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Cationic amphiphiles based on diethanolamine esters with amino acids in the polar block

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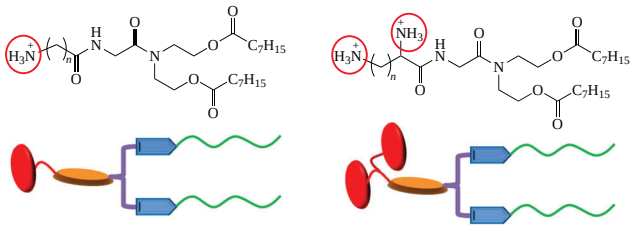
A series of new cationic amphiphiles based on diethanolamine esters with Gly, βAla, GABA, Lys, Orn amino acid residues in the hydrophilic block were prepared. The sample containing the Lys residue showed the best activity against gram-positive and gram-negative bacteria. For model membranes comprising combinations of DOPC, DOPG and DOPE phospholipids, the sample can electrostatically bind to the surface of a bacterial cell and destroy it by formation of through defects.



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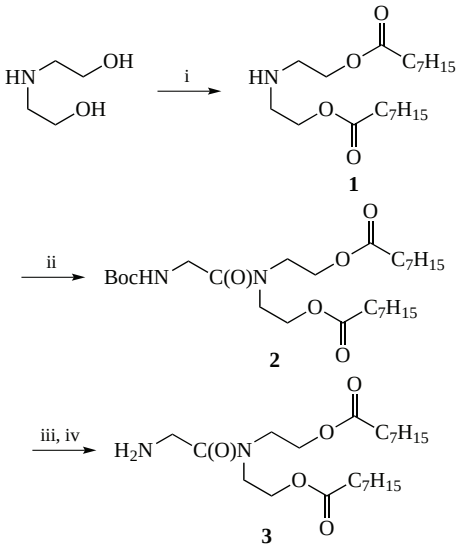
The antimicrobial resistance is recognized as a major public health problem worldwide since more and more infections caused by multidrug-resistant bacteria appear. Antimicrobial peptides (AMPs) have become a subject of keen interest in this context.[1] Studies on the activity of antimicrobial peptides have shown that there exist many factors affecting the specificity and biological activity of these agents. The cationic charge, secondary structure, hydrophobicity, and amphipathicity are crucial for their operation.[2–6] The specific features of AMP structure allows them to act on bacterial membranes selectively without affecting the eukaryotic cells' membranes. This selectivity is based on the difference between the compositions of bacterial and human cell membranes. Human cell membranes are generally dominated by zwitterionic lipids such as phosphatidylcholine and sphingomyelin; they are generally electroneutral whereas bacterial membranes are strongly charged negatively. The outer membrane of Gram-negative bacteria is rich in highly anionic lipopolysaccharides, whereas Gram-positive bacteria contain the anionic lipoteichoic acid (LTA).[7]

Despite the advantages of natural AMPs, their clinical use is hampered by a number of serious problems including toxicity, low *in vivo* efficiency, and high production costs.[8] These problems can be solved by development of synthetic peptidomimetics, *i.e.*, α-peptides, β-peptides, α-AA peptides, and low-molecular lipopeptidomimetics. Low-molecular lipopeptidomimetics are versatile molecules that can be readily modified resulting in new agents with enhanced therapeutic properties.[9] Cationic amphiphiles based on amino acids that mimic the structure and mechanism of action of antibacterial peptides are among their most promising representatives.[10–12] The positively charged amino acid residues in these molecules bind to the negatively charged outer membranes of bacteria through electrostatic interactions, which should lead to membrane destruction. They are biocompatible and easily penetrate into the bloodstream through the intestinal wall and are then transported from the bloodstream to almost any tissue and organ. A number of samples demonstrate high antibacterial efficiency against both Gram-positive and Gram-negative bacteria. Bacteria show low resistance to these agents. Moreover, it is noted that their production is relatively easy, which greatly reduces the costs and facilitates the scale up of their further production.[13–15]

