Supplementary Material (ESI) for Organic & Biomolecular Chemistry

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

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Supplementary Data

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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. CCl_4 *(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, CH_2Cl_2 was distilled from P_2O_5, DMF was distilled from K_2CO_3 and ninhydrin, *N*-methylpyrrolidinone was distilled from CaH_2 and ninhydrin. Styrene was distilled from CaH_2 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 F_{254} plates from *Merck*. Detection of the compounds was carried out by UV-light or dipping into solutions of $KMnO_4$ (1.5 g in 400 mL H_2O, 5 g $NaHCO_3$) or $Ce(SO_4)_2{\cdot}H_2O$ (10 g in 0.94 L H_2O, 60 mL *conc.* H_2SO_4, 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 $F_{254}S$ 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.

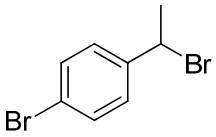
IR-spectra were recorded on a *Digilab Excalibur FTS 4000* device equipped with a *MKII Golden Gate Single Reflesction ATR System*. ^{1}H-NMR (300 and 400 MHz) and ^{13}C-NMR (75 and 100 MHz) spectra were recorded on a *Bruker DPX-300*, a *Bruker ARX-300* or a *Bruker AMX-400* spectrometer at room temperature (rt). Chemical shifts in ppm are referenced to the solvent residue peaks ($CHCl_3$ at 7.26 ppm for ^{1}H-NMR and at 77.00 ppm for ^{13}C-NMR;

CH_3OH 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 Punp 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-bromethyl)-benzene (26)

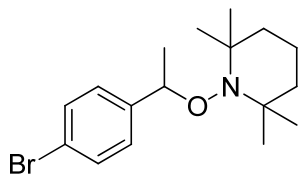


1-Bromo-4-ethyl-benzene (14.0 mL, 101 mmol, 1.0 eq.) was dissolved in CCl_4 (100 mL). The solution was warmed to 50 °C and illuminated by a light bulp (*Osram*, 500 W). Bromine (5.20 mL, 101 mmol, 1.0 eq.) was added over a period of 15 min. The reaction mixture was stirred at 50 °C for 1 h under illumination. Reaction mixture was washed with $NaHCO_3$ (*aq. sat.*, 3 × 20 mL) and NaCl (*aq. sat.*, 2 × 20 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The desired product was obtained as a pale yellow liquid (26.1 g, 98.9 mmol, 98%).

^{1}H-NMR (300 MHz, $CDCl_3$): 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, C*H*Br), 1.94 (*d*, *J* = 6.9 Hz, 3 H, CH_3).

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

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

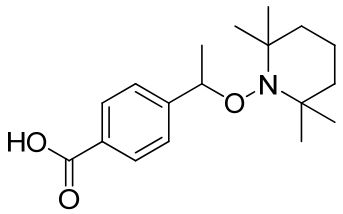


According to a procedure of *Matyjaszewski et al.*, bromide **26** (25.9 g, 98.3 mmol, 1.0 eq.), TEMPO (18.4 g, 118 mmol, 1.20 eq.), copper powder (6.56 g, 103 mmol, 1.05 eq.), $Cu(OTf)_2$ (0.36 g, 0.98 mmol, 0.01 eq.) and 4,4-di-*tert*-butyl-2,2-dipyridyl (1.05 g, 3.93 mmol, 0.04 eq.) were added to benzene (190 mL) and warmed to 75 °C for 16 h.[2] The reaction mixture was filtered through a plug of silica gel with CH_2Cl_2 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%).

^{1}H-NMR (300 MHz, $CDCl_3$): 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, C*H*O), 1.59 - 1.37 (*m*, 9 H, 3 × CH_2, CH_3CH), 1.27 (*s*, 3 H, CH_3), 1.15 (*s*, 3 H, CH_3), 1.02 (*s*, 3 H, CH_3), 0.65 (*s*, 3 H, CH_3).

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)

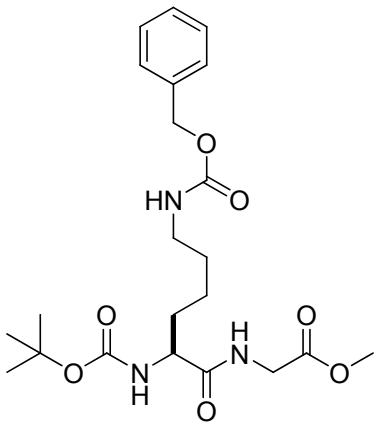


Alkoxyamine **27** (14.2 g, 41.8 mmol, 1.0 eq.) was dissolved in THF (170 mL) and *t*-BuLi (1.5 M in pentane, 55.7 mL, 83.6 mmol, 2.0 eq.) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 20 min and CO_2, dried by passing through conc. H_2SO_4, was passed through the reaction 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 CH_2Cl_2 (2 × 60 mL). The combined organic layers were dried over $MgSO_4$ 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%).

^{1}H-NMR (300 MHz, CD_3OD): 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, C*H*O), 2.12 - 1.81 (*m*, 6 H, 3 × CH_2), 1.77 (*d*, *J* = 6.4 Hz, 3 H, CHCH_3), 1.74 (*s*, 3 H, CH_3), 1.55 (*s*, 3 H, CH_3), 1.36 (*s*, 3 H, CH_3), 1.03 (*s*, 3 H, CH_3).

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

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

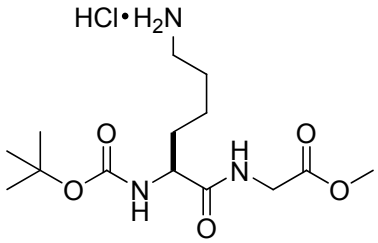


Boc-Lys(Cbz)-Gly-OH (9.51 g, 25.0 mmol, 1.0 eq.) and glycinmethylester hydrochloride (3.46 g, 27.5 mmol, 1.1 eq.) were dissolved in CH_2Cl_2 (120 mL). EDCI (5.75 g, 30.0 mmol, 1.2 eq.), HOBt (4.60 g, 30.0 mmol, 1.2 eq.) and NMM (6.40 mL, 57.5 mmol, 2.3 eq.) were added and the mixture was stirred at rt for 5 h. HCl (*aq*., 1 M, 20 mL) was added; the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (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*s*, 1454*m*, 1367*m*, 1245*s*, 1211*s*, 1165*s*, 1020*m*, 913*w*, 857*w*, 731*m*, 698*m*, 645*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.35 - 7.29 (*m*, 5 H, 5 × Aryl-*H*), 6.78 (*br s*, 1 H, CHCON*H*CH_2), 5.24 - 5.22 (*m*, 1 H, C*H*CO), 5.08 (*s*, 2 H, CH_2O), 5.05 - 5.00 (*m*, 1 H, CH_2N*H*CO_2), 4.13 (*br s*, 1 H, N*H*CO_2), 4.09 - 3.92 (*m*, 2 H, NHCH_2CO_2), 3.70 (*s*, 3 H, OCH_3), 3.22 - 3.15 (*m*, 2 H, CH_2N), 1.90 - 1.78 (*m*, 1 H, C*H*HCH), 1.70 - 1.61 (*m*, 1 H, C*H*HCH), 1.56 - 1.47 (*m*, 2 H, CH_2CH_2N), 1.45 - 1.36 (*m*, 11 H, CH_2CH_2CH, 3 × CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 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 (CH_2), 54.3 (CH), 52.4 (CH_3), 41.2 (CH_2), 40.5 (CH_2), 32.0 (CH_2), 29.6 (CH_2), 28.4 (3 × CH_3), 22.5 (CH_2). MS (ESI): 452 $[M+H]^+$, 474 $[M+Na]^+$, 925 $[2M+H]^+$. HRMS (ESI): calculated for $C_{22}H_{33}N_3O_7Na^+$: 474.2211, found: 474.2208. Anal. calculated for $C_{22}H_{33}N_3O_7$: C: 58.52, H: 7.37, N: 9.31, found: C: 58.54, H: 7.38, N: 9.21.

Boc-Lys(H×HCl)-Gly-OMe (7)

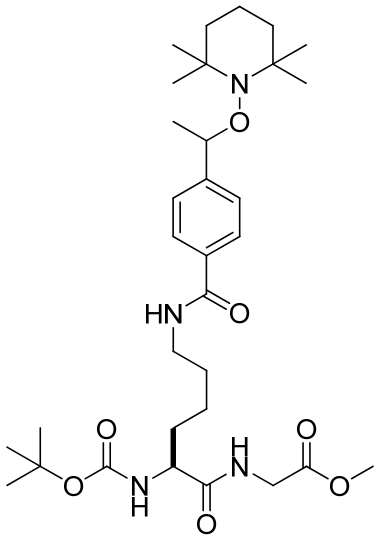


Dipeptide **28** (10.5 g, 23.2 mmol, 1.0 eq.) was dissolved in MeOH (200 mL). Pd/C (10% Pd, 1.24 g, 1.16 mmol, 0.05 eq.) and HCl (1.25 M in MeOH, 3.7 mL, 4.7 mmol, 0.2 eq.) were added and the flask was purged with hydrogen gas. The mixture was stirred for 16 h at rt under atmospheric pressure (balloon). 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*. ^{1}H-NMR (300 MHz, CD_3OD): δ = 4.11 - 4.06 (*m*, 1 H, C*H*CO), 4.06 - 3.89 (*m*, 2 H, CH_2NH_2), 3.73 (*s*, 3 H, OCH_3), 2.96 - 2.91 (*m*, 2 H, CH_2CO_2), 1.90 - 1.79 (*m*, 1 H, CHCH*H*), 1.74 - 1.62 (*m*, 3 H, CHCH*H*, CH_2CH_2N), 1.56 - 1.46 (*m*, 11 H, CH_2CH_2CH, 3 × CH_3). ^{13}C-NMR (75 MHz, CD_3OD): δ = 175.5 (C), 171.7 (C), 157.8 (C), 80.7 (C), 55.7 (CH), 52.6 (CH_3), 41.8 (CH_2), 40.6 (CH_2), 32.7 (CH_2), 28.7 (3 × CH_3),

28.3 (CH_2), 23.6 (CH_2). MS (ESI): 318 $[M+H]^+$, 340 $[M+Na]^+$, 635 $[2M+H]^+$, 657 $[2M+Na]^+$. HRMS (ESI): calculated for $C_{14}H_{27}N_3O_5H^+$: 318.2023, found: 318.2018.

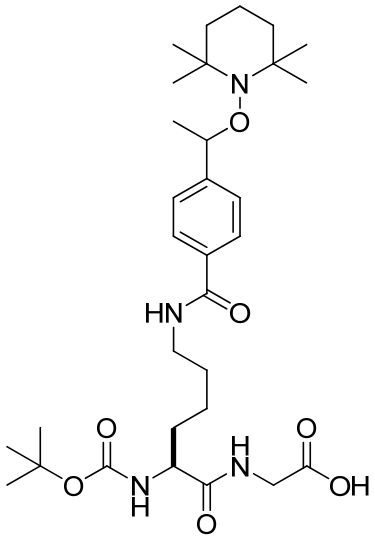
Boc-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly-OMe (1)



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 CH_2Cl_2 (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 CH_2Cl_2 (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 $MgSO_4$. The solvent was removed *in vacuo*. The crude product was purified by FC (CH_2Cl_2/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*w*, 771*w*, 707*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 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, CHCON*H*CH_2), 6.43 (*br s*, 1 H, CON*H*CH_2), 5.29 - 5.27 (*m*, 1 H, C*H*CO), 4.81 (*q*, *J* = 6.7 Hz, 1 H, C*H*CH_3), 4.15 (*br s*, 1 H, N*H*CO_2), 4.02 (*t*, *J* = 5.1 Hz, 2 H, NHCH_2CO_2), 3.71 (*s*, 3 H, OCH_3), 3.53 - 3.39 (*m*, 2 H, CH_2CH_2N), 1.98 - 1.85 (*m*, 1 H, CHCH*H*), 1.80 - 1.60 (*m*, 5 H, CHCH*H*, CH_2CH_2N, CH_2CH_2C), 1.51 - 1.42 (*m*, 18 H, CH_2CH_2CH, 2 × CH_2C, CH_3CH, C(CH_3)$_3$), 1.28 (*s*, 3 H, CH_3), 1.16 (*s*, 3 H, CH_3), 1.01 (*s*, 3 H, CH_3), 0.63 (*s*, 3 H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 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, CH_3), 41.2 (CH_2), 40.5 (CH_2), 39.3 (2 × CH_2), 32.0 (CH_2), 29.1 (CH_2), 28.4 (3 × CH_3), 23.7 (2 × CH_3), 22.5 (CH_2), 21.1 (CH_3), 20.4 (CH_3), 17.3 (CH_2), 14.2 (CH_3). MS (ESI): 314 $[M+H+Na]^{2+}$, 605 $[M+H]^+$, 627 $[M+Na]^+$, 1231 $[2M+Na]^+$. HRMS (ESI): calculated for $C_{32}H_{52}N_4O_7H^+$: 605.3909, found: 605.3904. Anal. calculated. for $C_{32}H_{52}N_4O_7 \times H_2O$: C: 61.74, H: 8.74, N: 9.00, found: C: 61.78, H: 8.58, N: 9.00.

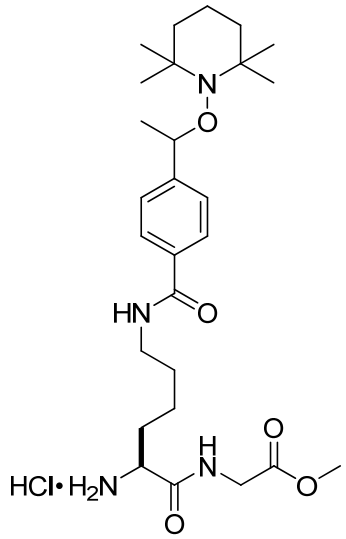
Boc-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly-OH (8b)



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. CH_2Cl_2 (50 mL) and HCl (*aq*., 1 M, 30 mL) were added, the phases were separated and the organic layer was extracted with CH_2Cl_2 (4×30 mL). The combined organic layers were dried over $MgSO_4$ 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*. ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.90 (*d*, *J* = 8.8 Hz, 2 H, 2 × Aryl-*H*), 7.59 (*d*, *J* = 8.4 Hz, 2 H, 2 × Aryl-*H*), 5.72 (*q*, *J* = 6.8 Hz, 1 H, C*H*CH_3), 4.12 - 4.06 (*m*, 1 H, C*H*CO), 3.93 (*q*, *J* = 17.8 Hz, 2 H, NHCH_2CO), 3.41 (*t*, *J* = 7.1 Hz, 2 H, CH_2NHCO), 2.14 - 1.61 (*m*, 16 H, CHCH_2, NCH_2CH_2, 2 × CCH_2, CH_2CH_2C, CHCH_3, CH_3), 1.53 (*s*, 3 H, CH_3), 1.51 - 1.46 (*m*, 2 H, C$H_2CH_2CH_2$N), 1.45 (*s*, 9 H, C(CH_3)$_3$), 1.33 (*s*, 3 H, CH_3), 1.03 (*s*, 3 H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 173.3 (C), 171.5 (2 × C), 167.0 (C), 144.2 (C), 134.7 (C), 127.9 (2 × CH), 127.1 (2 × CH), 85.1 (CH), 80.1 (C), 70.6 (C), 70.0 (C), 54.4 (CH), 41.4 (2 × CH_2), 39.5 (2 × CH_2), 37.2 (CH_2), 37.0 (CH_2), 29.0 (CH_3), 28.8 (CH_2), 28.4 (3 × CH_3), 23.5 (CH_3), 22.5 (CH_2), 21.2 (CH_3), 21.0 (CH_3), 15.8 (CH_3). MS (ESI): 307 $[M+H+Na]^{2+}$, 591 $[M+H]^+$, 613 $[M+Na]^+$, 1203 $[2M+Na]^+$. HRMS: calculated for $C_{31}H_{50}N_4O_7H^+$: 591.3752, found: 591.3754.

