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pH-Sensitive liposomes with embedded 3,7-diazabicyclo[3.3.1]nonane derivative

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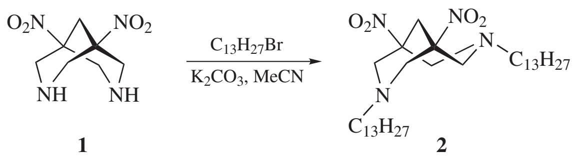
1,5-Dinitro-3,7-ditridecyl-3,7-diazabicyclo[3.3.1]nonane modulates the permeability of liposomal membranes as pH of surrounding aqueous solution decreases from 9 down to 5.

An important role in modern medicine belongs to nanocontainers capable of addressing drugs to target cells and tissues.[1] Spherical lipid vesicles (liposomes) are considered as a promising tool for drug delivery since the liposome can encapsulate water-soluble bioactive compounds in the inner water cavity and carry them to affected areas.[2–4] In order to increase the efficiency of therapeutic effect of liposomal drugs, the liposomes are modified so that they release their contents upon the change of external conditions (temperature, pH, radiation, *etc.*).[5,6] A pH-mediated drug release can be induced through the incorporation of a special amphiphilic compound ('conformational switch') which changes its conformation in response to the acidification of a surrounding solution.[7–9]

Earlier we have shown that liposomes with 3,7-dialkyl-3,7-diazabicyclo[3.3.1]nonan-9-ones incorporated into the lipid bilayer increase their permeability upon addition of bivalent copper salts.[10,11] The effect is due to the ability of such compounds to change their conformation from the chair-boat to chair-chair with different orientation of substituents after complexation with copper (2+) ions, that finally loosens the alkyl chain packing in the membrane and initiates a liposome content release.[12–17]

In this communication we describe the synthesis of 3,7-diazabicyclo[3.3.1]nonane-based conformational switch with two long alkyl substituents, preparation of liposomes with the switches embedded into the membranes and a release of the content from switch-containing liposomes in the acidic pH region.

The synthesis was performed[†] as outlined in Scheme 1. First, 1,5-dinitro-3,7-di-*tert*-butyl-3,7-diazabicyclo[3.3.1]nonane was synthesized by the Mannich reaction from nitromethane, *tert*-butylamine, and paraformaldehyde[18] and then converted to 1,5-dinitro-3,7-diazabicyclo[3.3.1]nonane **1**[19] by refluxing in concentrated hydrobromic acid with further treatment with NaOH solution. Finally, compound **1** was alkylated with 1-bromotridecane in absolute acetonitrile in the presence of K_2CO_3 thus resulting in 1,5-dinitro-3,7-ditridecyl-3,7-diazabicyclo[3.3.1]nonane **2**.



Scheme 1

Liposomes were prepared[20] from electroneutral phosphatidylcholine (PC) and the conformational switch **2** by sonicating[‡] the PC/**2** mixture in a 10^{-3} M buffer solution with pH 10. In order to control the permeability of liposomal membranes, the inner water cavity of liposomes additionally contained 1 M NaCl solution; a leakage of a NaCl solution from liposomes was measured using the conductivity method.[21]

The experiments were done as follows. The initial 10 mg ml^{-1} mixed PC/**2** liposome suspension was quickly diluted down to 1 mg ml^{-1} suspension with a 10^{-3} M buffer solution with a desirable pH value, and the conductivity of thus prepared pH-gradient liposomes was monitored. An ultimate level of conductivity, achieved 10 min after (G_{ult}), corresponded to the maximum NaCl amount capable of releasing from liposomes at a particular pH. A 100% conductivity ($G_{\max}$) was obtained when the suspension of NaCl-loaded liposomes was completely destroyed by addition of Triton X-100 surfactant. The conductivity for 1 mg ml^{-1} suspension of PC/**2** liposomes free of NaCl was taken as the initial level of conductivity (G_0) at each specific pH. A relative conductivity $(G_{\text{ult}} - G_0)/(G_{\max} - G_0)$ thus reflected a release of NaCl from the pH-dependent PC/**2** liposomes.

Figure 1 shows a time-dependent leakage of NaCl solution from the NaCl-loaded liposomes into a surrounding solution

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[†] *1,5-Dinitro-3,7-ditridecyl-3,7-diazabicyclo[3.3.1]nonane* **2**. The mixture of 1,5-dinitro-3,7-diazabicyclo[3.3.1]nonane **1** (0.1 g, 0.4 mmol), 1-bromotridecane (0.29 ml, 1 mmol) and K_2CO_3 (0.4 g, 3 mmol) was refluxed in MeCN for 12.5 h and then filtered. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel, the eluents were light petroleum, light petroleum–benzene (10:1, 3:1, 1:1) After evaporation of the solvent compound **2** was obtained, yield 40%, mp 47–48 °C. IR (ν/cm^{-1}): 1560, 1470 (NO_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, 6H, *J* 9 Hz), 1.28 (m, 40H), 1.47 (m, 4H), 2.49 (t, 4H, *J* 9 Hz), 2.69 (s, 2H), 2.86, 2.94 (2d, 8H, bispidine, J_{gem} 13 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.12, 22.69, 22.64, 27.18, 29.37, 29.66, 31.93, 35.00, 56.24, 58.99, 84.75. Found (%): C, 68.49; H, 11.11; N, 9.65. Calc. for $C_{33}H_{64}N_4O_4$ (%): C, 68.23; H, 11.20; N, 9.46.

