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# Stimulus-sensitive liposomal delivery system based on new 3,7-diaza bicyclo[3.3.1]nonane derivatives

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ABSTRACT

3,7-Diazabicyclo[3.3.1]nonane scaffold can serve as a basis for the design of molecular switches stimulating the fast release of water soluble compounds under the influence of external factors from the liposomal containers having those switches incorporated into the lipid bilayer. It was demonstrated that liposomes having 3,7-dihexadecyl-1,5-diphenyl-3,7-diazabicyclo[3.3.1]nonan-9-one (**3**) incorporated into the liposomal membrane sharply increase the permeability upon pH decrease from 7.4 to 6.5, and compound **3** can serve as a pH-sensitive agent in the bilayer of liposomal nanocontainers. Similar but less pronounced effect was shown for liposomes modified with 3,7-bis(methyldodecylaminoacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonane (**5**) and 3,7-didodecylsulfonyl-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one (**4**). The structure (morphology) and size of modified liposomes were studied with scanned transmission electron microscopy.

The development of techniques for the targeted delivery of biologically active compounds/"payloads" is of great importance for medical applications as well as for the design of new systems for the directed transport of various compounds in general.[1–4] The objects of our studies are liposomes – spherical lipid bilayer vesicles containing the aqueous core separated from the external liquid by the membrane.[5,6] Inclusion of amphiphilic compounds into the bilayer can change the permeability of the membrane either permanently or at a certain moment under the influence of external factors, depending on the embedded components. The liposomes modified with such compounds can be used for the targeted delivery/release of substances encapsulated in the internal liposomal volume.[7–10]

Earlier we have shown that various derivatives of 3,7-diazabicyclo [3.3.1]nonane (bispidine) with long alkyl substituents at nitrogen atoms incorporated into the lipid bilayer are capable to conformational reorganization under the protonation or formation of complexes with metal cations.[11–14]

It was demonstrated that bispidinone derivatives **1** with long-chain alkyl substituents at nitrogen atoms and methyls in positions 1,5 under the complexation with $Cu^{2+}$ ions change a chair-boat conformation for a chair-chair conformation (Scheme 1) and such conformational transition loosens the packing of the lipid tails in the lipid bilayer thus triggering the release of liposome content.[12–14]

These compounds proved to be good lipid bilayer modifiers for the design of stimulus-sensitive liposomal nanocontainers. However the bispidinone derivatives described earlier did not provide the quick enough release of the liposome content in the necessary pH range. But varying the substituents at nitrogen atoms $R^2$ and at carbon atoms $R^1$ and X, a wide range of compounds **2** (Scheme 2) selectively sensitive to the changes of various external factors (e.g. pH change or the presence of $Cu^{2+}$ ions) can be easily obtained.

The search for new bispidinone derivatives as molecular switches which are capable to promote the release of compounds from the stimulus-sensitive liposomes in the narrower necessary pH-range with higher kinetics as well as new metal cation sensitive modified liposomes is still a challenge.

In this paper we describe the conformational reorganization of four amphiphilic 3,7-diazabicyclo[3.3.1]nonane derivatives (compounds **1a, 3**–**5**) in the liposomal membrane upon pH change or addition of metal cations as well as the release of the content from stimulus-sensitive liposomes modified with those derivatives. The liposomes containing phosphatidylcholine (PC) and the appropriate bispidine derivative in 3:1 M ratio (for compounds **1a**, **3**, and **5**) were loaded with water-soluble fluorescent dye 5(6)-carboxyfluorescein (CF) in the concentration

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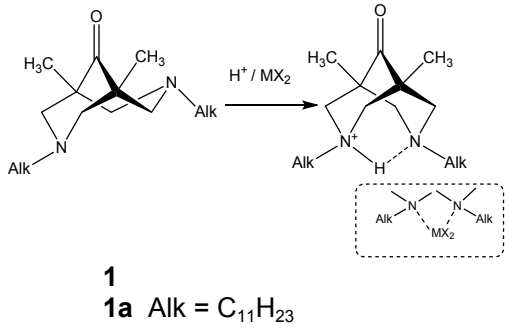
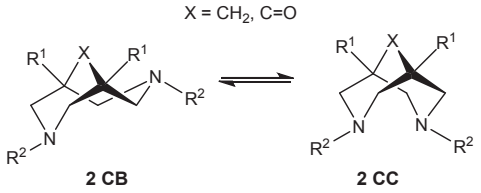


