Supplementary Information

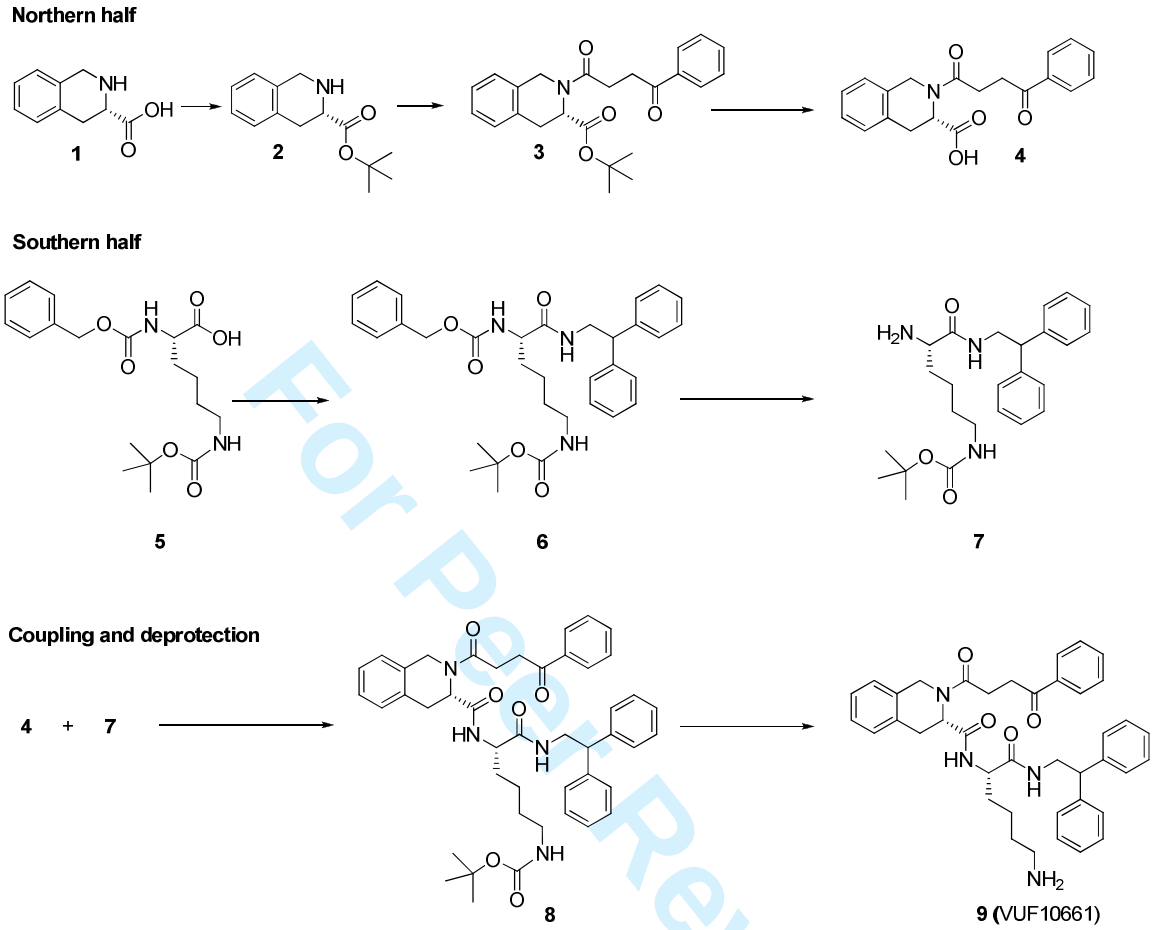


Figure S1. Synthesis scheme for VUF10661 (**9**).

