50 mmol, 3 eq.), and DIPEA (0.17 mL, 0.99 mmol, 6 eq.) were placed in a vial and dissolved in 4 mL of DMF. The solution was added to the SPE tube containing NH₂-(h-K*_x)_y-O-resin (0.17 mmol of oligomer grafted on the resin, 1 eq.). The reaction mixture was agitated for 1 h at room temperature with an orbital shaker. After filtering the solution, the resin was rinsed six times with DMF, three times with CH₂Cl₂, three times with MeOH, and six times with CH₂Cl₂. The Kaiser test was performed to monitor the consumption of the primary amine of NH₂-(h-K*_x)_y-O-resin: if the solution was blue, the coupling reaction was repeated.

Cleavage from the resin. The resin was transferred to a glass vial and a solution of CH₂Cl₂/TFE (4/1) was added. The solution was agitated at room temperature for 45 min and then the filtrate was collected. This step was performed four times and then the resin was washed three times with CH₂Cl₂. The filtrates were

combined and concentrated by rotary evaporation. The product was precipitated in Et₂O/*n*-hexane (2:1) and the suspension was centrifuged. The supernatant was removed to isolate the product, that was dialyzed against methanol for at least 24h. The solution was concentrated by rotary evaporation affording a brown solid that was dried under vacuum overnight.

N₃-h-K*₄-h-K*₄-h-K*₄-OH. 54 % yield. ¹H NMR (MeOD, 3.31 ppm): δ 8.57-7.73 (m, 16H, -**NH**-C(=O)- and -**CH**_{triazole}-), 6.70-6.09 (m, 12H, -**NH**(Boc)), 4.67-4.05 (m, 20H, -C(=O)-**CH**-NH-, -triazole-**CH**₂-NH-, and -CH₂-**CH**₂-triazole-), 3.22-2.81 (m, 26H, -CH-CH₂-CH₂-CH₂-**CH**₂-NH(Boc) and -**CH**₂-N₃), 2.41-2.18 (m, 6H, -NH-C(=O)-**CH**₂-), 2.07-1.23 (m, 198H, -CH-**CH**₂-**CH**₂-**CH**₂-CH₂-NH(Boc), -C(=O)-CH₂-**CH**₂-**CH**₂-**CH**₂-triazole-, -C(=O)-CH₂-**CH**₂-**CH**₂-**CH**₂-CH₂-N₃, and -**CH**₃ from Boc). HR ESI-MS (m/z): [M+2Na]²⁺ calculated from C₁₅₆H₂₈₁N₃₅O₄₀Na₂, 1642.5515; found, 1642.5532.

N₃-h-K*₆-h-K*₆-OH. 50 % yield. ¹H NMR (DMSO-*d*₆, 2.50 ppm): δ 8.51-7.58 (m, 14H, -**NH**-C(=O)- and -**CH**_{triazole}-), 6.92-6.24 (m, 12H, -**NH**(Boc)), 4.52-3.97 (m, 16H, -C(=O)-**CH**-NH-, -triazole-**CH**₂-NH-, and -CH₂-**CH**₂-triazole-), 3.31 (t, 2H, -**CH**₂-N₃), 3.00-2.76 (m, 24H, -CH-CH₂-CH₂-CH₂-**CH**₂-NH(Boc)), 2.00-2.23 (m, 4H, -NH-C(=O)-**CH**₂-), 1.91-1.12 (m, 192H, -CH-**CH**₂-**CH**₂-**CH**₂-CH₂-NH(Boc), -C(=O)-CH₂-**CH**₂-**CH**₂-**CH**₂-CH₂-triazole-, -C(=O)-CH₂-**CH**₂-**CH**₂-**CH**₂-CH₂-N₃, and -**CH**₃ from Boc). HR ESI (m/z): [M+Na+K]²⁺ calculated from C₁₄₇H₂₆₅N₃₁O₄₀NaK, 1575.4615; found, 1575.4624.