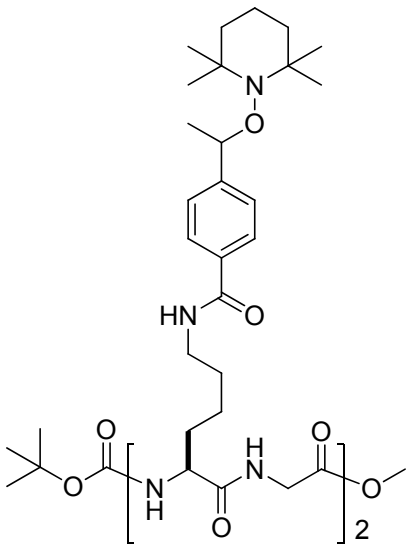
HCl×H-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly-OMe (8a)



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*s*, 1439*m*, 1384*w*, 1308*w*, 1210*m*, 940*w*, 856*w*. ^{1}H-NMR (300 MHz, CD_3OD): 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, C*H*CH_3), 4.13 - 3.93 (*m*, 3 H, C*H*CO, NHCH_2CO), 3.73 (*s*, 3 H, OCH_3), 3.45 (*t*, *J* = 6.9 Hz, 2 H, CH_2CH_2N), 2.26 - 1.87 (*m*, 6 H, CHCH_2, CH_2CH_2N, CH_2CH_2C), 1.81 - 1.65 (*m*, 10 H, 2 × CH_2C, CH_3, CH_3CH), 1.63 - 1.56 (*m*, 5 H, CH_2CH_2CH, CH_3), 1.36 (*s*, 3 H, CH_3), 1.08 (*s*, 3 H, CH_3). ^{13}C-NMR (75 MHz, CD_3OD): 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 (CH_3), 41.8 (CH_2), 40.4 (CH_2), 38.2 (CH_2), 38.1 (CH_2), 32.2 (CH_2), 30.0 (CH_2), 29.5 (CH_3), 29.3 (CH_3), 23.9 (CH_3), 23.0 (CH_2), 21.0 (CH_3), 20.8 (CH_3), 16.4 (CH_2). MS (ESI): 253 $[M+2H]^{2+}$, 505 $[M+H]^{+}$, 527 $[M+Na]^{+}$, 1009 $[2M+H]^{+}$, 1031 $[2M+Na]^{+}$. HRMS (ESI): calculated for $C_{27}H_{44}N_4O_5Na^{+}$: 527.3204, found: 527.3204.

Boc-bis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (9)

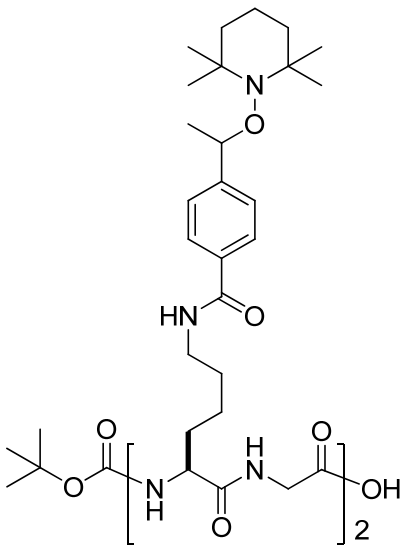


Acid **8b** (3.48 g, 5.90 mmol, 1.0 eq.) and amine hydrochloride **8a** (3.20 g, 5.90 mmol, 1.0 eq.) were dissolved in CH_2Cl_2 (60 mL). EDCI (1.36 g, 7.10 mmol, 1.2 eq.), HOBt (1.09 g, 7.10 mmol, 1.2 eq.) and NMM (1.4 mL, 13 mmol, 2.2 eq.) were added and the reaction mixture was stirred for 16 h at rt. HCl (*aq*., 1 M, 30 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (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 $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by FC (CH_2Cl_2/acetone 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*, 1018*w*, 908*m*, 853*w*, 730*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.78 - 7.74 (*m*, 4 H, 4 × Aryl-*H*), 7.62 (*br s*, 1 H, CH_2CONH), 7.56 - 7.52 (*m*, 1 H, $NHCH_2CO_2$), 7.44 (*br s*, 1 H, $NHCH_2CO$), 7.32 (*d*, *J* = 8.0 Hz, 4 H, 4 × Aryl-*H*), 6.96 (*br s*, 2 H, 2 × CH_2NHCO), 5.89 (*br s*, 1 H, CO_2NH), 4.79 (*q*, *J* = 6.4 Hz, 2 H, 2 × $CHCH_3$), 4.64 - 4.57 (*m*, 1 H, $CH_2CONHCH$), 4.27 - 4.18 (*m*, 1 H, CO_2NHCH), 4.12 - 3.87 (*m*, 4 H, 2 × $NHCH_2CO$), 3.65 (*s*, 3 H, OCH_3), 3.51 - 3.32 (*m*, 4 H, 2 × CH_2NHCOC), 2.00 - 1.33 (*m*, 39 H, 2 × $CHCH_2$, 2 × $CHCH_2CH_2$, 2 × $NHCH_2CH_2$, $C(CH_3)_3$, 2 × $CHCH_3$, 4 × CH_2C, 2 × CH_2CH_2C), 1.27 (*s*, 6 H, 2 × CH_3), 1.15 (*s*, 6 H, 2 × CH_3), 1.00 (*s*, 6 H, 2 × CH_3), 0.62 (*s*, 6 H, 2 × CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 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 (CH_3), 43.5 (CH_2), 41.2 (CH_2), 40.4 (4 × CH_2), 39.8 (CH_2), 39.7 (CH_2), 34.4 (4 × CH_3), 32.4 (CH_2), 32.1 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.4 (3 × CH_3), 23.7 (2 × CH_3), 22.9 (2 × CH_2), 20.4 (4 × CH_3), 17.3 (2 × CH_2). MS (ESI): 539 $[M+2H]^{2+}$, 550 $[M+H+Na]^{2+}$,1078 $[M+H]^+$, 1100 $[M+Na]^+$. HRMS (ESI):

calculated for $C_{58}H_{92}N_8O_{11}NaH^{2+}$: 550.3425, found: 550.3416. Anal. calculated for $C_{58}H_{92}N_8O_{11}$: C: 64.66, H: 8.61, N: 10.40, found: C: 64.34, H: 8.67, N: 10.30.

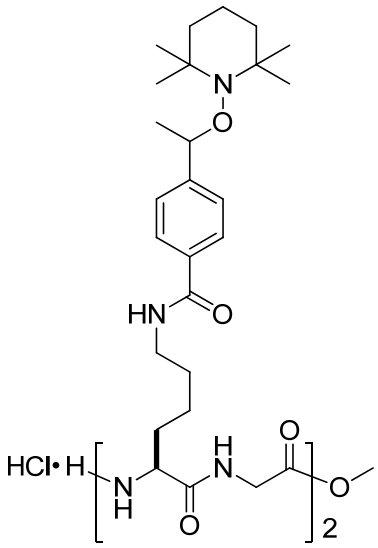
Boc-bis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OH (10b)



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_2Cl_2 (50 mL) and HCl (*aq*., 1 M, 50 mL) were added and the phases were separated. The aqueous layer was extracted with CH_2Cl_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_3OD): δ = 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 × C*H*CH_3), 4.44 (*dd*, J_1 = 5.1 Hz, J_2 = 8.7 Hz, 1 H, C*H*CO), 4.04 (*dd*, J_1 = 5.4 Hz, J_2 = 8.5 Hz, 1 H, C*H*CO), 3.98 - 3.84 (*m*, 4 H, 2 × NHCH_2CO), 3.45 - 3.40 (*m*, 4 H, 2 × CH_2NCO), 2.17 - 1.64 (*m*, 42 H, 2 × CHCH_2, 2 × CHCH_2CH_2, 2 × NHCH_2CH_2, 4 × CCH_2, 2 × CCH_2CH_2, 2 × CHCH_3, 4 × CCH_3), 1.47 (*s*, 9 H, C(CH_3)$_3$), 1.37 (*s*, 6 H, 2 × CCH_3), 1.06 (*s*, 6 H, 2 × CCH_3). ^{13}C-NMR (75 MHz, CD_3OD): δ = 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_2). MS (ESI): 532 $[M+2H]^{2+}$, 1064 $[M+H]^+$, 1086 $[M+Na]^+$. HRMS (ESI): calculated for $C_{57}H_{90}N_8O_{11}H^+$: 1063.6802, found: 1063.6810.

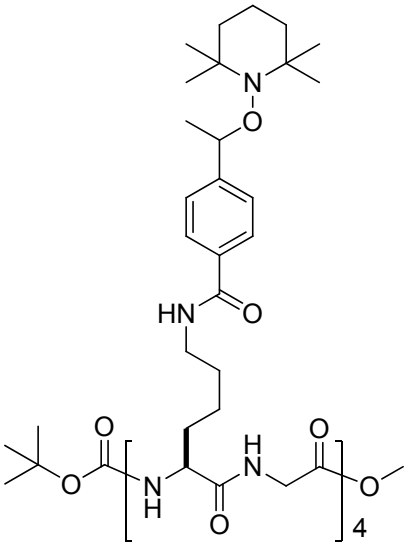
HCl×H-Bis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (10a)



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*, 1052*w*, 938*w*, 856*w*. ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.94 (*dd*, J_1 = 3.6 Hz, J_2 = 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 × C*H*CH_3), 4.44 (*dd*, J_1 = 5.3 Hz, J_2 = 8.4 Hz, 1 H, NHC*H*CO), 4.04 - 3.90 (*m*, 5 H, NHC*H*CO, 2 × NHCH_2CO), 3.79 (*s*, 3 H, OCH_3), 3.48 - 3.42 (*m*, 4 H, 2 × CH_2NCO), 2.23 - 1.55 (*m*, 42 H, 2 × CHCH_2, 2 × $CHCH_2CH_2$, 2 × $NHCH_2CH_2$, 4 × CCH_2, 2 × CCH_2CH_2, 2 × CHCH_3, 4 × CCH_3), 1.38 (*s*, 6 H, 2 × CCH_3), 1.08 (*s*, 6 H, 2 × CCH_3). ^{13}C-NMR (75 MHz, CD_3OD) : 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 (CH_3), 43.4 (CH_2), 41.8 (CH_2), 40.8 (CH_2), 40.4 (CH_2), 38.2 (2 × CH_2), 38.1 (2 × CH_2), 32.7 (CH_2), 32.1 (CH_2), 30.0 (2 × CH_2), 29.6 (2 × CH_3), 29.3 (2 × CH_3), 24.1 (CH_2), 23.9 (2 × CH_3), 23.2 (CH_2), 21.0 (2 × CH_3), 20.8 (2 × CH_3), 16.4 (2 × CH_2). MS (ESI): 489 $[M+2H]^{2+}$, 978 $[M+H]^{+}$, 1000 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{53}H_{84}N_8O_9H^{+}$: 977.6440, found: 977.6440.

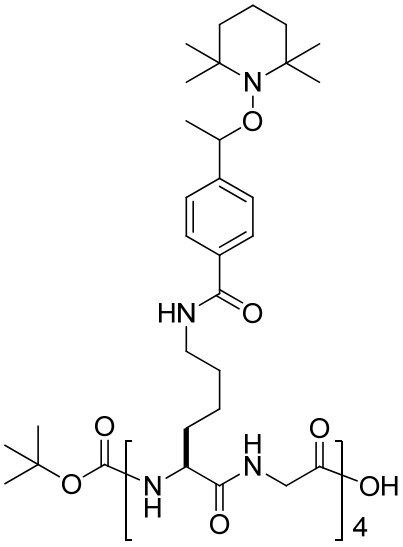
Boc-tetrakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (11)



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 (H_2O → H_2O/MeOH 1:1 → MeOH). Octapeptide **11** was obtained as a colorless solid (255 mg, 126 µmol, 70%).

Mp.: Decomp. above 240 °C. IR (neat): 3298*br m*, 2930*m*, 2264*w*, 2003*w*, 1654*s*, 1541*s*, 1377*w*, 1305*w*, 1181*w*, 856*w*, 771*w*. MS (ESI): 682 $[M+2H+Na]^{3+}$, 690 $[M+H+2Na]^{3+}$, 1012 $[M+2H]^{2+}$, 1023 $[M+H+Na]^{2+}$, 1034 $[M+2Na]^{2+}$, 2023 $[M+H]^{+}$, 2045 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{110}H_{172}N_{16}O_{19}NHNa^{2+}$: 1022.6475, found: 1022.6427. Due to low solubility of **11** in common solvents we did not obtain NMR-Spectra.

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

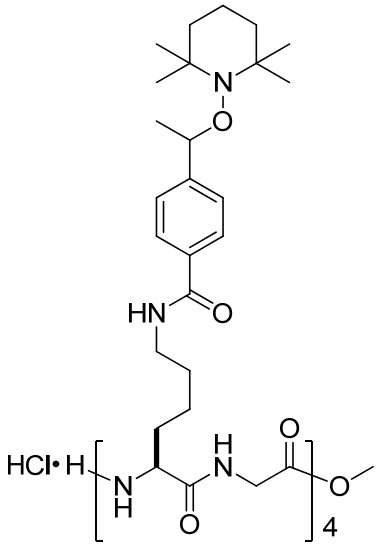


Octapeptide **11** (100 mg, 50.0 µmol, 1.0 eq.) was dissolved in MeOH (300 mL) in an ultrasonic bath. NaOH (*aq*., 1 M, 20 mL) was added and the reaction mixture was stirred for 40 h at rt. Formic acid (2.5 mL) was added and the volatile components were removed *in vacuo*. The crude product was purified by RP-FC (H_2O (200 mL) → MeOH). The desired product was obtained as a colorless solid (91 mg, 45 µmol, 91%).

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.

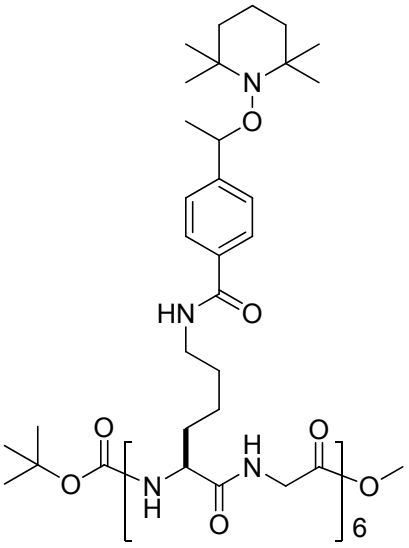
HCl×H-Tetrakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (12a)



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.

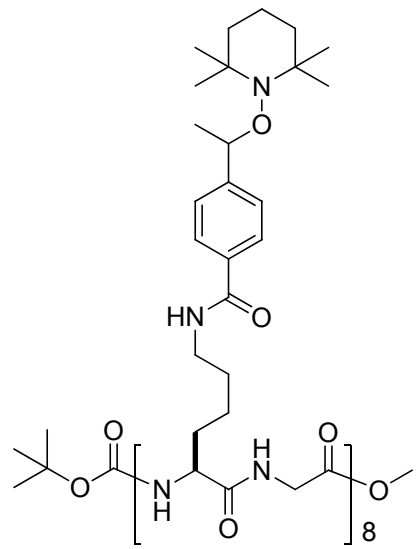
Boc-hexakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (13)



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.

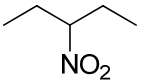
Boc-octakis[-Lys(4-[1-{2,2,6,6-tetramethylpiperidine-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (14)



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 $C_{214}H_{332}N_{32}O_{35}$: 3910.51774, found: 3910.51731, calculated from: 559.93891 $[M+7H]^{7+}$, 653.09434 $[M+6H]^{6+}$, 783.51189 $[M+5H]^{5+}$. Due to low solubility of **14** in common solvents we did not obtain NMR-Spectra.

3-Nitropentane (29)

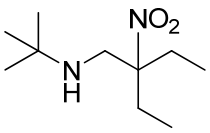


Sodium nitrite (44.2 g, 640 mmol, 1.6 eq.) was added to a solution of 3-bromopentane (50.0 mL, 400 mmol, 1.0 eq.) in DMSO (350 mL). The reaction mixture was stirred for 18 h at rt, cooled to 0 °C and water was added until the formed precipitate was dissolved. The mixture was extracted with pentane (3 × 60 mL). The combined extracts were washed with water (2 × 10 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The product was obtained as a pale blue liquid and used without further purification (29.8 g, 254 mmol, 64%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.36 - 4.27 (*m*, 1 H, C*H*), 1.99 - 1.91 (*m*, 2 H, CH_2), 1.82 - 1.75 (*m*, 2 H, CH_2), 0.95 (*t*, *J* = 7.4 Hz, 6 H, 2 × CH_3).

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

***N-tert*-Butyl-(2-ethyl-2-nitrobutyl)-amine (30)**

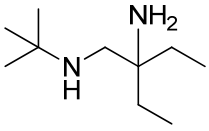


Formaldehyde (37% wt. in H_2O, 6.7 mL, 89 mmol, 1.0 eq.) was added slowly to *tert*-butylamine at 0 °C (9.5 mL, 89 mmol, 1.0 eq.). 3-Nitropentane (10.4 g, 89.0 mmol, 1.0 eq.) was added and the reaction mixture was stirred for 18 h at rt. Na_2SO_4 was added until a phase separation occured. The aqueous layer was removed and the organic layer was stirred for additional 5 d at rt. The mixture was dried over Na_2SO_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%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.93 (*s*, 2 H, C*H*$_2$NH), 1.97 (*q*, *J* = 7.6 Hz, 4 H, 2 × C*H*$_2$), 1.05 (*s*, 9 H, C(C*H*$_3$)$_3$), 0.85 (*t*, *J* = 7.5 Hz, 6 H, 2 × C*H*$_3$).

