Structural and spectroscopic studies on the formation of lipoplexes between DNA and cationic gemini surfactants*)

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Abstract: The process of complex formation between cationic gemini surfactants, 3,3'-[α,ω-(dioxaalkane)]bis(1-dodecylimidazolium) dichloride, with deoxyribonucleic acid (DNA) was studied. The study was performed for ten surfactants having spacer groups of different lengths used in 6 concentrations (5 mM, 2 mM, 1 mM, 0.5 mM, 0.2 mM, 0.1 mM) and a 6.5 μM DNA solution. The complex formation was verified by circular dichroism spectroscopy and gel electrophoresis. The complexes were found to be stable and the process of complex formation was reproducible, efficient and immediate.

Keywords: gene therapy, gemini surfactants, DNA, circular dichroism, gel electrophoresis.

Strukturalne i spektroskopowe badania procesu formowania lipopleksów DNA z kationowymi surfaktantami gemini

Streszczenie: Zbadano proces kompleksowania kationowych surfaktantów typu gemini — dichlorków 3,3'-[α,ω-(dioxaalkane)]bis(1-dodecyloimidazoliowych) z kwasem deoksyrybonukleinowym (DNA). Wykorzystano dziesięć surfaktantów różniących się długością grupy łącznikowej, w postaci roztworów o 6 różnych stężeniach (5 mM, 2 mM, 1 mM, 0,5 mM, 0,2 mM, 0,1 mM), oraz 6,5 μM roztwór DNA. Za pomocą spektroskopii dichroizmu kołowego oraz elektroforezy żelowej wykazano, iż zachodzi proces kompleksowania tych cząsteczek. Stwierdzono, że otrzymane kompleksy są stabilne a proces ich powstawania — natychmiastowy, wydajny i powtarzalny.

Słowa kluczowe: terapia genowa, surfaktanty gemini, DNA, dichroizm kołowy, elektroforeza żelowa.

Natural, non-viral systems for nucleic acid transfections are based on lipid, and in particular phospholipid, systems [1—5]. Lipids are able to make complexes with nucleic acids (lipoplexes) and different structural forms, mainly micelles [6, 7]. The natural structural forms of lipids, such as mono- and multilayer liposomes, can facilitate the transport of genetic material across the cell membrane. However, effective complexation between lipids and the negatively charged genetic material requires them to have a resultant positive charge. To satisfy this requirement, mixtures of cationic surfactants with phospholipids are very often used [1]. Gemini surfactants are particularly promising for this application as they show high biocompatibility and low toxicity [8—10]. These properties are essential for their application as gene vectors as biocompatibility with the cellular environment ensures no immunological response in the cell and thus permits non-invasive introduction of the therapeutic substance [11, 12]. This property is the main reason for the growing interest in gemini surfactants at research centres all over the world. The primary aim is to find methods allowing the use of gemini surfactants in medicine as auxiliary agents and gene vectors in gene therapy [13]. Besides the use in DNA transfer, gemini surfactants are also potentially useful in siRNA therapy [14].

The realisation of this aim should be preceded by a thorough investigation that would establish the ways of effective formation of stable complexes between the therapeutic material and the gene vector. In this paper, the process of complex formation between ten selected gemini surfactants and low molecular weight DNA is analysed.

EXPERIMENTAL PART

Materials

— A series of ten gemini surfactants based on 3,3'-[α,ω-(dioxaalkane)]bis(1-dodecylimidazolium) dichloride (C12JCn, n = 2—12), where n stands for the num-

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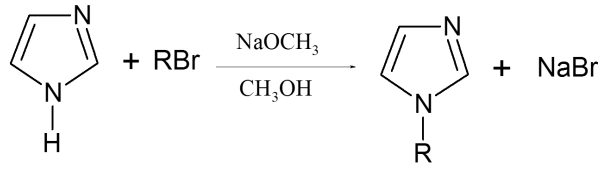
ber of methylene groups in the spacer group, were studied. The surfactant molecules were made of hydrophilic imidazolium rings separated by a linker (spacer group) of different lengths. The choice of surfactant molecules of such geometry was made to permit a controlled modification of the distance between the positively charged groups interacting with DNA. The hydrophobic group was dodecyl as it is most often used in systems applied as nucleic acid vectors [15, 16].