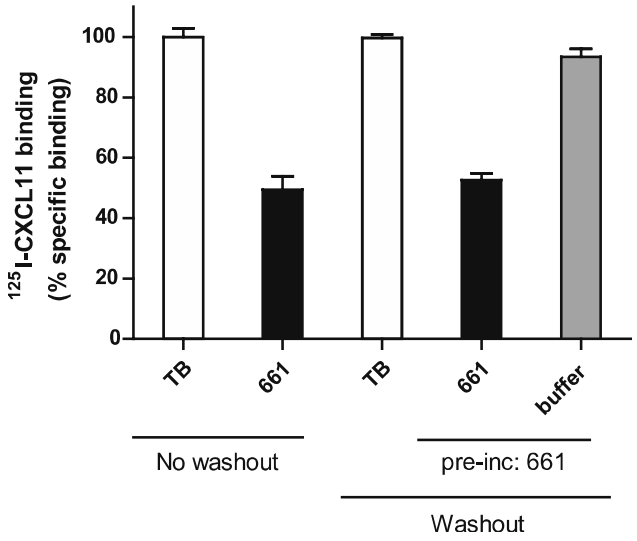


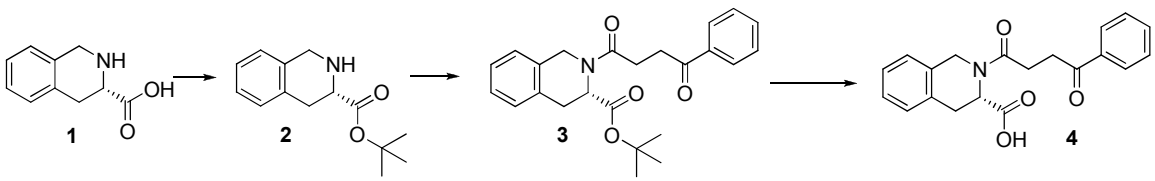
Figure S2

VUF10661 washout experiment using HEK293 cells stably expressing CXCR3 receptors. The cells were pretreated for 1 hour with buffer (TB) or 500 nM of VUF10661 (black and gray bars). Next, indicated cells were washed 10 times 3 min with buffer. Subsequently, 500 pM of ^{125}I-CXCL11 was added to all cells, in the presence (black) or absence (white and gray bars) of 500 nM of VUF10661 (black bars).

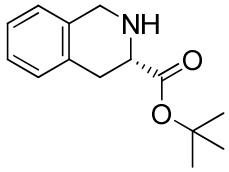
General synthetic procedures.

THF, toluene, ether and CH_2Cl_2 were distilled freshly from drying agent, all other solvents were used as received. Unless specified otherwise, all chemicals were purchased from Aldrich and used as received. Z-Lys(Boc)-OH was purchased from Fluorochem and used as received. All reactions were carried out under an inert atmosphere. TLC analyses were performed with Merck F254 Alumina Silica Plates using UV visualization. Column purifications were carried out using Silicycle Ultra Pure Silica Gel. HRMS spectra were recorded using a Finnigat Mat 900 spectrometer in EI mode. The ^{1}H-, ^{13}C- and 2D spectra were recorded on a Bruker 200, 250 or 400 MHz spectrometer. High-temperature NMR spectra were recorded on a Bruker 400 MHz spectrometer. Systematic names for molecules according to IUPAC rules were generated using the Beilstein AutoNom program.

Synthesis of 'northern half'

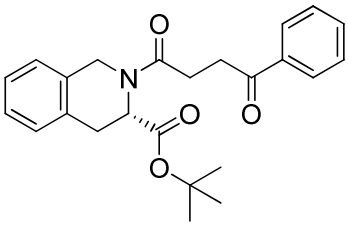


tert-butyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**2**).



Acid **1** (10.0 g, 56.5 mmol) was suspended in dry DCM (350 mL). Conc. sulfuric acid (8 mL) was added and the suspension became almost clear. The solution was slowly cooled to -20 °C and 2-methylpropene (82 g, 1461 mmol) was introduced. A septum, pierced with a needle, was placed on the flask. The solution was allowed to slowly warm to r.t. and stirred for 16 h. The suspension was slowly added to an ice-cold 5 % Na_2CO_3 solution. The solution was washed with DCM. The organic layer was dried over Na_2SO_4, filtered and evaporated to afford **2** as a colorless oil (3.0 g, 23%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.18-7.02 (m, 4H), 4.34 (d, 1H), 4.19 (d, 1H), 3.92-3.83 (m, 1H), 3.25-3.03 (m, 2H), 1.45 (s, 9H).