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

N-tert-Butyl-2-ethylbutan-1,2-diamine (31)

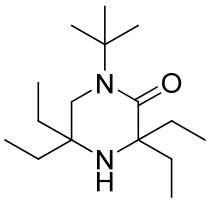


Amine **30** (14.0 g, 69.0 mmol, 1.0 eq.) was dissolved in a mixture of HOAc (100 mL) and H_2O (150 mL). While cooling in an ice bath, zinc powder (27.1 g, 414 mmol, 6.0 eq.) was added and the reaction mixture was stirred at rt for 2 h. The excess of zinc was filtered off and solid NaOH was added to the filtrate until the solution turned basic. The mixture was extracted with Et_2O (3 × 200 mL) and the extracts were dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the product was obtained as a colorless liquid (10.9 g, 63.3 mmol, 92%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.36 (*s*, 2 H, C*H*$_2$N), 1.40 - 1.29 (*m*, 4 H, 2 × C*H*$_2$), 1.07 (*s*, 9 H, C(C*H*$_3$)$_3$), 0.82 (*t*, *J* = 7.6 Hz, 6 H, 2 × C*H*$_3$).

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

1-*tert*-Butyl-3,3,5,5-tetraethyl-2-piperazinone (32)

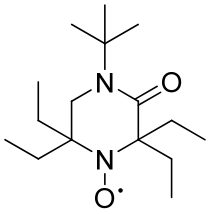


KOH (18.5 g, 329 mmol, 5.3 eq.) powder was added slowly at 10 °C to a mixture of diamine **31** (10.7 g, 62.2 mmol, 1.00 eq.), 3-pentanone (99.0 mL, 933 mmol, 15.0 eq.) and $CHCl_3$ (8.0 mL, 0.1 mol, 1.6 eq.). After stirring for for 18 h at rt, the reaction mixture filtered. The filtrate was evaporated 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%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.15 (*s*, 2 H, C*H*$_2$N), 1.58 (*q*, *J* = 7.4 Hz, 4 H, 2 × C*H*$_2$), 1.43 - 1.34 (*m*, 4 H, 2 × C*H*$_2$), 1.41 (*s*, 9 H, C(C*H*$_3$)$_3$), 0.86 (*t*, *J* = 7.4 Hz, 6 H, 2 × C*H*$_3$), 0.83 (*t*, *J* = 7.4 Hz, 6 H, 2 × C*H*$_3$).

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)

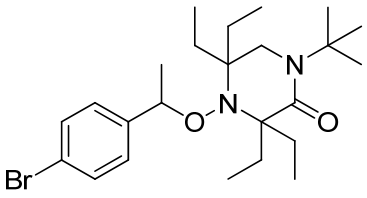


Piperazinone **32** (2.44 g, 9.08 mmol, 1.0 eq.) was dissolved in EtOAc (20 mL). Peroxyacetic (39% wt. in HOAc, 2.31 mL, 13.6 mmol, 1.5 eq.) acid was added at 0 °C. The reaction mixture was stirred for 16 h at rt. Pentane (60 mL) was added and the mixture was washed with $NaHCO_3$ (*aq*. *sat*., 3 × 40 mL). The organic layer was dried over $MgSO_4$ 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%).

MS (ESI): 284 $[M+H]^+$, 306 $[M+Na]^+$, 589 $[2M+Na]^+$. HRMS: calculated. for $C_{16}H_{31}N_2O_2Na^+$: 306.2278, found: 306.2275.

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)

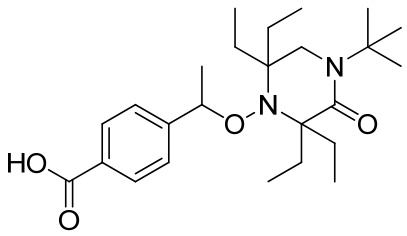


According to a procedure of *Matyjaszewski et al.*, bromide **26** (2.02 g, 7.66 mmol, 1.0 eq.), nitroxide **15** (2.17 g, 7.66 mmol, 1.0 eq.), copper powder (511 mg, 8.04 mmol, 1.05 eq.), $Cu(OTf)_2$ (28.0 mg, 80.0 µmol, 0.01 eq.) and 4,4-di-*tert*-butyl-2,2-dipyridyl (80.0 mg, 310 µmol, 0.04 eq.) were 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 CH_2Cl_2 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%).

^{1}H-NMR (300 MHz, $CDCl_3$): 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, C*H*CH_3), 3.21 - 2.95 (*m*, 2 H, CH_2N), 2.16 - 1.50 (*m*, 7 H, 2 × CH_2, CHCH_3), 1.44 - 1.38 (*m*, 13 H, 2 × CH_2, C(CH_3)$_3$), 1.08 - 0.93 (*m*, 6 H, 2 × CH_3), 0.86 - 0.60 (*m*, 6 H, 2 × CH_3).

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)

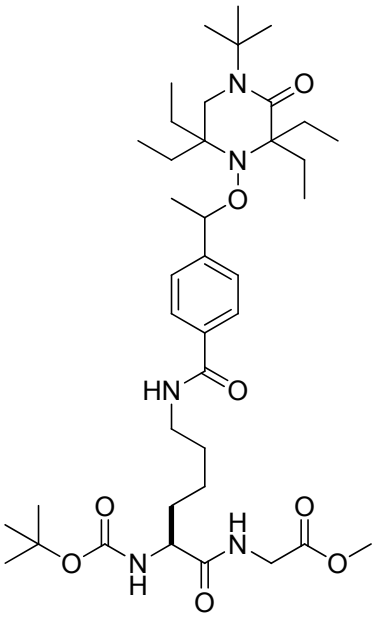


Alkoxyamine **33** (9.50 g, 20.3 mmol, 1.0 eq.) was dissolved in THF (90 mL) and *t*-BuLi (1.5 M in pentane, 27.1 mL, 40.6 mmol, 2.0 eq.) was added slowly at -78 °C. The reaction mixture was stirred at -78 °C for 20 min. CO_2, dried by passing through conc. H_2SO_4, was passed through the reaction mixture 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 CH_2Cl_2 (2 × 40 mL). The combined organic layers were dried over $MgSO_4$ 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*. ^{1}H-NMR (300 MHz, $CDCl_3$): 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, C*H*CH_3), 3.23 - 2.96 (*m*, 2 H, CH_2N), 2.23 - 1.23 (*m*, 11 H, 4 × CH_2, CHCH_3), 1.39 (*s*, 9 H, C(CH_3)$_3$), 1.10 - 0.95 (*m*, 6 H, 2 × CH_3), 0.87 - 0.58 (*m*, 6 H, 2 × CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 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 (CH_2), 46.1 (CH_2), 34.9 (CH_2), 33.4 (CH_2), 29.4 (CH_2), 29.0 (CH_2), 28.3 (6 × CH_3), 26.9 (CH_2), 26.7 (CH_2), 24.7 (2 × CH_2), 23.3 (CH_3), 22.3 (CH_3), 11.8 (CH_3), 11.3 (CH_3), 9.7 (CH_3), 9.4 (CH_3), 9.2 (2 × CH_3), 8.4 (CH_3), 7.7 (CH_3). MS (ESI): 433 $[M+H]^+$, 455 $[M+Na]^+$, 888 $[2M+Na]^+$, 1320 $[3M+Na]^+$. HRMS (ESI): calculated for $C_{25}H_{10}N_2O_4H^+$: 433.3061, found: 433.3065.

Anal. calculated for $C_{25}H_{10}N_2O_4$: C: 69.41, H: 9.32, N: 6.48, found: C: 69.13, H: 9.37, N: 6.33.

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

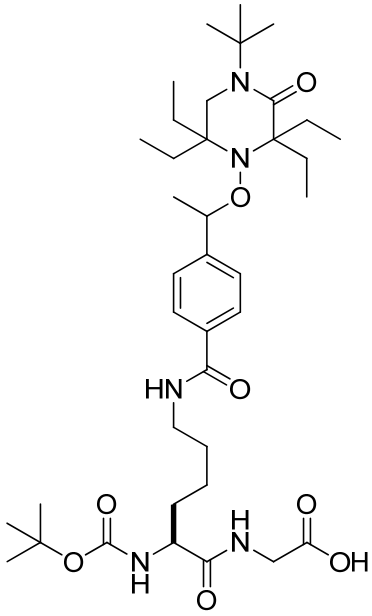


Acid **34** (9.07 g, 21.0 mmol, 1.1 eq.), amine hydrochloride **7** (6.73 g, 19.1 mmol, 1.0 eq.), EDCI (4.38 g, 22.9 mmol, 1.2 eq.), HOBt (3.50 g, 22.9 mmol, 1.0 eq.) and NMM (4.63 mL, 41.9 mmol, 2.2 eq.) were dissolved in CH_2Cl_2 (100 mL) and the reaction mixture was stirred for 16 h at rt. After addition of HCl (*aq*., 0.5 M, 60 mL) the phases were separated. The aqueous layer was extracted with CH_2Cl_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_2Cl_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, CHCON*H*), 6.44 (*br s*, 1 H, CH_2NHCO), 5.27 - 5.24 (*m*, 1 H, C*H*CO), 4.78 - 4.69 (*m*, 1 H, C*H*CH_3), 4.19 - 4.10 (*m*, 1 H, N*H*CO_2), 4.02 (*t*, *J* = 5.2 Hz, 2 H, NHCH_2CO_2), 3.71 (*s*, 3 H, OCH_3), 3.51 - 3.44 (*m*, 2 H, CH_2CH_2N), 3.21 - 3.00 (*m*, 2 H, CH_2N), 1.98 - 1.56 (*m*, 11 H, CH_2CH_2N, CH_2CH, $CHCH_2CH_2$, CH_2CH_3, $CHCH_3$), 1.50 - 1.42 (*m*, 15 H, 3 × CH_2CH_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 $[2M+Na]^+$. HRMS (ESI): calculated for

$C_{39}H_{65}N_5O_8Na^+$: 754.4725, found: 754.4712. Anal. calculated for $C_{39}H_{65}N_5O_8$: C: 63.99, H: 8.95, N: 9.57, found: C: 63.59, H: 9.07, N: 9.34.

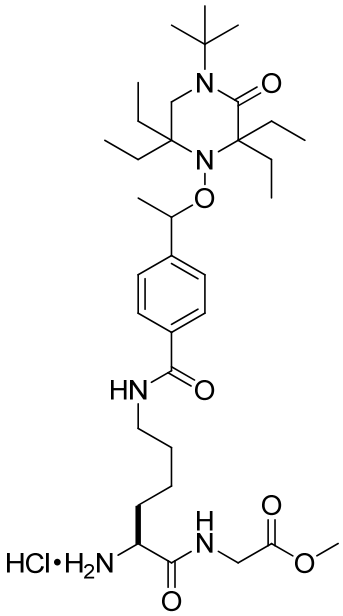
Boc-Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly-OH (35)



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 CH_2Cl_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 (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*. ^{1}H-NMR (300 MHz, $CDCl_3$): 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, C*H*CH_3), 4.05 - 4.01 (*m*, 1 H, C*H*CO), 3.95 - 3.79 (*m*, 2 H, NHCH_2CO), 3.35 (*t*, *J* = 6.7 Hz, 2 H, CH_2CH_2N), 3.18 - 3.02 (*m*, 2 H, CH_2N), 2.15 - 1.53 (*m*, 11 H, CH_2CH, CH_2CH_2N, $CHCH_2CH_2$, CH_2CH_3, CHCH_3), 1.46 - 1.35 (*m*, 24 H, 3 × CH_2CH_3, OC(CH_3)$_3$, NC(CH_3)$_3$), 1.09 - 0.95 (*m*, 6 H, 2 × CH_3), 0.82 - 0.59 (*m*, 6 H, 2 × CH_3). ^{13}C-NMR (300 MHz, $CDCl_3$): 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 (CH_2), 46.0 (CH_2), 41.3 (CH_2), 39.6 (CH_2), 35.4 (CH_2), 33.6 (CH_2), 32.2 (CH_2), 28.4 (CH_3), 28.3 (CH_3), 26.8 (CH_2), 24.6 (CH_2), 23.3 (CH_3), 22.6 (CH_2), 22.2 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 9.7 (CH_3), 9.4 (CH_3), 9.2 (CH_3), 8.3 (CH_3), 7.7 (CH_3). MS (ESI): 718 $[M+H]^+$, 740 $[M+Na]^+$, 1434 $[2M+H]^+$. HRMS (ESI): calculated for: $C_{38}H_{63}N_5O_8Na^+$: 740.4569, found: 740.4554.

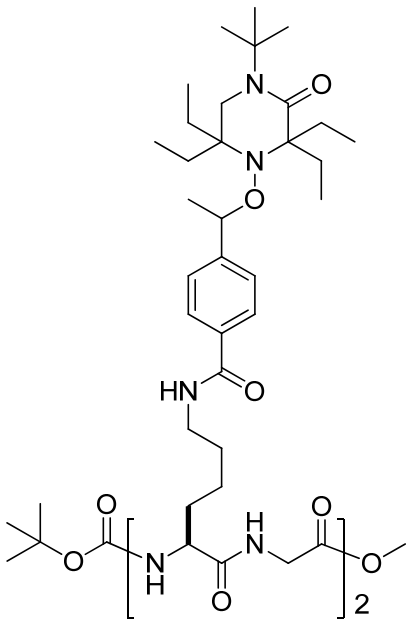
HCl×H-Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly-OMe (36)



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_3OD): δ = 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, C*H*CH_3), 4.05 - 3.85 (*m*, 3 H, C*H*CO, NHC*H*$_2$CO), 3.65 (*s*, 3 H, OC*H*$_3$), 3.37 (*t*, 2 H, *J* = 7.1 Hz, CH_2CH_2N), 3.16 - 3.00 (*m*, 2 H, C*H*$_2$N), 2.11 - 1.34 (*m*, 26 H, C*H*$_2$CH, C*H*$_2$$CH_2N$, $CHCH_2CH_2$, 4 × C*H*$_2$$CH_3$, CHC*H*$_3$, C(C*H*$_3$)$_3$), 1.08 - 0.94 (*m*, 6 H, 2 × C*H*$_3$), 0.80 - 0.73 (*m*, 3 H, C*H*$_3$), 0.65 - 0.57 (*m*, 3 H, C*H*$_3$). ^{13}C-NMR (75 MHz, CD_3OD): δ = 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 $C_{34}H_{57}N_5O_6H^+$: 632.4381, found: 632.4382.

Boc-bis[Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (17)

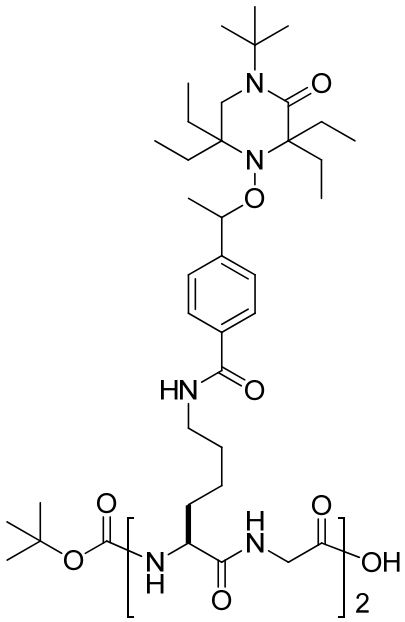


Acid **35** (2.26 g, 3.14 mmol, 1.0 eq.) and amine hydrochloride **36** (2.10 g, 3.14 mmol, 1.0 eq.) were dissolved in CH_2Cl_2 (40 mL). EDCI (722 mg, 3.77 mmol, 1.2 eq.), HOBt (577 mg, 3.77 mmol, 1.2 eq.) and NMM (0.76 mL, 6.91 mmol, 2.2 eq.) were added and the reaction mixture was stirred for 16 h at rt. HCl (*aq*., 1 M, 20 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (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 $MgSO_4$ and the solvent was evaporated *in vacuo*. Purification was carried out by FC (CH_2Cl_2/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*. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.78 (*d*, *J* = 8.1 Hz, 4 H, 4 × Aryl-*H*), 7.48 (*br s*, 1 H, CH_2CONH), 7.41 (*br s*, 1 H, $NHCH_2CO_2$), 7.32 - 7.30 (*m*, 5 H, 4 × Aryl-*H*, $NHCH_2CO$), 6.89 (*br s*, 2 H, 2 × CH_2NH), 5.77 (*br s*, 1 H, CO_2NH), 4.77 - 4.68 (*m*, 2 H, 2 × $CHCH_3$), 4.55 - 4.48 (*m*, 1 H, $CH_2CONHCH$), 4.14 - 4.08 (*m*, 1 H, CO_2NHCH), 4.03 - 3.84 (*m*, 4 H, 2 × $NHCH_2CO$), 3.66 (*s*, 3 H, OCH_3), 3.48 - 3.34 (*m*, 4 H, 2 × CH_2CH_2NH), 3.21 - 2.95 (*m*, 4 H, 2 × CH_2N), 2.16 - 1.37 (*m*, 61 H, 2 × CH_2CH_2N, 2 × CH_2CH, 2 × $CHCH_2CH_2$, 8 × CH_2CH_3, 2 × $CHCH_3$, $OC(CH_3)_3$, 2 × $NC(CH_3)_3$), 1.08 - 0.94 (*m*, 12 H, 4 × CH_3), 0.83 - 0.59 (*m*, 12 H, 4 × CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 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 (CH_3), 47.1 (CH_2), 46.1 (CH_2), 43.5 (CH_2), 41.3 (CH_2), 39.6 (2 × CH_2), 34.8 (2 × CH_2), 33.6 (CH_2), 31.6 (CH_2), 31.4 (CH_2), 29.3 (2 × CH_2), 29.2 (2 × CH_2), 28.5 (3 × CH_3), 28.3 (6 × CH_3), 27.0 (CH_2), 26.7 (CH_2), 24.7 (2 × CH_2), 23.4 (2 × CH_3), 22.3

(CH_2), 11.8 (CH_3), 11.4 (CH_3), 9.7 (CH_3), 9.5 (2 × CH_3), 9.2 (CH_3), 8.4 (CH_3), 7.8 (CH_3). MS (ESI): 688 $[M+2Na]^{2+}$, 1354 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{72}H_{118}N_{10}O_{13}Na^{+}$: 1353.8772, found: 1353.8782. Anal. calculated for $C_{72}H_{118}N_{10}O_{13}\times H_2O$: C: 64.07, H: 8.96, N: 10.38. found: C: 64.37, H: 8.97, N: 10.27.