— Low molecular weight DNA from salmon sperm of mean molar mass ~ 120 kDa and length of about 185 base pairs was purchased from SIGMA-ALDRICH.

Preparation of gemini surfactants

1-Dodecylimidazole was synthesized according to the procedure described earlier [17]. Sodium (2.3 g/mol) was added to 75 cm^3 of anhydrous methanol and imidazole (0.1 mol) was also dissolved. The appropriate amount of



$R = C_{12}H_{25}$

Scheme A

1-bromododecane (0.11 mol) was added and the mixture was stirred for 6 h at 65 °C. Precipitated sodium bromide was filtered and the methanol removed. The final product, 1-dodecylimidazole (see Scheme A), was twice distilled under reduced pressure. The reaction yield was 69 %.

α,ω-Di(chloromethoxy)alkanes were obtained (Scheme B) by passing HCl gas through a mixture of formaldehyde and the appropriate α,ω-diol according to a procedure described earlier [18]. All α,ω-di(chloromethoxy)alkanes readily hydrolyzed in the presence of a small amount of water to form HCl, which in turn gives the 1-dodecylimidazolium hydrochlorides. The separation of the quaternization product and hydrochloride is practically impossible. For this reason, quaternization reactions with α,ω-di(chloromethoxy)alkanes were conducted under strictly anhydrous conditions:

$$HO(CH_2)_nOH + 2\,CH_2O + 2\,HCl \rightarrow$$
$$\rightarrow ClCH_2O(CH_2)_nOCH_2Cl + 2\,H_2O$$

n = 2—12

Scheme B

Gemini surfactants based on 3,3'-[α,ω-(dioxaalkane)]bis(1-dodecylimidazolium) dichloride were prepared in the reaction of 1-dodecylimidazoles with α,ω-di(chloromethoxy)alkanes (Scheme C).

Dry DMF was a convenient solvent from which the products were isolated as a white powder. Unfortunately, all obtained chlorides were very hygroscopic but they remained stable in air, in aqueous solution, and in common organic solvents. They showed high solubility in common alcohols, DMSO, acetone, and chloroform but were immiscible in hexane and ethyl acetate.

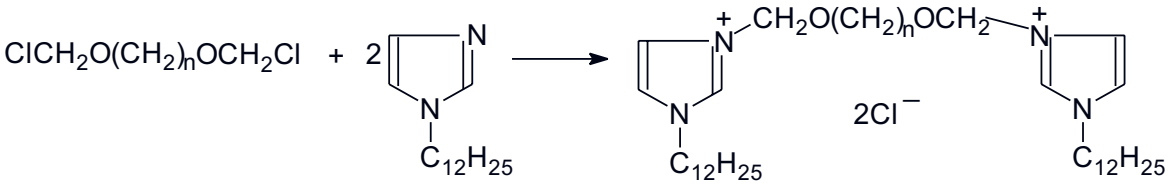
Preparation of test samples

The solutions to be studied contained 6.5 μM DNA and the surfactants were used at concentrations (0.1, 0.2, 0.5, 1, 2 and 5 mM) prepared in phosphate buffer (10 mM sodium phosphate, pH 7.0).

Methods of testing

Circular dichroism

The effectiveness of complex formation between the studied surfactants and low molecular weight DNA was examined by circular dichroism (CD) spectroscopy on a Jasco 815 spectrometer (Jasco Inc. Japan). The spectral



C12JCn, n = 2—12

Scheme C

range of measurements was 220—340 nm, scanning rate 50 nm/min and spectral width 0.5 nm. Measurements were made in a nitrogen atmosphere with a flow rate of 5 cm^3/min. The samples to be studied were placed in thermostated (20 °C) quartz cells of optical path length 0.5 mm. For each sample, five independent spectra were taken and then averaged. All data were analysed using Spectra Manager II (Jasco) and Origin software.

Electrophoresis

Electrophoretic separation was performed in a horizontal system with 1 % agarose gels at 120 V, in TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8), with ethidium bromide (EtBr) at a concentration 0.5 μg/cm^3 used as a fluorescent dye. The results permitted a verification of complex formation between the surfactants and DNA and an evaluation of the yield of the process. The gels were visualized using a standard 300 nm UV transilluminator.

RESULTS AND DISCUSSION

The CD spectra of DNA-surfactant mixtures are presented in Figs 1—4.