4.6. Transfection experiments

Cell culture. The Human colorectal carcinoma cell line HCT116 was cultured at 37 °C in a humid atmosphere (5% CO₂) in RPMI 1640 (Roswell Park Memorial Institute) supplemented with 2 mM L-glutamine, 100 units mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin and 10% fetal calf serum (FCS).

Preparation of the DNA complexes. The polymer/DNA complexes were prepared by mixing equal volumes of DNA and polymer in 150 mM NaCl: 1.5 µg of DNA in 25 µL of 150 mM NaCl were mixed with increasing amounts of carrier in 25 µL NaCl 150 mM. After stirring, the mixture was kept for 20 min at room temperature allowing the formation of the complexes. Serum-free culture medium was then added to obtain a final volume of 500 µL; next, 250 µL were added to each well of the duplicate.

Complexes with the helper lipid DOPE were generated in the same way as described above except that DOPE was added to the oligomer before addition of DNA (unless otherwise stated). For the oligomer/DNA/JTS-1 complexes the following procedure was used: oligomer/DNA complexes were generated in 150 mM NaCl as described above. After a maturation step of 15 min, 10 µg of JTS-1 peptide was added, and the complexes were allowed to stay for 10 min at room temperature before they were diluted in culture medium.

The cationic lipid DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methyl-sulfate) was used at an amine to phosphate (N/P) ratio of 3. N/P ratio is used to indicate the amount of lipid used for a transfection; N/P=1 corresponds to the amount required to neutralize all of the negative charges carried by the phosphate groups of the plasmid. For example, 1 µg of DNA corresponds to 3 nmol of negative charges; the N/P ratio is calculated by considering the fact that 1 molecule of DOTAP carries 1 "N".

Zeta potential. The surface charge of the formulation 20 µg **K4x3**/1.5 µg DNA +/- 10 µg JTS-1 in 15 mM NaCl was determined by using a Zetasizer Nano ZS (Malvern Instruments).

DNA transfection. The plasmid pSMD2Luc Δ ITR 7.6 kbp was used in the transfection assays; it encodes for the *luciferase* gene pGL2 under the control of the human cytomegalovirus (CMV) immediate. All transfection experiments were performed in 48-well plates. The cells were seeded into the plates one day before transfection, in order to have a confluency of 60-80%. After removing the culture medium, the complexes were deposited into the wells and the plates were placed into the incubator. After a period of 2-3 h of incubation, the transfection medium was replaced by fresh medium containing 10% serum. Then, the cells were incubated at 37 °C until the end of the experiment.

Luciferase activity. The luciferase assay was performed as previously described.[50] Briefly, to determine the luciferase activity, the cells were first lysed after the transfection step by adding 100 µL of lysis buffer/well after having removed the culture medium. Next, the lysates were transferred into Eppendorf tubes and centrifuged for 5 min at 18,000 g. Then 1-5 µL of the supernatant were deposited in wells of a white 96 well-plate. The reading of the luciferase activity with the luminometer was realized after successive addition of 50 µL assay buffer and 50 µL of a luciferin solution. The results are expressed in relative light units (RLU) and are normalized for the amount of proteins present in each sample and are expressed in RLU/1s/mg protein. The amount of protein was determined by a Bradford assay (Biorad protein assay kit, Biorad). This measurement was made with 4 µL of supernatant in a transparent 96 well-plate. To each sample was then added 200 µL of Bradford reagent previously diluted at 1/5 in MilliQ water. The colorimetric assay was done by measuring the optical density (OD) at 595 nm using a spectrophotometer (SP Safas 2000). The amount of protein was determined using a calibration curve realized in the same plate with albumin.

5. DATA AVAILABILITY

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Clothilde Le Guen: Methodology, Investigation. **Candice Dussouillez:** Investigation. **Antoine Kichler:** Supervision, Visualization. **Delphine Chan-Seng:** Funding acquisition, Conceptualization, Supervision, Visualization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

This work was funded by the LabEx Emerging Investigators Grant 2017 from the Fondation pour la Recherche en Chimie that supported the postdoctoral position of CLG. The authors thank the mass spectrometry facilities of the University of Strasbourg and the Cronenbourg NMR core facilities.