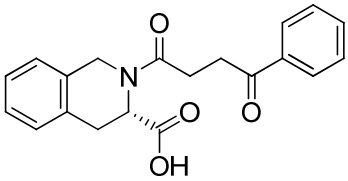
tert-butyl 2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**3**).



Amine **2** (4.0 g, 17.2 mmol) and 3-benzoylpropionic acid (3.08 g, 17.2 mmol) were dissolved in dry DCM (250 mL). EDCI (3.28 g, 17.2 mmol) was added and the solution was stirred for 16 h at r.t. The organic layer was washed with water and sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and evaporated to afford a yellow oil. The crude product was purified using flash column chromatography (EtOAc:Hex:TEA 30:69:1) to afford a yellow oil (3.4 g, 50%).

The ^{1}H-NMR spectrum shows two rotamers in a ratio of 1 : 0.6, causing several peaks to be visible as sets. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.01 (d, 2H), 7.53-7.39 (m, 3H), 7.19-7.10 (m, 4H), 5.39-5.34 (m, 1H of rotamer 1), 4.92 – 4.87 (m, 1H of rotamer 2), 4.87-4.53 (m, 2H), 3.59-2.62 (several m, 6H), 1.22 (s, 9H of rotamer 1), 1.21 (s, 9H of rotamer 2).

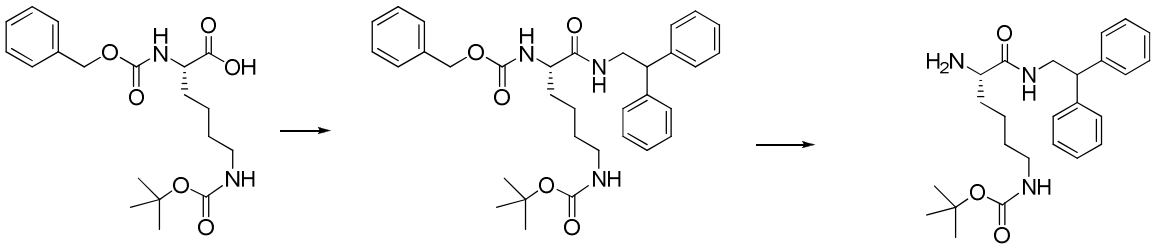
2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4**).



Protected acid **3** (3.4 g, 8.6 mmol) was dissolved in formic acid (50 mL).The mixture was stirred for 4 h at r.t. An additional 15 mL of formic acid was added and after 1 h the reaction was complete. Formic acid was evaporated to afford a light yellow powder (2.5 g, 86%).

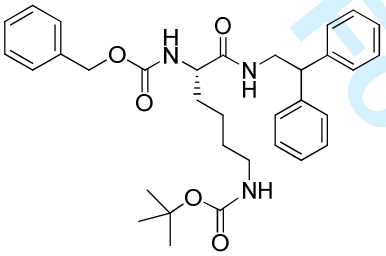
The ^{1}H-NMR spectrum shows two rotamers in a ratio of 1 : 0.25, causing several peaks to be visible as sets. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.80 (br s, 1H), 7.97 (d, 2H), 7.60-7.38 (m, 3H), 7.29-7.04 (m, 4H), 5.42-5.36 (m, 1H of rotamer 1), 5.07-5.01 (m, 1H of rotamer 2), 4.87-4.51 (m, 2H), 3.58-2.62 (several m, 6H). LR-MS: calc for M+1: 338.14, found: 338.1.

Synthesis of 'southern half'



5 **6** **7**

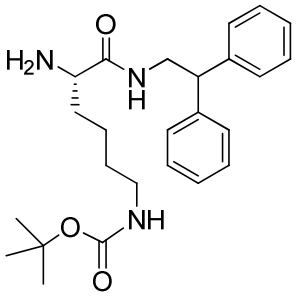
tert-butyl 5-(benzyloxycarbonylamino)-6-(2,2-diphenylethylamino)-6-oxo-hexylcarbamate (**6**).



Z-Lys(Boc)-OH (**5**, 5.0 g, 13.1 mmol) and 2,2-diphenylethylamine (2.8 g, 14.2 mmol) were dissolved in dry DCM 100 mL). EDCI (2.8 g, 14.6 mmol) was added and the solution was stirred overnight at r.t. The organic layer was washed with water and sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and evaporated to afford an off-white solid. The solid was recrystallized from hexane/ethyl acetate to afford fluffy white crystals (6.14 g, 84%).

