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Amphiphilic adducts of myrcene and N-substituted maleimides as potential drug delivery agents

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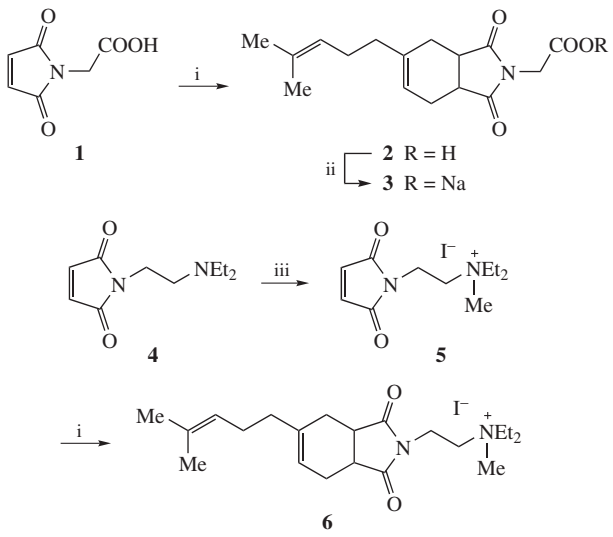
The title drug delivery compounds with pharmacophoric moieties were synthesized, and their interaction with model biomembranes (dipalmitoylphosphatidylcholine vesicles) was examined.

Targeted drug delivery is of major current interest to medicine, specifically in oncology.[1,2] The design of drugs capable of crossing cell membranes is the main problem in this field.[3] The current synthesis of pharmacologically active compounds involves the formation of hybrid molecular structures[4,5] or molecular conjugates[6–11] with different combinations of pharmacophoric functions and properties.[12,13]

Amphiphilic compounds with spatially separated hydrophilic and lipophilic fragments tend to exhibit membranotropic activity. Thus, there are great prospects in the modification of natural products, especially isoprenoids.[14–16] Furthermore, isoprenoid hydrocarbon frame structures have found application as membrane anchors in the design of modulators for membrane-integrated proteins.[17,18]

Therefore, we hypothesized that the introduction of biologically active fragments into terpenoid hydrocarbon structures may lead to their targeted delivery into subcellular compartments. To verify this hypothesis, we synthesized amphiphilic compounds containing a terpenoid hydrocarbon chain. Our aim was to determine how different hydrophilic fragments in terpenoid derivatives affect their ability to interact with a phospholipid membrane. Based on monoterpene β-myrcene, we prepared compounds containing carboxyl (**2**), carboxylate (**3**) and tertiary ammonium (**6**) groups using the Diels–Alder click reaction between N-substituted maleimides **1** and **5** and β-myrcene (Scheme 1).[†]



Scheme 1 *Reagents and conditions*: i, β-myrcene, THF, 12 h; ii, abs. THF, Na, 6 h; iii, MeCN, MeI, 20 °C.

[†] *2-[5-(4-Methylpent-3-en-1-yl)-1,3-dioxo-3a,4,7,7a-tetrahydro-1*H*-isoindol-2(3*H*)-yl]acetic acid* **2**. Imide **1** (0.20 g, 1.3 mmol) was dissolved in 5 ml of THF. Then 0.34 ml (2.0 mmol) of myrcene was added to this solution. The mixture was left overnight at room temperature. Thereafter, THF was removed in a vacuum and the solid residue was recrystallized from *n*-hexane to yield 0.28 g (74%) of compound **2**, mp 84–85 °C. ^{1}H NMR ($CDCl_3$) δ: 1.59 (s, 3H, Me), 1.68 (s, 3H, Me), 2.03 (m, 4H, CH_2), 2.26 (m, 2H, CH_2), 2.56 (m, 2H, CH_2), 3.17 (ddd, 2H, CH, $^3J_{HH}$ 9.2 Hz, $^3J_{HH}$ 6.3 Hz, $^3J_{HH}$ 3.4 Hz), 4.25 [s, 2H, C(O)CH_2N], 5.03 (br. s, 1H, =CH), 5.57 (br. s, 1H, =CH). ^{13}C NMR ($CDCl_3$) δ: 17.7, 24.0, 25.7, 25.9, 27.5, 37.2, 39.2, 39.5, 39.8, 119.8, 123.6, 131.9, 140.2, 171.5, 179.2, 179.4. IR (ν/cm^{-1}): 1748, 1676 (C=O), 2728, 2601, 2520 (COOH). MS (MALDI-TOF), *m/z*: 291.9 $[M+H]^+$, 313.8 $[M+Na]^+$ (calc. for $[M^+]$, *m/z* 291.2). Found (%): C, 65.72; H, 7.07; N, 5.10. Calc. for $C_{16}H_{21}NO_4$ (%): C, 65.96; H, 7.27; N, 4.81.