REFERENCES

- [1] R.B. Merrifield, Solid phase peptide synthesis. I. The synthesis of a tetrapeptide, *J. Am. Chem. Soc.* 85 (1963) 2149-2154.
- [2] R. Behrendt, P. White, J. Offer, Advances in Fmoc solid-phase peptide synthesis, *J. Pept. Sci.* 22 (2016) 4-27.
- [3] J.D. Watson, F.H.C. Crick, Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid, *Nature* 171 (1953) 737-738.
- [4] C.B. Reese, Oligo- and poly-nucleotides: 50 years of chemical synthesis, *Org. Biomol. Chem.* 3 (2005) 3851-3868.
- [5] S. Danishefsky, K. McClure, J. Randolph, R. Ruggeri, A strategy for the solid-phase synthesis of oligosaccharides, *Science* 260 (1993) 1307-1309.
- [6] C.S. Bennett, Principles of modern solid-phase oligosaccharide synthesis, *Org. Biomol. Chem.* 12 (2014) 1686-1698.
- [7] J.-F. Lutz, Defining the field of sequence-controlled polymers, *Macromol. Rapid Commun.* 38 (2017) 1700582.
- [8] S.C. Solleder, R.V. Schneider, K.S. Wetzel, A.C. Boukis, M.A.R. Meier, Recent progress in the design of monodisperse, sequence-defined macromolecules, *Macromol. Rapid Commun.* 38 (2017) 1600711.

- [9] P. Nanjan, M. Porel, Sequence-defined non-natural polymers: Synthesis and applications, *Polym. Chem.* 10 (2019) 5406-5424.
- [10] M.J. Austin, A.M. Rosales, Tunable biomaterials from synthetic, sequence-controlled polymers, *Biomater. Sci.* 7 (2019) 490-505.
- [11] C. Yang, K.B. Wu, Y. Deng, J. Yuan, J. Niu, Geared toward applications: A perspective on functional sequence-controlled polymers, *ACS Macro Lett.* 10 (2021) 243-257.
- [12] Y. Wang, E. Wagner, Non-viral targeted nucleic acid delivery: Apply sequences for optimization, *Pharmaceutics* 12 (2020) 888.
- [13] D.L. McKenzie, E. Smiley, K.Y. Kwok, K.G. Rice, Low molecular weight disulfide cross-linking peptides as nonviral gene delivery carriers, *Bioconjugate Chem.* 11 (2000) 901-909.
- [14] M.L. Read, K.H. Bremner, D. Oupický, N.K. Green, P.F. Searle, L.W. Seymour, Vectors based on reducible polycations facilitate intracellular release of nucleic acids, *J. Gene Med.* 5 (2003) 232-245.
- [15] Q. Leng, P. Scaria, J. Zhu, N. Ambulos, P. Campbell, A.J. Mixson, Highly branched HK peptides are effective carriers of siRNA, *J. Gene Med.* 7 (2005) 977-986.
- [16] M. Colin, S. Moritz, P. Fontanges, M. Kornprobst, C. Delouis, M. Keller, A.D. Miller, J. Capeau, C. Coutelle, M.C. Brahimi-Horn, The nuclear pore complex is involved in nuclear transfer of plasmid DNA condensed with an oligolysine–RGD peptide containing nuclear localisation properties, *Gene Ther.* 8 (2001) 1643-1653.
- [17] A.M.R. Haines, A.S. Irvine, A. Mountain, J. Charlesworth, N.A. Farrow, R.D. Husain, H. Hyde, H. Ketteringham, R.H. McDermott, A.F. Mulcahy, T.L. Mustoe, S.C.H. Reid, M. Rouquette, J.C. Shaw, D.R. Thatcher, J.H. Welsh, D.E. Williams, W. Zauner, R.O. Phillips, CL22 – a novel cationic peptide for efficient transfection of mammalian cells, *Gene Ther.* 8 (2001) 99-110.
- [18] Z. Liu, Z. Zhang, C. Zhou, Y. Jiao, Hydrophobic modifications of cationic polymers for gene delivery, *Prog. Polym. Sci.* 35 (2010) 1144-1162.
- [19] M. Kurisawa, M. Yokoyama, T. Okano, Transfection efficiency increases by incorporating hydrophobic monomer units into polymeric gene carriers, *J. Controlled Release* 68 (2000) 1-8.
- [20] F. Richter, P. Mapfumo, L. Martin, J.I. Solomun, F. Hausig, J.J. Frietsch, T. Ernst, S. Hoepfner, J.C. Brendel, A. Traeger, Improved gene delivery to K-562 leukemia cells by lipoic acid modified block copolymer micelles, *J. Nanobiotechnol.* 19 (2021) 70.
- [21] R.N. Johnson, D.S.H. Chu, J. Shi, J.G. Schellinger, P.M. Carlson, S.H. Pun, HPMA-oligolysine copolymers for gene delivery: Optimization of peptide length and polymer molecular weight, *J. Controlled Release* 155 (2011) 303-311.

