Aggregation Behaviour of Peptide-Polymer Conjugates Containing Linear Peptide Backbones and Multiple Polymer Side Chains Prepared by Nitroxide-Mediated Radical Polymerization

Michael Möller, a Carsten Hentschel, b Lifeng Chi, b and Armido Studer* a

a Organisch-Chemisches Institut and NRW Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstraße 40, 48149 Münster (Germany). Fax: (+49)251-833-6523; Tel: (+49)251-833-3291; E-mail: studer@uni-muenster.de

b Physikalisches Institut, Westfälische Wilhelms-Universität Münster, Wilhelm-Klemm-Straße 10, 48149 Münster (Germany). Fax: (+49)251-833-3602; Tel: (+49)251-833-3651; E-mail: chi@uni-muenster.de

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General

Reactions involving air or moisture sensitive starting materials, intermediates or products were performed in Schlenk flasks, which have been flame-dried in high vacuum and flushed with argon. CCl₄ (Acros), methanol (MeOH, 99.9 %, Extra Dry, AcroSeal®), DMSO (Acros) and acetic acid (HOAc, Acros) were used as received. Ethyl acetate (EtOAc) was distilled without drying reagent. All other solvents were freshly distilled under argon atmosphere or in vacuum from drying reagents. Benzene was distilled from Na, THF was distilled from Na/K, CH₂Cl₂ was distilled from P₂O₅, DMF was distilled from K₂CO₃ and ninhydrin, N-methylpyrrolidinone was distilled from CaH₂ and ninhydrin. Styrene was distilled from CaH₂ and N-isopropylacrylamide (NIPAM) was crystallized twice from n-pentane to remove stabilizers and stored at -30 °C under argon atmosphere. All other chemicals were used as received from the suppliers (Acros, Aldrich, Bachem, Fluka).

Solvents for flash column chromatography (FC), crystallizations and extractions have been distilled once, MTBE was previously dried over KOH. FC was performed using silica gel 60 (40-63 μm, Merck) applying an overpressure of up to 0.4 bar. Thin layer chromatography (TLC) was performed using silica gel 60 F₂₅₄ plates from Merck. Detection of the compounds was carried out by UV-light or dipping into solutions of KMnO₄ (1.5 g in 400 mL H₂O, 5 g NaHCO₃) or Ce(SO₄)₂·H₂O (10 g in 0.94 L H₂O, 60 mL conc. H₂SO₄, 25 g, phosphormolybdic acid hydrate). Reversed-phase flash chromatography (RP-FC) was performed using silica gel 90 C-18 reversed phase (40-63 μm, Fluka). Reversed-phase TLC was carried out using silica gel 60 RP-18 F₂₅₄S plates from Merck and substances were detected by UV-light (λ = 254 nm). Samples for spin-coating were dissolved with a concentration of 1 mg/mL. A droplet of 10 μL was spread on a silicon wafer and left for 20 seconds. Spin-coating was performed on a self-prepared spin-coating apparatus with a rotation speed of 5000 rounds per minute.

IR-spectra were recorded on a Digilab Excalibur FTS 4000 device equipped with a MKII Golden Gate Single Reflection ATR System. ¹H-NMR (300 and 400 MHz) and ¹³C-NMR (75 and 100 MHz) spectra were recorded on a Bruker DPX-300, a Bruker ARX-300 or a Bruker AMX-400 spectrometer at room temperature (rt). Chemical shifts in ppm are referenced to the solvent residue peaks (CHCl₃ at 7.26 ppm for ¹H-NMR and at 77.00 ppm for ¹³C-NMR;
CH$_3$OH at 4.87 ppm for $^1$H-NMR and at 49.00 ppm for $^{13}$C-NMR) as internal standards. Mass spectrometry was performed on a Bruker Daltronics MicroTof, a Waters-Micromass Quatro LCZ or an Orbitrap LTQ XL for ESI-MS and HRMS. Elemental analyses were performed on a Vario EL III (Elemental). Melting points were determined with a Stuart SMP10 and are uncorrected.

Gel permeation chromatography (GPC) was performed on a system consisting of a Knauer HPLC Pump 64 and a Knauer Differential Refractometer (λ = 950 ± 30 nm). Analysis was carried out using PSS WinGPC Compact V.720 software (Polymer Standards Service) based upon calibration with polystyrene standards (Polymer Laboratories Polystyrene Medium MW Calibration Kit S-M-10) or poly(methyl methacrylate) standards (Polymer Laboratories Poly(methyl methacrylate) Medium MW Calibration Kit S-M-10). Polystyrene chromatography was performed with a set of two PLgel MIXED-C columns (300 × 7.5 mm, particle size: 5 μm Polymer Laboratories, linear range of molecular weight: 200-2,000,000 g/mol) in THF (distilled over KOH and FeSO$_4$ and degassed by bubbling argon for at least 30 min). PNIPAM chromatography was carried out using a set of two Shodex GPC KF-804L columns (300 × 8 mm, particle size: 7 μm, range of molecular weight: < 400,000 (polystyrene)) in DMF (HPLC-grade, purchased by Roth, degassed by bubbling argon for at least 30 min) containing LiBr (c = 0.01 M) at 40 °C (warmed by an oven). Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZEN 3600 and analyzed with Malvern Zetasizer Nano software, version 5.03. THF was purified as for GPC, water was purified as for HPLC. Atomic force microscopy (AFM) was performed on a Park Systems XE-100 microscope in tapping mode at resonance frequency. The used cantilever was a rectangular silicon cantilever (PPP-NRHR-W) from Nanosensors. Typical tip radius was < 10 nm, typical force constant 42 N/m and typical resonance had a frequency of 330 KHz. Typical values were taken from data sheet.
Synthetic procedures

1-Bromo-4-(1-bromomethyl)-benzene (26)

1-Bromo-4-ethyl-benzene (14.0 mL, 101 mmol, 1.0 eq.) was dissolved in CCl₄ (100 mL). The solution was warmed to 50 °C and illuminated by a light bulb (Osram, 500 W). Bromine (5.20 mL, 101 mmol, 1.0 eq.) was added over a period of 15 min. The reaction mixture was stirred at 50 °C for 1 h under illumination. Reaction mixture was washed with NaHCO₃ (aq. sat., 3 × 20 mL) and NaCl (aq. sat., 2 × 20 mL), dried over MgSO₄ and the solvent was removed in vacuo. The desired product was obtained as a pale yellow liquid (26.1 g, 98.9 mmol, 98%).

^1H-NMR (300 MHz, CDCl₃): δ = 7.41 - 7.38 (m, 2 H, 2 × Aryl-H), 7.25 - 7.22 (m, 2 H, 2 × Aryl-H), 5.07 (q, J = 6.9 Hz, 1 H, CHBr), 1.94 (d, J = 6.9 Hz, 3 H, CH₃).

The spectroscopic data are in accordance with those reported in the literature.[1]

1-[1-(4-Bromophenyl)ethoxy]-2,2,6,6-tetramethylpiperidine (27)

According to a procedure of Matyjaszewski et al., bromide 26 (25.9 g, 98.3 mmol, 1.0 eq.), TEMPO (18.4 g, 118 mmol, 1.20 eq.), copper powder (6.56 g, 103 mmol, 1.05 eq.), Cu(O Tf)₂ (0.36 g, 0.98 mmol, 0.01 eq.) and 4,4-di-tert-butyl-2,2-dipyridyl (1.05 g, 3.93 mmol, 0.04 eq.) were added to benzene (190 mL) and warmed to 75 °C for 16 h.[2] The reaction mixture was filtered through a plug of silica gel with CH₂Cl₂ as solvent. The solvent was removed in vacuo and the crude product was purified by FC (pentane/MTBE 20:1). Alkoxyamine 27 as obtained as a colorless solid (28.5 g, 83.8 mmol, 85%).

^1H-NMR (300 MHz, CDCl₃): δ = 7.44 - 7.41 (m, 2 H, 2 × Aryl-H), 7.20 - 7.18 (m, 2 H, 2 × Aryl-H), 4.74 (q, J = 6.8 Hz, 1 H, CHO), 1.59 - 1.37 (m, 9 H, 3 × CH₂, CH₃CH), 1.27 (s, 3 H, CH₃), 1.15 (s, 3 H, CH₃), 1.02 (s, 3 H, CH₃), 0.65 (s, 3 H, CH₃).
The spectroscopic data are in accordance with those reported in the literature.[3]

4-[1-(2,2,6,6-Tetramethylpiperidine-1-yloxy)-ethyl]-benzoic acid (5)

Alkoxyamine 27 (14.2 g, 41.8 mmol, 1.0 eq.) was dissolved in THF (170 mL) and t-BuLi (1.5 M in pentane, 55.7 mL, 83.6 mmol, 2.0 eq.) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 20 min and CO₂, dried by passing through conc. H₂SO₄, was passed through the reaction mixture over a period of 30 min at -78 °C. The mixture was warmed to rt and HCl (aq., 1 M) was added until the mixture was acidic. The phases were separated and the aqueous layer was extracted with CH₂Cl₂ (2 × 60 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed under reduced pressure. Purification was carried out by crystallization in EtOAc at -18 °C and delivered acid 5 as a colorless solid (12.8 g, 41.8 mmol, 100%).

¹H-NMR (300 MHz, CD₃OD): δ = 8.11 (d, J = 8.4 Hz, 2 H, 2 × Aryl-H), 7.62 (d, J = 8.3 Hz, 2 H, 2 × Aryl-H), 5.68 (q, J = 6.4 Hz, 1 H, CHO), 2.12 - 1.81 (m, 6 H, 3 × CH₂), 1.77 (d, J = 6.4 Hz, 3 H, CHCH₃), 1.74 (s, 3 H, CH₃), 1.55 (s, 3 H, CH₃), 1.36 (s, 3 H, CH₃), 1.03 (s, 3 H, CH₃).