The purpose of this work was to synthesize a series of new cationic amphiphiles and study their properties. The series is based on diethanolamine diesters with aliphatic carboxylic acids. The hydrophilic moiety is represented by various amino acids (Gly, βAla, GABA, Lys, Orn) linked with the hydrophobic block through the Gly residue. Analysis of literature data indicates that compounds with hydrophilic–lipophilic balance (HLB) values in the range of 4–7 are the most promising candidates possessing antibacterial activity.[13] In view of this, the HLB values were evaluated for 30 potential structures in the 'ACD/Labs, LogP' program at the preliminary stage of molecular design. It was found that the greatest contribution to this index in the presence of amino acids with different polarity should be made by hydrophobicity, which depends on the chain length of the carboxylic acid residue in the corresponding block. Compounds with saturated fatty acid residues such as octanoic acid $C_8H_{16}O_2$ were found to be the most promising representatives based on HLB (Table 1).

Table 1 HLB evaluation for the compounds under study.

Compound	Structural formula	HLB
5a	Gly-Gly-DEA-(OOC-C_7H_{15})$_2$	4.99
5b	βAla-Gly-DEA-(OOC-C_7H_{15})$_2$	4.86
5c	GABA-Gly-DEA-(OOC-C_7H_{15})$_2$	5.07
7a	Lys-Gly-DEA-(OOC-C_7H_{15})$_2$	4.98
7b	Orn-Gly-DEA-(OOC-C_7H_{15})$_2$	5.06

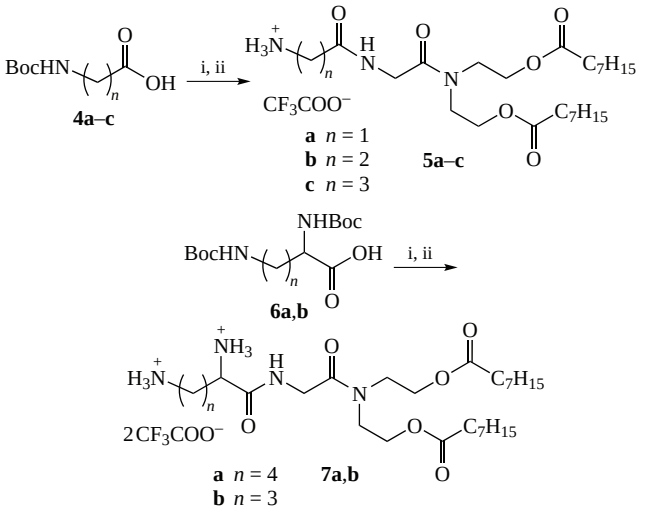


Scheme 1 *Reagents and conditions*: i, $C_7H_{15}CO_2H$, TsOH; ii, $BocNHCH_2CO_2H$, DCC, DMAP, CH_2Cl_2; iii, CF_3CO_2H, CH_2Cl_2; iv, 5% $NaHCO_3$ (aq.).

The synthesis of the target compounds can be subdivided into three main stages: building the hydrophobic block; addition of the linker; and addition of the corresponding amino acids. The hydrophobic block **1** was obtained by esterification of diethanolamine with octanoic acid in the presence of *p*-toluenesulfonic acid (Scheme 1).[16] Subsequently, glycine linker was attached to amino diester **1** by reacting with BocGly whose free carboxylic group was activated by the carbodiimide method.[14] Final N-deprotection afforded the required glycine amide derivative **3**.

Extension of the amino acid sequence in compound **3** (Scheme 2) and formation of the hydrophilic moiety, *i.e.*, attachment of BocGly, BocβAla, BocGABA, Boc_2Lys and Boc_2Orn, **4a–c** and **6a**,**b**, respectively, were performed using a different activating urethane reagent (HBTU) in the presence of DIPEA.[17] The structures of the target compounds **5a–c** and **7a**,**b** were confirmed by NMR spectroscopy and mass spectrometry.

The resulting compounds were tested for activity toward gram-positive bacteria, *Bacillus subtilis* 534, and gram-negative ones, *Escherichia coli* M17, by the technique of sequential dilutions with a broth that makes it possible to determine the accurate value of the minimum inhibiting concentration (MIC). The best results were shown by compounds **7a** and **7b** containing Lys and Orn terminal residues, respectively, with a MIC of



Scheme 2 *Reagents and conditions*: i, compound **3**, HBTU, DIPEA; ii, CF_3CO_2H.