Boc-bis[Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OH (37)

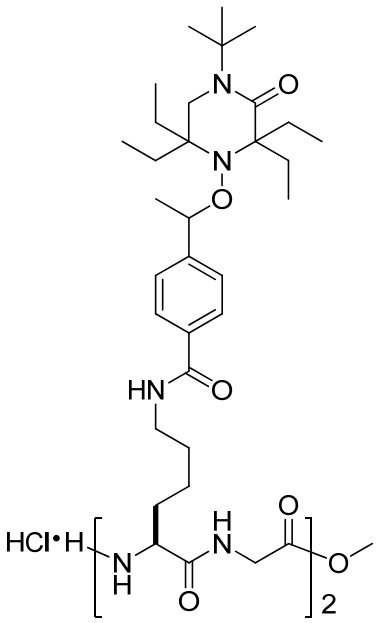


NaOH (*aq*., 0.25 M, 15 mL) was added to a solution of tetrapeptide **17** (450 mg, 0.34 mmol, 1.0 eq.) in MeOH (25 mL). The reaction mixture was stirred for 16 h at rt. HCl (*aq*., 1 M, 10 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 30 mL). The combined organic phases were dried over $MgSO_4$ 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*. ^{1}H-NMR (400 MHz, CD_3OD): 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 × C*H*CH_3), 4.43 (*dd*, J_1 = 5.0 Hz, J_2 = 8.7 Hz, 1 H, NHC*H*CO), 4.05 - 3.99 (*m*, 1 H, NHC*H*CO), 3.94 - 3.83 (*m*, 4 H, 2 × NHCH_2CO), 3.40 (*t*, *J* = 5.9 Hz, 4 H, 2 × CH_2CH_2N), 3.30 - 3.07 (*m*, 4 H, 2 × CH_2N), 2.17 - 1.29 (*m*, 61 H, 2 × C$H_2$$CH_2$N, 2 × C$H_2$CH, 2 × CHC$H_2CH_2$, 8 × C$H_2$$CH_3$, 2 × CHC$H_3$, OC(C$H_3$)$_3$, 2 × NC(C$H_3$)$_3$), 1.14 - 1.01 (*m*, 12 H, 4 × CH_3), 0.89 - 0.80 (*m*, 6 H, 2 × CH_3), 0.73 - 0.63 (*m*, 6 H, 2 × CH_3). ^{13}C-NMR (100 MHz, CD_3OD): 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 (CH_2), 43.7 (CH_2), 41.8 (CH_2), 40.7 (CH_2), 40.5 (CH_2), 36.0 (CH_2), 34.7 (CH_2), 32.5 (CH_2), 30.4 (CH_2), 30.2 (CH_2), 30.1 (2 × CH_2), 29.9 (CH_2), 28.7 (3 × CH_3), 28.4 (6 × CH_3), 27.8 (2 × CH_2), 27.6 (CH_2), 27.0 (CH_3), 25.7 (2 × CH_2), 24.1 (2 × CH_2), 23.5 (CH_3), 22.4

(CH_3), 20.8 (CH_3), 12.1 (CH_3), 11.7 (CH_3), 9.9 (CH_3), 9.7 (CH_3), 9.6 (CH_3), 8.7 (CH_3), 8.1 (CH_3). MS (ESI): 681 $[M+2Na]^{2+}$, 1340 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{71}H_{116}N_{10}O_{13}Na^{+}$: 1339.8616, found: 1339.8587.

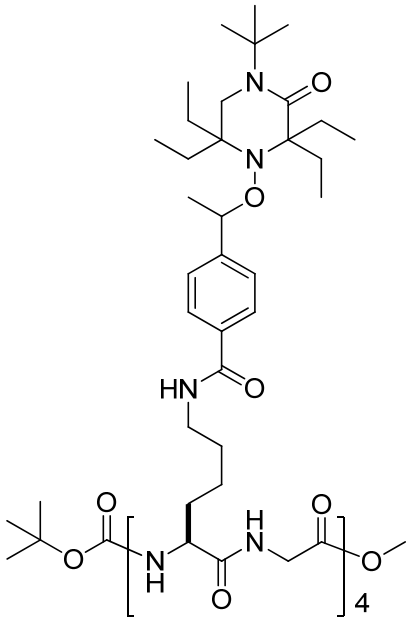
HCl×H-Bis[Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (38)



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 (0.95g, 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*m*, 952*w*, 855*w*, 762*w*, 671*w*. ^{1}H-NMR (300 MHz, CD_3OD): 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 × C*H*CH_3), 4.43 (*dd*, J_1 = 5.3 Hz, J_2 = 8.6 Hz, 1 H, C*H*CO), 4.12 - 3.84 (*m*, 5 H, C*H*CO, 2 × NHCH_2CO), 3.72 (*s*, 3 H, OCH_3), 3.47 - 3.39 (*m*, 4 H, 2 × CH_2CH_2N), 3.24 - 3.07 (*m*, 4 H, 2 × CH_2N), 2.19 - 1.32 (*m*, 52 H, 2 × C$H_2$$CH_2$N, 2 × C$H_2$CH, 2 × CHC$H_2CH_2$, 8 × C$H_2$$CH_3$, 2 × CHC$H_3$, 2 × NC(C$H_3$)$_3$), 1.15 - 1.01 (*m*, 12 H, 4 × CH_3), 0.88 - 0.80 (*m*, 6 H, 2 × CH_3), 0.73 - 0.64 (*m*, 6 H, 2 × CH_3). ^{13}C-NMR (100 MHz, CD_3OD): 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 (CH_3), 48.3 (CH_2), 47.5 (CH_2), 43.4 (CH_2), 41.8 (2 × CH_2), 40.7 (CH_2), 40.4 (CH_2), 36.0 (CH_2), 32.7 (CH_2), 32.1 (CH_2), 30.2 (2 × CH_2), 30.0 (2 × CH_2), 28.4 (6 × CH_3), 27.9 (CH_2), 27.7 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 24.1 (CH_2), 23.5 (CH_3), 23.3 (CH_2), 22.4 (CH_3), 12.2 (CH_3), 11.8 (CH_3), 9.9 (CH_3), 9.8 (CH_3), 9.7 (2 × CH_3), 8.6 (CH_3), 8.1 (2 × CH_3). MS (ESI): 627 $[M+Na+H]^{2+}$, 1232 $[M+H]^{+}$, 1254 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{67}H_{110}N_{10}O_{11}Na^{+}$: 1253.8248, found: 1253.8250.

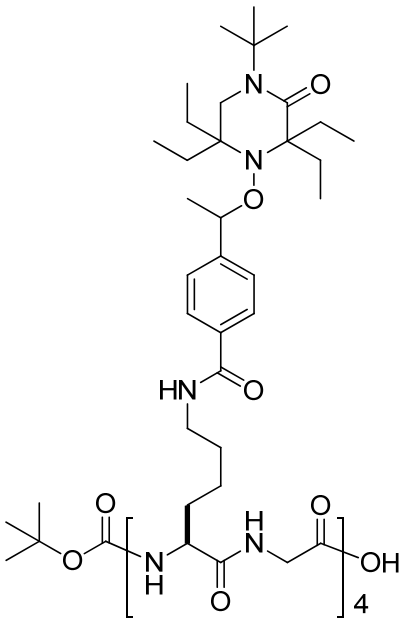
Boc-tetrakis[Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (18)



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 (H_2O → H_2O/MeOH 1:1 → H_2O/MeOH 1:4 → H_2O/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*, 933*w*, 940*w*, 914*w*, 853*m*, 768*w*, 685*m*. MS (ESI): 1288 $[M+2Na]^{2+}$, 2553 $[M+Na]^{+}$. HRMS (ESI): calculated for $C_{138}H_{224}N_{20}O_{23}Na_2^{2+}$: 1287.8379, found: 1287.8414. Due to low solubility of **18** in common solvents we did not obtain NMR-Spectra.

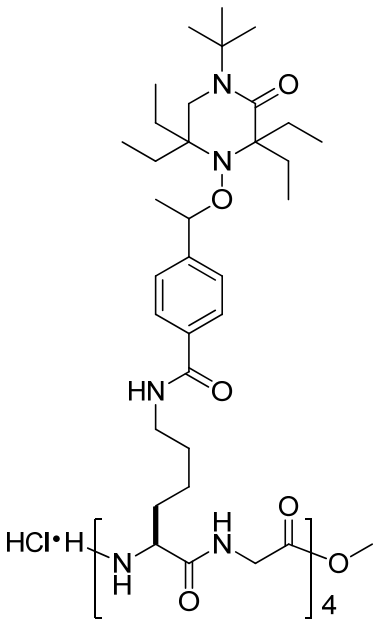
Boc-tetrakis[Lys(4-[1-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OH (39)



Octapeptide **18** (127 mg, 50.0 µmol, 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_2O (200 mL) → MeOH). Acid **39** was isolated as a colorless solid (118 mg, 46.9 µmol, 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 $C_{137}H_{222}N_{20}O_{23}Na_2^{2+}$: 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-{1-*tert*-butyl-3,3,5,5-tetraethylpiperazin-2-one-1-yloxy}-ethyl]-benzamide)-Gly]-OMe (40)

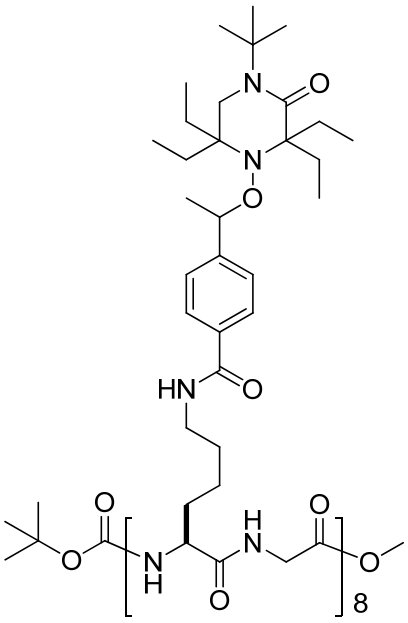


Octapeptide **18** (127 mg, 50.0 µmol, 1.0 eq.) was suspended in MeOH (5 mL) and a methanolic solution of HCl (1.25 M in MeOH, 500 µL, 630 µmol, 12.5 eq.) was added. The reaction mixture was stirred for 48 h at rt and the volatile components were removed *in vacuo*. Amine hydrochloride **40** was obtained as a colorless solid (123 mg, 50.0 µmol, 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-{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 $[M+3H]^{3+}$.

Polymerization procedures

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

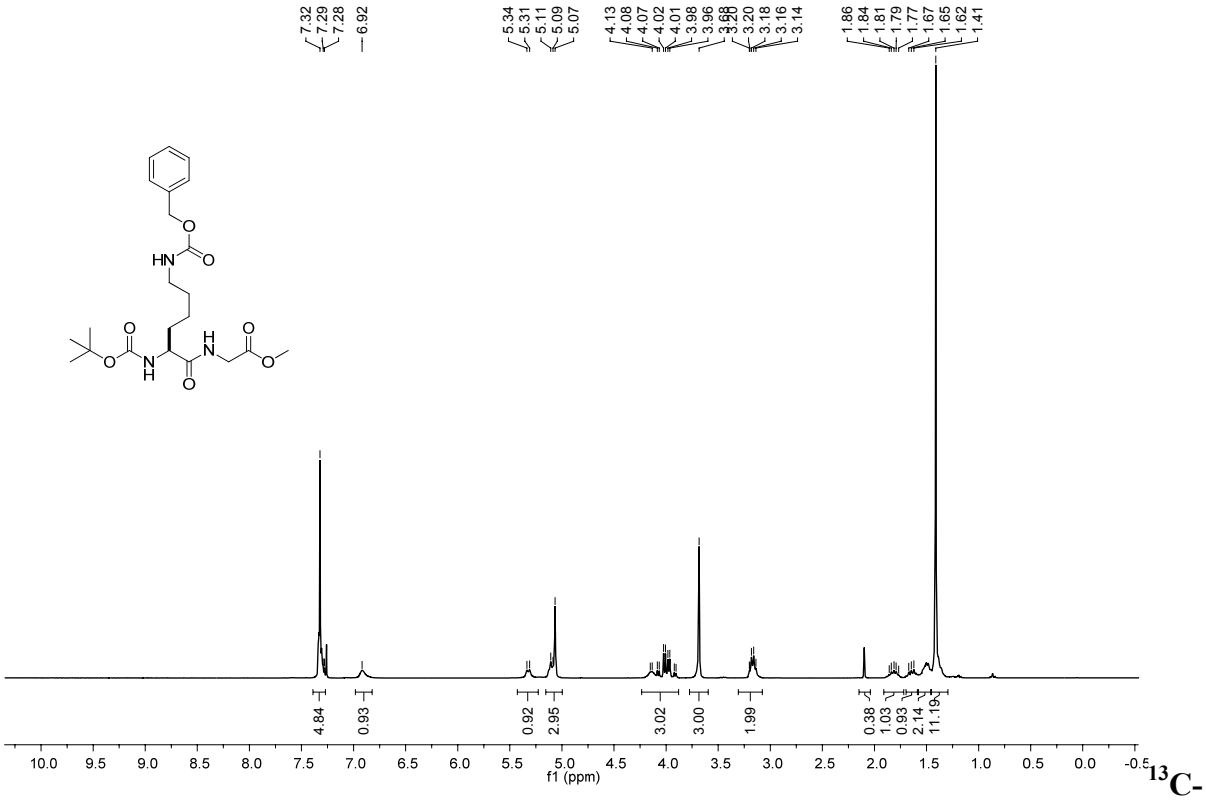
In a *Schlenk*-tube the peptide initiator (0.125 mol% to 1.0 mol%) was suspended in styrene (250 µL, 2.19 mmol, 1.0 eq.). The suspension was degassed by three freeze-thaw cycles under cooling in liquid nitrogen. The reaction mixture was heated in an oil bath at 125 °C for the desired time. The tube was cooled to rt, the polymer was dissolved in CH_2Cl_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 ($M_{n,theo.}$) was calculated from yield. Experimental molecular weight ($M_{n,exp.}$) and polydispersity index (PDI) were determined by GPC.