They confirm complex formation between gemini surfactants and the DNA used for the study and indicate that the complexes appear to be stable. The spectrum of pure DNA in solution shows a positive maximum at about 280 nm, with a negative minimum near 245 nm. The crossover point between them is at about 260 nm. These values imply that pure DNA in solution occurs in the dextrorotatory B-DNA form [19], which is the expected natural conformation of the native, fully hydrated double helix DNA structure. With increasing surfactant concentration, the CD spectrum is shifted towards longer wavelengths (Δλ) from about 245 nm to 250 nm. After the addition of a surfactant to the DNA solution, the positively charged surfactant groups interacted with the negatively charged polyanionic DNA molecules and, consequently, the hydrophobic components of the surfactant were exposed towards the solution. The B-DNA form remains in the solution probably up to the surfactant concentration c = 0.5 mM and 1 mM, as above this concentration the positive and the negative bands disappear in the CD spectra. From the point of view of gene therapy applica-

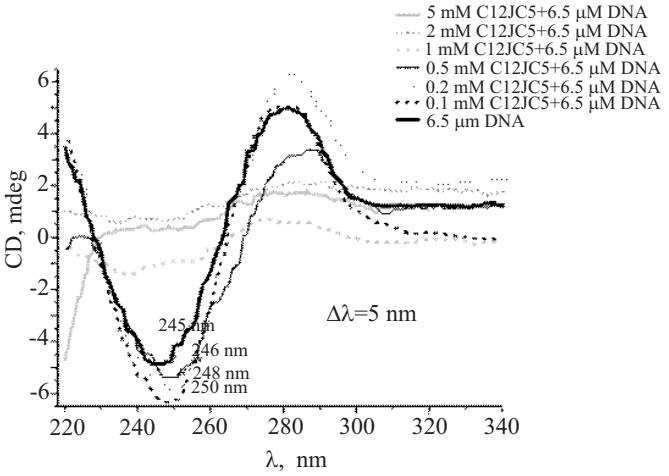


Fig. 2. CD spectra of C12JC5-DNA complexes as a function of light wavelength; a shift by Δλ towards longer wavelengths with increasing surfactant concentration is marked

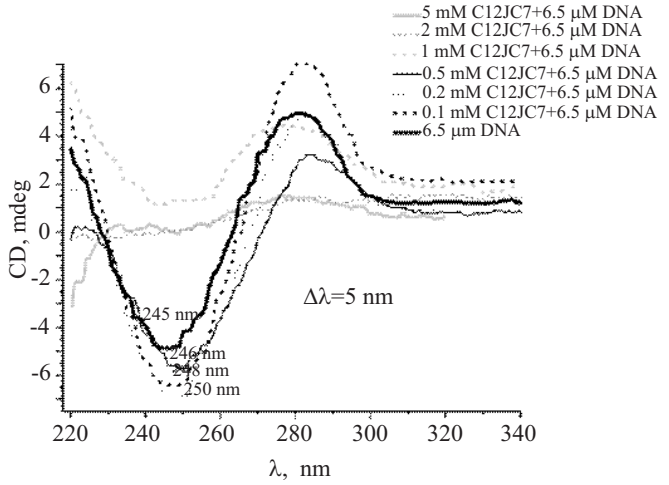


Fig. 3. CD spectra of C12JC7-DNA complexes as a function of light wavelength; a shift by Δλ towards longer wavelengths with increasing surfactant concentration is marked

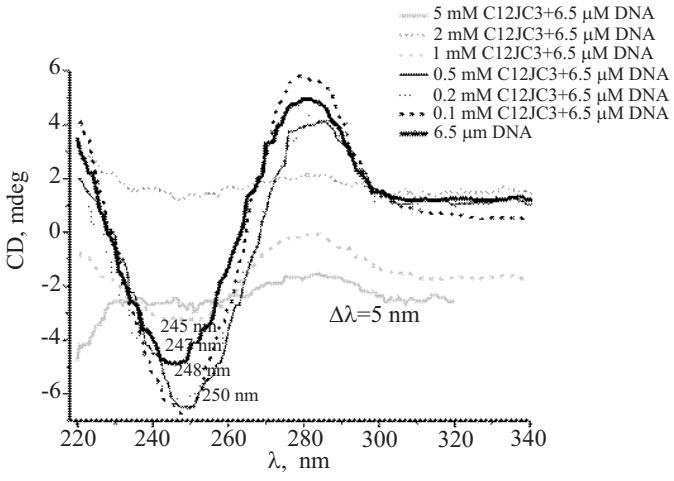


Fig. 1. CD spectra of C12JC3-DNA complexes as a function of light wavelength; a shift by Δλ towards longer wavelengths with increasing surfactant concentration is marked