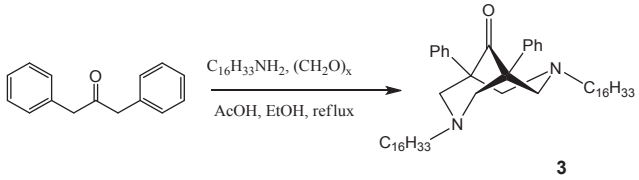

**1**

**1a** Alk = $C_{11}H_{23}$

**Scheme 1.** Chair-boat CB to chair-chair CC switching under external factors.



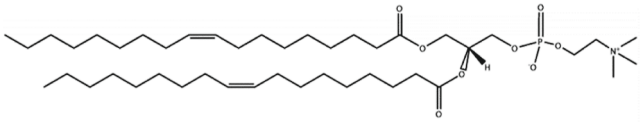

**Scheme 2.** Chair-boat CB/chair-chair CC equilibrium.



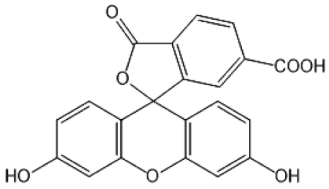

**3**

**Scheme 3.** Synthesis of compound **3**.

exceeding its self-quenching concentration (see details in Experimental Section in Supplementary Data).



**Phosphatidylcholine (PC)**




**5(6)-Carboxyfluorescein (CF)**

The leakage of CF from liposomes was accompanied by its dilution in the outer volume and was registered as the increase in fluorescence intensity. To obtain the maximal release the liposomes were destroyed by the addition of Triton X-100 detergent.

Compound **3** (3,7-dihexadecyl-1,5-diphenyl-3,7-diazabicyclo[3.3.1] nonan-9-one) was obtained by Mannich reaction starting from hexadecylamine acetate, paraformaldehyde and 1,3-diphenylpropan-2-one according to the modified method[15] proposed by Chiavarelli (Scheme 3). It should be noted that despite Mannich reaction is a convenient one-step approach for the synthesis of bispidinones, the increase of the length of substituents leads to emulsion formation and in contrast to[15] the chromatographic isolation of the product was necessary.

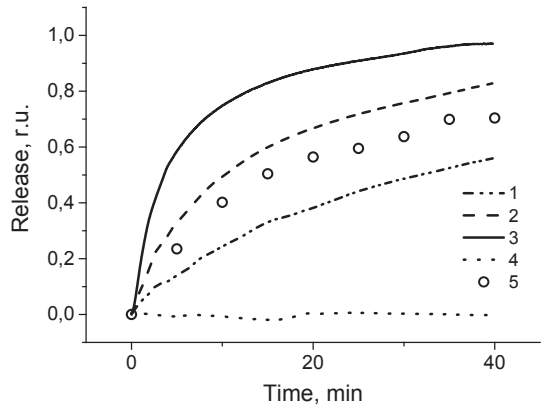



**Fig. 1.** Time-dependent normalized release of CF from PC/**3** liposomes in the presence of $Cu^{2+}$ 0.4 (*1*), 0.53 (*2*), 0.66 (*3*) mM and without $Cu^{2+}$ (*4*), and from PC/**1a** liposomes in the presence of 0.66 (5) mM of $Cu^{2+}$. Concentration of liposomes 0.5 mg/mL. t = 22 °C, pH = 7.4.

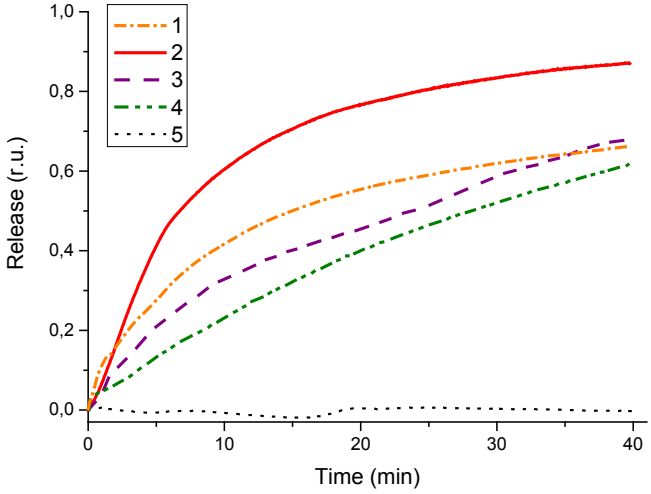
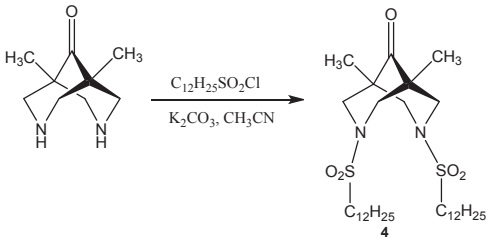


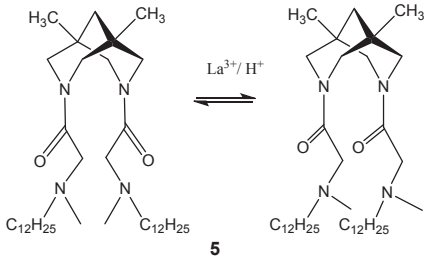

**Fig. 2.** Release from PC/**1a** (*1*), PC/**3** (*2*), PC/**4** (*3*), PC/**5** (*4*) liposomes at pH 6.5. Control experiment – PC/**3** (*5*) pH 7.4. t = 22 °C.