^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.33-7.10 (m, 15H), 6.08-5.95 (m, 1H), 5.39-5.25 (m, 1H), 5.00 (s, 2H), 4.61-4.45 (m, 1H), 4.15 (t, 1H), 4.04-3.69 (m, 3H), 3.07-2.95 (m, 2H), 1.39 (s, 9H), 1.64-1.11 (several m, 6H). LR-MS M+1: Calc 338.13, found 338.1.

tert-butyl 5-amino-6-(2,2-diphenylethylamino)-6-oxohexylcarbamate (**7**).

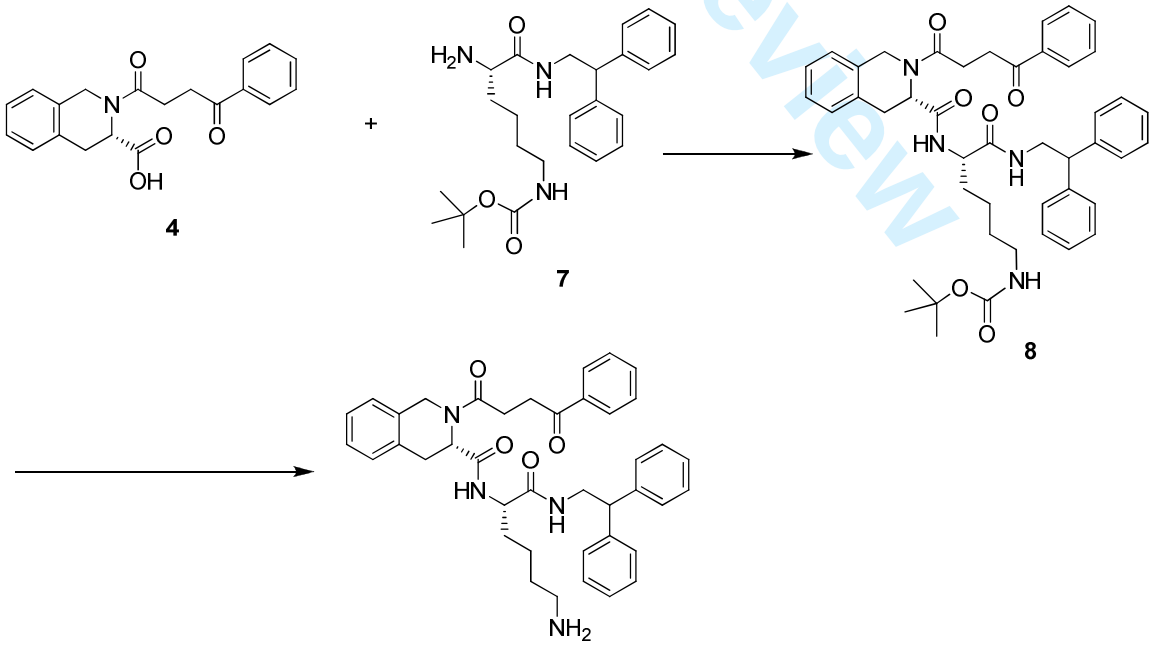


Compound **6** (2.20 g, 3.9 mmol) was dissolved in EtOAc (100 mL). 10 % Pd/C (200 mg) was added and the mixture was stirred for 16 h at r.t under a hydrogen atmosphere (1 bar). The catalyst was removed by filtration over hyflo gel. The solvent was evaporated to afford a colorless oil, which crystallized over 2 days to afford a white solid (1.6 g, 96 %).

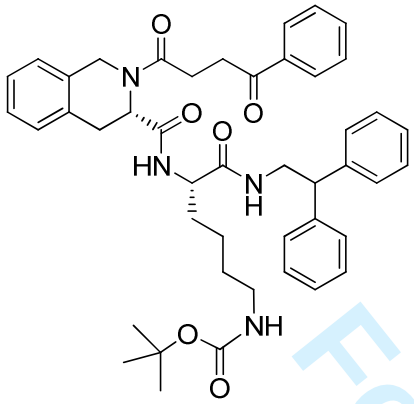
^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.31-7.09 (m, 10 H), 4.60-4.50 (m, 1H), 4.17 (t, 1H), 3.94-3.78 (m, 2H), 3.25-3.18 (m, 1H), 3.09-2.94 (m, 2H), 1.73-1.48 (m, 2H), 1.40 (s, 9H), 1.40-1.10 (m, 4H).

