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# Ornithine and lysine based lipotripeptides: synthesis and comparison of transfection efficiency

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**The derivatives of aliphatic tripeptides containing L-ornithine, L-lysine and diesters of L-glutamic acid were synthesized, the physicochemical properties of dispersions based on them were examined and the dependence of transfection efficiency on the amphiphile : DNA ratio and the structure of the peptides was evaluated.**

The synthesis and examination of cationic peptide-saturated gene delivery vectors are a rapidly developing field. These compounds are capable of packing effectively DNA molecules and protecting them from nucleases. A natural character of structure components provides a successful entry into the cell.[1–5] One of the modern trends in the development of new synthetic transfection mediators concerns cationic amphiphiles with multivalent cationic group and a long hydrocarbon chain.[6–8] Previously, we synthesized a series of cationic lipodipeptides and studied their properties.[9]

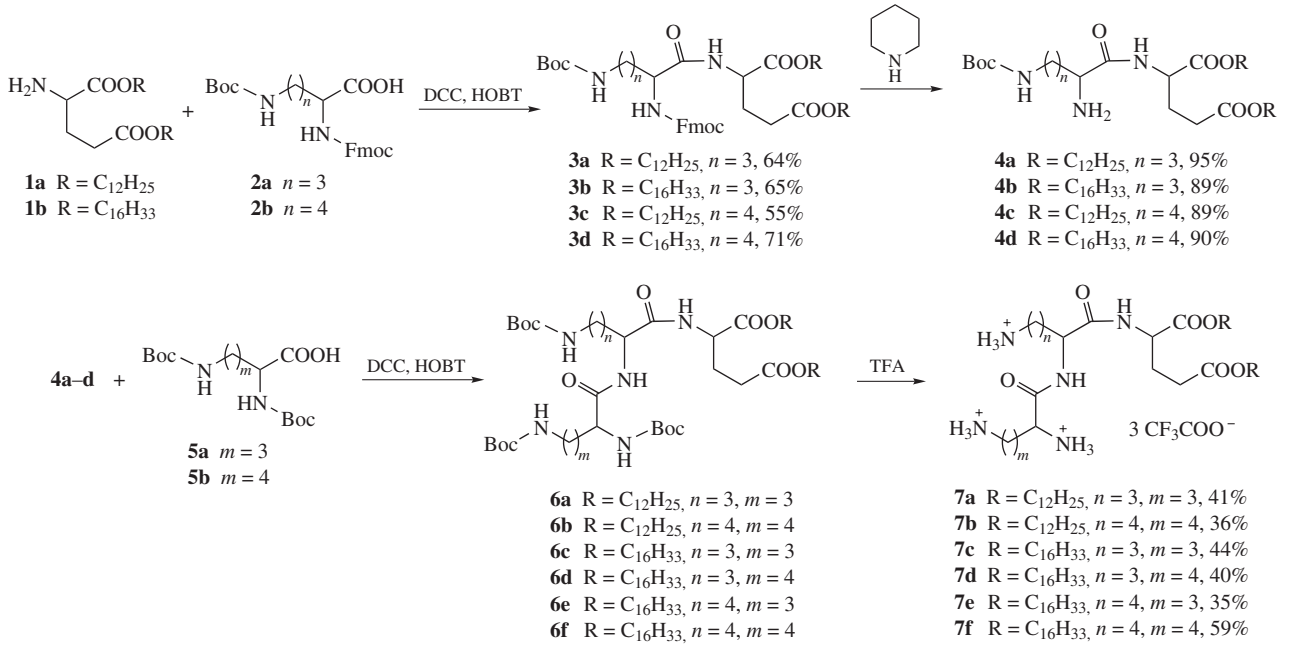
To increase the density of positive charge on the particle surface and the transfection efficiency, we proposed the following protocol for preparing amphiphilic tripeptides (Scheme 1). The synthesis included the following steps: activation of the carboxyl groups of Fmoc-Lys (Boc) or Fmoc-Orn (Boc) and formation of the peptide bond with diesters of L-glutamic acid; then removal of Fmoc protecting group from the obtained dipeptide derivative; activation of the carboxyl groups of Boc2Lys or Boc2Orn and formation of the peptide bond to obtain tripeptides derivatives; and removal of protecting group to get lipotripeptides.†

Formation of amide bond between protected activated amino acid derivatives and esters of L-glutamic acid was carried out in a $CHCl_3$–DMF mixture. The reaction products were isolated by preparative TLC on silica gel. Removing Fmoc protecting groups was performed by the action of piperidine in DMF.