1.56 μg ml^{-1}. Samples **5a–c** demonstrated lower activity with MIC values higher than 6.25 μg ml^{-1}. Positive control was provided by vancomycin, a glycopeptide antibiotic whose antibacterial activity toward gram-positive bacteria amounted to 0.5 μg ml^{-1}.

Low-molecular amphiphiles belong to membrane-active compounds whose main mechanism of antibacterial action involves the creation of certain defects in the cell structure.[18] The ability of compound **7a** to interact with model lipid membranes was studied to identify the expected effect. Membranes were prepared from combinations of phospholipids DOPC, DOPG and DOPE typical of bacterial cells. The results of this experiment indicate that the sample generates through-defects in the membrane with a lipid composition DOPC : DOPG : DOPE = = 60 : 20 : 20 mol% at a concentration equal to the MIC. This process was observed as jumps of the membrane conductance with an amplitude of (1.7 ± 0.6) nS for 600 s [(Figure 1(*a*)]. The average lifetime of defects was ~1700 ms [(Figure 1(*d*)], after which the membrane recovered and its conductivity returned to the original level.

To study the capability of **7a** to bind to a lipid bilayer by electrostatic interactions, the molar content of the DOPG lipid in the membrane was varied. No defects formed throughout the experiment on an uncharged membrane with a DOPC : DOPE ratio of 80 : 20 mol%, while the membrane conductivity remained unchanged [see Figure 1(*b*)]. On membranes with the composition DOPC : DOPG : DOPE = 40 : 40 : 20 mol%, formation of defects was observed as conductivity jumps with an amplitude of (1.9 ± 0.5) nS for 500 s, which then led to total membrane failure and growth of its conductivity to the instrument limit in 200 s [(Figure 1(*c*)].

These experiments showed that the sample of interest could interact with the lipid membrane through electrostatic forces, and an increase in the content of the charged lipid (DOPG) resulted in small through defects along with the total destruction of the membrane. This may serve as indirect evidence that the mechanism of cell death under the action of amphiphile **7a** is based on the destruction of the bacterial membrane upon adsorption.

In summary, we have synthesized a series of amino acid derivatives containing diethanolamine ester moiety. The

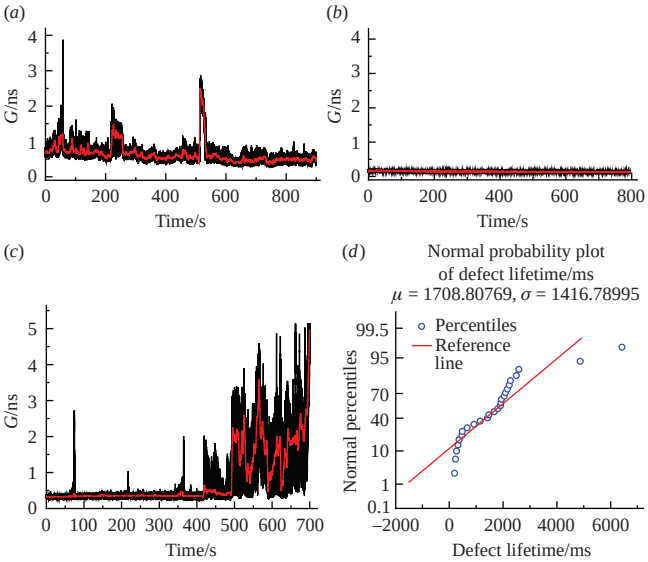


Figure 1 Typical plots of **7a** absorption on a membrane depending on its lipid composition (the number of independent experiments for each lipid composition is 6). Lipid composition: (*a*) DOPC : DOPG : DOPE = = 60 : 20 : 20 mol%; (*b*) DOPC : DOPE = 80 : 20 mol%; (*c*) DOPC : DOPG : DOPE = = 40 : 40 : 20 mol%. (*d*) Distribution of defect existence lifetimes in a membrane with lipid composition DOPC : DOPG : DOPE = 60 : 20 : 20 mol%.

antibacterial activity of the cationic amphiphiles against Gram-positive and Gram-negative bacteria was estimated. Electrical conductivity measurements were used to show the ability of the sample containing the Lys residue (MIC 1.56 μg ml^{-1}) to interact with model lipid membranes with formation of through defects therein.

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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.2023.02.029.

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