- [22] S.S. Parelkar, D. Chan-Seng, T. Emrick, Reconfiguring polylysine architectures for controlling polyplex binding and non-viral transfection, *Biomaterials* 32 (2011) 2432-2444.
- [23] D. Chan-Seng, J.-F. Lutz, Primary structure control of oligomers based on natural and synthetic building blocks, *ACS Macro Lett.* 3 (2014) 291-294.
- [24] D. Chan-Seng, J.-F. Lutz, Solid-phase synthesis as a tool for the preparation of sequence-defined oligomers based on natural amino acids and synthetic building blocks, *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, American Chemical Society 2014, pp. 103-116.
- [25] D. Chan-Seng, J. Louwsma, J.-F. Lutz, S. Joly, Synthesis of macromolecules containing phenylalanine and aliphatic building blocks, *Macromol. Rapid Commun.* 39 (2018) 1700764.
- [26] J. Louwsma, A. Carvalho, J.-F. Lutz, S. Joly, D. Chan-Seng, Adsorption of phenylalanine-rich sequence-defined oligomers onto Kevlar fibers for fiber-reinforced polyolefin composite materials, *Polymer* 217 (2021) 123465.
- [27] J.P. Tam, J. Xu, K.D. Eom, Methods and strategies of peptide ligation, *Pept. Sci.* 60 (2001) 194-205.
- [28] T. Kimmerlin, D. Seebach, '100 years of peptide synthesis': ligation methods for peptide and protein synthesis with applications to β -peptide assemblies, *J. Pept. Res.* 65 (2005) 229-260.
- [29] A. Kwok, D. McCarthy, S.L. Hart, A.D. Tagalakis, Systematic comparisons of formulations of linear oligolysine peptides with siRNA and plasmid DNA, *Chem. Biol. Drug Des.* 87 (2016) 747-763.
- [30] F. Albeicicio, R. Chinchilla, D.J. Dodsworth, C. Nájera, New trends in peptide coupling reagents, *Org. Prep. Proced. Int.* 33 (2001) 203-303.
- [31] E. Wagner, C. Plank, K. Zatloukal, M. Cotten, M.L. Birnstiel, Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: Toward a synthetic virus-like gene-transfer vehicle, *Proc. Natl. Acad. Sci. U. S. A.* 89 (1992) 7934-7938.
- [32] P. Erbacher, A.C. Roche, M. Monsigny, P. Midoux, Glycosylated polylysine/DNA complexes: Gene transfer efficiency in relation with the size and the sugar substitution level of glycosylated polylysines and with the plasmid size, *Bioconjugate Chem.* 6 (1995) 401-410.
- [33] P. Erbacher, A.C. Roche, M. Monsigny, P. Midoux, Putative role of chloroquine in gene transfer into a human hepatoma cell line by DNA/lactosylated polylysine complexes, *Exp. Cell Res.* 225 (1996) 186-194.
- [34] H. Farhood, N. Serbina, L. Huang, The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer, *Biochim. Biophys. Acta, Biomembr.* 1235 (1995) 289-295.
- [35] Z. Du, M.M. Munye, A.D. Tagalakis, M.D.I. Manunta, S.L. Hart, The role of the helper lipid on the DNA transfection efficiency of lipopolyplex formulations, *Sci. Rep.* 4 (2014) 7107.