LR-MS M+1: Calc 426.26, found 426.3

Coupling of 'southern and northern halves' and deprotection



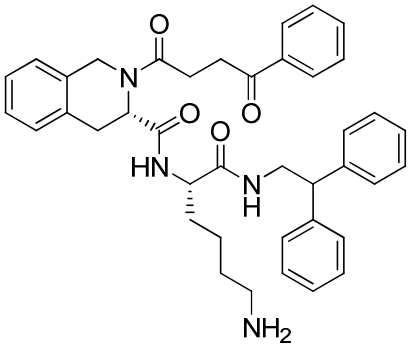
Tert-butyl-6-(2,2-diphenylethylamino)-6-oxo-5-(2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamido)hexylcarbamate (**8**).



Acid **4** (1.2 g, 3.56 mmol) and amine **7** (1.5 g, 3.52 mmol) were dissolved in dry DCM (100 mL). EDCI (740 mg, 3.86 mmol) was added and the solution was stirred for 16 h at r.t. The resulting yellow solution was washed with water and sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, filtered and evaporated to afford a yellow oil. The product was triturated with a EtOAc:hexane (50:50) mixture. The precipitate was recrystallized from refluxing hexane/EtOAc to afford white crystals (1.32 g, 50%) in two batches. The recrystallisation step was crucial to remove impurities that could not be removed otherwise (i.e. chromatography) and co-elute with the product in the next synthetic step.

^{1}H-NMR assignment was complex due to presence of rotamers in a ~1 : 3 ratio. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.05 (d, 2H of rotamer 1), 7.95 (d, 2H of rotamer 2), 7.65 – 7.45 (m, 3H), 7.32 – 7.06 (m, 14H), 6.71 (d, 1H of rotamer 1), 6.58 (d, 1H of rotamer 2), 6.47 – 6.31 (m, 1H), 5.05 – 4.65 (m, 3H), 4.62 – 4.00 (m, 3H), 3.78 – 3.58 (m, 2H), 3.58 – 2.90 (several m, 6H), 2.67 – 2.44 (m, 1H), 1.90-1.58 (m, 2H), 1.49 (s, 9H), 1.40-1.17 (m, 2H), 0.95-0.72 (m, 2H). LR-MS M+1: Calc 745.39, found 745.4.

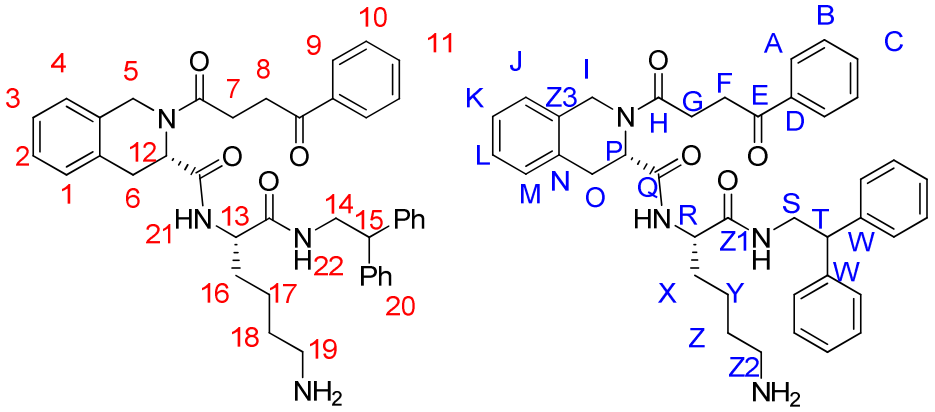
N-(6-amino-1-(2,2-diphenylethylamino)-1-oxohexan-2-yl)-2-(4-oxo-4-phenylbutanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**9**, VUF10661)