It was shown earlier that 1,5-diphenyl-3,7-diazabicyclo[3.3.1] nonan-9-one with two methyl substituents at the nitrogen atoms adopts preferably a chair–boat conformation both in the crystalline state and in solution.[11,16,17] Such compounds can change their conformation from a chair-boat to a chair-chair after complexation with copper ions[18] or after protonation of one of the nitrogen atoms. It was logical to expect the same conformational transitions for the bispidine derivatives with longer alkyl substituents.

Liposomes containing compound **3** (PC/**3**) showed the release sensitivity to the presence of $Cu^{2+}$ and $H^+$ ions. Fig. 1 represents the kinetic curves of the release from PC/**3** induced by the addition of different concentrations of $Cu^{2+}$. The control experiment in the absence of $Cu^{2+}$ (curve *4*) did not show any dye leakage. Addition of $Cu^{2+}$ solution to the liposomal suspension led to a rapid release of encapsulated CF. The increase in metal ion concentration led to more prominent release – both the initial rate and the maximal release increased. The PC/**3** liposomes demonstrated significantly better release properties than the earlier described liposomes modified with derivatives **1**[12] – compare curves *3* and *5* in Fig. 1. Thus, PC/**3** liposomes may find promising applications as $Cu^{2+}$-sensitive liposomal containers for bioactive




**Scheme 4.** Synthesis of compound **4**.




**Scheme 5.** Anti-parallel and parallel arrangement of acyl substituents in molecule 5.

compounds.

Compound **3** differs from **1** by 1,5-substituents, thus, we also expected for PC/**3** liposomes some pH sensitivity (as it was shown earlier for other bispidine derivatives in Ref. 14). Fig. 2 shows the kinetic release profile from PC/**3** liposomes (curve *2*) under the decrease of pH value from 7.4 to 6.5 with the initial release rate 0.088 $min^{-1}$. The control experiment, with no change of pH value (pH 7.4), was also performed (curve *5*), and showed no release from liposomes.

Compound **4** was synthesized from 1,5-dimethyl-3,7-diazabicyclo [3.3.1]nonan-9-one according to Scheme 4. The liposomes with incorporated compound **4** (PC/**4**) seemed to be more defective than PC/**3** on the stage of preparation. The basic fluorescence after the separation of unloaded CF was higher than that for PC/**3**. We suggested that compound **4** made the liposomal membrane more rigid and it was easily disrupted while passing through the column.

The conformational preferences for compound **4** are not quite clear, however somewhat analogous 3,7-ditosyl-1,5-diphenyl-3,7-diazabicyclo[3.3.1]nonan-9-one adopts in a crystalline state a chair-boat conformation.[19] It was interesting to study the influence of compound **4** on the formation and stability of PC/**4** liposomes as well as their possible pH sensitivity (for the protonation of sulfonamides in strongly acidic media see, e.g.).[20]

It is worth noting that the molar fraction of compound **4** in PC/**4** liposomes was not 0.25, our experiments have shown that the liposomes without defects can be obtained only with 10% concentration of substituted bispidine **4**. The high basic fluorescence has not let us make a convincing conclusion about the sensitivity of PC/**4** liposomes to $Cu^{2+}$ ions, however we succeeded to detect the pH sensitivity. The decrease of pH value from 7.4 to 6.50 induced CF leakage from PC/**4** liposomes (Fig. 2, curve *3*). The initial release rate for PC/**4** liposomes (0.045 $min^{-1}$) was about 2 times slower than that for PC/**3** ones (compare curves *3* and *2* in Fig. 2). Also, the release from PC/**4** did not reach the maximal value in 40 min after the pH change. The nature of PC/**4** pH sensitivity is not quite clear. Probably, it is related to poor compatibility of compound **4** and PC lipid. Thus, even a slight change in the media (such as pH or ionic strength) can destabilize the system crucially.

