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Water-soluble meroterpenes containing an aminoglyceride fragment with geraniol residues: synthesis and membranotropic properties

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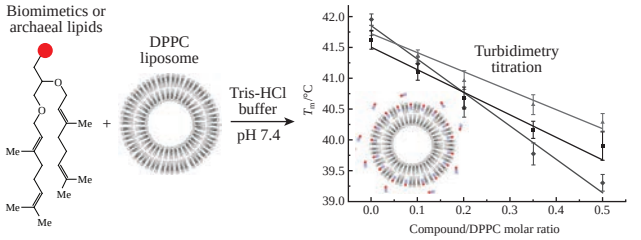
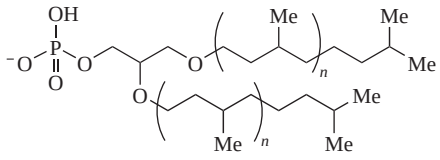
A number of new membrane anchors based on water-soluble aminoglycerides with geraniol fragments have been synthesized. A biomimetic approach was used based on the design of meroterpenes structurally similar to archaeal lipids. Turbidimetry and laser Doppler microelectrophoresis showed that the synthesized compounds were incorporated into unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles.

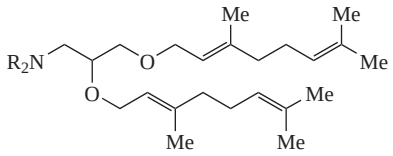


It is known that archaea[1] belong to the earliest forms of life and exist in almost all niches of the biosphere. In contrast to bacteria and eukaryotes, the archaeal cell membranes contain lipids that have ether type of bonds instead of ester ones and contain phosphoglycerides of isoprenoid alcohols or glycols.[2] Archaea are present in humans and other organisms and no clear evidence has been found that they may be pathogens or parasites.[3,4] Liposomes consisting of archaeal lipids **A** are termed archaeosomes and used for drug delivery into cells.[5,6]

The ability of archaea to be embedded and to survive in multicellular organisms allows us to put forward the idea of constructing the lipid membrane anchors as structural biomimetics of archaeal lipids with their potential use in drug delivery. In this paper we report on the application of this biomimetic approach to the design of meroterpenes structurally similar to archaeal lipids and having the properties of membrane anchor **B**.



A



B

Earlier it was shown that terpene fragments can not only be embedded in the membrane bilayer, but also dilute the bilayer.[7] This makes it possible for the substance to penetrate into the cell[2,8,9] and is important as well for the delivery of pharmacophores both on the surface of the cell membrane and inside the cell itself.

Consideration of archaeal lipids structure **A** allowed us to propose a model fragment for the membrane anchor **B**. In this model a monoterpene alcohol (*e.g.* geraniol) moiety was selected as the lipophilic part and the tertiary amino group was selected as its hydrophilic counterpart. The tertiary amino group can subsequently be modified for example by calixarene[10] or in general by any pharmacophore fragment. Geraniol has attracted attention due to its biologically significant properties,[11–15] including membranotropic ones.[16] The design of geraniol-based amphiphilic lipid-like meroterpene structure should help to confirm its effective interaction with the lipid bilayer. Thus, the fragment of geraniol can combine membrane-anchoring[17,18] as well as pharmacophoric functions.

Aminoglycerols **4a–c** and their precursors† were synthesized using published methods (Scheme 1).[19–22] Then according to the Williamson reaction, aminoglycerides **6a–c** with geraniol fragment were obtained.‡

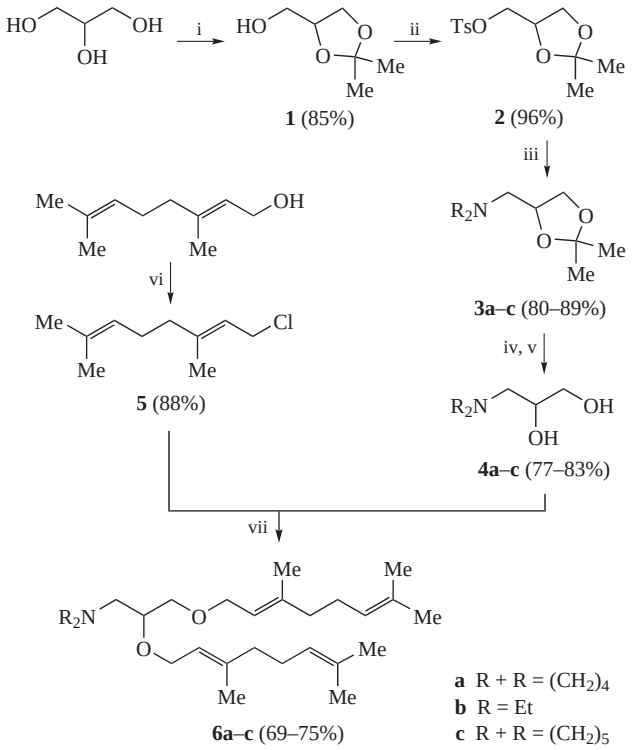
We used a system consisting of unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles with 100 nm diameter[23]