*Sodium 2-[5-(4-methylpent-3-en-1-yl)-1,3-dioxo-3a,4,7,7a-tetrahydro-1*H*-isoindol-2(3*H*)-yl]acetate* **3**. Sodium (0.1 g, 4.30 mmol) was added to the solution of compound **2** (0.10 g, 0.34 mmol) in 5 ml of abs. THF. The mixture was stirred for 6 h and then filtered. The filtrate was evaporated and solvent traces were removed in a high vacuum. Yield of compound **3** was 0.10 g (93%). ^{1}H NMR (DMSO-d_6) δ: 1.54 (s, 3H, Me), 1.62 (s, 3H, Me), 1.91 (m, 4H, CH_2), 1.98 (m, 2H, CH_2), 2.16 (m, 2H, CH_2), 2.30 (m, 2H, CH_2), 3.06 (ddd, 2H, CH, $^3J_{HH}$ 9.1 Hz, $^3J_{HH}$ 6.0 Hz, $^3J_{HH}$ 3.0 Hz), 3.56 (s, 2H, CH_2), 4.97 (br. s, 1H, =CH), 5.47 (br. s, 1H, =CH). ^{13}C NMR (DMSO-d_6) δ: 22.8, 28.7, 30.6, 30.7, 31.2, 32.2, 42.1, 43.7, 44.2, 47.7, 125.1, 129.0, 136.0, 144.6, 173.5, 184.7, 184.8. MS (MALDI-TOF), *m/z*: 313.8 $[M+H]^+$, 335.8 $[M+Na]^+$ (calc. for $[M^+]$, *m/z* 313.1). Found (%): C, 61.10; H, 6.34; N, 4.54. Calc. for $C_{16}H_{20}NNaO_4$ (%): C, 61.33; H, 6.43; N, 4.47.

*N,N-Diethyl-N-methyl-2-[5-(4-methylpent-3-en-1-yl)-1,3-dioxo-3a,4,7,7a-tetrahydro-1*H*-isoindol-2(3*H*)-yl]ethanaminium iodide* **6**. Myrcene (5 ml, 29.4 mmol) was added to the solution of compound **5** (0.3 g, 0.88 mmol) in 20 ml of THF. The mixture was left at room temperature for 24 h. Thereafter, THF was removed in a vacuum and the solid residue was recrystallized from ethyl acetate to yield 0.35 g (83%) of compound **6**, mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ: 1.44 (t, 6H, Me, $^3J_{HH}$ 7.25 Hz), 1.58 (s, 3H, Me), 1.66 (s, 3H, Me), 2.00 (m, 4H, CH_2), 2.21 (m, 2H, CH_2), 2.53 (m, 2H, CH_2), 3.28 (ddd, 2H, CH, $^3J_{HH}$ 9.3 Hz, $^3J_{HH}$ 6.9 Hz, $^3J_{HH}$ 2.2 Hz), 3.32 (s, 3H, $^+$N–Me), 3.69 (m, 6H, $^+$N–CH_2), 3.89 (t, 2H, CH_2N, $^3J_{HH}$ 7.2 Hz), 5.00 (br. s, 1H, =CH), 5.53 (br. s, 1H, =CH). ^{13}C NMR ($CDCl_3$) δ: 8.5, 17.8, 23.9, 25.7, 26.1, 27.3, 32.4, 37.4, 39.6, 40.0, 48.4, 56.7, 57.7, 120.0, 123.4, 132.1, 140.5, 179.9, 180.0, 181.7. IR (ν/cm^{-1}): 1767, 1695 (C=O). MS (MALDI-TOF), *m/z*: 346.7 $[M-I]^+$ (calc. for $[M^+]$: *m/z* 474.2). Found (%): C, 52.92; H, 7.32; N, 6.09; I, 26.69. Calc. for $C_{21}H_{35}IN_2O_2$ (%): C, 53.16; H, 7.44; N, 5.90; I, 26.75.