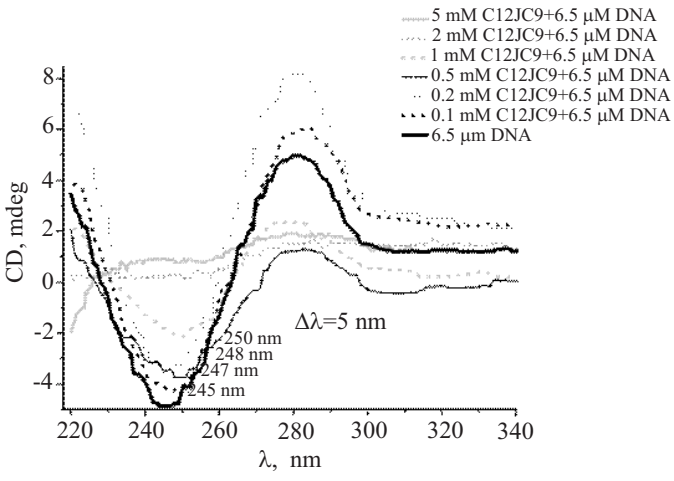


Fig. 4. CD spectra of C12JC9-DNA complexes as a function of light wavelength; a shift by Δλ towards longer wavelengths with increasing surfactant concentration is marked

tion, it would be most desirable that the DNA molecule, as a result of interaction with the surfactant, would assume the Ψ DNA form, which is strongly condensed. On the basis of our results, we could conclude the formation of complexes, but the CD spectra analysis of the complexes did not indicate the formation of the Ψ DNA form. The changes in the CD spectra could be caused by changes in the hydration shell of the DNA phosphate groups upon their interactions with the positively charged surfactant groups. This interaction could also bring about disturbances in the geometry of DNA nitrogen bases. A similar effect was observed earlier [20] for the DNA lipoplexes with 1,5-bis (1-imidazolilo-3-decyloxymethyl) pentane dichloride. In other CD spectroscopic studies on systems based on dodecyltrimethylammonium bromide (DTAB) as a DNA complexing agent, similar changes in the CD spectra were observed upon complexation yet they could not be assigned to conformational changes from B-DNA to A-DNA or from B-DNA to Z-DNA [19, 21]. Similar effects have also been reported by Chang et al. [19] in an investigation of DNA interactions with polyamines.

The results of electrophoretic separation in 1 % agarose gel are shown in Figs 5—9.

After electrophoresis of the studied complexes, a decrease in fluorescence intensity was taken as a measure of the capacity of the gemini surfactant to form its complex with DNA. According to the results, the addition of C12JC4 and C12JC6 surfactants almost totally inhibited the electrophoretic mobility of DNA molecules at a concentration of 2 mM, which means that the negative charge of DNA was neutralised because of the formation of stable DNA/gemini surfactant complexes. For the other studied surfactants, the effect of complexation was also observed but it was not so strong. Other surfactants form stable complexes only at a concentration of 5 mM. The differences in the level of complexation are related to the geometry of surfactant and in particular to the length of the spacer group.