The spectroscopic data are in accordance with those reported in the literature.[4]

Boc-Lys(Cbz)-Gly-OMe (28)

Boc-Lys(Cbz)-Gly-OH (9.51 g, 25.0 mmol, 1.0 eq.) and glycinmethylester hydrochloride (3.46 g, 27.5 mmol, 1.1 eq.) were dissolved in CH₂Cl₂ (120 mL). EDCI (5.75 g, 30.0 mmol, 1.2 eq.), HOBT (4.60 g, 30.0 mmol, 1.2 eq.) and NMM (6.40 mL, 57.5 mmol, 2.3 eq.) were added and the mixture was stirred at rt for 5 h. HCl (aq., 1 M, 20 mL) was added; the phases were separated and the aqueous layer was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were washed with NaOH (aq., 0.25 M, 40 mL) and NaCl (aq. sat., 30 mL) and the
solvent was removed *in vacuo*. Dipeptide 28 was obtained as a colorless solid (10.5 g, 23.3 mmol, 93%).

Mp.: 85 °C. IR (neat): 3318 w, 2938 w, 2362 w, 1667 s, 1520 m, 1454 m, 1367 m, 1245 s, 1211 s, 1165 s, 1020 m, 913 w, 857 w, 731 m, 698 m, 645 m. ¹H-NMR (300 MHz, CDCl₃): δ = 7.35 - 7.29 (m, 5 H, 5 × Aryl-H), 6.78 (br s, 1 H, CHCONH₂CH₂), 5.24 - 5.22 (m, 1 H, CHCO), 5.08 (s, 2 H, CH₂O), 5.05 - 5.00 (m, 1 H, CH₂NHCO₂), 4.13 (br s, 1 H, NHCO₂), 4.09 - 3.92 (m, 2 H, NHCH₂CO₂), 3.70 (s, 3 H, OCH₃), 3.22 - 3.15 (m, 2 H, CH₂N), 1.90 - 1.78 (m, 1 H, CHHCH), 1.70 - 1.61 (m, 1 H, CHHCH), 1.56 - 1.47 (m, 2 H, CH₂CH₂N), 1.45 - 1.36 (m, 11 H, CH₂CH₂CH₃, 3 × CH₃). ¹³C-NMR (75 MHz, CDCl₃): δ = 172.6 (C), 170.3 (C), 156.7 (C), 155.9 (C), 136.8 (C), 128.6 (2 × CH), 128.2 (3 × CH), 80.3 (C), 66.8 (CH₂), 54.3 (CH), 52.4 (CH₃), 41.2 (CH₂), 40.5 (CH₂), 32.0 (CH₂), 29.6 (CH₂), 28.4 (3 × CH₃), 22.5 (CH₂). MS (ESI): 452 [M+H]+, 474 [M+Na]+, 925 [2M+H]+. HRMS (ESI): calculated for C₂₂H₃₃N₃O₇Na+: 474.2211, found: 474.2208. Anal. calculated for C₂₂H₃₃N₃O₇: C: 58.52, H: 7.37, N: 9.31, found: C: 58.54, H: 7.38, N: 9.21.

**Boc-Lys(HxHCl)-Gly-OMe (7)**

Dipeptide 28 (10.5 g, 23.2 mmol, 1.0 eq.) was dissolved in MeOH (200 mL). Pd/C (10% Pd, 1.24 g, 1.16 mmol, 0.05 eq.) and HCl (1.25 M in MeOH, 3.7 mL, 4.7 mmol, 0.2 eq.) were added and the flask was purged with hydrogen gas. The mixture was stirred for 16 h at rt under atmospheric pressure (balloon). HCl (1.25 M in MeOH, 14.8 mL, 18.6 mmol, 0.8 eq.) was added. The reaction mixture was filtered and the solvent was evaporated *in vacuo*. The desired product was obtained as a colorless solid (8.19 g, 23.2 mmol, 100%).

Mp.: 62 °C. IR (neat): 3296 br m, 3063 m, 2936 m, 1655 s, 1520 s, 1367 m, 1225 m, 1163 s, 1086 m, 1047 m, 859 w, 653 m, 561 m. ¹H-NMR (300 MHz, CD₃OD): δ = 4.11 - 4.06 (m, 1 H, CHCO), 4.06 - 3.89 (m, 2 H, CH₂NH₂), 3.73 (s, 3 H, OCH₃), 2.96 - 2.91 (m, 2 H, CH₂CO₂), 1.90 - 1.79 (m, 1 H, CHCHH), 1.74 - 1.62 (m, 3 H, CHCHH, CH₂CH₂N), 1.56 - 1.46 (m, 11 H, CH₂CH₂CH₂, 3 × CH₃). ¹³C-NMR (75 MHz, CD₃OD): δ = 175.5 (C), 171.7 (C), 157.8 (C), 80.7 (C), 55.7 (CH), 52.6 (CH₃), 41.8 (CH₂), 40.6 (CH₂), 32.7 (CH₂), 28.7 (3 × CH₃), 22.5 (CH₂).

**Boc-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly-OMe (1)**

Dipeptide 7 (10.2 g, 28.9 mmol, 1.0 eq.), alkoxyamine 5 (9.70 g, 31.8 mmol, 1.1 eq.), EDCI (6.64 g, 34.7 mmol, 1.2 eq.), HOBt (5.31 g, 34.7 mmol, 1.2 eq.) and NMM (7.00 mL, 63.5 mmol, 2.2 eq.) were dissolved in CH₂Cl₂ (150 mL) and the reaction mixture was stirred for 16 h at rt. HCl (1 M aq., 100 mL) was added and the phases were separated. The aqueous layer was extracted with CH₂Cl₂ (4 × 50 mL). The combined organic layers were washed with NaOH (aq., 0.25 M, 50 mL) and NaCl (aq. sat., 50 mL) and dried over MgSO₄. The solvent was removed in vacuo. The crude product was purified by FC (CH₂Cl₂/acetone 5:1) and the desired product was obtained as a colorless solid (13.9 g, 23.0 mmol, 80%).

Mp.: 82 °C. IR (neat): 3315 br m, 2931 m, 1641 s, 1538 s, 1454 m, 1366 m, 1303 m, 1245 m, 1171 s, 1062 w, 1018 w, 935 w, 854 v, 771 w, 707 w. ¹H-NMR (300 MHz, CDCl₃): δ = 7.73 (d, J = 7.9 Hz, 2 H, 2 × Aryl-H), 7.35 (d, J = 7.7 Hz, 2 H, 2 × Aryl-H), 6.84 (br s, 1 H, CHCONHCH₂), 6.43 (br s, 1 H, CONHCH₂), 5.29 - 5.27 (m, 1 H, CHCO), 4.81 (q, J = 6.7 Hz, 1 H, CHCH₃), 4.15 (br s, 1 H, NHCO₂), 4.02 (t, J = 5.1 Hz, 2 H, NHCH₂CO₂), 3.71 (s, 3 H, OCH₃), 3.53 - 3.39 (m, 2 H, CH₂CH₂N), 1.98 - 1.85 (m, 1 H, CHCHH), 1.80 - 1.60 (m, 5 H, CHCHH, CH₂CH₂N, CH₂CH₂C), 1.51 - 1.42 (m, 18 H, 2 × CH₂CH₂CH₂, 2 × CH₂C, CH₃CH, C(CH₃)₃), 1.28 (s, 3 H, CH₃), 1.16 (s, 3 H, CH₃), 1.01 (s, 3 H, CH₃), 0.63 (s, 3 H, CH₃).

¹³C-NMR (75 MHz, CDCl₃): δ = 172.9 (C), 170.2 (C), 167.8 (C), 155.9 (C), 149.5 (C), 133.1 (C), 127.0 (2 × CH), 126.6 (2 × CH), 83.0 (CH), 60.4 (C), 59.7 (2 × C), 52.3 (CH, CH₃), 41.2 (CH₂), 40.5 (CH₂), 39.3 (2 × CH₂), 32.0 (CH₂), 29.1 (CH₂), 28.4 (3 × CH₃), 23.7 (2 × CH₃), 22.5 (CH₂), 21.1 (CH₃), 20.4 (CH₃), 17.3 (CH₂), 14.2 (CH₃). MS (ESI): 314 [M+H+Na]²⁺, 605 [M+H]^+, 627 [M+Na]^+, 1231 [2M+Na]^+. HRMS (ESI): calculated for C_{32}H_{52}N_{4}O_{7}H^+: 605.3909, found: 605.3904. Anal. calculated. for C_{32}H_{52}N_{4}O_{7}·H₂O: C: 61.74, H: 8.74, N: 9.00, found: C: 61.78, H: 8.58, N: 9.00.
Boc-Lys(4-{1-{2,2,6,6-tetramethylpiperidine-1-yl oxy}-ethyl]-benzamide)-Gly-OH (8b)

NaOH (aq., 0.25 M, 65 mL) was added to a solution of dipeptide 1 (4.00 g, 6.62 mmol, 1.0 eq.) in MeOH (110 mL). The reaction mixture was stirred for 16 h at rt. CH₂Cl₂ (50 mL) and HCl (aq., 1 M, 30 mL) were added, the phases were separated and the organic layer was extracted with CH₂Cl₂ (4 x 30 mL). The combined organic layers were dried over MgSO₄ and the solvent was evaporated in vacuo. Acid 8b was obtained as a colorless solid (3.85 g, 6.52 mmol, 98%).