The next step was the formation of peptide bond between aliphatic derivatives of dipeptides and derivatives of activated Boc-amino acid in a mixture of chloroform and DMF. The reaction products were isolated by preparative TLC on silica gel. The *tert*-butyloxycarbonyl protective group was removed by anhydrous trifluoroacetic acid.

The series of modified lipotripeptides with different amino acid sequences in polar part and different lengths of the hydrocarbon chain in the hydrophobic part was subjected to physicochemical and biological studies.

To characterize obtained aggregates by the diameter of their particles, we used photonic correlation spectroscopy. The particles were found to have an average diameter of 100 nm or less. For the effective gene delivery into cells, the bilayer of the aggregates should exist in a liquid crystalline state, and a phase transition temperature (PTT) must range around the physiological temperature (~37 °C).[10,11] Results of the experiments showed that the PTT of tripeptide derivatives with aliphatic chain length of 16




**1a** R = $C_{12}H_{25}$
**1b** R = $C_{16}H_{33}$
**2a** *n* = 3
**2b** *n* = 4

**3a** R = $C_{12}H_{25}$, *n* = 3, 64%
**3b** R = $C_{16}H_{33}$, *n* = 3, 65%
**3c** R = $C_{12}H_{25}$, *n* = 4, 55%
**3d** R = $C_{16}H_{33}$, *n* = 4, 71%

**4a** R = $C_{12}H_{25}$, *n* = 3, 95%
**4b** R = $C_{16}H_{33}$, *n* = 3, 89%
**4c** R = $C_{12}H_{25}$, *n* = 4, 89%
**4d** R = $C_{16}H_{33}$, *n* = 4, 90%

**4a–d** +
**5a** *m* = 3
**5b** *m* = 4

**6a** R = $C_{12}H_{25}$, *n* = 3, *m* = 3
**6b** R = $C_{12}H_{25}$, *n* = 4, *m* = 4
**6c** R = $C_{16}H_{33}$, *n* = 3, *m* = 3
**6d** R = $C_{16}H_{33}$, *n* = 3, *m* = 4
**6e** R = $C_{16}H_{33}$, *n* = 4, *m* = 3
**6f** R = $C_{16}H_{33}$, *n* = 4, *m* = 4

**7a** R = $C_{12}H_{25}$, *n* = 3, *m* = 3, 41%
**7b** R = $C_{12}H_{25}$, *n* = 4, *m* = 4, 36%
**7c** R = $C_{16}H_{33}$, *n* = 3, *m* = 3, 44%
**7d** R = $C_{16}H_{33}$, *n* = 3, *m* = 4, 40%
**7e** R = $C_{16}H_{33}$, *n* = 4, *m* = 3, 35%
**7f** R = $C_{16}H_{33}$, *n* = 4, *m* = 4, 59%

**Scheme 1**

**Table 1** Characteristics of aqueous dispersions for obtained peptide derivatives.

| Compound | | Diameter[a]/nm | PTT/°C | Transfected cells (%) |
|---|---|---|---|---|
| $OrnOrnGlu(C_{12})_2$ | **7a** | 79 | 36 | 65 |
| $LysLysGlu(C_{12})_2$ | **7b** | 83 | 36 | 53 |
| $OrnOrnGlu(C_{16})_2$ | **7c** | 97 | 42 | 74 |
| $OrnLysGlu(C_{16})_2$ | **7d** | 100 | 42 | 69 |
| $LysOrnGlu(C_{16})_2$ | **7e** | 99 | 42 | 61 |
| $LysLysGlu(C_{16})_2$ | **7f** | 100 | 43 | 56 |

[a] Total amount of particles of the specified size was >95%.

carbon atoms was 42 °C and for the chain length of 12 atoms the PTT was 36 °C (Table 1). In this work we found that PTT of our lipotripeptides had no significant effect on the transfection activity.

An important characteristic of colloidal dispersions is their stability during storage. The time dependence of optical density was determined for the aqueous dispersions of synthesized compounds. We found that the deviation of the values during one month did not exceed 5%.

To study the transfection efficiency of synthesized compounds, the percentage of transfected cells of human embryonic kidney HEK293 was determined.‡ The plasmid of green fluorescent protein pUCHR IRES GFP was added to human embryonic kidney cells HEK293. To reveal the most effective conditions the transfection mixture was prepared using various ratios of plasmid/lipopeptide, μg/μg: 1:2, 1:4, 1:8, 1:16, 1:32. In case of lipotripeptides the highest amount of transfected cells was observed when using 8 μg lipopeptide per 1 μg plasmid (Figure 1).