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

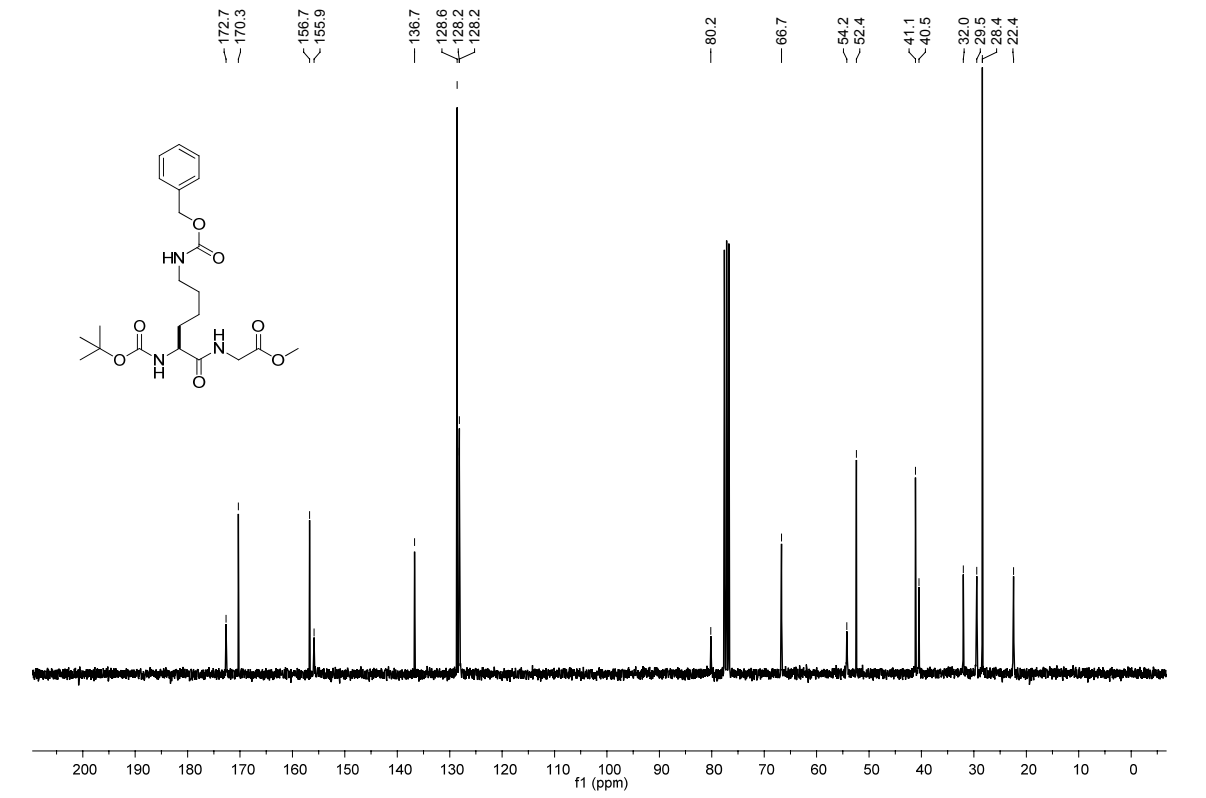
The peptide initator (0.5 mol% or 1.0 mol%) was suspended in benzene (1 mL) and NIPAM (201 mg, 1.78 mmol, 1.0 eq.) was added. Argon was passed through this suspension via a syringe for 1 min. The tube was sealed tightly and heated at 125 °C in an oil bath for the desired time. After cooling to rt, the 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 ($M_{n,theo.}$) was calculated from yield. Experimental molecular weight ($M_{n,exp.}$) and polydispesrity index (PDI) were determined by GPC.