† *1-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]piperidine* **3c**:[21] yield 2.87 g (80%). ^{1}H NMR ($CDCl_3$) δ: 1.36 (s, 3H, Me), 1.41 (s, 3H, Me), 1.44 (m, 2H, $CH_2CH_2CH_2$), 1.59 (m, 4H, $CH_2CH_2CH_2$), 2.43 (m, 2H, CH_2N), 2.48 (m, 2H, CH_2N), 2.54 (m, 2H, CH_2N), 3.59 (m, 1H, OCH*H*), 4.07 (m, 1H, OC*H*H), 4.29 (m, 1H, CH).

3-(Piperidin-1-yl)propane-1,2-diol **4c**:[21] yield 1.84 g (77%). ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 4H, CH_2NCH_2, ^3J 7.0 Hz), 1.46 (m, 2H, $CH_2CH_2CH_2$) 1.61 (m, 4H, $CH_2CH_2CH_2$), 2.37 (dd, 1H, NCH*H*, ^3J 4.6 Hz, ^2J 13.1 Hz), 2.43 (br. s, 1H, OH), 2.55 (dd, 1H, NC*H*H, ^3J 9.5 Hz, ^2J 12.5 Hz), 2.62 (br. s, 1H, OH), 3.52 (dd, 1H, OCH*H*, ^3J 4.5 Hz, ^2J 11.4 Hz), 3.72 (m, 1H, OC*H*H), 3.85 (m, 1H, CH).

For characteristics of compounds **1**, **2**, **3a**,**b**, **4a**,**b** and **5**, see Online Supplementary Materials.

‡ *General procedure for the preparation of compounds* **6a–c**. Sodium hydride (0.6 g, 25 mmol) was added to a solution of aminoglycerol **4a–c** (10 mmol) in THF (20 ml) with stirring, and the resulting solution was additionally stirred for 15 min prior to the formation of the alcoholate. Then, a solution of geranylchloride **5** (5.17 ml, 28 mmol) in THF (15 ml) was added dropwise, and the mixture was refluxed for 24 h. The flask was cooled to room temperature and ice-cold water (40 ml) was added. The resulting mixture was extracted with dichloromethane (3 × 20 ml), solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with hexane–ethylacetate (3 : 1) (for **6a**,**b**) and hexane–acetone (3 : 1) (for **6c**) as eluents. The products were obtained as yellow (**6a**,**c**) or yellow-orange (**6b**) oil.



Scheme 1 *Reagents and conditions*: i, Me_2CO, TsOH, room temperature; ii, TsCl, pyridine, room temperature; iii, R_2NH, 100 °C; iv, 1 M HCl, room temperatute; v, MeONa, MeOH, room temperature; vi, CCl_4, PPh_3, reflux; vii, NaH, abs. THF, reflux.

to evaluate quantitatively the compounds **6a–c** interaction with phospholipid bilayer by the turbidimetry method.[23] It is well known that binding of amphiphilic substances with a bilayer[24] is accompanied by a change in the packing density of lipids, which ultimately can lead to solubilization of the vesicles.[25] The gel–liquid crystals phase transition temperature T_m is a sensitive indicator for the lipid molecules state in the bilayer.[25] In this study T_m was determined by measuring the optical density of the aqueous lipid dispersion with temperature increase[25] (see Online Supplementary Materials). This approach is not affected by scattering particles, because the phase transition is recorded as a sharp decrease in absorption within a certain narrow temperature range typical of the chosen lipid. Therefore, it is useful for screening the membrane-active compounds.

Figure 1 shows the dependence of the vesicle phase transition temperature T_m on the amount of **6a–c** introduced, which is linear up to a compound/DPPC molar ratio of 1 : 2. A decrease in T_m with an increase in the concentration of compounds **6a–c** indicates their interaction with DPPC vesicles. As well, a decrease of T_m may be caused by disorder in the packaging of the lipid acyl chains, which testifies to the incorporation of **6a–c** into the lipophilic part of the bilayer.[26] Finally, the linear character of