[‡] *Liposome preparation.* Appropriate amounts of PC and **2** solutions in chloroform (3:1 molar ratio) were mixed in a flask, after which the solvent was evaporated *in vacuo*. A thin lipid film was dispersed for 400 s in a hydrocarbonate buffer (pH 10, 10^{-4} M) additionally containing NaCl with a 4700 Cole-Parmer ultrasonic homogenizer. Liposome samples were separated from titanium dust by centrifugation for 5 min at 10000 rpm and dialyzed. Liposomes with a molar fraction of compound **2** equal to 0.25 were thus obtained. The size (hydrodynamic diameter) of vesicles fluctuated from sample to sample but was retained within 80–130 nm range.

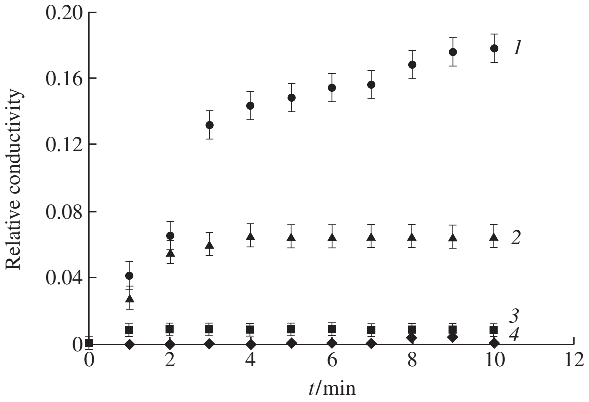


Figure 1 Time-dependent change in conductivity of the NaCl-loaded PC/**2** liposome suspension *vs*. pH of the outer solution. (*1*) 10^{-3} M acetate buffer, pH 5, (*2*) 10^{-3} M TRIS buffer, pH 7, (*3*) 10^{-3} M TRIS buffer, pH 8, (*4*) 10^{-3} M borate buffer, pH 9.

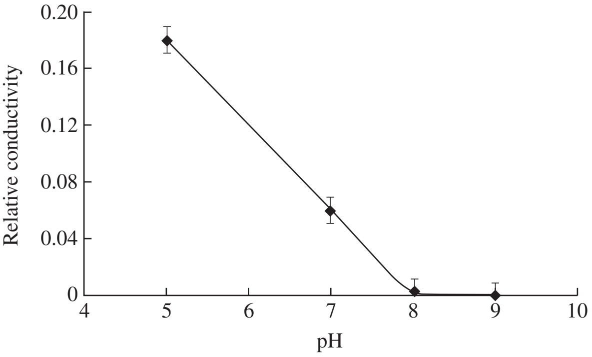
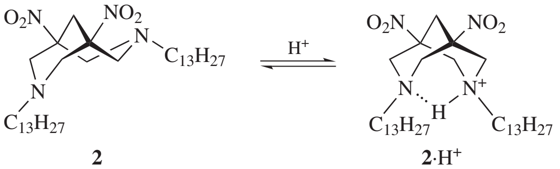


Figure 2 The ultimate conductivity of the NaCl-loaded PC/**2** suspension *vs*. pH of the outer solution.

whose pH varied from 9 down to 5. No leakage of NaCl from the PC/**2** liposomes was detected in surrounding solutions with pH 9 and 8 (curves *4* and *3*). A decrease in the pH of the surrounding solution down to 7 had risen the total conductivity (curve *2*), thus showing the formation of defects in the liposomal membrane and leakage of NaCl. Further decrease in the outside pH down to 5 resulted in a progressive growth of the released NaCl (curve *1*). In the latter two cases, the release was fast and completed within a few minutes after changing pH of the outer solution. The ultimate NaCl release is shown in Figure 2. Importantly, liposomes deprive of the conformational switch (composed of neutral PC alone) and loaded with NaCl solution do not leak either at pH 7 or 5.

A pH-induced release of NaCl solution from the NaCl-loaded PC/**2** liposomes can be attributed to structural rearrangements in the molecules of **2** incorporated into the liposomal membrane. It has been shown earlier that 3,7-diazabicyclo[3.3.1]nonan-9-one derivatives with two methyl substituents at nitrogen atoms change their chair-boat conformation to chair-chair conformation when protonated.[12,13,16,17] The same conformational transition can be reasonably expected in compound **2** (Scheme 2). According to this scheme, the molecules of **2**, embedded into the liposomal membrane, are in a chair-boat conformation with distant alkyl substituents in slightly alkaline (pH 9 and 8) solutions. Both alkyl radicals are incorporated into the liposomal membrane with no defects in the mixed PC/**2** packing and no release of encapsulated NaCl solution. Decrease of surrounding solution pH down to 7 and to 5 is accompanied by the protonation of molecules **2** that causes them to adopt chair-chair conformation with different orientation of alkyl chains. This conformational re-organization loosens the packing of alkyl chains in the membrane and initiates a leakage of NaCl solution from inside liposomes.



2 **2**·H$^+$

Scheme 2

Thus, we have shown that 1,5-dinitro-3,7-ditridecyl-3,7-diazabicyclo[3.3.1]nonane incorporated into the liposomal membrane increases the liposome permeability upon decreasing pH from 9 to 5.

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