^{1}H-NMR and ^{13}C-NMR spectra of new compounds

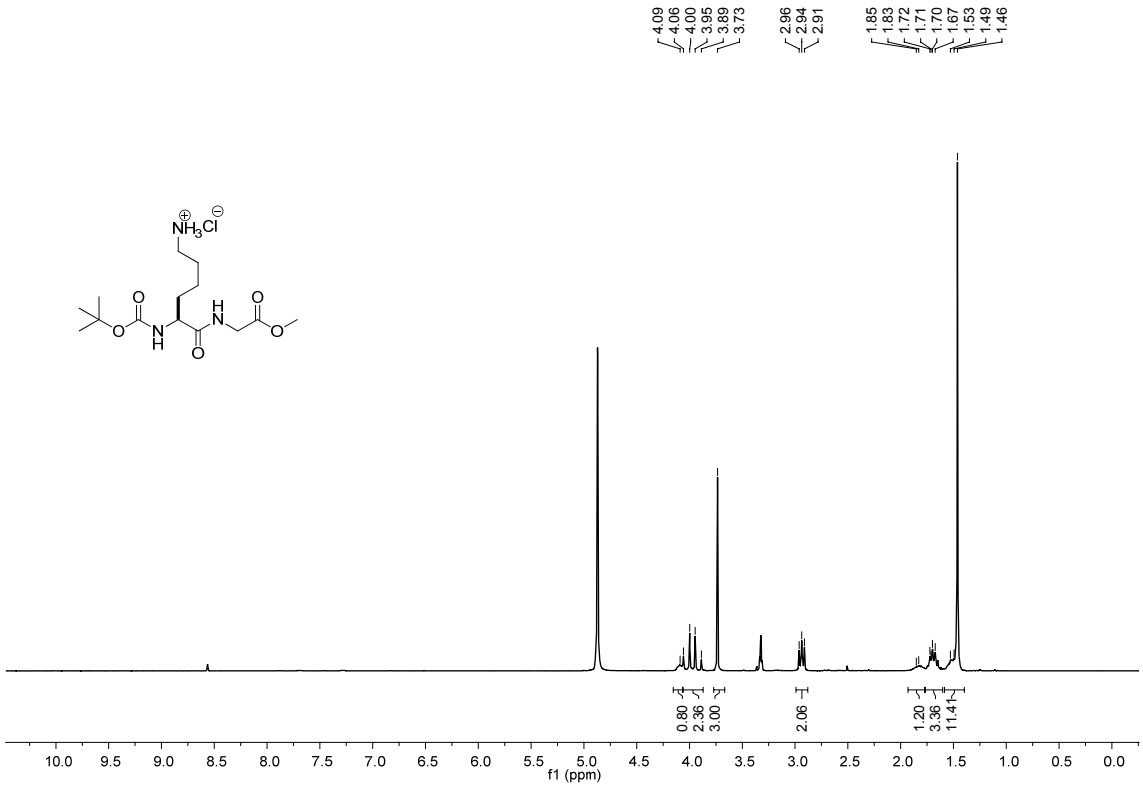
^{1}H-NMR spectrum of 28



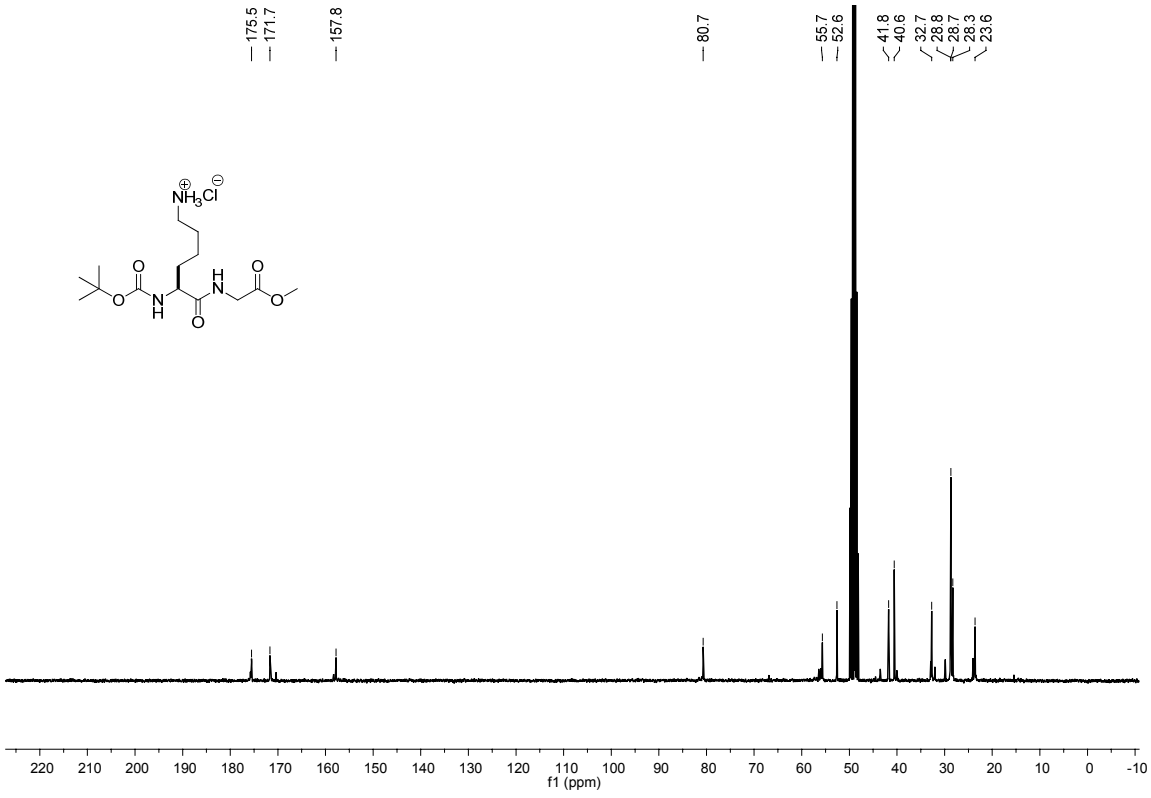
^{13}C-NMR spectrum of 28



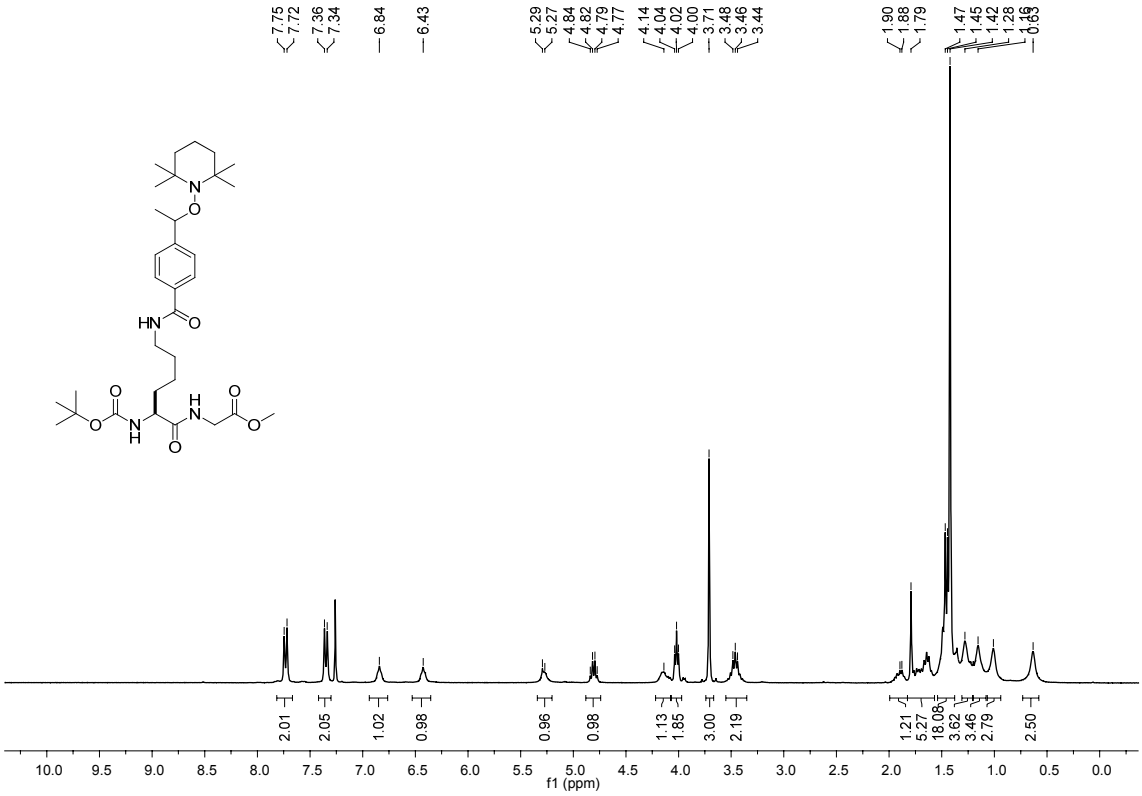
^{1}H-NMR spectrum of 7



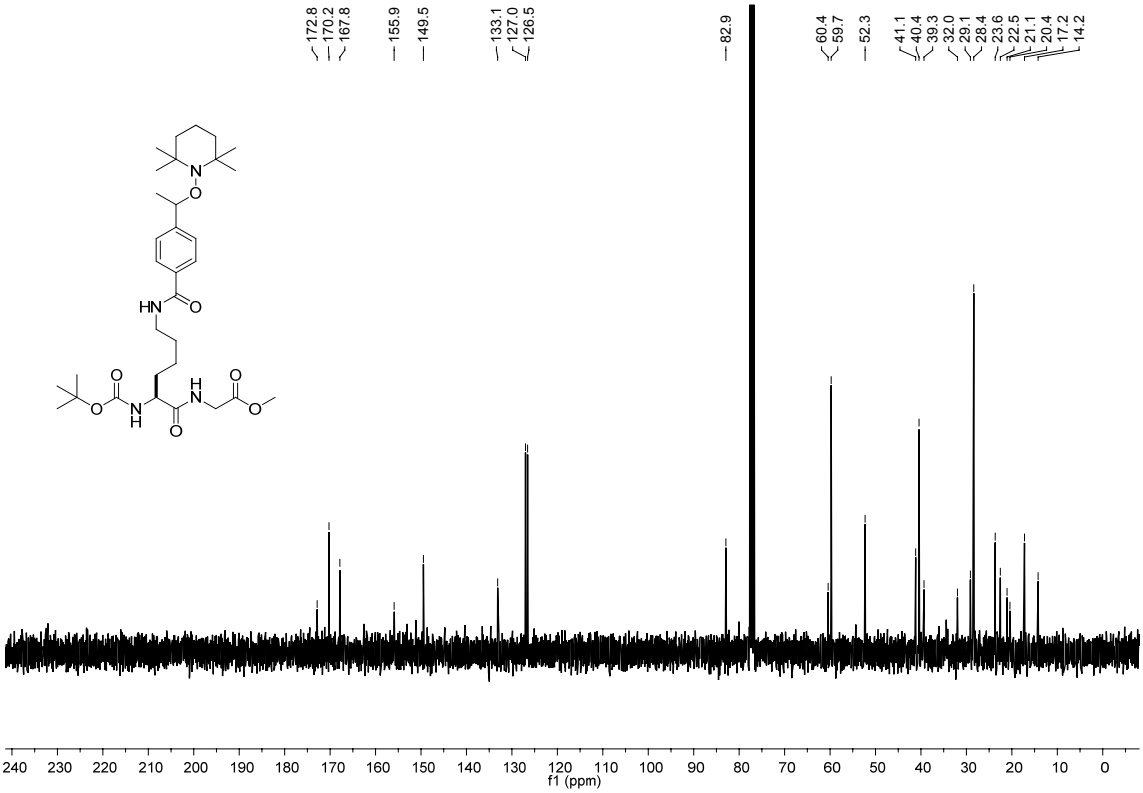
^{13}C-NMR spectrum of 7



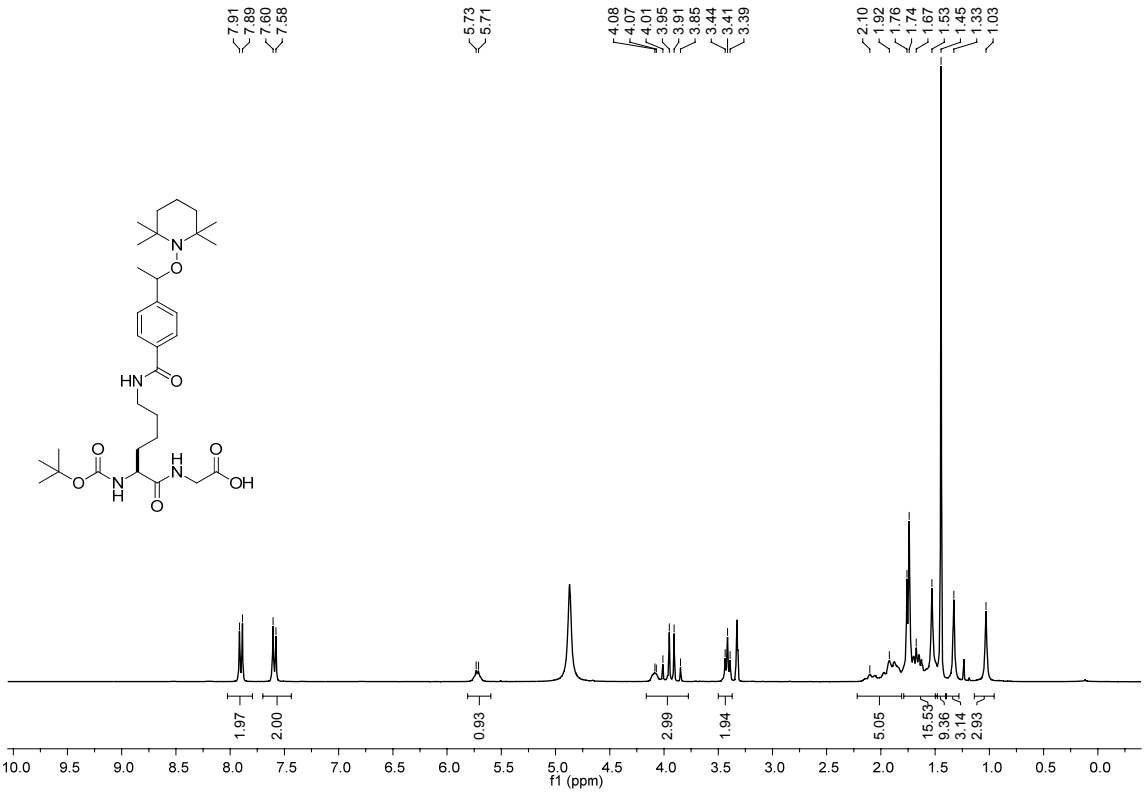
^{1}H-NMR spectrum of 1



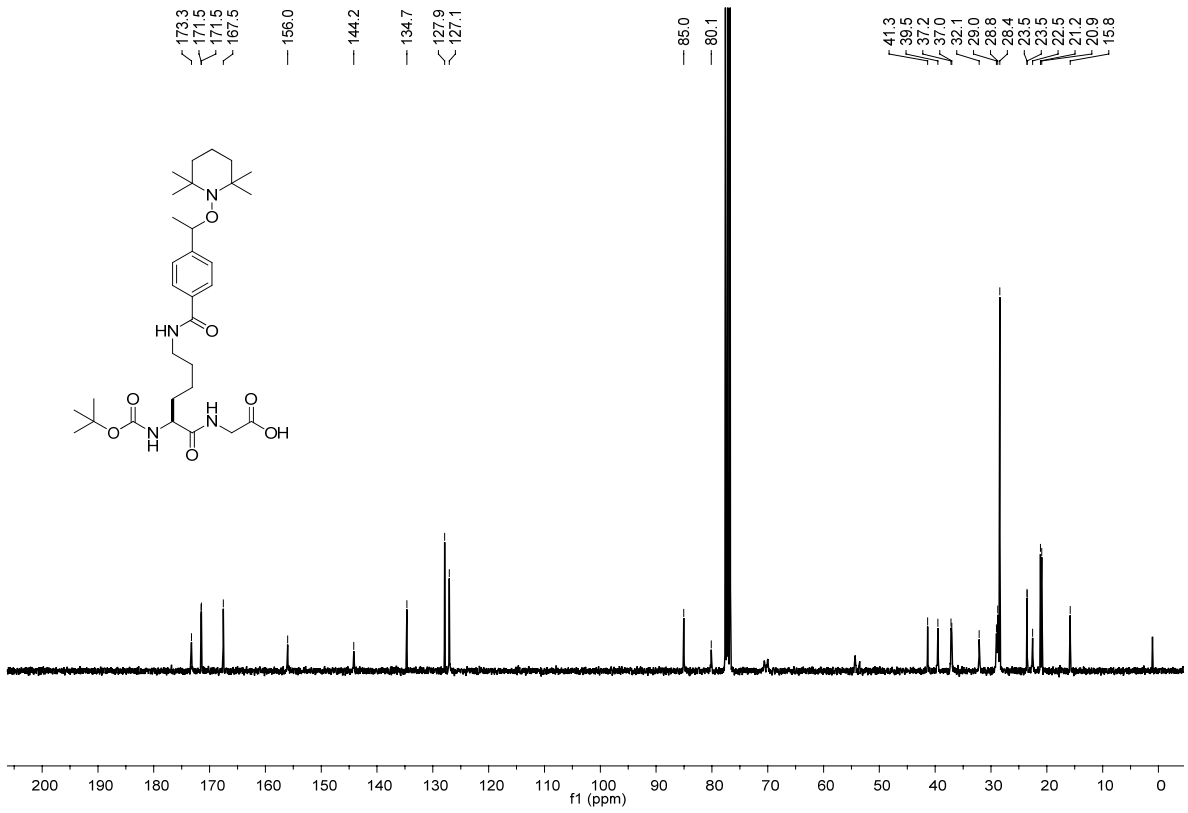
^{13}C-NMR spectrum of 1



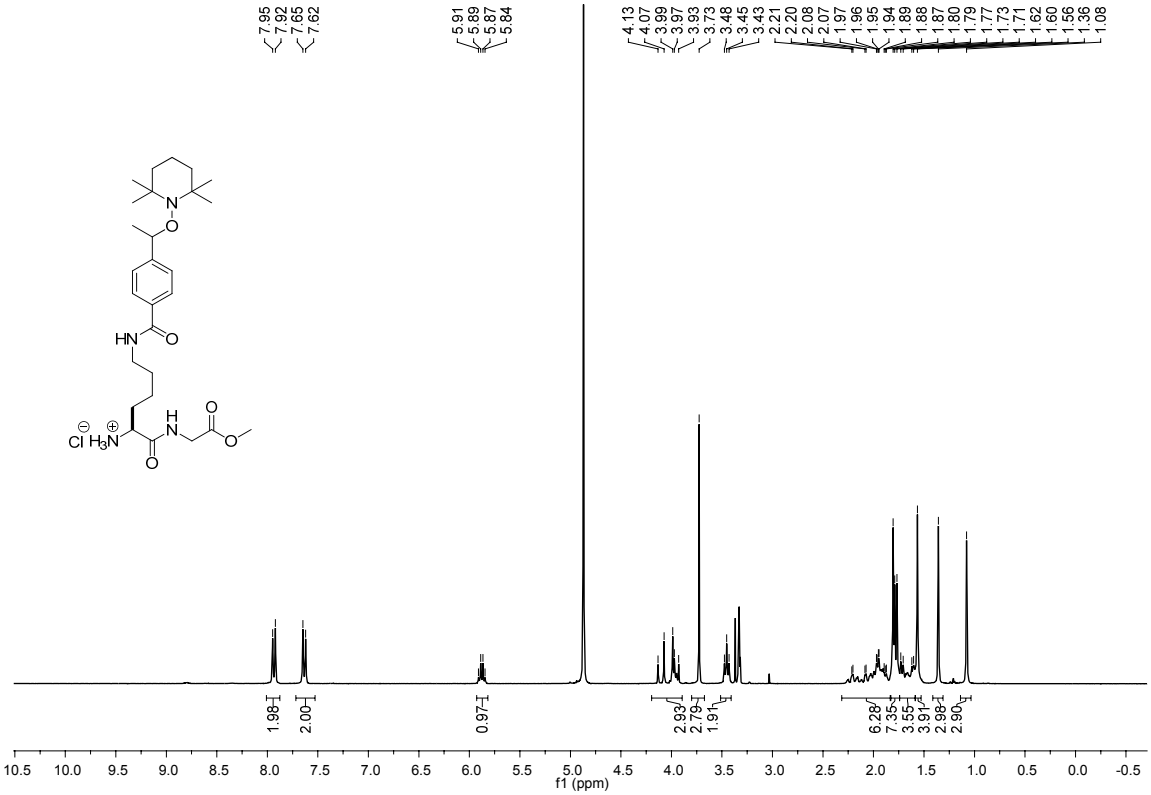
^{1}H-NMR spectrum of 8b