Mp.: 126 °C. IR (neat): 3301 br w, 2936 w, 1705 s, 1643 s, 1542 s, 1504 s, 1438 m, 1367 m, 1361 w, 1306 m, 1248 m, 1168 s, 1120 w, 1051 m, 972 w, 912 s, 854 m, 772 w, 728 s, 645 m. ¹H-NMR (300 MHz, CD₃OD): δ = 7.90 (d, J = 8.8 Hz, 2 H, 2 x Aryl-H), 7.59 (d, J = 8.4 Hz, 2 H, 2 x Aryl-H), 5.72 (q, J = 6.8 Hz, 1 H, CHCH₃), 4.12 - 4.06 (m, 1 H, CHCO), 3.93 (q, J = 17.8 Hz, 2 H, NHCH₂CO), 3.41 (t, J = 7.1 Hz, 2 H, CH₂NHCO), 2.14 - 1.61 (m, 16 H, CHCH₂, NCH₂CH₂, 2 x CCH₂, CH₂CH₂C, CHCH₃, CH₃), 1.53 (s, 3 H, CH₃), 1.51 - 1.46 (m, 2 H, CH₂CH₂CH₂N), 1.45 (s, 9 H, C(CH₃)₃), 1.33 (s, 3 H, CH₃), 1.03 (s, 3 H, CH₃). ¹³C-NMR (75 MHz, CDCl₃): δ = 173.3 (C), 171.5 (2 x C), 167.0 (C), 144.2 (C), 134.7 (C), 127.9 (2 x CH), 127.1 (2 x CH), 85.1 (CH), 80.1 (C), 70.6 (C), 70.0 (C), 54.4 (CH), 41.4 (2 x CH₂), 39.5 (2 x CH₂), 37.2 (CH₂), 37.0 (CH₂), 29.0 (CH₃), 28.8 (CH₂), 28.4 (3 x CH₃), 23.5 (CH₃), 22.5 (CH₂), 21.2 (CH₃), 21.0 (CH₃), 15.8 (CH₃). MS (ESI): 307 [M+H+Na]⁺, 591 [M+H]⁺, 613 [M+Na]⁺, 1203 [2M+Na]⁺. HRMS: calculated for C₃₁H₅₀N₄O₇H⁺: 591.3752, found: 591.3754.
**HCb×H-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly-OMe** *(8a)*

Dipeptide 1 (1.31 g, 2.17 mmol, 1.0 eq.) was added to a methanolic solution of HCl (1.25 M in MeOH, 8 mL) and the mixture was stirred for 16 h at rt. The solvent was removed *in vacuo* and the desired product was obtained as a colorless solid (1.18 g, 2.17 mmol, 100%).

Mp.: 86 °C. IR (neat): 3385 *m* *br*, 3259 *m* *br*, 2945 *s*, 2362 *w*, 1744 *m*, 1686 *m*, 1638 *s*, 1548 *m*, 1384 *w*, 1308 *w*, 1210 *m*, 940 *w*, 856 *w*. ¹H-NMR (300 MHz, CD₃OD): δ = 7.93 (*d*, *J* = 8.2 Hz, 2 H, *2 × Aryl-H), 7.63 (*d*, *J* = 8.2 Hz, 2 H, *2 × Aryl-H), 5.88 (*q*, *J* = 6.5 Hz, 1 H, CHCH₃), 4.13 - 3.93 (*m*, 3 H, CHCO, NHCH₂CO), 3.73 (s, 3 H, OCH₃), 3.45 (*t*, *J* = 6.9 Hz, 2 H, CH₂CH₂N), 2.26 - 1.87 (*m*, 6 H, CHCH₂, CH₂CH₂N, CH₂CH₂C), 1.81 - 1.65 (*m*, 10 H, 2 × CH₂C, CH₃, CH₂CH), 1.63 - 1.56 (*m*, 5 H, CH₂CH₂CH, CH₃), 1.36 (s, 3 H, CH₃), 1.08 (s, 3 H, CH₃). ¹³C-NMR (75 MHz, CD₃OD): δ = 172.4 (C), 170.7 (C), 169.5 (C), 145.4 (C), 136.1 (C), 128.9 (2 × CH), 128.0 (2 × CH), 87.3 (CH), 72.8 (C), 72.2 (C), 54.3 (CH), 52.7 (CH₃), 41.8 (CH₂), 40.4 (CH₂), 38.2 (CH₂), 38.1 (CH₂), 32.2 (CH₂), 30.0 (CH₂), 29.5 (CH₃), 29.3 (CH₃), 23.9 (CH₃), 23.0 (CH₂), 21.0 (CH₃), 20.8 (CH₃), 16.4 (CH₂). MS (ESI): 253 [M+2H]²⁺, 505 [M+H]⁺, 527 [M+Na]⁺, 1009 [2M+H]⁺, 1031 [2M+Na]⁺. HRMS (ESI): calculated for C₂₇H₄₄N₄O₅Na⁺: 527.3204, found: 527.3204.
Boc-bis[-Lys(4-[[1-[[2,2,6,6-tetramethylpiperidine-1-yloxy]-ethyl]-benzamide]-Gly]-OMe (9)

Acid 8b (3.48 g, 5.90 mmol, 1.0 eq.) and amine hydrochloride 8a (3.20 g, 5.90 mmol, 1.0 eq.) were dissolved in CH₂Cl₂ (60 mL). EDCI (1.36 g, 7.10 mmol, 1.2 eq.), HOBt (1.09 g, 7.10 mmol, 1.2 eq.) and NMM (1.4 mL, 13 mmol, 2.2 eq.) were added and the reaction mixture was stirred for 16 h at rt. HCl (aq., 1 M, 30 mL) was added and the phases were separated. The aqueous layer was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic layers were washed with NaOH (aq., 0.25 M, 40 mL) and NaCl (aq. sat., 30 mL) and dried over MgSO₄. The solvent was removed under reduced pressure and the crude product was purified by FC (CH₂Cl₂/aceton 3:1 → 1:1). Tetrapeptide 9 was obtained as a colorless solid (4.91 g, 4.56 mmol, 77%).

Mp.: 144 °C. IR (neat): 3297 br m, 2933 m, 1749 w, 1632 s, 1535 s, 1455 w, 1364 m, 1254 m, 1179 m, 1063 m, 1018w, 908m, 853w, 730s. ¹H-NMR (300 MHz, CDCl₃): δ = 7.78 - 7.74 (m, 4 H, 4 × Aryl-H), 7.62 (br s, 1 H, CH₂CONH), 7.56 - 7.52 (m, 1 H, NH₂CH₂CO₂), 7.44 (br s, 1 H, NHCH₂CO), 7.32 (d, J = 8.0 Hz, 4 H, 4 × Aryl-H), 6.96 (br s, 2 H, 2 × CH₂NHO), 5.89 (br s, 1 H, CO₂NH), 4.79 (q, J = 6.4 Hz, 2 H, 2 × CHCH₃), 4.64 - 4.57 (m, 1 H, CH₂CONHC₂H), 4.27 - 4.18 (m, 1 H, CO₂NH₂C₂H), 3.65 (s, 3 H, OCH₃), 3.51 - 3.32 (m, 4 H, 2 × CH₂NH₂CO₂C), 2.00 - 1.33 (m, 39 H, 2 × CHCH₂, 2 × CH₂CH₂CH₂, 2 × NHCH₂CH₂, C(CH₃)₃, 2 × CHCH₃, 4 × CH₂C, 2 × CH₂CH₂C), 1.27 (s, 6 H, 2 × CH₃), 1.15 (s, 6 H, 2 × CH₃), 1.00 (s, 6 H, 2 × CH₃), 0.62 (s, 6 H, 2 × CH₃). ¹³C-NMR (75 MHz, CDCl₃): δ = 173.6 (C), 172.5 (C), 170.2 (C), 169.4 (C), 168.0 (2 × C), 156.3 (C), 149.5 (C), 149.4 (C), 133.1 (C), 133.0 (C), 127.2 (4 × CH), 126.6 (4 × CH), 83.0 (2 × CH), 80.0 (C), 59.9 (4 × C), 57.8 (CH), 53.2 (CH), 52.3 (CH₃), 43.5 (CH₂), 41.2 (CH₂), 40.4 (4 × CH₂), 39.8 (CH₂), 39.7 (CH₂), 34.4 (4 × CH₃), 32.4 (CH₂), 32.1 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.4 (3 × CH₃), 23.7 (2 × CH₃), 22.9 (2 × CH₂), 20.4 (4 × CH₃), 17.3 (2 × CH₂). MS (ESI): 539 [M+2H]²⁺, 550 [M+H+Na]²⁺, 1078 [M+H]⁺, 1100 [M+Na]⁺. HRMS (ESI):
calculated for C$_{58}$H$_{92}$N$_8$O$_{11}$NaH$_2$: 550.3425, found: 550.3416. Anal. calculated for C$_{58}$H$_{92}$N$_8$O$_{11}$: C: 64.66, H: 8.61, N: 10.40, found: C: 64.34, H: 8.67, N: 10.30.

**Boc-bis[-Lys(4-{1-2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OH (10b)**

NaOH (0.25 M aq., 50 mL) was added to a solution of tetrapeptide 9 (1.70 g, 1.57 mmol, 1.0 eq.) in MeOH (80 mL). The reaction mixture was stirred for 16 h at rt. CH$_2$Cl$_2$ (50 mL) and HCl (aq., 1 M, 50 mL) were added and the phases were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (4 × 50 mL). The combined organic layers were dried over MgSO$_4$ and the solvent was removed in vacuo. The product was obtained as a colorless solid (1.47 g, 1.38 mmol, 88%).

Mp.: 172 °C. IR (neat): 3288 br m, 2929 m, 2364 w, 1646 s, 1541 s, 1440 m, 1384 m, 1306 m, 1247 m, 1169 s, 1052 w, 938 w, 855 w, 733 s. $^1$H-NMR (300 MHz, CD$_3$OD): $\delta$ = 7.93 ($d$, $J$ = 7.6 Hz, 4 H, 4 × Aryl-H), 7.62 ($d$, $J$ = 8.3 Hz, 4 × Aryl-H), 5.75 ($q$, $J$ = 6.2 Hz, 2 H, 2 × CHCH$_3$), 4.44 ($dd$, $J_1$ = 5.1 Hz, $J_2$ = 8.7 Hz, 1 H, CHCO), 4.04 ($dd$, $J_1$ = 5.4 Hz, $J_2$ = 8.5 Hz, 1 H, CHCO), 3.98 - 3.84 ($m$, 4 H, 2 × NHC$_2$CO), 3.45 - 3.40 ($m$, 4 H, 2 × CH$_2$NCO), 2.17 - 1.64 ($m$, 42 H, 2 × CHCH$_2$, 2 × CHCH$_2$CH$_2$, 2 × NHCH$_2$CH$_2$, 4 × CCH, 2 × CCH$_2$CH$_2$, 2 × CHCH$_3$, 4 × CCH$_3$), 1.47 ($s$, 9 H, C(C$_3$)$_3$), 1.37 ($s$, 6 H, 2 × CCH$_3$), 1.06 ($s$, 6 H, 2 × CCH$_3$). $^{13}$C-NMR (75 MHz, CD$_3$OD): $\delta$ = 176.0 (C), 174.5 (C), 172.6 (C), 171.6 (C), 169.4 (2 × C), 158.1 (C), 145.5 (2 × C), 136.1 (2 × C), 128.9 (4 × CH), 128.0 (4 × CH), 87.4 (2 × CH), 80.7 (C), 56.4 (4 × C), 54.6 (2 × CH), 43.7 (CH$_2$), 41.8 (CH$_2$), 40.6 (CH$_2$), 40.7 (CH$_2$), 38.5 (2 × CH$_2$), 38.4 (2 × CH$_2$), 32.6 (2 × CH$_2$), 30.1 (2 × CH$_2$), 29.9 (2 × CH$_3$), 29.7 (2 × CH$_3$), 28.8 (3 × CH$_3$), 24.2 (2 × CH$_2$), 23.9 (2 × CH$_3$), 20.9 (2 × CH$_3$), 20.8 (2 × CH$_3$), 16.6 (2 × CH$_3$). MS (ESI): 532 [M+2H]$^{2+}$, 1064 [M+H]$^+$, 1086 [M+Na]$^+$. HRMS (ESI): calculated for C$_{57}$H$_{90}$N$_8$O$_{11}$H$: 1063.6802$, found: 1063.6810.
HCl×H-Bis-[Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (10a)

Tetrapeptide 9 (1.08 g, 1.00 mmol, 1.0 eq.) was added to a methanolic solution of HCl (1.25 M in MeOH, 4.0 mL, 5.0 mmol, 5.0 eq.) and the resulting solution was stirred for 16 h at rt. The volatile components were evaporated in vacuo. Amine hydrochloride 10a was obtained as a colorless solid (1.01 g, 1.00 mmol, 100%).