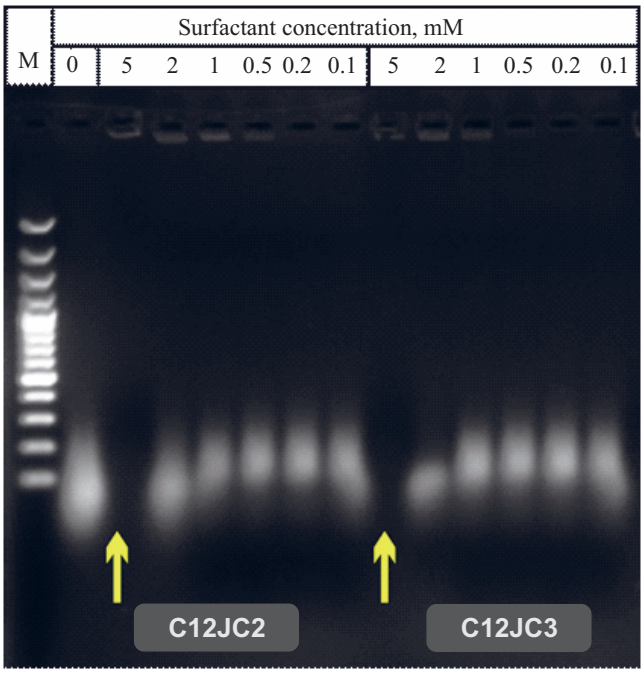


Fig. 5. Electrophoretic separation of C12JC2-DNA and C12JC3--DNA complexes in 1 % agarose gel; the lipoplexes are marked by arrows, *M* is a DNA molecular weight standard

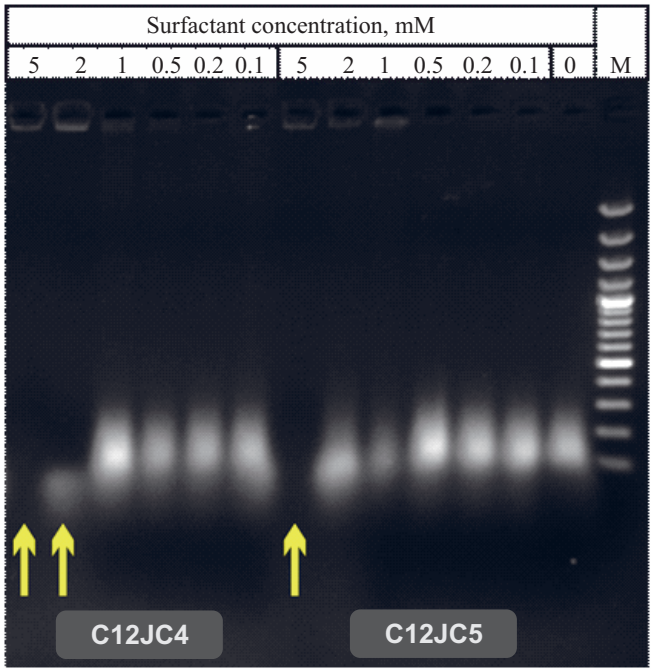


Fig. 6. Electrophoretic separation of C12JC4-DNA and C12JC5--DNA complexes in 1 % agarose gel; the lipoplexes are marked by arrows, *M* is a DNA molecular weight standard

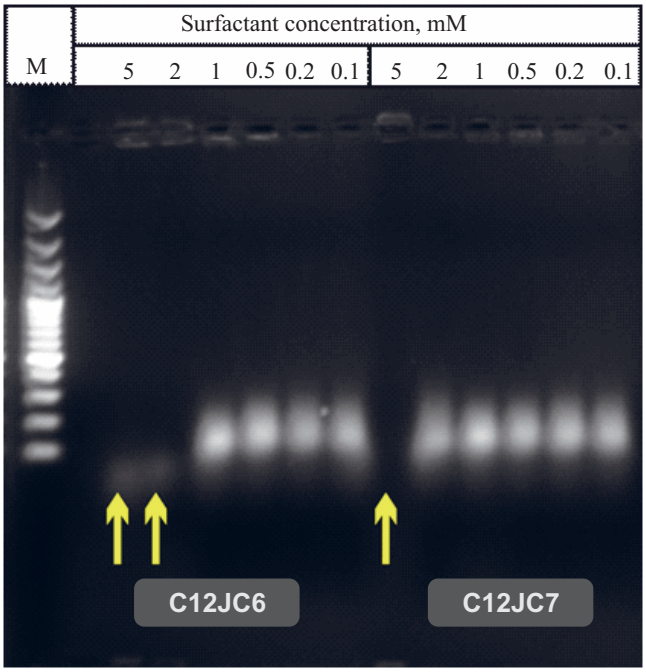


Fig. 7. Electrophoretic separation of C12JC6-DNA and C12JC7--DNA complexes in 1 % agarose gel; the lipoplexes are marked by arrows, *M* is a DNA molecular weight standard

