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# Bispidinone-based molecular switches for construction of stimulus-sensitive liposomal containers

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ABSTRACT

It is demonstrated that new lipid-like amphiphilic compounds, derivatives of 3,7-diazabicyclo[3.3.1]nonan-9-one (bispidinone) with long alkyl substituents, can be integrated into liposomal membranes. They can serve as molecular switches changing the conformation from the chair–boat to chair–chair on addition of an aqueous solution of a bivalent copper salt, and thus enhancing the permeability of the lipid bilayer of liposomes and the release of encapsulated compounds.

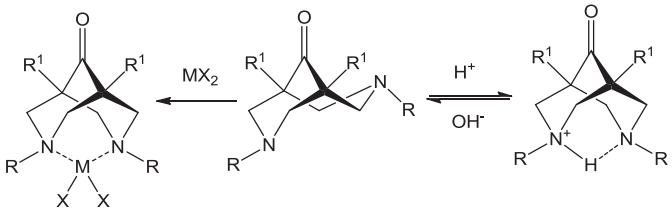


## 1. Introduction

Efficacy of drug delivery is a key problem of modern pharmacology.[1] Liposomes, spherical lipid bilayer vesicles are widely used for the controllable encapsulation and release of drugs: hydrophilic compounds can be dissolved in the inner water cavity, while the hydrophobic ones are incorporated into the lipid bilayer.[2] Liposomal containers protect the encapsulated drugs from degradative enzymes and thus enhance the circulation time and bioavailability of drugs.[3] A therapeutic effect of a drug significantly increases if drug leakage from the liposome occurs in the vicinity of a target cell or organ. The leakage can be initiated by changing temperature or pH, and by enzymatic cleavage of labile chemical bonds as well.[4–9]

In the present article we describe the synthesis of lipid-like amphiphilic compounds capable of incorporating into the lipid bilayer and serving as 'molecular switches' undergoing conformational reorganization in the presence of bivalent copper ions. This is accompanied by the formation of defects in the lipid packing and a sharp increase in the permeability of a liposomal membrane. The liposomal containers with such stimulus-sensitive molecular switches can be applied for the encapsulation and subsequent release of drugs, which control the copper level in patients with various pathologies, e.g., hepato-cerebral dystrophy (Wilson disease).[10–12]

The molecular switches were designed on the basis of 3,7-diazabicyclo[3.3.1]nonan-9-one (bispidinone) scaffold. It has been shown earlier that bispidinone derivatives with two short alkyl substituents at nitrogen atoms adopt a chair–boat (CB) conformation **1** in alkaline media while a chair–chair (CC) conformation (**2**, **3**) with different orientation of substituents is preferable in the acidic media or after complexation with bivalent metal cations.[13–22] It was natural to expect the same conformational transitions for bispidinone derivatives with longer lipophilic substituents. In this work bispidinone-based molecular switches with long alkyl substituents were synthesized and studied.




**3** **1** **2**

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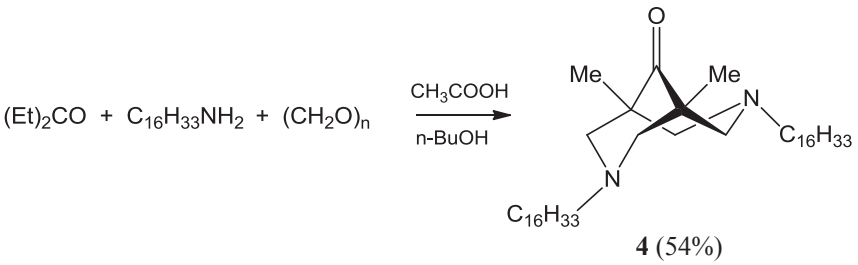
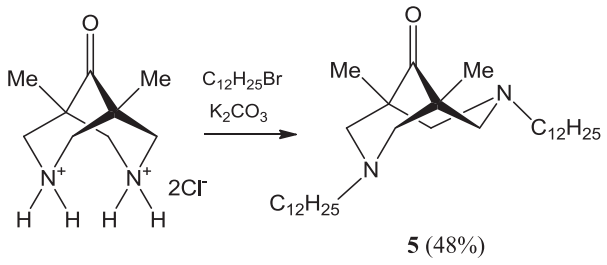
## 2. Results and discussion

*N*,*N*′-Dihexadecyl bispidinone derivative (**4**) was obtained by the Mannich reaction (analogously to bispidinones with 'short' substituents[17]) from diethylketone, hexadecylamine, and paraformaldehyde with 54% yield when the reaction mixture was refluxed in acetic acid/*n*-butanol (Scheme 1).




**Scheme 1.**

An alternative approach was used for the synthesis of bispidinone *N*,*N*′-didodecyl derivative (**5**) from 1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one dihydrochloride.[23] The alkylation was performed in mild conditions with the use of $K_2CO_3$ in anhydrous $CH_3CN$; the best result (48% yield) was obtained when stirring the reaction mixture for several days at 50 °C (Scheme 2).