Mp.: 157 °C. IR (neat): 3277 br m, 2941 m, 2867 w, 2362 w, 2337 w, 1737 w, 1649 s, 1544 s, 1385 w, 1308 w, 938 w, 856 w. 1H-NMR (300 MHz, CD3OD): δ = 7.94 (dd, J1 = 3.6 Hz, J2 = 8.2 Hz, 4 H, 4 × Aryl-H), 7.66 - 7.63 (m, 4 H, 4 × Aryl-H), 5.84 (q, J = 6.4 Hz, 2 H, 2 × CHCH3), 4.44 (dd, J1 = 5.3 Hz, J2 = 8.4 Hz, 1 H, NHCHCO), 4.04 - 3.90 (m, 5 H, NHCHCO, 2 × NHCH2CO), 3.79 (s, 3 H, OCH3), 3.48 - 3.42 (m, 4 H, 2 × CH2NCO), 2.23 - 1.55 (m, 42 H, 2 × CHCH2, 2 × CHCH2CH2, 2 × NHCH2CH2, 4 × CCH2, 2 × CCH2CH2, 2 × CHCH2CH3, 4 × CCH3), 1.38 (s, 6 H, 2 × CCH3), 1.08 (s, 6 H, 2 × CCH3). 13C-NMR (75 MHz, CD3OD) : δ = 174.7 (C), 171.5 (C), 171.1 (C), 170.9 (C), 169.5 (C), 169.4 (C), 145.3 (2 × C), 136.2 (C), 136.1 (C), 129.0 (4 × CH), 128.0 (4 × CH), 87.5 (2 × CH), 72.9 (2 × C), 72.2 (2 × C), 54.6 (CH), 54.5 (CH), 52.6 (CH3), 43.4 (CH2), 41.8 (CH2), 40.8 (CH2), 40.4 (CH2), 38.2 (2 × CH2), 38.1 (2 × CH2), 32.7 (CH2), 32.1 (CH2), 30.0 (2 × CH2), 29.6 (2 × CH3), 29.3 (2 × CH3), 24.1 (CH2), 23.9 (2 × CH3), 23.2 (CH2), 21.0 (2 × CH3), 20.8 (2 × CH3), 16.4 (2 × CH2). MS (ESI): 489 [M+2H]2+, 978 [M+H]+, 1000 [M+Na]+. HRMS (ESI): calculated for C53H84N8O9H+: 977.6440, found: 977.6440.
Boc-tetrakis[-Lys(4-{1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (11)

Acid 10b (190 mg, 179 µmol, 1.0 eq.) and amine hydrochloride 10a (181 mg, 179 µmol, 1.0 eq.) were dissolved in DMF (2 mL). EDCI (51.0 mg, 269 µmol, 1.5 eq.), HOBt (33.0 mg, 215 µmol, 1.2 eq.) and NMM (49.0 µL, 448 µmol, 2.5 eq.) were added and the reaction mixture was stirred for 16 h at rt. The solvent was removed in vacuo and the crude product was purified by RP-FC (H₂O → H₂O/MeOH 1:1 → MeOH). Octapeptide 11 was obtained as a colorless solid (255 mg, 126 µmol, 70%).


Boc-tetrakis[-Lys(4-{1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OH (12b)

Octapeptide 11 (100 mg, 50.0 µmol, 1.0 eq.) was dissolved in MeOH (300 mL) in an ultrasonic bath. NaOH (aq., 1 M, 20 mL) was added and the reaction mixture was stirred for 40 h at rt. Formic acid (2.5 mL) was added and the volatile components were removed in vacuo. The crude product was purified by RP-FC (H₂O (200 mL) → MeOH). The desired product was obtained as a colorless solid (91 mg, 45 µmol, 91%).
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Mp.: Decomp. above 200 °C. IR (neat): 3288 br m, 2929 m, 1654 s, 1539 s, 1454 m, 1385 m, 1304 m, 1256 m, 1179 w, 1133 w, 1094 m, 1063 m, 1019 w, 935 w, 854 w, 770 w, 661 m. MS (ESI): 670 [M+3H]^3+, 677 [M+2H+Na]^3+, 685 [M+H+2Na]^3+, 692 [M+3Na]^3+, 1005 [M+2H]^2+, 1016 [M+H+Na]^2+, 1027 [M+2Na]^2+, 2009 [M+H]^+, 2031 [M+Na]^+. HRMS (ESI): calculated for C_{109}H_{170}N_{16}O_{19}HNa_{2}+: 1015.6397, found: 1015.6398.

Due to low solubility of 12b in common solvents we did not obtain NMR-Spectra.

**HClxH-Tetrakis-[Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (12a)**

Octapeptide 11 (100 mg, 0.050 mmol, 1.0 eq.) was suspended in MeOH (5 mL) and a methanolic solution of HCl (1.25 M in MeOH, 0.50 mL, 0.63 mmol, 12.5 eq.) was added. The reaction mixture was stirred for 48 h at rt. The solvent was removed in vacuo and amine hydrochloride 12a was obtained as a colorless solid (97 mg, 50 μmol, 100%).

Mp.: Decomp. above 200 °C. IR (Film): 3302 br m, 2935 m, 1656 s, 1452 s, 1439 m, 1385 m, 1306 w, 1093 w, 938 w, 855 w, 590 w. MS (ESI): 641 [M+3H]^3+, 962 [M+2H]^2+, 973 [M+H+Na]^2+, 984 [M+2Na]^2+, 1923 [M+H]^+, 1945 [M+Na]^+. HRMS (ESI): calculated for C_{105}H_{164}N_{16}O_{17}HNa_{2}+: 972.6213, found: 972.6243. Due to low solubility of 12a in common solvents we did not obtain NMR-Spectra.
Boc-hexakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (13)

Acid 12b (100 mg, 50.0 μmol, 1.0 eq.) and amine hydrochloride 10a (50.0 mg, 50.0 μmol, 1.0 eq.) were dissolved in DMF (3 mL). HATU (29 mg, 75 μmol, 1.5 eq.), HOAt (10 mg, 75 μmol, 1.5 eq.) and DIPEA (44.0 μL, 250 μmol, 5.0 eq.) were added and the reaction mixture was stirred for 16 h at rt. MeOH (10 mL) and acetone (10 mL) were added and the product was crystallized overnight at 4 °C. The crystallization process was repeated once. Dodecanpeptide 13 was obtained as a colorless solid (111 mg, 37.4 μmol, 75%).

Mp.: Decomp. above 240 °C. IR (neat): 3285 br w, 2931 w, 1627 s, 1525 s, 1439 m, 1361 m, 1301 m, 1210 m, 1181 m, 1062 s, 1017 m, 935 m, 852 m, 707 m. HRMS (ESI): calculated for C_{162}H_{252}N_{24}O_{27}: 2965.90783, found: 2965.91262, calculated from: 495.49288 [M+6H]^{6+}, 594.18985 [M+5H]^{5+}, 742.73611 [M+4H]^{4+}, 989.97935 [M+3H]^{3+}, 1484.96729 [M+2H]^{2+}. Due to low solubility of 13 in common solvents we did not obtain NMR-Spectra.

Boc-octakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (14)

Acid 12b (73 mg, 36 μmol, 1.0 eq.) and amine hydrochloride 12a (71 mg, 36 μmol 1.0 eq.) were dissolved in N-methylpyrrolidone (5 mL). HATU (21 mg, 54 μmol, 1.5 eq.), HOAt (7.4 mg, 54 μmol, 1.5 eq.) and DIPEA (32 μL, 0.18 mmol, 5.0 eq.) were added and the reaction mixture was stirred for 16 h at rt. MeOH (20 mL) was added and the product was crystallized at 4 °C. The crystallization process was repeated once and the product was obtained as a colorless solid (88 mg, 22 μmol, 62%).
Mp.: Decomp. above 260 °C. IR (neat): 3286 br w, 2931 w, 1626 s, 1522 s, 1439 m, 1361 m, 1302 m, 1210 m, 1133 m, 1062 s, 1018 m, 935 m, 852 m, 653 m. HRMS (ESI): calculated for C\textsubscript{214}H\textsubscript{332}N\textsubscript{32}O\textsubscript{35}: 3910.51774, found: 3910.51731, calculated from: 559.93891 [M+7H]\textsuperscript{7+}, 653.09434 [M+6H]\textsuperscript{6+}, 783.51189 [M+5H]\textsuperscript{5+}. Due to low solubility of 14 in common solvents we did not obtain NMR-Spectra.