In our previous work[21] we described 3,7-bis(diethylaminoacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonanes and their ability to undergo the conformational reorganization under the influence of solvent polarity, protonation or complexation with lanthanum cations which

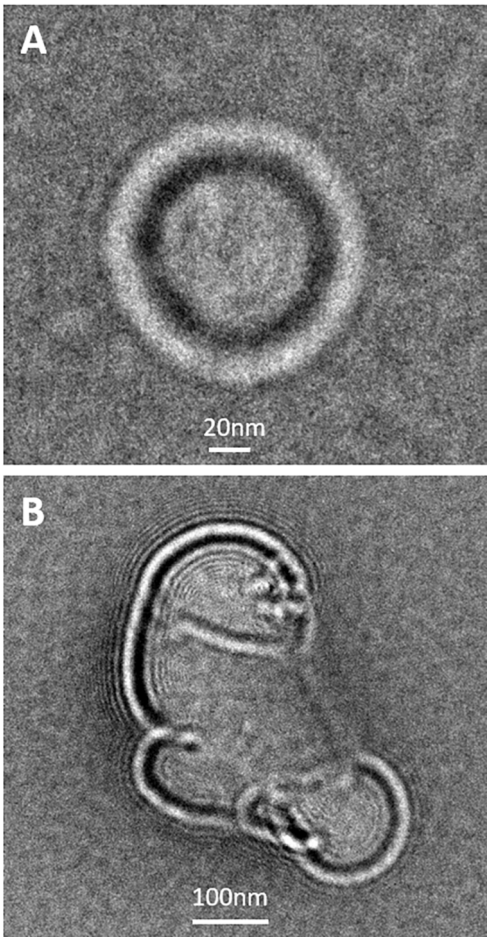



**Fig. 3.** TEM images of liposomes (A and B) modified with compound **5**.

was proved by means of NMR titration techniques, and ability of amphiphilic derivatives **5** to be embedded into the liposomal membranes. Here we performed the experiments on the stability of modified by amphiphilic compound **5** liposomes under the influence of external factors – upon the pH change and addition of lanthanum ions (Scheme 5).

Indeed, the change of pH value to 6.50 induced the release of CF from PC/**5** liposomes (Fig. 2, curve *4*) with the initial release rate 0.012 $min^{-1}$. The proof of the $La^{3+}$ sensitivity of PC/**5** liposomes was a challenge as we were limited in pH range. On the one hand, in alkaline media $La^{3+}$ precipitates as $La(OH)_3$ ($pK_b = 3.30$), on the other hand, in acidic media PC/**5** liposomes leak due to the protonation (Fig. 2, curve *4*). Moreover the $La^{3+}$ concentration had to be controlled properly and could not exceed 21 μM ($K_{sp} = 2 \times 10^{-19}$). But, hopefully, even with those limitations we succeeded to register 6% CF leakage after the addition of $La^{3+}$ ions to PC/**5** liposomes as compared to PC/**1a** and PC/**4** liposomes (see Supplementary Data, Fig. S1).

In addition we made the experiments on stabilization-destabilization of liposomes modified by 3,7-diundecyl-1,5-dimethyl-3,7-diazabicyclo [3.3.1]nonan-9-one **1a**. The synthesis of 3,7-dialkyl-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one homologs and the sensitivity of compound **1a** to $Cu^{2+}$ ions was described earlier.[12] This compound should not be sensitive to the presence of $La^{3+}$ ions, and that was proved by the absence of CF leakage upon the addition of $LaCl_3$ solution to the liposomal suspension. On the other hand, the decrease of pH was accompanied by the protonation of molecules **1a** that forces them to adopt a chair-chair conformation according to Scheme 1. This resulted in the formation of defects in the lipid bilayer and leakage of CF with initial rate 0.074 $min^{-1}$ (Fig. 2, curve *1*).

The structure (morphology) and size of liposomes modified with compound **5** were studied with scanned transmission electron microscopy (TEM)[22] with CsI as a contrast with accelerating voltage 200 kV (in STEM Titan Themis Z) and are shown in Fig. 3.

Microphotographs show the objects of individual liposomes (A), as well as destruction during TEM image and adhesion of liposomes (B). The measured thickness of the lipid bilayer and the diameter of the liposome are shown.

In summary, varying the substituents of 3,7-diazabicyclo[3.3.1]nonane scaffold it is possible to synthesize molecular switches which can stimulate the fast release of water soluble compounds from liposomal containers under the influence of various external factors. Liposomes modified by 3,7-dialkyl-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one may find promising applications as $Cu^{2+}$-sensitive and pH-sensitive liposomal containers for bioactive compounds. We also have shown that 3,7-dialkyl-1,5-diphenyl-3,7-diazabicyclo[3.3.1]nonan-9-one **3** incorporated into the liposomal membrane increases the liposome permeability upon pH decrease from 7.4 to 6.5. It was confirmed that 1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonane derivatives with methyldodecylaminoacetyl substituents at nitrogen atoms and sulfonamide derivatives of 1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one could serve as pH-sensitive agents in liposomal nanocontainers.

## 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.

## Acknowledgement

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## Appendix A. Supplementary data

Supplementary data experimental section, characterization data including $^{1}H$ NMR, $^{13}C$ NMR spectra for all isolated compounds, additional supporting information to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127871.

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