The influence of the amino acid sequences in the polar part on the transfection efficiency has also been demonstrated. The highest percentage of transfected cells was achieved in the case of only lipotripeptides bearing L-ornithine derivatives in the polar head group (Figure 2).

† *Didodecyl* N-*[*Nα*-(L-ornithyl)]-L-ornithyl-L-glutamate tris(hydrotrifluoroacetate)* **7a**. 1-Hydroxybenzotriazole hydrate (HOBT) (0.142 g, 1.05 mmol) in 3 ml of DMF and a cold solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (0.217 g, 1.05 mmol) in 6 ml of THF with active stirring was added to the solution of *Nα*-Boc,*Nε*-Fmoc-L-ornithine **2a** (0.45 g, 1.05 mmol) in 6 ml of THF. The mixture was stirred for 2 h on cooling, the precipitate was filtered off. Then didodecyl L-glutamate **1a** (0.507 g, 1.05 mmol) in 6 ml of THF was added. The mixture was stirred at room temperature for 5 h, the solvent was removed *in vacuo*. The product was purified by column chromatography in hexane–ethyl acetate (8:1).

The obtained compound **3a** (0.300 g, 0.337 mmol) was mixed with 2 ml 20% piperidine in DMF. The mixture was stirred at room temperature for 20 min. The obtained product **4a** was purified by preparative TLC in toluene–chloroform–2-butanone–2-propanol (10:6:3:1).

For spectral characteristics of compounds **3a** and **4a**, see Online Supplementary Materials.

HOBT (0.044 g, 0.327 mmol) in 2 ml of DMF and a cold solution of DCC (0.06 g, 0.327 mmol) in 8 ml of chloroform were added with active stirring to the solution of *Nα*-Boc,*Nε*-Boc-L-ornithine **5a** (0.35 g, 1.05 mmol) in 4 ml of chloroform. The mixture was stirred for 3 h under cooling and 1 h at room temperature, the precipitate was filtered off. The progress of the reaction was examined by TLC. Further, 0.200 g (0.287 mmol) of **4a** in 3 ml of THF was added to the above obtained solution. The mixture was stirred at room temperature for 8 h, the solvent was removed *in vacuo*. The residue (0.2 g) was dissolved in 1 ml of DCM and 2 ml of $CF_3COOH$ and this was stirred at room temperature for 8 h. The solvents were removed *in vacuo*. The obtained product **7a** was purified by preparative TLC in toluene–chloroform–2-butanone–2-propanol (10:6:3:1), and then methanol–chloroform (2:1) ($R_f$ 0.68). Yield 0.083 g (41%). MALDI-MS, *m*/*z*: 713.598 $[M]^+$ (calc., *m*/*z*: 713.603).

Compounds **7b**–**f** were synthesized similarly.

‡ For experimental details, see Online Supplementary Materials.

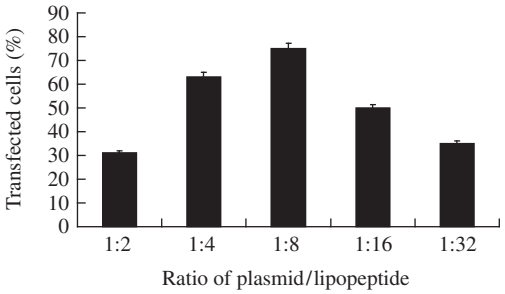



**Figure 1** Dependence of the transfection efficiency for various ratios of plasmid/lipopeptide (μg/μg).

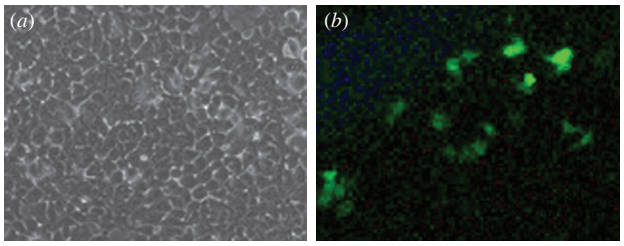



**Figure 2** (*a*) Optical and (*b*) confocal microscopy of transfected cells using $OrnOrnGlu(C_{16})$ and pUCHR IRES GFP.

In conclusion, syntheses of derivatives of tripeptides with OrnOrnGlu, LysLysGlu, OrnLysGlu, LysOrnGlu amino acid sequences and C12 or C16 hydrocarbon chains were developed; new lipotripeptides were prepared. Data on physicochemical properties of the dispersions based on obtained compounds, and the effect of plasmid/lipopeptide ratios and structure of peptides on the transfection efficacy show that the synthesized compounds are prospective for further studying and practical applications.

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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.2014.09.004.

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