3-Nitropentane (29)

\[
\text{NO}_2
\]
Sodium nitrite (44.2 g, 640 mmol, 1.6 eq.) was added to a solution of 3-bromopentane (50.0 mL, 400 mmol, 1.0 eq.) in DMSO (350 mL). The reaction mixture was stirred for 18 h at rt, cooled to 0 °C and water was added until the formed precipitate was dissolved. The mixture was extracted with pentane (3 × 60 mL). The combined extracts were washed with water (2 × 10 mL), dried over MgSO\textsubscript{4} and the solvent was removed \textit{in vacuo}. The product was obtained as a pale blue liquid and used without further purification (29.8 g, 254 mmol, 64%).

\textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}): \(\delta = 4.36 - 4.27 \) (\textit{m}, 1 H, CH), 1.99 - 1.91 (\textit{m}, 2 H, CH\textsubscript{2}), 1.82 - 1.75 (\textit{m}, 2 H, CH\textsubscript{2}), 0.95 (\textit{t}, \(J = 7.4\) Hz, 6 H, 2 × CH\textsubscript{3}).

The spectroscopic data are in accordance with those reported in the literature.[5]

\textit{N-tert-Butyl-(2-ethyl-2-nitrobutyl)-amine} (30)

\[
\text{NH}
\]
Formaldehyde (37% wt. in H\textsubscript{2}O, 6.7 mL, 89 mmol, 1.0 eq.) was added slowly to \textit{tert}-butylamine at 0 °C (9.5 mL, 89 mmol, 1.0 eq.). 3-Nitropentane (10.4 g, 89.0 mmol, 1.0 eq.) was added and the reaction mixture was stirred for 18 h at rt. Na\textsubscript{2}SO\textsubscript{4} was added until a phase separation occurred. The aqueous layer was removed and the organic layer was stirred for additional 5 d at rt. The mixture was dried over Na\textsubscript{2}SO\textsubscript{4} and the crude product was purified by distillation (86 °C, 5.3 mbar). Amine 30 was obtained as a colorless liquid (14.1 g, 69.6 mmol, 78%).
1H-NMR (300 MHz, CDCl\textsubscript{3}): \(\delta = 2.93\ (s,\ 2\ H,\ CH_2NH)\), 1.97 \((q,\ J = 7.6\ Hz,\ 4\ H,\ 2\times CH_2)\), 1.05 \((s,\ 9\ H,\ C(CH_3)_3)\), 0.85 \((t,\ J = 7.5\ Hz,\ 6\ H,\ 2\times CH_3)\).

The spectroscopic data are in accordance with those reported in the literature.\cite{5}

\textbf{N-tert-Butyl-2-ethylbutan-1,2-diamine (31)}

\[
\text{N}
\begin{align*}
\text{H} & \quad \text{NH}_2 \\
\text{H} & \quad \text{H}
\end{align*}
\]

Amine 30 (14.0 g, 69.0 mmol, 1.0 eq.) was dissolved in a mixture of HOAc (100 mL) and H\textsubscript{2}O (150 mL). While cooling in an ice bath, zinc powder (27.1 g, 414 mmol, 6.0 eq.) was added and the reaction mixture was stirred at rt for 2 h. The excess of zinc was filtered off and solid NaOH was added to the filtrate until the solution turned basic. The mixture was extracted with Et\textsubscript{2}O (3 \times 200 mL) and the extracts were dried over MgSO\textsubscript{4}. The solvent was evaporated \textit{in vacuo} and the product was obtained as a colorless liquid (10.9 g, 63.3 mmol, 92%).

1H-NMR (300 MHz, CDCl\textsubscript{3}): \(\delta = 2.36\ (s,\ 2\ H,\ CH_2N)\), 1.40 - 1.29 \((m,\ 4\ H,\ 2\times CH_2)\), 1.07 \((s,\ 9\ H,\ C(CH_3)_3)\), 0.82 \((t,\ J = 7.6\ Hz,\ 6\ H,\ 2\times CH_3)\).

The spectroscopic data are in accordance with those reported in the literature.\cite{5}

\textbf{1-tert-Butyl-3,3,5,5-tetraethyl-2-piperazinone (32)}

KOH (18.5 g, 329 mmol, 5.3 eq.) powder was added slowly at 10 ℃ to a mixture of diamine 31 (10.7 g, 62.2 mmol, 1.00 eq.), 3-pentanone (99.0 mL, 933 mmol, 15.0 eq.) and CHCl\textsubscript{3} (8.0 mL, 0.1 mol, 1.6 eq.). After stirring for 18 h at rt, the reaction mixture filtered. The filtrate was evaporated to dryness and the crude product was purified by FC (pentane/MTBE 10:1). Piperazinone 32 was obtained as a pale yellow oil (10.1 g, 37.6 mol, 60%).
1H-NMR (300 MHz, CDCl3): δ = 3.15 (s, 2 H, CH2N), 1.58 (q, J = 7.4 Hz, 4 H, 2 × CH2),
1.43 - 1.34 (m, 4 H, 2 × CH2), 1.41 (s, 9 H, C(CH3)3), 0.86 (t, J = 7.4 Hz, 6 H, 2 × CH3), 0.83
(t, J = 7.4 Hz, 6 H, 2 × CH3).

The spectroscopic data are in accordance with those reported in the literature.[5]

1-tert-Butyl-3,3,5,5-tetraethylpiperazin-2-one-4-oxyl radical (15)

Piperazinone 32 (2.44 g, 9.08 mmol, 1.0 eq.) was dissolved in EtOAc
(20 mL). Peroxyacetic (39% wt. in HOAc, 2.31 mL, 13.6 mmol, 1.5 eq.)
acid was added at 0 °C. The reaction mixture was stirred for 16 h at rt.
Pentane (60 mL) was added and the mixture was washed with NaHCO3
(aq. sat., 3 × 40 mL). The organic layer was dried over MgSO4 and the solvent was
evaporated in vacuo. Purification was carried out by FC (pentane/EtOAc 10:1) and delivered
nitroxide 15 as red oil (2.47g, 8.71 mmol, 96%).


The spectrometric data are in accordance with those reported in the literature.[5]

4-[1-(4-Bromophenyl)-ethoxy]-1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one (33)

According to a procedure of Matyjaszewski et al., bromide 26
(2.02 g, 7.66 mmol, 1.0 eq.), nitroxide 15 (2.17 g, 7.66 mmol,
1.0 eq.), copper powder (511 mg, 8.04 mmol, 1.05 eq.),
Cu(OTf)2 (28.0 mg, 80.0 µmol, 0.01 eq.) and 4,4-di-tert-butyl-
2,2-dipyrindyl (80.0 mg, 310 µmol, 0.04 eq.) were suspended in benzene (15 mL) and the
mixture was warmed to 75 °C for 16 h. The crude mixture was filtered through a plug of silica
with CH2Cl2 as eluent. The solvent was evaporated in vacuo and the crude product was
purified by FC (pentane/MTBE 20:1). Alkoxyamine 33 was isolated as a colorless solid
(3.29 g, 7.04 mmol, 92%).
1H-NMR (300 MHz, CDCl₃): δ = 7.44 (d, J = 8.4 Hz, 2 H, 2 × Aryl-H), 7.16 (d, J = 8.1 Hz, 2 H, 2 × Aryl-H), 4.70 - 4.60 (m, 1 H, CH₃), 3.21 - 2.95 (m, 2 H, 2 × CH₂N), 2.16 - 1.50 (m, 7 H, 2 × CH₂, CHCH₃), 1.44 - 1.38 (m, 13 H, 2 × C₂H₂, C(CH₃)₃), 1.08 - 0.93 (m, 6 H, 2 × CH₃), 0.86 - 0.60 (m, 6 H, 2 × CH₃).

The spectrometric data are in accordance with those reported in the literature.⁶

**4-[1-(1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy)-ethyl]-benzoic acid (34)**

Alkoxyamine 33 (9.50 g, 20.3 mmol, 1.0 eq.) was dissolved in THF (90 mL) and t-BuLi (1.5 M in pentane, 27.1 mL, 40.6 mmol, 2.0 eq.) was added slowly at -78 °C. The reaction mixture was stirred at -78 °C for 20 min. CO₂, dried by passing through conc. H₂SO₄, was passed through the reaction mixture for 30 min at -78 °C. The mixture was warmed to rt and HCl (aq., 1 M) was added until the mixture turned acidic. The phases were separated and the aqueous layer was extracted with CH₂Cl₂ (2 × 40 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed in vacuo. Purification was carried out by crystallization in EtOAc at -18 °C and afforded the desired product as a colorless solid (7.71 g, 17.8 mmol, 88%).

Mp.: 157 °C. IR (neat): 2973 s, 2668 w, 1691 s, 1641 s, 1456 m, 1420 s, 1366 m, 1283 s, 1205 s, 1063 m, 994 w, 912 m, 858 m, 778 w, 731 s, 645 w. ¹H-NMR (300 MHz, CDCl₃): 8.07 (d, J = 8.3 Hz, 2 H, 2 × Aryl-H), 7.39 (d, J = 8.6 Hz, 2 H, 2 × Aryl-H), 4.82 - 4.73 (m, 1 H, CHCH₃), 3.23 - 2.96 (m, 2 H, CH₂N), 2.23 - 1.23 (m, 11 H, 4 × CH₂, CHCH₃), 1.39 (s, 9 H, C(CH₃)₃), 1.10 - 0.95 (m, 6 H, 2 × CH₃), 0.87 - 0.58 (m, 6 H, 2 × CH₃). ¹³C-NMR (75 MHz, CDCl₃): doubled set of resonance obtained, δ = 172.9 (C), 172.8 (C), 171.4 (C), 171.3 (C), 150.6 (C), 150.3 (C), 130.3 (4 × CH), 128.6 (C), 128.3 (C), 127.1 (2 × CH), 126.8 (2 × CH), 82.8 (CH), 82.6 (CH), 73.6 (C), 73.2 (C), 62.8 (C), 62.5 (C), 57.3 (2 × C), 47.1 (CH₂), 46.1 (CH₂), 34.9 (CH₂), 33.4 (CH₂), 29.4 (CH₂), 29.0 (CH₂), 28.3 (6 × CH₃), 26.9 (CH₂), 26.7 (CH₂), 24.7 (2 × CH₂), 23.3 (CH₃), 22.3 (CH₃), 11.8 (CH₃), 11.3 (CH₃), 9.7 (CH₃), 9.4 (CH₃), 9.2 (2 × CH₃), 8.4 (CH₃), 7.7 (CH₃). MS (ESI): 433 [M+H]⁺, 455 [M+Na]⁺, 888 [2M+Na]⁺, 1320 [3M+Na]⁺. HRMS (ESI): calculated for C₂₅H₁₀N₂O₄H⁺: 433.3061, found: 433.3065.