- [36] C. Douat, C. Aisenbrey, S. Antunes, M. Decossas, O. Lambert, B. Bechinger, A. Kichler, G. Guichard, A cell-penetrating foldamer with a bio reducible linkage for intracellular delivery of DNA, *Angew. Chem. Int. Ed.* 54 (2015) 11133-11137.
- [37] M. Bornerie, A. Brion, G. Guichard, A. Kichler, C. Douat, Delivery of siRNA by tailored cell-penetrating urea-based foldamers, *Chem. Commun.* 57 (2021) 1458-1461.
- [38] A. Kichler, I. Freulon, V. Boutin, R. Mayer, M. Monsigny, P. Midoux, Glycofection™ in the presence of anionic fusogenic peptides: A study of the parameters affecting the peptide-mediated enhancement of the transfection efficiency, *J. Gene Med.* 1 (1999) 134-143.
- [39] R. Kircheis, A. Kichler, G. Wallner, M. Kurs, M. Ogris, T. Felzmann, M. Buchberger, E. Wagner, Coupling of cell-binding ligands to polyethylenimine for targeted gene delivery, *Gene Ther.* 4 (1997) 409-418.
- [40] P. Midoux, C. Mendes, A. Legrand, J. Raimond, R. Mayer, M. Monsigny, A.C. Roche, Specific gene transfer mediated by lactosylated poly-L-lysine into hepatoma cells, *Nucleic Acids Res.* 21 (1993) 871-878.
- [41] C. Plank, B. Oberhauser, K. Mechtler, C. Koch, E. Wagner, The influence of endosome-disruptive peptides on gene transfer using synthetic virus-like gene transfer systems, *J. Biol. Chem.* 269 (1994) 12918-12924.
- [42] A. Kichler, K. Mechtler, J.-P. Behr, E. Wagner, Influence of membrane-active peptides on lipospermine/DNA complex mediated gene transfer, *Bioconjugate Chem.* 8 (1997) 213-221.
- [43] S. Simões, V. Slepishkin, R. Gaspar, M.C. Pedrosa de Lima, N. Düzgüneş, Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusogenic peptides, *Gene Ther.* 5 (1998) 955-964.
- [44] S. Takahashi, Conformation of membrane fusion-active 20-residue peptides with or without lipid bilayers. Implication of α -helix formation for membrane fusion, *Biochemistry* 29 (1990) 6257-6264.
- [45] S. Gottschalk, J.T. Sparrow, J. Hauer, M.P. Mims, F.E. Leland, S.L. Woo, L.C. Smith, A novel DNA-peptide complex for efficient gene transfer and expression in mammalian cells, *Gene Ther.* 3 (1996) 448-457.
- [46] R. Leventis, J.R. Silvius, Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles, *Biochim. Biophys. Acta, Biomembr.* 1023 (1990) 124-132.
- [47] A. Kichler, C. Leborgne, J. März, O. Danos, B. Bechinger, Histidine-rich amphipathic peptide antibiotics promote efficient delivery of DNA into mammalian cells, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 1564-1568.

- [48] P. Midoux, M. Monsigny, Efficient gene transfer by histidylated polylysine/pDNA complexes, *Bioconjugate Chem.* 10 (1999) 406-411.
- [49] E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides, *Anal. Biochem.* 34 (1970) 595-598.
- [50] A. Kichler, C. Leborgne, P.B. Savage, O. Danos, Cationic steroid antibiotics demonstrate DNA delivery properties, *J. Controlled Release* 107 (2005) 174-182.