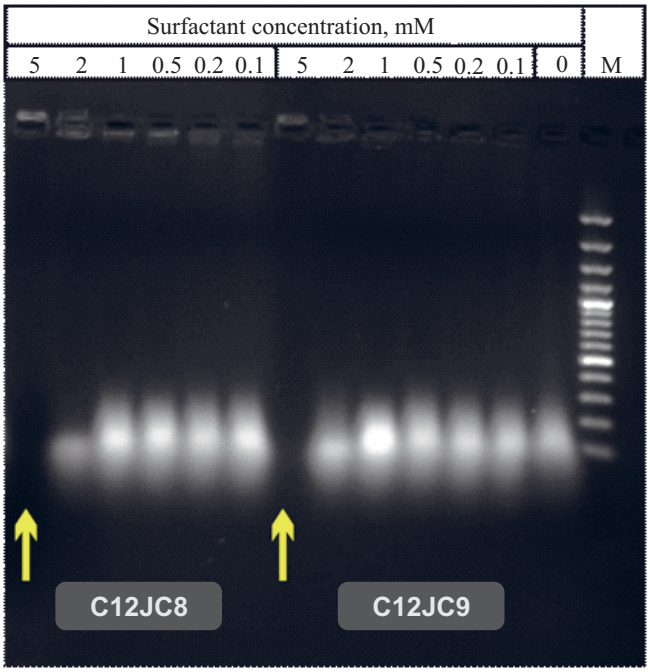


Fig. 8. Electrophoretic separation of C12JC8-DNA and C12JC9--DNA complexes in 1 % agarose gel; the lipoplexes are marked by arrows, *M* is a DNA molecular weight standard

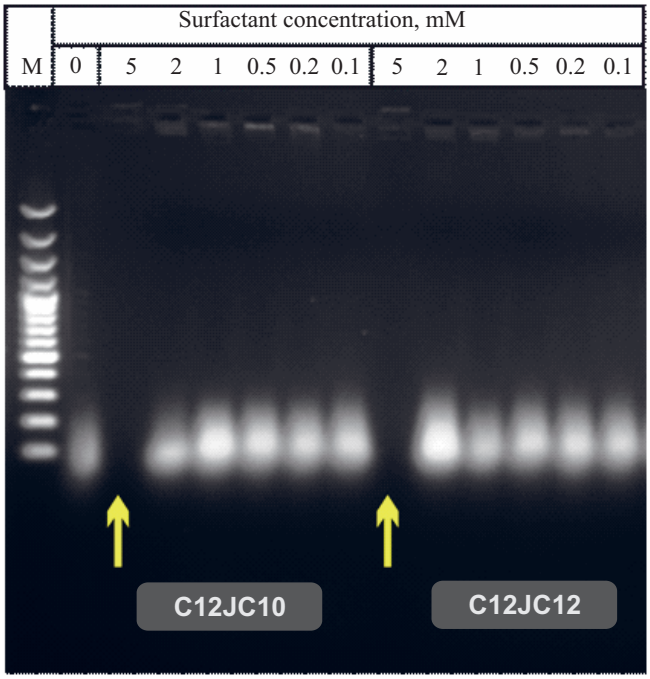


Fig. 9. Electrophoretic separation of C12JC10-DNA and C12JC12--DNA complexes in 1 % agarose gel; the lipoplexes are marked by arrows, *M* is a DNA molecular weight standard

As follows from the results of electrophoresis, the strongest complexes are formed for the surfactant with a spacer group made of 4 methylene groups. The shortest spacer group is the least advantageous as it probably disturbs the geometry of the dimeric forms of the surfactant molecules. The reduced electrophoretic mobility, observed for the studied lipoplexes, was also noted for other DNA delivery systems formulated on gemini surfactants [22].

CONCLUSIONS

The above presented results of CD spectroscopy and gel electrophoresis have definitely shown that the surfactants studied form stable complexes with low molecular weight DNA. The process of complex formation is most effective for the charge ratio (p/n) of surfactant: DNA of about 4:1. The observed changes in CD spectra as a result of complexation should be assigned to local disturbances in the geometry of nitrogen bases in DNA rather then to the disturbances in the helical DNA structure. To confirm the potential biomedical applications of the studied gemini surfactants, further studies of DNA transfection efficiency are necessary.

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