Protected compound **8** (300 mg, 0.40 mmol) was dissolved in dioxane (12 mL). A solution of HCl in dioxane (4 M, 4 mL) was added and the solution was stirred overnight at r.t. The HCl was removed by bubbling nitrogen gas through the solution. Dioxane was evaporated by rotary evaporation and the residue was partitioned between DCM and 2 M Na_2CO_3 solution. The water layer was washed with DCM and the organic layer was dried over Na_2SO_4, filtered and evaporated to afford a yellow oil. The crude oil was dissolved in little DCM. Et_2O was added dropwise to the solution at 0 °C. Then, the mixture was briefly stored in the freezer. The precipitated product was recovered by filtration and washed with icecold ether to afford an off-white powder. The filtrate was concentrated *in vacuo* and the crude oil was again dissolved in little DCM and the above procedure was repeated. This afforded a second batch as a white powder. Both batches were extensively dried, affording a total yield of 90 mg (35%) of VUF10661 (**9**) as a white powder.

A ^{1}H-NMR spectrum at room temperature (400 MHz, $CDCl_3$) afforded very complex signals due to the presence of rotamers in a ~1 : 2.5 ratio.

^{1}H-NMR (400 MHz, d_6-DMSO, **130 °C**) δ 7.92 (d, 2H), 7.61 (t, 1H), 7.49 (t, 2H), 7.30-7.08 (m, 15H), 4.92 (t, 1H), 4.81 (d, 1H), 4.59 (br d, 1H), 4.19 (t, 1H), 4.07-3.98 (m, 1H), 3.81-3.71 (m, 1H), 3.64-3.54 (m, 1H), 3.30 (t, 2H), 3.15-3.01 (m, 2H), 2.95-2.70 (m, 2H + water), 2.41 (t, 2H), 1.48-1.38 (m, 1H), 1.35-1.22 (m, 1H), 1.21-1.11 (m, 2H), 1.05-0.88 (m, 2H).



2D COSY (400 MHz, d_6-DMSO, **130 °C**). Observed relevant couplings: H_9 x H_{10}, H_9 x H_{11} (w), H_{11} x H_{10}, H_{20} x H_{15} (w), H_{12} x H_6, H_{5a} x H_{5b}, H_{13} x H_{16}, H_{15} x H_{14}, H_{14a} x H_{14b}, H_8 x H_7, H_{19} x H_{18}, H_{16} x H_{17}, H_{16a} x H_{16b}, H_{18} x H_{17}, H_{22} x H_{14}, many aromatic couplings are obscured.

2D HSQC (400 MHz, d_6-DMSO, **130 °C**). Observed relevant couplings: H_{11} X C_C, H_{10} X C_B, H_9 X C_A, H_{12} X C_P, H_{13} X C_R, H_{15} X C_T, H_{5a} X C_I, H_{5b} X C_I, H_{14a} X C_S, H_{14b} X C_S, H_{19} X C_{Z2}, H_8 X C_F, H_{18} X C_Z, H_{16a} X C_X, H_{16b} X C_X, H_6 X C_O, H_7 X C_G, H_{17} X C_Y, many aromatic couplings are obscured.

2D HMBC (400 MHz, d_6-DMSO, **130 °C**). Additional relevant couplings: H_9 X C_E, H_8 X C_E, H_8 X C_H, H_{5a} X C_H, H_{14} X C_{Z1}, H_6 X C_Q, H_{14} X C_W, H_{15} X C_W.

A ^{13}C-NMR spectrum recorded at room temperature was complex due to the presence of rotamers. ^{13}C-NMR (400 MHz, d_6-DMSO, 25 °C) δ 200.1, 173.5, 172.3, 171.2, 170.3, 141.9, 135.8, 133.9, 133.7, 132.4, 132.1, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.90, 127.9, 127.4, 126.8, 126.5, 126.3, 126.2, 125.9, 76.2, 54.5, 52.9, 50.0, 46.5, 43.6, 41.4, 34.4, 32.5, 30.7, 30.4, 27.0, 22.3, 22.0.

LC-MS (detection at 250 nm): > 95 %.

HR-MS: Calc for M+1 ($C_{40}H_{45}N_4O_4^+$): 645.3435, found 645.3445.