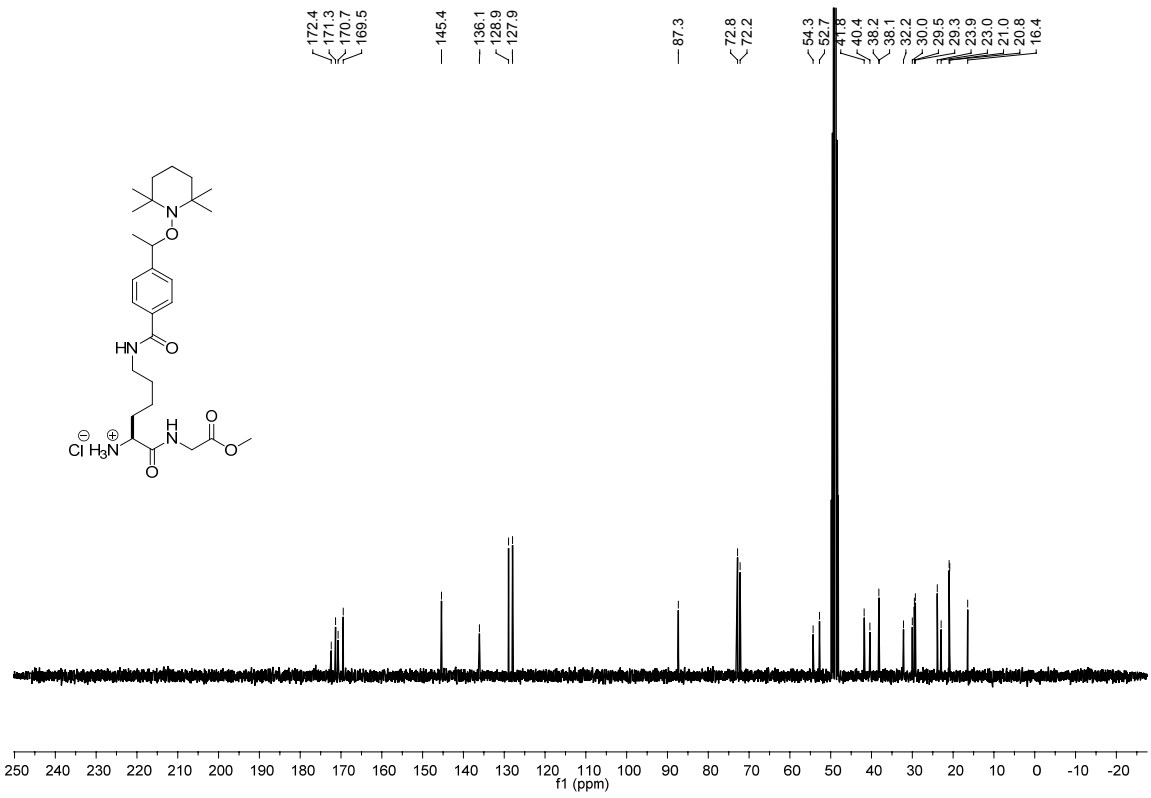
^{13}C-NMR spectrum of 8b



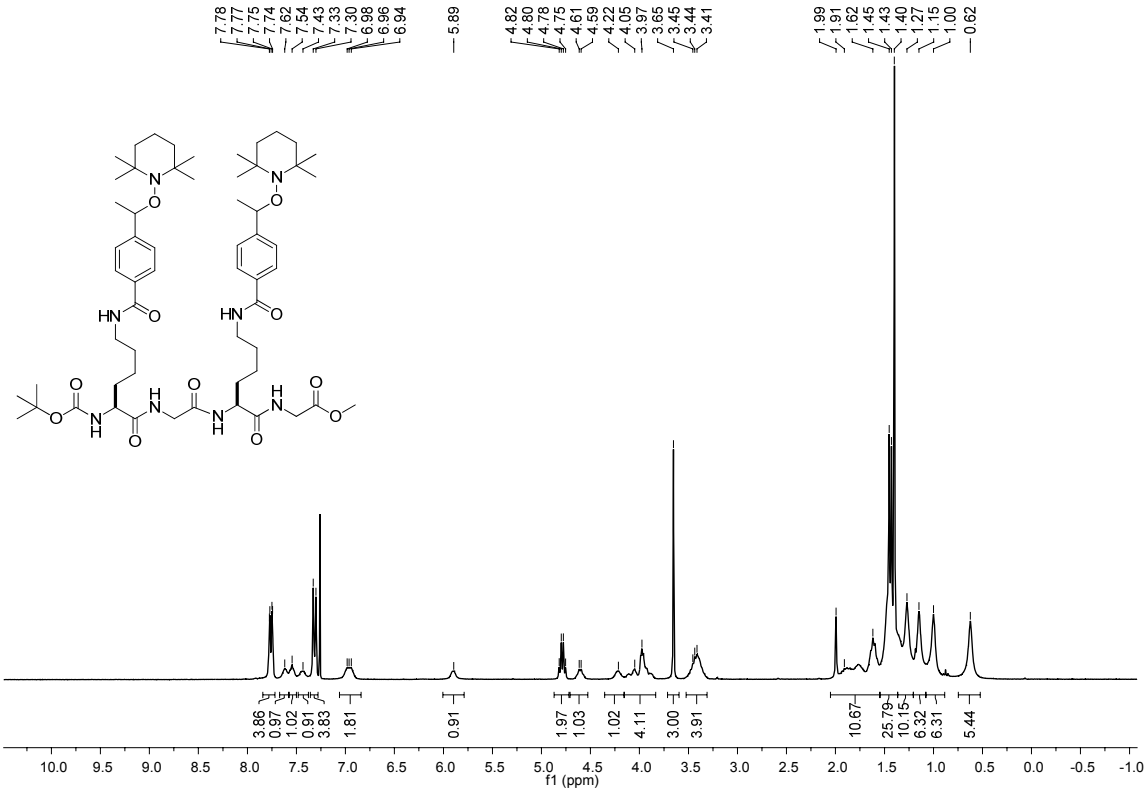
^{1}H-NMR spectrum of 8a



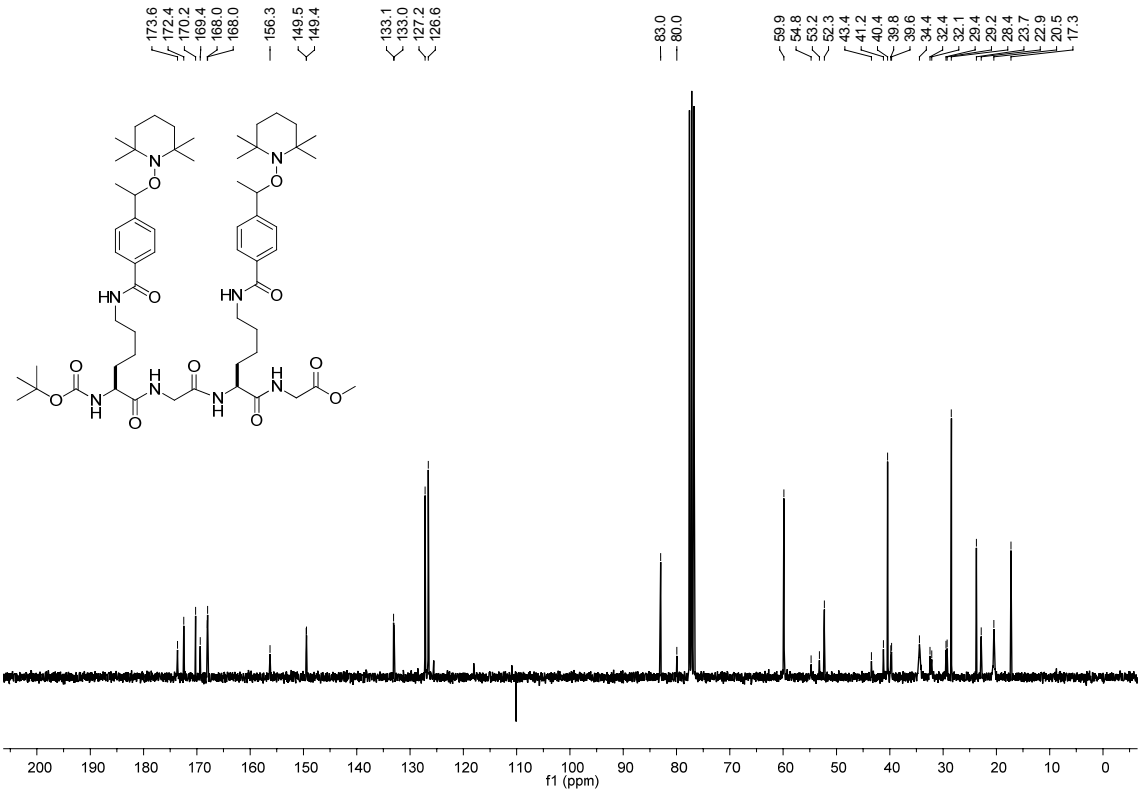
^{13}C-NMR spectrum of 8a



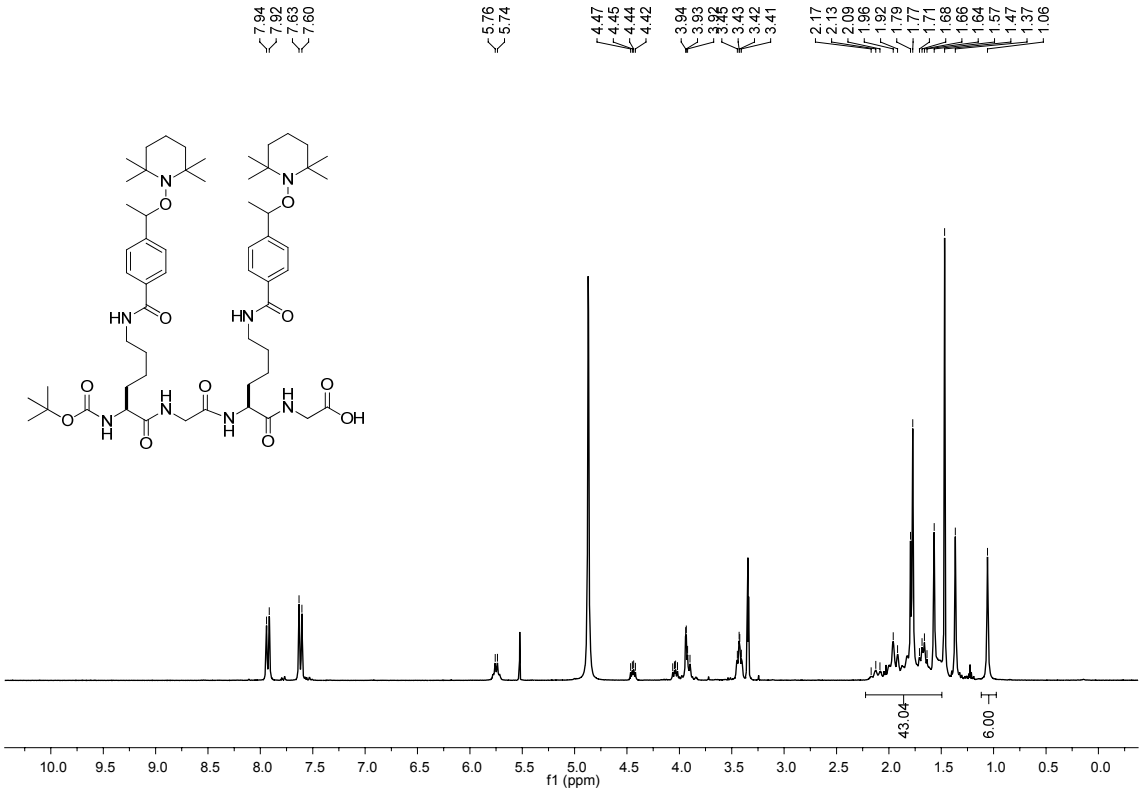
^{1}H-NMR spectrum of 9



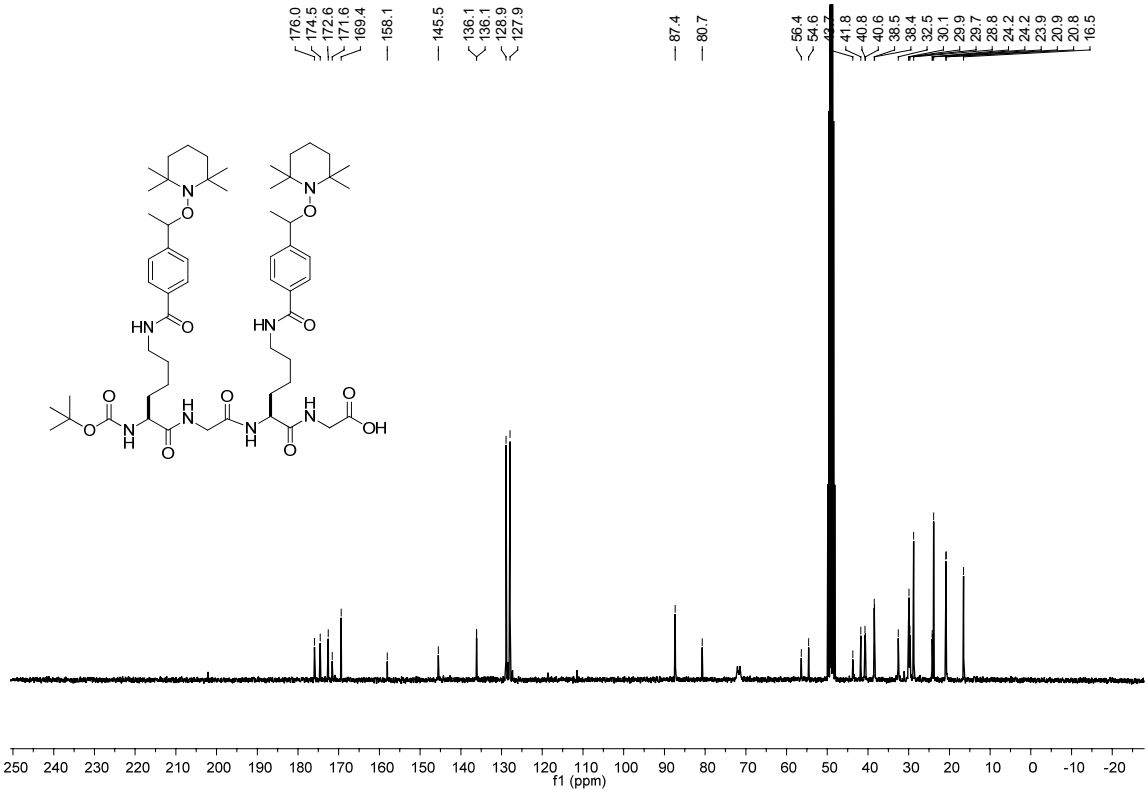
^{13}C-NMR spectrum of 9



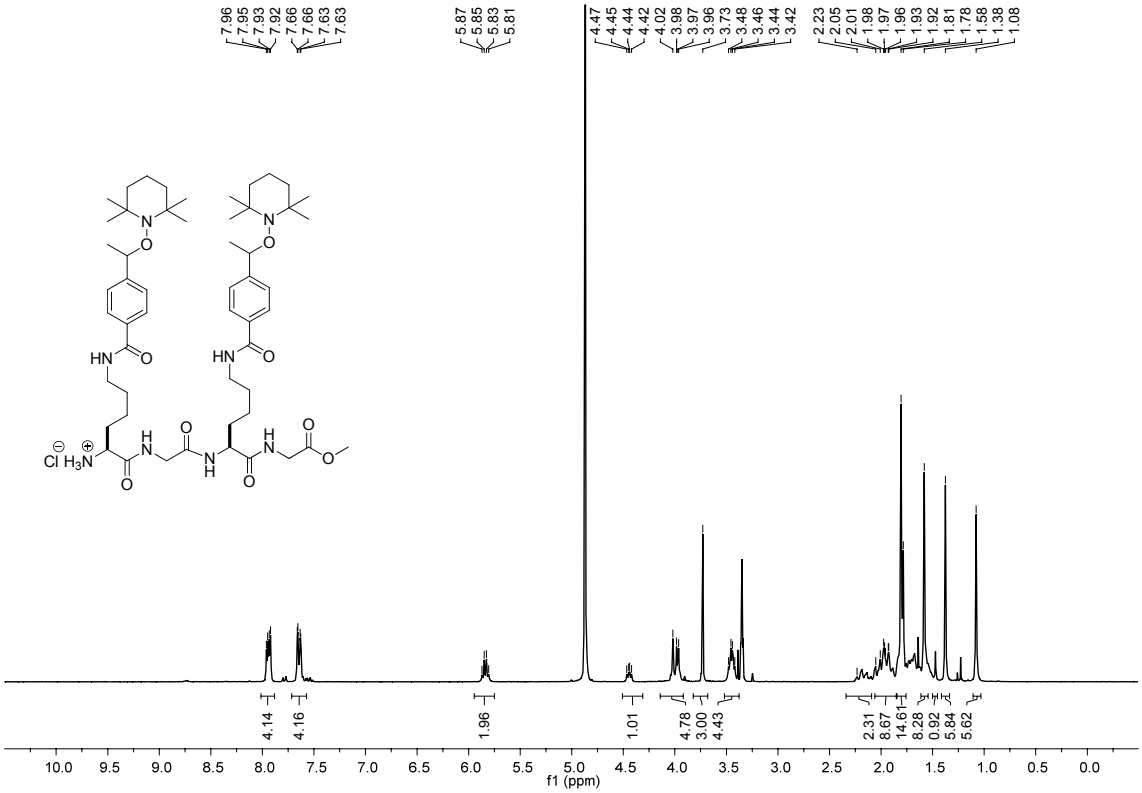
^{1}H-NMR spectrum of 10b



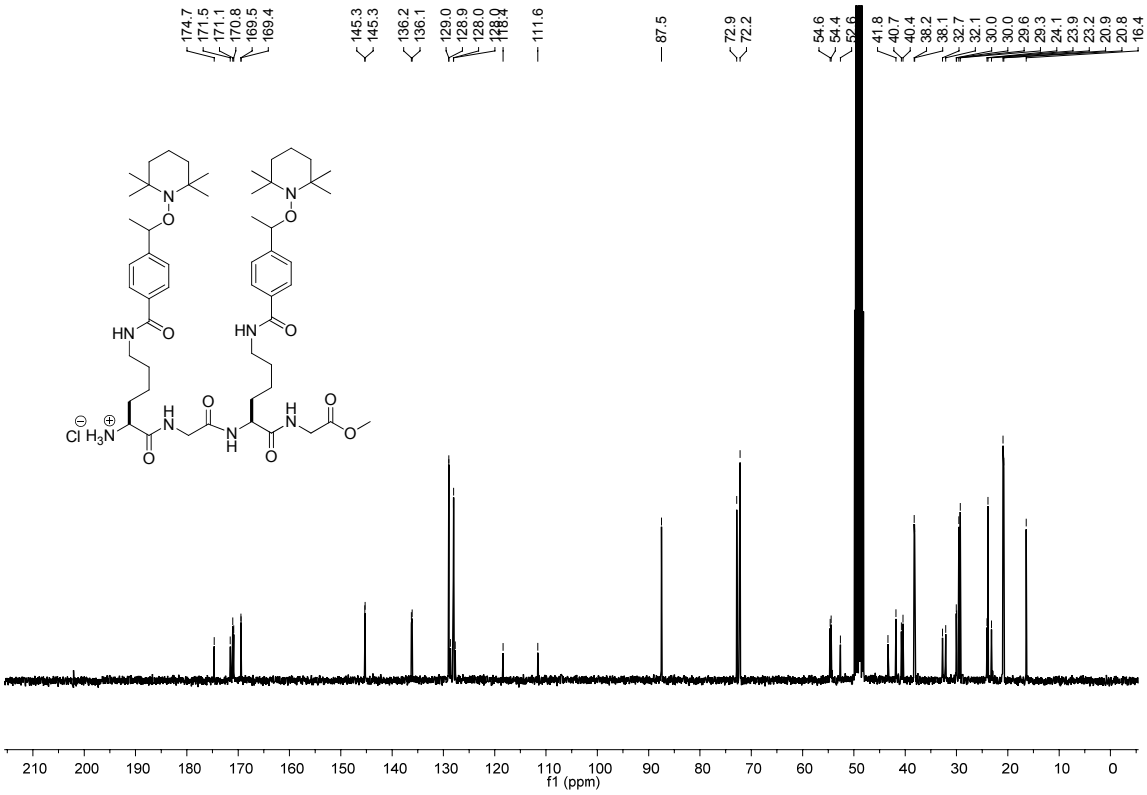
^{13}C-NMR spectrum of 10b



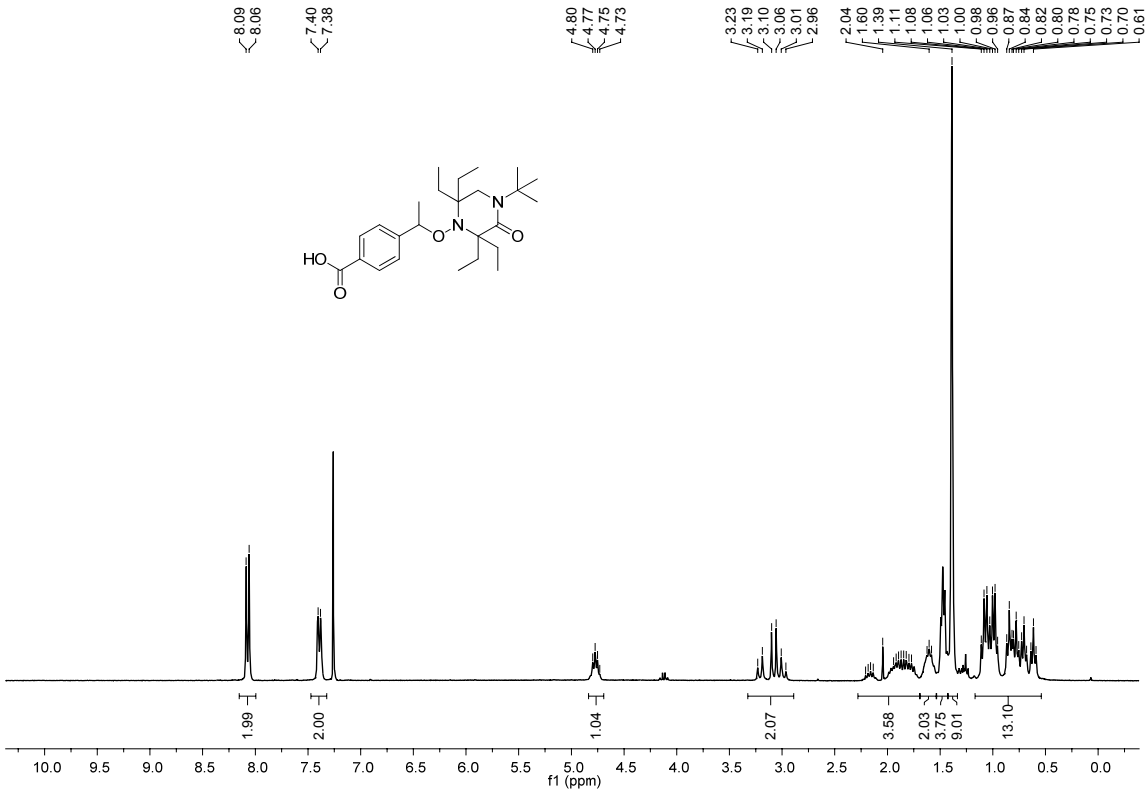