**Boc-Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly-OMe (16)**

Acid 34 (9.07 g, 21.0 mmol, 1.1 eq.), amine hydrochloride 7 (6.73 g, 19.1 mmol, 1.0 eq.), EDCI (4.38 g, 22.9 mmol, 1.2 eq.), HOBt (3.50 g, 22.9 mmol, 1.0 eq.) and NMM (4.63 mL, 41.9 mmol, 2.2 eq.) were dissolved in CH$_2$Cl$_2$ (100 mL) and the reaction mixture was stirred for 16 h at rt. After addition of HCl (aq., 0.5 M, 60 mL) the phases were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic layers were washed with NaOH (aq., 0.25 M, 40 mL) and NaCl (aq. sat., 50 mL), dried over MgSO$_4$ and the solvent was evaporated in vacuo.

Purification by FC (CH$_2$Cl$_2$/acetone 5:1) delivered the desired product as a colorless solid (8.87 g, 12.1 mmol, 63%).

Mp.: 88 °C. IR (neat): 3313 br m, 2973 s, 1642 s, 1538 s, 1455 m, 1366 s, 1305 m, 1205 s, 1170 s, 1062 m, 1018 w, 915 w, 855 m, 735 m, 625 w, 568 w. $^1$H-NMR (300 MHz, CDCl$_3$): 7.75 (d, J = 8.1 Hz, 2 H, 2 × Aryl-H), 7.32 (d, J = 8.0 Hz, 2 H, 2 × Aryl-H), 6.82 (br t, J = 5.3 Hz, 1 H, CHCONH), 6.44 (br s, 1 H, CH$_2$NHC), 5.27 - 5.24 (m, 1 H, CHCO), 4.78 - 4.69 (m, 1 H, CHCH$_3$), 4.19 - 4.10 (m, 1 H, NHCO$_2$), 4.02 (t, J = 5.2 Hz, 2 H, NHCH$_2$CO$_2$), 3.71 (s, 3 H, OCH$_3$), 3.51 - 3.44 (m, 2 H, CH$_2$CH$_2$N), 3.21 - 3.00 (m, 2 H, CH$_2$N), 1.98 - 1.56 (m, 11 H, CH$_2$CH$_2$N, CH$_2$CH, CHCH$_2$CH$_2$, CH$_2$CH$_3$, CHCH$_3$), 1.50 - 1.42 (m, 15 H, 3 × CH$_2$CH$_3$, OC(CH$_3$)$_3$), 1.37 (s, 9 H, NC(CH$_3$)$_3$), 1.09 - 0.94 (m, 6 H, 2 × CH$_3$), 0.85 - 0.60 (m, 6 H, 2 × CH$_3$). $^{13}$C-NMR (75 MHz, CDCl$_3$): 172.7 (C), 170.2 (C), 167.7 (C), 156.0 (C), 148.0 (C), 133.6 (C), 127.1 (2 × CH), 126.7 (2 × CH), 110.1 (C), 82.7 (CH), 80.3 (C), 73.7 (C), 62.8 (C), 57.3 (C), 54.4 (CH), 52.4 (CH$_3$), 47.1 (CH$_2$), 46.2 (CH$_2$), 41.3 (CH$_2$), 39.4 (CH$_2$), 34.8 (CH$_2$), 33.6 (CH$_2$), 31.8 (CH$_2$), 29.2 (CH$_2$), 28.4 (CH$_3$), 28.3 (CH$_3$), 27.0 (CH$_2$), 24.7 (CH$_2$), 23.3 (CH$_3$), 22.6 (CH$_3$), 11.8 (CH$_3$), 11.3 (CH$_3$), 9.7 (CH$_3$), 9.4 (CH$_3$), 9.2 (CH$_3$), 8.4 (CH$_3$), 7.8 (CH$_3$). MS (ESI): 754 [M+Na]$^+$, 1486 [2M+Na]$^{2+}$. HRMS (ESI): calculated for

**Boc-Lys(4-[^1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy]-ethyl]-benzamide)-Gly-OH (35)**

NaOH (aq., 0.25 m, 50 mL) was added to a solution of dipeptide 16 (3.55 g, 4.85 mmol, 1.0 eq.) in MeOH (80 mL). The reaction mixture was stirred for 18 h at rt. HCl (aq., 1 M, 30 mL) was added and the mixture was extracted with CH₂Cl₂ (4 × 50 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed in vacuo. The product was obtained as a colorless solid (3.39 g, 4.72 mmol, 97%).

Mp.: 97 °C. IR (neat): 3320 br w, 2974 m, 2245 w, 1635 s, 1540 s, 1455 m, 1366 m, 1308 m, 1203 m, 1167 s, 1061 w, 1018 w, 910 s, 854 w, 729 s, 646 m. ¹H-NMR (300 MHz, CDCl₃): δ = 7.77 (d, J = 8.5 Hz, 2 H, 2 × Aryl-H), 7.39 - 7.35 (m, 2 H, 2 × Aryl-H), 4.80 - 4.72 (m, 1 H, CHCH₃), 4.05 - 4.01 (m, 1 H, CHCO), 3.95 - 3.79 (m, 2 H, NHCH₂CO), 3.35 (t, J = 6.7 Hz, 2 H, CH₂CH₂N), 3.18 - 3.02 (m, 2 H, CH₂N), 2.15 - 1.53 (m, 11 H, CH₂CH, CH₂CH₂N, CHCH₂CH₂, CH₂CH₃, CHCH₃), 1.46 - 1.35 (m, 24 H, 3 × CH₂CH₃, OC(CH₃)₃, NC(CH₃)₃), 1.09 - 0.95 (m, 6 H, 2 × CH₃), 0.82 - 0.59 (m, 6 H, 2 × CH₃). ¹³C-NMR (300 MHz, CDCl₃): 173.2 (C), 172.9 (C), 171.9 (C), 168.1 (C), 156.2 (C), 147.8 (C), 133.4 (C), 127.1 (2 × CH), 126.7 (2 × CH), 82.6 (CH), 80.2 (C), 73.5 (C), 62.5 (C), 57.3 (C), 54.3 (CH), 47.0 (CH₂), 46.0 (CH₂), 41.3 (CH₂), 39.6 (CH₂), 35.4 (CH₂), 33.6 (CH₂), 32.2 (CH₂), 28.4 (CH₃), 28.3 (CH₃), 26.8 (CH₂), 24.6 (CH₂), 23.3 (CH₃), 22.6 (CH₂), 22.2 (CH₃), 11.8 (CH₃), 11.4 (CH₃), 9.7 (CH₃), 9.4 (CH₃), 9.2 (CH₃), 8.3 (CH₃), 7.7 (CH₃). MS (ESI): 718 [M+H]⁺, 740 [M+Na]⁺, 1434 [2M+H]⁺. HRMS (ESI): calculated for: C₃₈H₆₃N₅O₈Na⁺: 740.4569, found: 740.4554.
**HCl×H-Lys(4-[1-{1-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-
benzamide)-Gly-OMe (36)**

Dipeptide 16 (2.5 g, 3.4 mmol, 1.0 eq.) was added to a methanolic solution of HCl (1.25 M in MeOH, 12 mL, 15 mmol, 4.4 eq.) and the mixture was stirred for 16 h at rt. The solvent was removed *in vacuo* and amine hydrochloride was obtained as a colorless solid (2.28 g, 3.40 mmol, 100%).

Mp.: 92 °C. IR (neat): 3329 br s, 2973 s, 2363 w, 1747 m, 1646 s, 1547 m, 1458 s, 1366 m, 1309 m, 1210 s, 1063 w, 994 w, 855 w, 770 w.

$^1$H-NMR (300 MHz, CD$_3$OD): $\delta =$ 7.77 ($d$, $J = 8.2$ Hz, 2 H, 2 × Aryl-H), 7.39 - 7.34 ($m$, 2 H, 2 × Aryl-H), 4.81 - 4.70 ($m$, 1 H, CHCH$_3$), 4.05 - 3.85 ($m$, 3 H, CHCO, NHCH$_2$CO), 3.65 ($s$, 3 H, OCH$_3$), 3.37 ($t$, 2 H, $J = 7.1$ Hz, CH$_2$CH$_2$N), 3.16 - 3.00 ($m$, 2 H, CH$_2$N), 2.11 - 1.34 ($m$, 26 H, CH$_2$CH, CH$_2$CH$_2$N, CHCH$_2$CH$_2$, 4 × CH$_2$CH$_3$, CHCH$_3$, C(CH$_3$)$_3$), 1.08 - 0.94 ($m$, 6 H, 2 × CH$_3$), 0.80 - 0.73 ($m$, 3 H, CH$_3$), 0.65 - 0.57 ($m$, 3 H, CH$_3$). $^{13}$C-NMR (75 MHz, CD$_3$OD): $\delta =$ 174.6 (C), 172.5 (C), 171.4 (C), 170.0 (C), 149.1 (C), 135.0 (C), 128.3 (2 × CH), 127.9 (2 × CH), 84.0 (CH), 74.7 (C), 63.6 (C), 58.7 (C), 54.3 (CH), 52.8 (CH$_3$), 41.8 (CH$_2$), 40.4 (CH$_2$), 36.2 (CH$_2$), 34.9 (CH$_2$), 32.2 (CH$_2$), 30.3 (CH$_2$), 30.1 (CH$_2$), 28.4 (CH$_3$), 27.7 (CH$_2$), 25.8 (CH$_2$), 23.5 (CH$_3$), 23.0 (CH$_2$), 22.3 (CH$_3$), 12.2 (CH$_3$), 11.8 (CH$_3$), 9.9 (CH$_3$), 8.6 (CH$_3$), 8.0 (CH$_3$). MS (ESI): 632 [M+H]$^+$, 654 [M+Na]$^+$. HRMS (ESI): calculated for C$_{34}$H$_{57}$N$_5$O$_6$H$: 632.4381, found: 632.4382.
Boc-bis[Lys(4-[1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy]-ethyl]-benzamide)-Gly]-OMe (17)

Acid 35 (2.26 g, 3.14 mmol, 1.0 eq.) and amine hydrochloride 36 (2.10 g, 3.14 mmol, 1.0 eq.) were dissolved in CH₂Cl₂ (40 mL). EDCI (722 mg, 3.77 mmol, 1.2 eq.), HOBt (577 mg, 3.77 mmol, 1.2 eq.) and NMM (0.76 mL, 6.91 mmol, 2.2 eq.) were added and the reaction mixture was stirred for 16 h at rt. HCl (aq., 1 M, 20 mL) was added and the phases were separated. The aqueous layer was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic layers were washed with NaOH (aq., 0.25 M, 30 mL) and NaCl (aq. sat., 30 mL), dried over MgSO₄ and the solvent was evaporated in vacuo. Purification was carried out by FC (CH₂Cl₂/acetone 3:1 → 1:1) and afforded the desired product as a colorless solid (3.07 g, 2.31 mmol, 73%).