1-(2,3-Bisgeranyloxypropyl)piperidine **6c**: yield 3.02 g (70%). ^{1}H NMR ($CDCl_3$) δ: 1.60 (m, 6H, $CH_2CH_2CH_2$), 1.66 (s, 6H, Me), 1.68 (m, 16H, Me, CH_2N), 1.83 (m, 2H, $CHCH_2O$), 2.09 (m, 10H, CH_2CH_2 and NCH_2), 3.98 (d, 4H, OCH_2, ^3J 6.8 Hz), 5.08 (m, 2H, =CH), 5.26 (m, 1H, CH), 5.44 (m, 2H, =CH). ^{13}C NMR ($CDCl_3$) δ: 16.48, 17.70, 23.03, 25.72, 26.39, 32.46, 39.63, 44.47, 45.50, 66.41, 113.30, 120.99, 121.54, 124.06, 131.63, 140.03. IR (ν/cm^{-1}): 3365 (=CH), 2967 (Me), 2928 (Me), 2857 (CH_2), 1720 (=CH), 1670 (C=C), 1446 (=CH), 1376 (Me), 1256 (C–N), 1140, 1065, 928 (C–O–C), 902 (=CH), 570 (=CH). MS (MALDI), *m*/*z*: 432.5 $[M+H]^+$, 465.5 $[M+Na]^+$, 469.5 $[M+K]^+$ (calc., *m*/*z*: 431.4 $[M]^+$). Found (%): C, 77.02; H, 10.98; N, 3.56. Calc. for $C_{28}H_{49}NO_2$ (%): C, 77.90; H, 11.44; N, 3.24.

For characteristics of compounds **6a**,**b**, see Online Supplementary Materials.

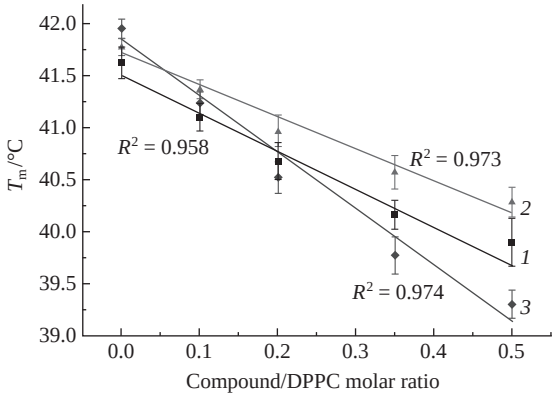


Figure 1 Plot of T_m *vs.* compound/DPPC molar ratio: (*1*) **6a**, (*2*) **6b** and (*3*) **6c**.

the dependence indicates that during the experiment there was no solubilization (destruction) of the lipid vesicles in the studied concentration range.

The ζ-potential of DPPC vesicles is a valuable indicator for the incorporation of foreign compounds into the lipid bilayer. It depends weakly on the concentration and type of common electrolyte in the solution.[27] When embedded in the bilayer, compounds **6a–c** should normally change the ζ-potential of the vesicles. The results of the ζ-potential measurement for compound/DPPC mixtures in different ratio (see Online Supplementary Materials) are shown in Figure 2. The point 0.0 on the abscissa axis corresponds to the ζ-potential value for the pristine DPPC vesicle suspension (~1.5 mV) which is consistent with the published data.[26] Compounds **6a**,**b** decrease the ζ-potential of the DPPC vesicles by about 3 mV, while **6c** decreases its value by 6.5 mV when the molar ratio 1 : 2 (compound/DPPC) is reached. Such relatively small changes in the ζ-potential indicate the preservation of the lipid bilayer structure. The dependence reaches plateau with an increase of the molar ratio of compound/DPPC to the value above 1 : 2, which implies the saturation of the vesicles in agreement with the data obtained by turbidimetry.

Thus, for the first time, a biomimetic approach to the design of membrane anchors was proposed and developed based on the construction of compounds structurally similar to archaeal lipids on an aminoglyceride platform with geraniol fragments. New lipid-like water-soluble meroterpenes capable of incorporating into the phospholipid bilayer were synthesized. The ability of the compounds to interact with the lipid bilayer of model DPPC membranes has been studied by turbidimetry as well as by laser Doppler microelectrophoresis. The results unambiguously demonstrate the ability of the synthesized compounds to be inserted into the DPPC membranes. The ζ-potential of the DPPC vesicles is changed due to meroterpenes binding to the DPPC vesicles. The range of the ζ-potential change (~1.5–6.5 mV)

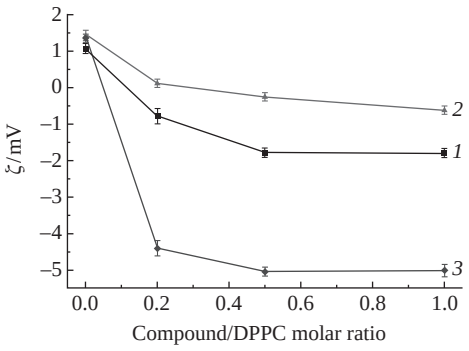


Figure 2 ζ-potential of DPPC vesicles in the presence of (*1*) **6a**, (*2*) **6b** and (*3*) **6c**.

confirms the preservation of the vesicles lipid bilayer structure. It has been shown that for the molar ratio above 1 : 2 (meroterpene/DPPC) the model vesicles are saturated by the studied compound without destruction of the lipid bilayer. These results can be applied to the design of new classes of effective non-toxic agents for targeted delivery of various pharmaceutical preparations.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.01.008.

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