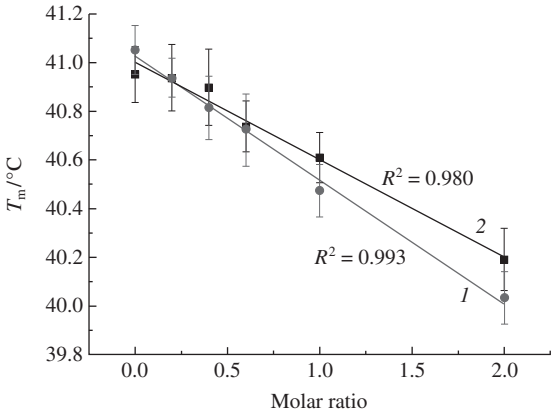


Figure 1 T_m (DPPC vesicles) *vs.* the molar ratios of (*1*) compound **2**/lipid and (*2*) compound **3**/lipid.

Precursor imides **1**, **4** and **5** were synthesized as previously described.[19–21] To study the membrane anchoring ability of the compounds, a model system consisting of unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles with a diameter of 100 nm was used as a cell membrane mimics.‡ The binding of amphiphilic compounds can be accompanied by changes in lipid packing density, which can further result in the complete restructuring of vesicles.[22] The phase transition temperature T_m (gel–liquid crystalline) is a sensitive indicator of the status of lipid molecules in the bilayer.[23] T_m was determined by changes in the turbidity of an aqueous lipid dispersion with increasing the temperature.[24] This method is convenient because it is independent of the influence of scattering particles since the phase transition is recorded as a sharp decrease in absorbance within a defined narrow temperature range typical of a selected lipid. This simple method can be used for screening membrane-active compounds while the mechanism of the interaction can be studied in more detail.

Figure 1 shows the titration curves of a lipid vesicle suspension with the concentrated solutions of test compounds **2** and **3**. The almost linear relations between these two compounds and the phospholipid indicate that the test compounds do not solubilize vesicles, as is typical of other surfactants.[25–27] As a result, they cannot degrade biomembranes. Variations in the concentration of compound **6** did not affect T_m which remained unchanged within the limits of experimental error.

The decrease in the T_m testifies to the weakening of interactions between lipid molecules resulted from the anchoring of compounds **2** and **3** into the lipid bilayer. No concentration influence observed for compound **6** on T_m is an indication of the lack of interaction with the lipid bilayer. This can be due to the counterion present in compound **6** because a large counterion hinders the incorporation of amphiphilic compounds into a lipid bilayer.[27]

Thus, we found that the synthesized compounds interact with a membrane surface. This interaction depends on the polar fragment of the amphiphilic molecule. Polar fragments containing carboxylic and carboxylate groups interact with membranes, whereas those containing the tertiary ammonium group with a large iodide counterion do not interact. The obtained results confirm our hypothesis for membranotropic terpenoids, which offers new prospects in drug development for targeted delivery.

‡ *Determination of the phase transition temperatures of DPPC vesicles.* The vesicles were prepared by dissolving 26 mg (0.035 mmol) of DPPC in 0.5 ml of chloroform. The solvent was gently evaporated to obtain a thin lipid film. The lipid was hydrated using 0.5 ml of aqueous buffer [0.1 mM tris(hydroxymethyl)aminomethane, HCl, 0.15 mM NaCl, pH 7.4] with constant shaking at 50 °C for 1 h to give a crude lipid suspension. Unilamellar vesicles of DPPC were prepared by extrusion of the hydrated lipid suspension using an Avanti Mini-Extruder fitted with a 100 nm diameter pore size polycarbonate filter.

The lipid phase transition temperature was determined by measuring the turbidity of diluted lipid suspensions (0.7 mM) on a Shimadzu UV-3600 spectrophotometer at 400 nm. The titration of lipid suspensions with the test compounds was carried out in quartz cells with a 10 mm optical path length. In order to reduce experimental error, concentrated solutions (100 mg cm^{-3}) in distilled water (compounds **3** and **6**) and methanol (compound **2**) were added to 3 ml of lipid suspension (0.7 mM).

The T_m of lipid was determined by measuring the absorbance of samples at an interval of 0.3 °C within a temperature range from 39 to 43.5 °C. Samples were thermostated for 3 min before each measurement using a Shimadzu TCC-240A temperature controlled cell holder. Thus, the average heating rate was 0.1 K min^{-1}.

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