Mp.: 131 °C. IR (neat): 3294 br m, 2972 m, 1755 w, 1633 s, 1538 s, 1455 m, 1365 m, 1305 m, 1206 s, 1174 s, 1062 m, 993 w, 915 w, 853 m, 735 s, 701 m, 570 w. ¹H-NMR (300 MHz, CDCl₃): δ = 7.78 (d, J = 8.1 Hz, 4 H, 4 × Aryl-H), 7.48 (br s, 1 H, CH₂CON), 7.41 (br s, 1 H, NHCH₂CO₂), 7.32 - 7.30 (m, 5 H, 4 × Aryl-H, NHCH₂CO), 6.89 (br s, 2 H, 2 × CH₂NH), 5.77 (br s, 1 H, CO₂NH), 4.77 - 4.68 (m, 2 H, 2 × CH₃), 4.55 - 4.48 (m, 1 H, CH₂CONHC), 4.14 - 4.08 (m, 1 H, CO₂NH), 4.03 - 3.84 (m, 4 H, 2 × NHCH₂CO), 3.66 (s, 3 H, OCH₃), 3.48 - 3.34 (m, 4 H, 2 × CH₂CH₂NH), 3.21 - 2.95 (m, 4 H, 2 × CH₂N), 2.16 - 1.37 (m, 61 H, 2 × CH₂CH₂N, 2 × CH₂CH, 2 × CHCH₂CH₂, 8 × CH₂CH₃, 2 × CHCH₃, OC(CH₃)₃, 2 × NC(CH₃)₃), 1.08 - 0.94 (m, 12 H, 4 × CH₃), 0.83 - 0.59 (m, 12 H, 4 × CH₃). ¹³C-NMR (75 MHz, CDCl₃): δ = 173.7 (C), 172.7 (2 × C), 172.3 (C), 170.3 (C), 169.5 (C), 167.9 (2 × C), 156.4 (C), 147.8 (2 × C), 133.7 (2 × C), 127.2 (4 × CH), 127.0 (2 × CH), 126.7 (2 × CH), 82.7 (2 × CH), 80.4 (C), 73.8 (C), 73.4 (C), 62.9 (C), 62.7 (C), 57.3 (2 × C), 55.2 (CH), 53.3 (CH), 52.3 (CH₃), 47.1 (CH₂), 46.1 (CH₂), 43.5 (CH₂), 41.3 (CH₂), 39.6 (2 × CH₂), 34.8 (2 × CH₂), 33.6 (CH₂), 31.6 (CH₂), 31.4 (CH₂), 29.3 (2 × CH₂), 29.2 (2 × CH₂), 28.5 (3 × CH₃), 28.3 (6 × CH₃), 27.0 (CH₂), 26.7 (CH₂), 24.7 (2 × CH₂), 23.4 (2 × CH₃), 22.3
Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Boc-bis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl)-benzamide)-Gly]-OH (37)

NaOH (aq., 0.25 m, 15 mL) was added to a solution of tetrapeptide 17 (450 mg, 0.34 mmol, 1.0 eq.) in MeOH (25 mL). The reaction mixture was stirred for 16 h at rt. HCl (aq., 1 M, 10 mL) was added and the mixture was extracted with CH₂Cl₂ (4 × 30 mL). The combined organic phases were dried over MgSO₄ and the solvent was removed in vacuo. Acid 37 was obtained as a colorless solid (436 mg, 330 μmol, 97%).

Mp.: 146 °C. IR (neat): 3444 br w, 2973 m, 2481 br w, 1638 s, 1570 w, 1456 s, 1366 m, 1246 w, 1205 m, 1170 m, 1065 w, 1016 w, 855 w, 769 w, 707 w, 470 m. ¹H-NMR (400 MHz, CD₃OD): δ = 7.82 (d, J = 7.7 Hz, 4 H, 4 × Aryl-H), 7.44 - 7.39 (m, 4 H, 4 × Aryl-H), 4.87 - 4.77 (m, 2 H, 2 × CH(CH₃)), 4.43 (dd, J₁ = 5.0 Hz, J₂ = 8.7 Hz, 1 H, NHCHCO), 4.05 - 3.99 (m, 1 H, NHCHCO), 3.94 - 3.83 (m, 4 H, 2 × NHCH₂CO), 3.40 (t, J = 5.9 Hz, 4 H, 2 × CH₂CH₂N), 3.30 - 3.07 (m, 4 H, 2 × CH₂NH), 2.17 - 1.29 (m, 61 H, 2 × CH₂CH₂N, 2 × CH₂CH, 2 × CHCH₂CH₂, 8 × CH₂CH₃, 2 × CHCH₃, 2 × NC(CH₃)₃), 1.14 - 1.01 (m, 12 H, 4 × CH₃), 0.89 - 0.80 (m, 6 H, 2 × CH₃), 0.73 - 0.63 (m, 6 H, 2 × CH₃).

¹³C-NMR (100 MHz, CD₃OD): δ = 175.9 (C), 174.7 (C), 174.6 (C), 174.4 (C), 172.6 (C), 171.5 (C), 169.9 (2 × C), 158.0 (C), 149.0 (C), 148.8 (C), 147.6 (C), 135.0 (C), 134.9 (C), 129.5 (CH), 128.6 (CH), 128.5 (CH), 128.3 (2 × CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 83.9 (CH), 83.7 (CH), 74.6 (C), 74.1 (C), 63.8 (C), 63.5 (C), 58.6 (C), 56.3 (CH), 54.5 (CH), 47.3 (CH₂), 43.7 (CH₂), 41.8 (CH₂), 40.7 (CH₂), 40.5 (CH₂), 36.0 (CH₂), 34.7 (CH₂), 32.5 (CH₂), 30.4 (CH₂), 30.2 (CH₂), 30.1 (2 × CH₂), 29.9 (CH₂), 28.7 (3 × CH₃), 28.4 (6 × CH₃), 27.8 (2 × CH₂), 27.6 (CH₂), 27.0 (CH₃), 25.7 (2 × CH₂), 24.1 (2 × CH₂), 23.5 (CH₃), 22.4
HCl·H-Bis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]benzamide)-Gly]-OMe (38)

Tetrapeptide 17 (1.00 g, 0.75 mmol, 1.0 eq.) was added to a methanolic solution of HCl (1.25 M in MeOH, 3.00 mL, 3.75 mmol, 5.0 eq.) and the resulting mixture was stirred for 16 h at rt. The solvent was evaporated in vacuo and amine hydrochloride 38 was obtained as a colorless solid (0.95 g, 0.75 mmol, 100%).

Mp.: 110 °C. IR (neat): 3313 br w, 2970 m, 2938 m, 2363 w, 1746 w, 1652 s, 1544 s, 1459 s, 1367 m, 1309 m, 1225 m, 1122 w, 952 w, 855 w, 762 w, 671 w. 1H-NMR (300 MHz, CD3OD): δ = 7.85 - 7.80 (m, 4 H, 4 × Aryl-H), 7.45 - 7.41 (m, 4 H, 4 × Aryl-H), 4.84 - 4.77 (m, 2 H, 2 × CH3CO), 4.43 - 4.77 (m, 2 H, 2 × CHCH2, 4.43 (dd, J1 = 5.3 Hz, J2 = 8.6 Hz, 1 H, CHCO), 4.12 - 3.84 (m, 5 H, CHCO, 2 × NHCH2CO), 3.72 (s, 3 H, OCH3), 3.47 - 3.39 (m, 4 H, 2 × CH2CH2N), 3.24 - 3.07 (m, 4 H, 2 × CH2N), 2.19 - 1.32 (m, 52 H, 2 × CH2CH2N, 2 × CH2CH, 2 × CHCH2CH2, 8 × CH2CH3, 2 × CHCH3, 2 × NC(CH3)3), 1.15 - 1.01 (m, 12 H, 4 × CH3), 0.88 - 0.80 (m, 6 H, 2 × CH3), 0.73 - 0.64 (m, 6 H, 2 × CH3). 13C-NMR (100 MHz, CD3OD): δ = 174.7 (C), 174.6 (C), 171.5 (C), 171.0 (C), 170.9 (C), 169.9 (C), 149.2 (C), 149.1 (2 × C), 149.0 (C), 135.0 (C), 134.9 (C), 134.7 (C), 134.6 (C), 128.3 (4 × CH), 127.9 (4 × CH), 84.0 (2 × CH), 74.7 (C), 74.3 (C), 63.9 (CH), 58.7 (2 × C), 54.5 (CH), 52.7 (CH3), 48.3 (CH2), 47.5 (CH2), 43.4 (CH2), 41.8 (2 × CH2), 40.7 (CH2), 40.4 (CH2), 36.0 (CH2), 32.7 (CH2), 32.1 (CH2), 30.2 (2 × CH2), 30.0 (2 × CH2), 28.4 (6 × CH3), 27.9 (CH2), 27.7 (CH2), 25.9 (CH2), 25.8 (CH2), 24.1 (CH2), 23.5 (CH3), 23.3 (CH2), 22.4 (CH3), 12.2 (CH3), 11.8 (CH3), 9.9 (CH3), 9.8 (CH3), 9.7 (2 × CH3), 8.6 (CH3), 8.1 (2 × CH3). MS (ESI): 627 [M+Na+H]2+, 1232 [M+H]+, 1254 [M+Na]+. HRMS (ESI): calculated for C67H110N10O11Na+: 1253.8248, found: 1253.8250.
Boc-tetrakis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperezin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (18)

Acid 37 (200 mg, 150 µmol, 1.0 eq.) and amine hydrochloride 38 (192 mg, 150 µmol, 1.0 eq.) were dissolved in DMF (2.5 mL). EDCI (44.0 mg, 230 µmol, 1.5 eq.), HOBt (28.0 mg, 180 µmol, 1.2 eq.) and NMM (42.0 µL, 400 µmol, 2.5 eq.) were added and the reaction mixture was stirred for 16 h at rt. The solvent was removed under reduced pressure and the crude product was purified by RP-FC (H₂O → H₂O/MeOH 1:1 → H₂O/MeOH 1:4 → H₂O/MeOH 1:9 → MeOH). Octapeptide 18 was obtained as a colorless solid (256 mg, 10.1 µmol, 67%).

