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

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Materials and methods

General

All reagents and organic solvents were purchased from Acros, Aldrich, or Reachim in reagent grade or better and used without further purification. Column chromatography was accomplished on silica gel 60 (60−200 mesh). Thin layer preparative chromatography (TLPC) was accomplished on silica gel 60 (2−25 mesh). IR spectra were recorded on a Bruker FTIR EQUINOX 55 spectrometer. NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d6 solutions. MALDI mass spectrometry (MALDI-MS) was performed on a MALDI mass spectrometer Ultraflex (Bruker Daltonics GmbH).

Didodecyl N-*(*Nε-*Boc*,Nα-*Fmoc-L-ornithyl)-L-glutamate* **3a**, yield 0.602g (64%), R_f 0.82 (toluene−chloroform−methyl ethyl ketone−isopropanol, 10:6:3:1). IR (in film, ν_{max}, cm^{-1}): 3400 (NH), 2910 (CH), 2902 (CH), 1741 (C=O), 1664(C=O), 1640 (NH), 1374 (CH), 1200, 1100 (C–O). ^{1}H NMR (δ, ppt): 0.85 (6 H, t, $2CH_3$), 1.25 (36 H, c, $18CH_2$), 1.45 (9 H, c, 2 $C(CH_3)_3$), 1.63–1.82 (4 H, m, 2 CH_2), 2.28 (2 H, m, 2 CH_2), 2.50 (2 H, t, CH_2COO), 3.8 (4 H, m, $2OCH_2$), 4.25 (1 H, m), 4.35 (4 H, m, CH_2), 5.6 (1 H, d, CONH), 7.08 (2H, td), 7.44 (2 H, tt), 7.55 (dc), 7.77 (2H, m).

Didodecyl N-*(*Nε-*Boc-L-ornithyl)-L-glutamate* **4a**, yield 0.223g (95%), R_f 0.52 (methanol−chloroform, 4:1). IR (in film, ν_{max}, cm^{-1}): 3331 (NH), 2942 (CH), 2828 (CH), 1725 (C=O), 1660 (C=O), 1640 (NH), 1358 (CH), 1209 (CF), 1107 (C–O), 959, 828, 792, 715. ^{1}H NMR (DMSO-d_6, δ, ppt): 0.85 (6 H, t, 2 CH_3), 1.23 (36 H, c, 18 CH_2), 1.44 (9 H, c, 2 $C(CH_3)_3$), 1.53 (6 H, m, $3CH_2$), 1.77-1.91 (4 H, m, $2CH_2$), 2.84 (2 H, t, CH_2COO), 4.0 (4 H, m), 4.28 (1 H, m, CH), 7.3 (5.6 H, c, $2NH_3^+$).

The study of transfection efficiency of lipopeptides

Before transfection, cells were seeded in 24-well plate at 1×105 cells/well in a volume of 0.7 ml/well of DMEM medium with 300 mg/l glutamine, 80 mg/l gentamicin and 10% fetal calf serum. A few hours prior to transfection, medium was replaced with DMEM with 300 mg/l glutamine and 10% fetal calf serum. Transfection mixture was prepared in DMEM: 1 mcg plasmid pEGPP-n1, carrying a reporter gene GFP was added to 50 mcl of DMEM, transfection agent in 50 mcl of DMEM was used in ratios plasmid/lipopeptide mg/mg: 1:2, 1:4, 1: 8, 1:16, 1:32, thus the volume of the transfection mix was 100 microliters. A mixture of 2 mcl of commercial transfection reagent Lipofectamine 2000 (Invitrogen) and 1 g pEGPP-n1 at the same total volume of 100 mcl DMEM was used as positive control. The mixture was stirred and incubated at room temperature for 30 min and added dropwise to the wells with HEK293T. Cells were incubated at 37 ° C, 5% CO_2. After 4 h, the medium was exchanged for a new one. Transfection efficiency was assessed at 12, 24, 48, 72 h of incubation with a fluorescence microscope and by flow cytometry (Cytometer FACScan, BectonDickinson, USA) by the amount of GFP^+-cells.