**Scheme 2.**

A suspension of unilamellar liposomes was prepared by sonication[2,24] of a mixture composed of zwitter-ionic egg yolk lecithin (EL) and either compound **4** or **5**. The molar fraction of **4** or **5** in the **4**(**5**)/EL mixture was equal to 0.25. The hydrodynamic diameter of liposomes with embedded **4**(**5**) was found to fluctuate from sample to sample but always fell into the 80–130 nm range; for each specific liposome sample the polydispersity index did not exceed 0.05. The integrity of mixed liposomes was controlled by the fluorescence. The liposomes were obtained with water-soluble carboxyfluorescein (CF) inside, whose concentration exceeded its self-quenching concentration. A leakage of CF from liposomes was accompanied by its dilution and increase in its fluorescence intensity. The result was represented as: $I$(r.u.)=$(I-I_0)/(I_{max}-I_0)$, where $I$ is current fluorescence, $I_0$ is the fluorescence of the initial CF-loaded liposomes and $I_{max}$ is the fluorescence of the liposomes destroyed in the presence of Triton X-100 detergent.

Fig. 1a shows how the fluorescence of CF-loaded **5**/EL liposome suspension changed when $CuSO_4$ solution was added. In the control experiment no increase in the CF-fluorescence of the CF-loaded liposome suspension was detected within a 4-h interval (**curve 1**) thus indicating no spontaneous release of the dye from the initial CF-loaded **5**/EL liposomes. Addition of a $0.15\times10^{-4}$ M $CuSO_4$ solution to the liposome suspension gave rise to significant fluorescence intensity (**curve 2**); more added $CuSO_4$ solution resulted in sharper fluorescence increase (**curve 3**). These results indicated that a $CuSO_4$ solution induced a release of CF from **5**/EL liposomes to the surrounding solution; a major part of CF leaked from liposomes within 1.5 h after $CuSO_4$ addition. Addition of a $CuSO_4$ solution to a suspension of CF-loaded **4**/EL liposomes was also accompanied by a CF release; though the effect was somewhat less pronounced (Fig. 1b). In contrast to this, CF-loaded EL liposomes

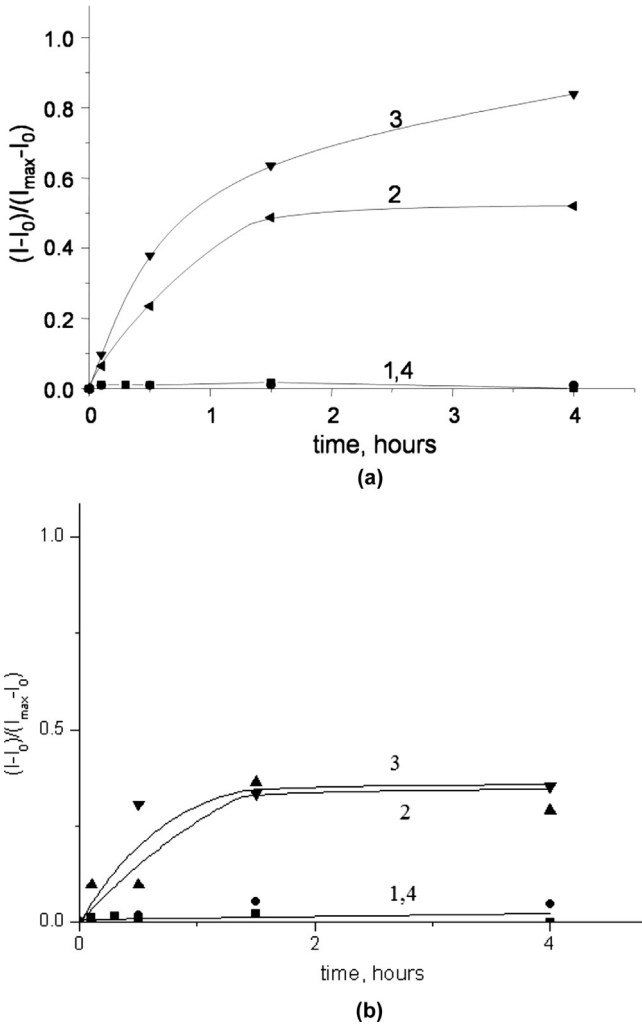



**Fig. 1.** Time-dependent change in the fluorescence intensity of CF-loaded liposome suspension. (a) **5**/EL liposomes (1), **5**/EL liposomes+$0.15\times10^{-4}$ M $CuSO_4$ (2), **5**/EL liposomes+$1.4\times10^{-4}$ M $CuSO_4$ (3) and EL liposomes+$1.4\times10^{-4}$ M $CuSO_4$ (4), [**5**]=$3.3\times10^{-4}$ M (1–3); $10^{-2}$ M borate buffer, pH 9. (b) **4**/EL liposomes (1), **4**/EL liposomes+$0.45\times10^{-4}$ M $CuSO_4$ (2), **4**/EL liposomes+$1.4\times10^{-4}$ M $CuSO_4$ (3) and EL liposomes+$1.4\times10^{-4}$ M $CuSO_4$ (4), [**4**]=$3.3\times10^{-4}$ M (1–3); $10^{-2}$ M borate buffer, pH 9.