Mp.: Decomp. above 230 °C. IR (neat): 3283 br w, 2970 w, 2928 w, 1624 s, 1538 s, 1455 m, 1365 m, 1305 m, 1207 m, 1175 m, 1152 m, 1062 m, 1175 m, 1152 m, 1062 m, 933 w, 940 w, 853 m, 768 m, 685 m. MS (ESI): 1288 [M+2Na]²⁺, 2553 [M+Na]⁺. HRMS (ESI): calculated for C₁₃₈H₂₂₄N₂₀O₂₃Na₂²⁺: 1287.8379, found: 1287.8414. Due to low solubility of 18 in common solvents we did not obtain NMR-Spectra.
Boc-tetrakis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OH (39)

Octapeptide 18 (127 mg, 50.0 \( \mu \)mol, 1.0 eq.) was dissolved in MeOH (300 mL) under ultrasonification. NaOH (aq., 1 M, 20 mL) was added and the reaction mixture was stirred for 40 h at rt. Formic acid (2.5 mL) was added and the solvent removed under reduced pressure. The crude product was purified by RP-FC (H\(_2\)O (200 mL) → MeOH). Acid 39 was isolated as a colorless solid (118 mg, 46.9 \( \mu \)mol, 94%).

Mp.: Decomp. above 230 °C. IR (neat): 3296 br w, 2971 m, 2930 m, 2880 m, 1639 s, 1539 s, 1456 m, 1365 m, 1307 m, 1244 m, 1206 m, 1150 m, 1062 m, 1018 m, 993 m, 938 w, 915 w, 854 m, 767 m, 696 m. MS (ESI): 1281 [M+2Na]\(^{2+}\). HRMS (ESI): calculated for C\(_{137}\)H\(_{222}\)N\(_2\)O\(_{23}\)Na\(_2\): 1280.8301, found: 1280.8309. Due to low solubility of 39 in common solvents we did not obtain NMR-Spectra.

HCl×H-Tetrakis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (40)

Octapeptide 18 (127 mg, 50.0 \( \mu \)mol, 1.0 eq.) was suspended in MeOH (5 mL) and a methanolic solution of HCl (1.25 M in MeOH, 500 \( \mu \)L, 630 \( \mu \)mol, 12.5 eq.) was added. The reaction mixture was stirred for 48 h at rt and the volatile components were removed in vacuo. Amine hydrochloride 40 was obtained as a colorless solid (123 mg, 50.0 \( \mu \)mol, 100%).

Mp.: Decomp. above 195 °C. IR (neat): 3286 br w, 2971 m, 2937 m, 2879 w, 1639 s, 1538 s, 1503 s, 1456 m, 1413 m, 1364 m, 1306 s, 1281 m, 1205 s, 1150 m, 1063 m, 1017 w, 994 w, 940 w,
915 m, 854 m, 768 w. MS (ESI): 1227 [M+Na+H]^{2+}, 1238 [M+2Na]^{2+}, 2453 [M+Na]. HRMS (ESI): calculated for C_{133}H_{216}N_{20}O_{23}HNa^{2+}: 1226.8207, found: 1226.8204. Due to low solubility of 40 in common solvents we did not obtain NMR-Spectra.

**Boc-octakis[Lys(4-{1-tert-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (19)**

Acid 39 (112 mg, 44.5 µmol, 1.0 eq.) and amine hydrochloride 40 (110 mg, 44.5 µmol, 1.0 eq.) were dissolved in N-methylpyrrolidinone (2.5 mL). HATU (25.0 mg, 65.7 µmol, 1.5 eq.), HOAt (9.00 mg, 67.0 µmol, 1.5 eq.) and DIPEA (39.0 µL, 220 µmol, 5.0 eq.) were added and the reaction mixture was stirred for 16 h at rt. Acetone (10 mL) and MeOH (10 mL) were added and the product was crystallized at 4 °C. The crystallization process was repeated once and the desired product was obtained as a colorless solid (121 mg, 24.4 µmol, 55%).

Mp.: Decomp. above 210 °C. IR (neat): 3292 m, 2969 w, 2937 w, 2881 w, 1700 w, 1626 s, 1517 s, 1437 m, 1411 m, 1363 w, 1302 m, 1262 m, 1208 m, 1147 m, 1063 s, 1014 s, 913 m, 852 m, 804 m. HRMS (ESI): calculated for C_{270}H_{436}N_{40}O_{43}: 4927.31600. found: 4927.32243, calculated from: 1233.58752 [M+4H]^{4+}, 1644.44763 [M+3H]^{3+}. 

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Polymerization procedures

**General procedure for the polymerization of styrene with initiators 1, 9, 11, 13, 14**

In a *Schlenk*-tube the peptide initiator (0.125 mol% to 1.0 mol%) was suspended in styrene (250 μL, 2.19 mmol, 1.0 eq.). The suspension was degassed by three freeze-thaw cycles under cooling in liquid nitrogen. The reaction mixture was heated in an oil bath at 125 °C for the desired time. The tube was cooled to rt, the polymer was dissolved in CH$_2$Cl$_2$ and transferred to an open flask. Unreacted monomer was removed in a vacuum cabinet for at least 18 h at 60 °C. Conversion was determined gravimetrically. Theoretical molecular weight ($M_{n,\text{theo}}$) was calculated from yield. Experimental molecular weight ($M_{n,\text{exp}}$) and polydispersity index (PDI) were determined by GPC.

**General procedure for the polymerization of NIPAM with initiators 16, 17, 18, 19**

The peptide initiator (0.5 mol% or 1.0 mol%) was suspended in benzene (1 mL) and NIPAM (201 mg, 1.78 mmol, 1.0 eq.) was added. Argon was passed through this suspension via a syringe for 1 min. The tube was sealed tightly and heated at 125 °C in an oil bath for the desired time. After cooling to rt, the mixture was dissolved in a small amount of acetone and the polymer was precipitated by the addition of diethylether. Conversion was determined gravimetrically. Theoretical molecular weight ($M_{n,\text{theo}}$) was calculated from yield. Experimental molecular weight ($M_{n,\text{exp}}$) and polydispersity index (PDI) were determined by GPC.
$^{1}$H-NMR and $^{13}$C-NMR spectra of new compounds

$^{1}$H-NMR spectrum of 28

$^{13}$C-NMR spectrum of 28

NMR spectrum of 28
$^1$H-NMR spectrum of 7

![H-NMR spectrum](image)

$^{13}$C-NMR spectrum of 7

![C-NMR spectrum](image)
$^1$H-NMR spectrum of 1

$^{13}$C-NMR spectrum of 1

$^1$H-NMR spectrum of 8b
$^{13}$C-NMR spectrum of 8a

$^1$H-NMR spectrum of 9
13C-NMR spectrum of 9

1H-NMR spectrum of 10b
$^{13}$C-NMR spectrum of 10b

$^{1}$H-NMR spectrum of 10a
$^{13}$C-NMR spectrum of 10a

$^1$H-NMR spectrum of 34
13C-NMR spectrum of 34

1H-NMR spectrum of 16
$^{13}$C-NMR spectrum of 16

$^1$H-NMR spectrum of 35
13C-NMR spectrum of 36

1H-NMR spectrum of 17
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\[ \begin{array}{cccccc}
13C-NMR spectrum of 17 \\
\end{array} \]

\[ \begin{array}{cccccc}
1H-NMR spectrum of 37 \\
\end{array} \]
$^1$H-NMR spectrum of 38

$^{13}$C-NMR spectrum of 37
13C-NMR spectrum of 38
Polymerization results

Polymerization of styrene, $T = 125 \, ^\circ\text{C}$, neat, sealed tube.
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<th>Time (h)</th>
<th>Conv. (%)</th>
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Polymerization of NIPAM, at 125 °C, 1.78 M in benzene, sealed tube.
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Proof of livingness of polymerizations

The controlled character of styrene polymerizations mediated by initiator 9

Initiator 9 was used in the general polymerization procedure for styrene with a concentration of 0.5 mol% for 3, 6, 12, 18 and 24 h respectively. Conversion was determined as a function of time and the molecular weight and analyzed as a function of monomer conversion. Both plots show a typical linear behavior as expected for a controlled process.

Figure 1: Monomer conversion vs. time (styrene, initiator 9, 0.5 mol%, 125 °C).
Figure 2: $M_n,\text{exp.}$ vs. monomer conversion (styrene, initiator 9, 0.5 mol%, 125 °C).

Proof of control/livingness of NIPAM polymerizations using initiator 17

NIPAM polymerization was carried out in the described manner with initiator 17 with a concentration of 0.5 mol% with respect to NIPAM at 125 °C for 3, 6, 9, 12 and 24 h. Conversion was determined as a function of time and the molecular weight and analyzed as a function of monomer conversion. Both plots show a typical linear behavior as expected for a controlled process.
**Figure 3:** Monomer conversion vs. time (NIPAM, initiator 17, 0.5 mol%, 125 °C).

**Figure 4:** Molecular weight vs. conversion (NIPAM, initiator 17, 0.5 mol%, 125 °C).
DLS-data of peptide-PNIPAM conjugates

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All samples were measured with a concentration of 1 mg/mL of the conjugate in water. Between the measurements, samples were equilibrated for 5 minutes at the determined temperature.

References