^{1}H-NMR spectrum of 10a



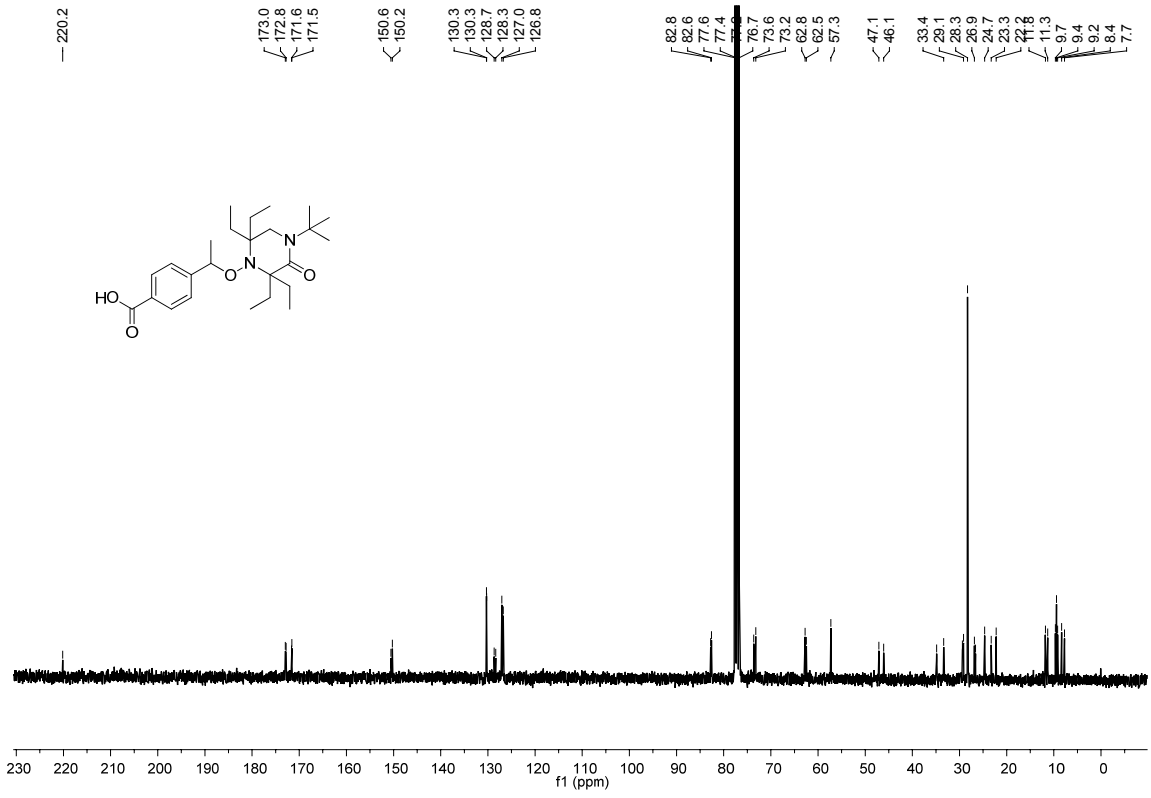
^{13}C-NMR spectrum of 10a



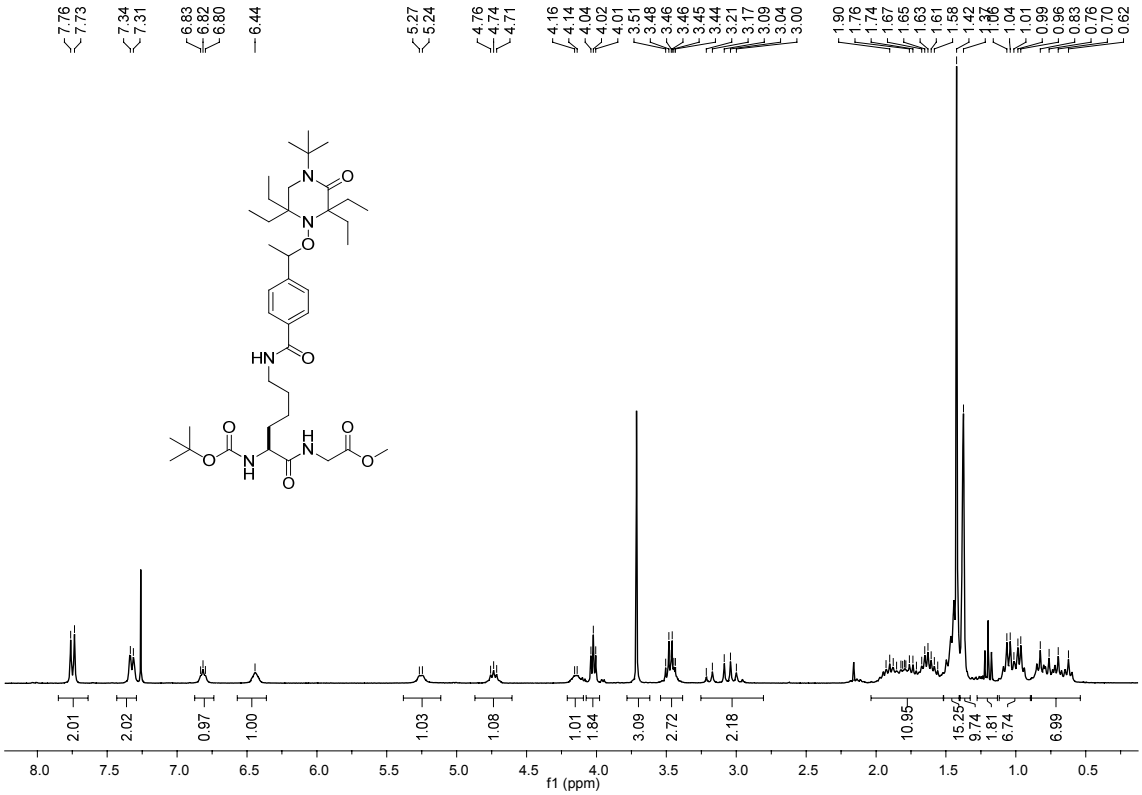
^{1}H-NMR spectrum of 34



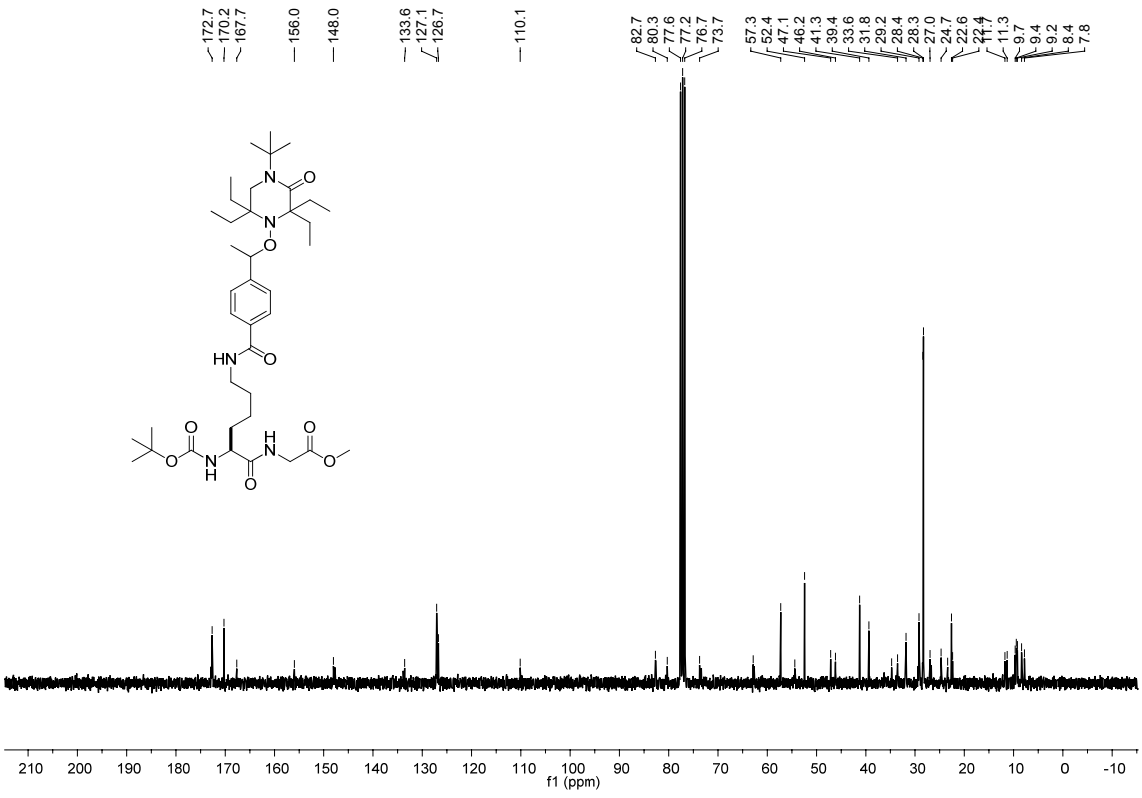
^{13}C-NMR spectrum of 34



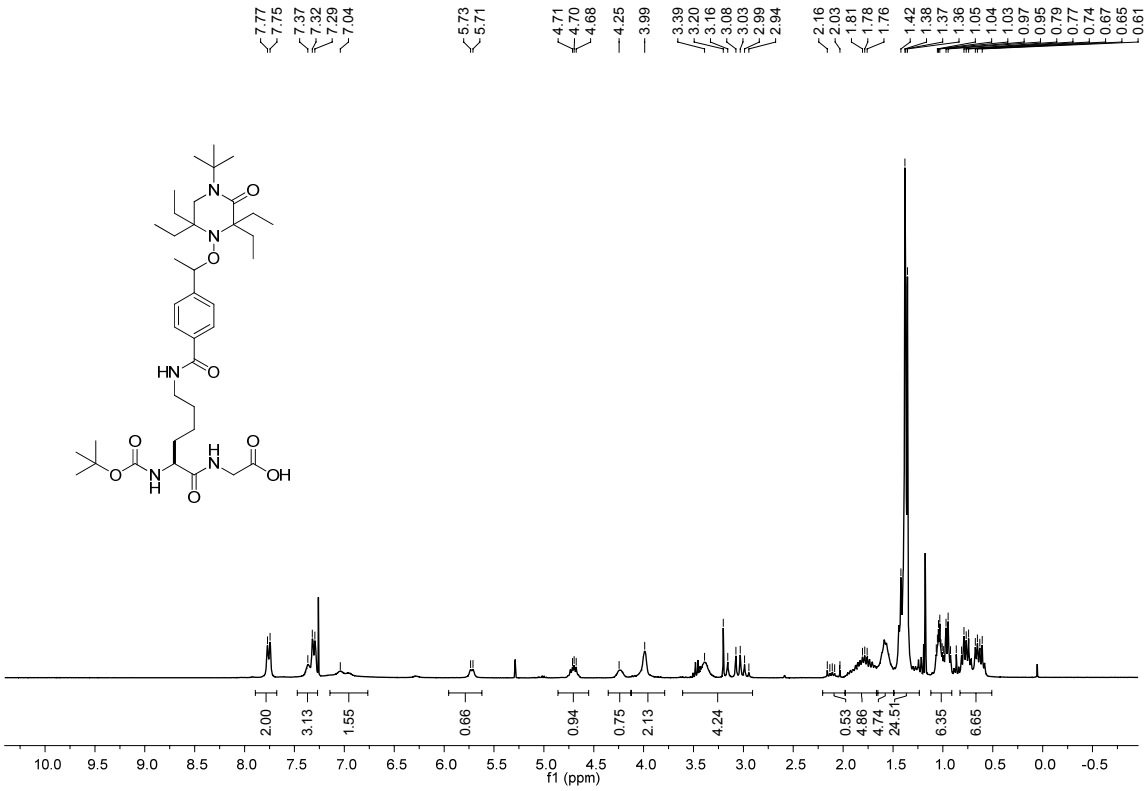
^{1}H-NMR spectrum of 16



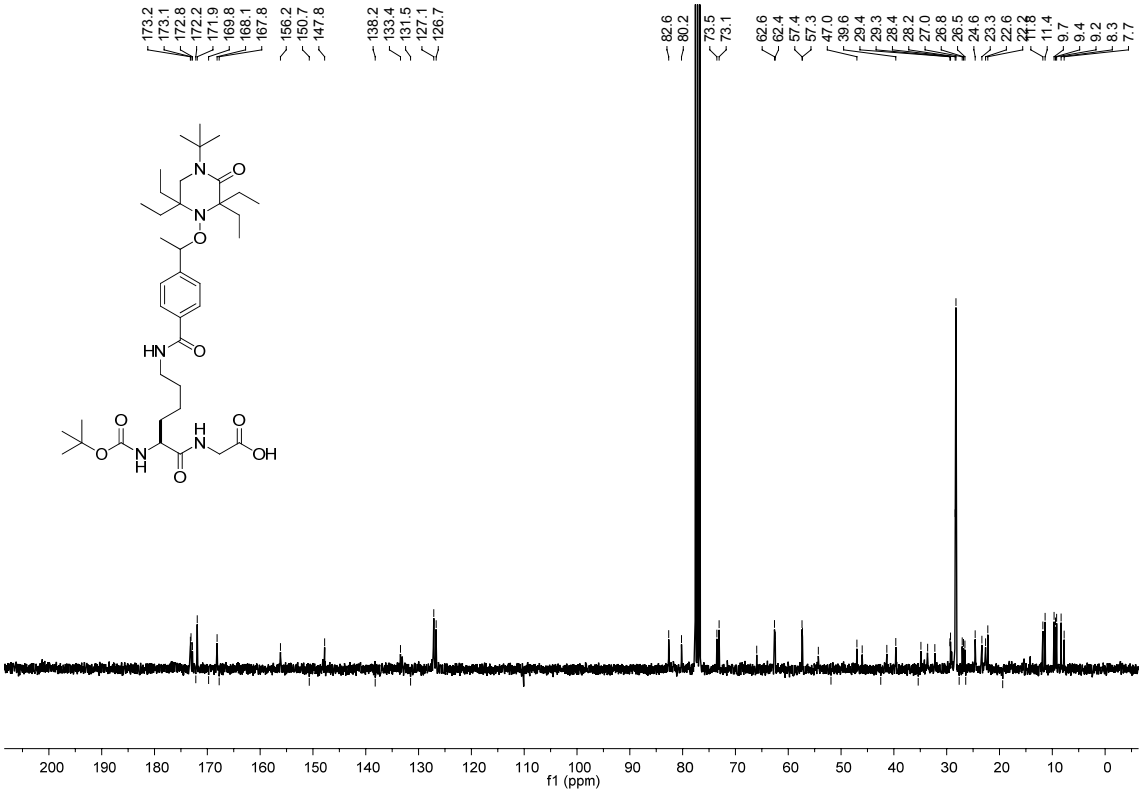
^{13}C-NMR spectrum of 16



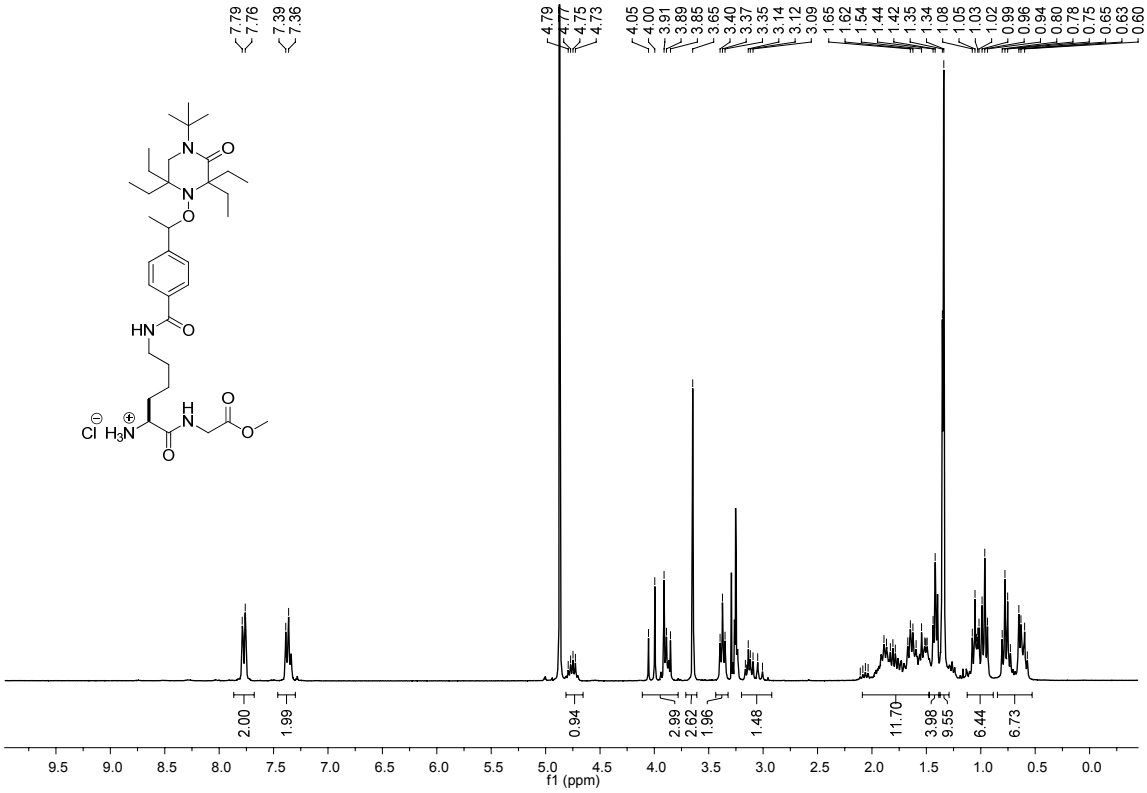
^{1}H-NMR spectrum of 35



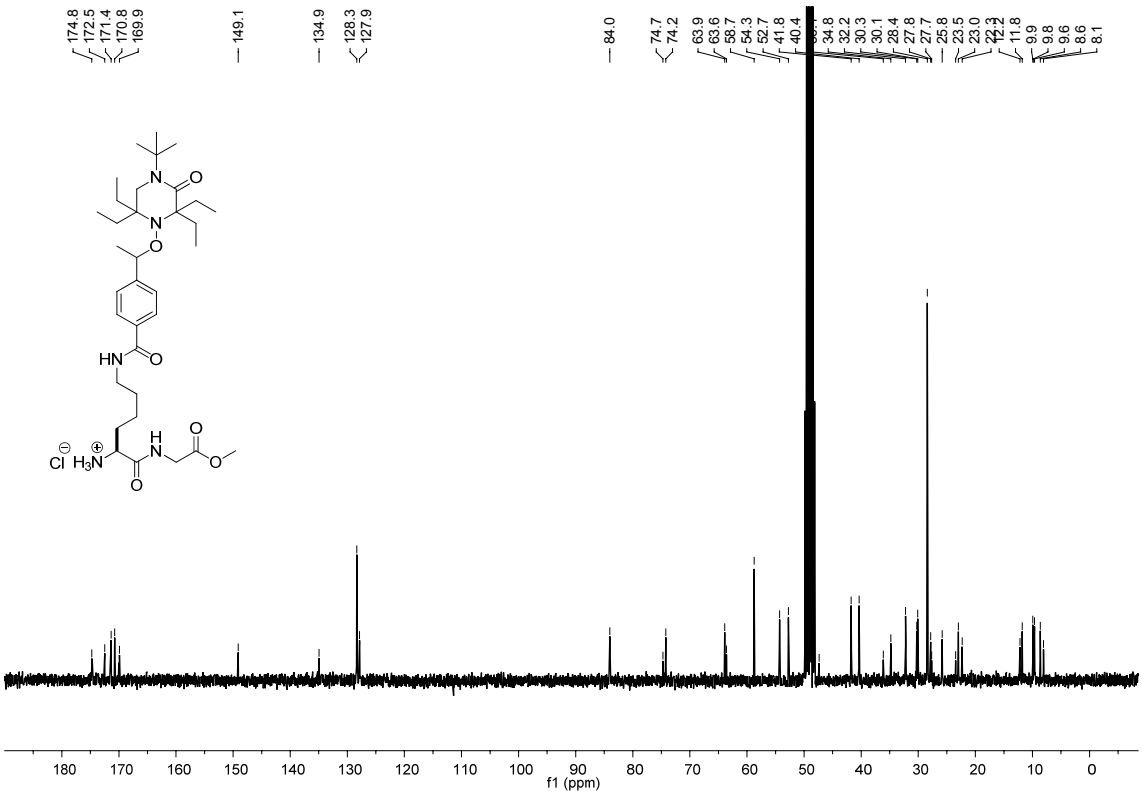
^{13}C-NMR spectrum of 35



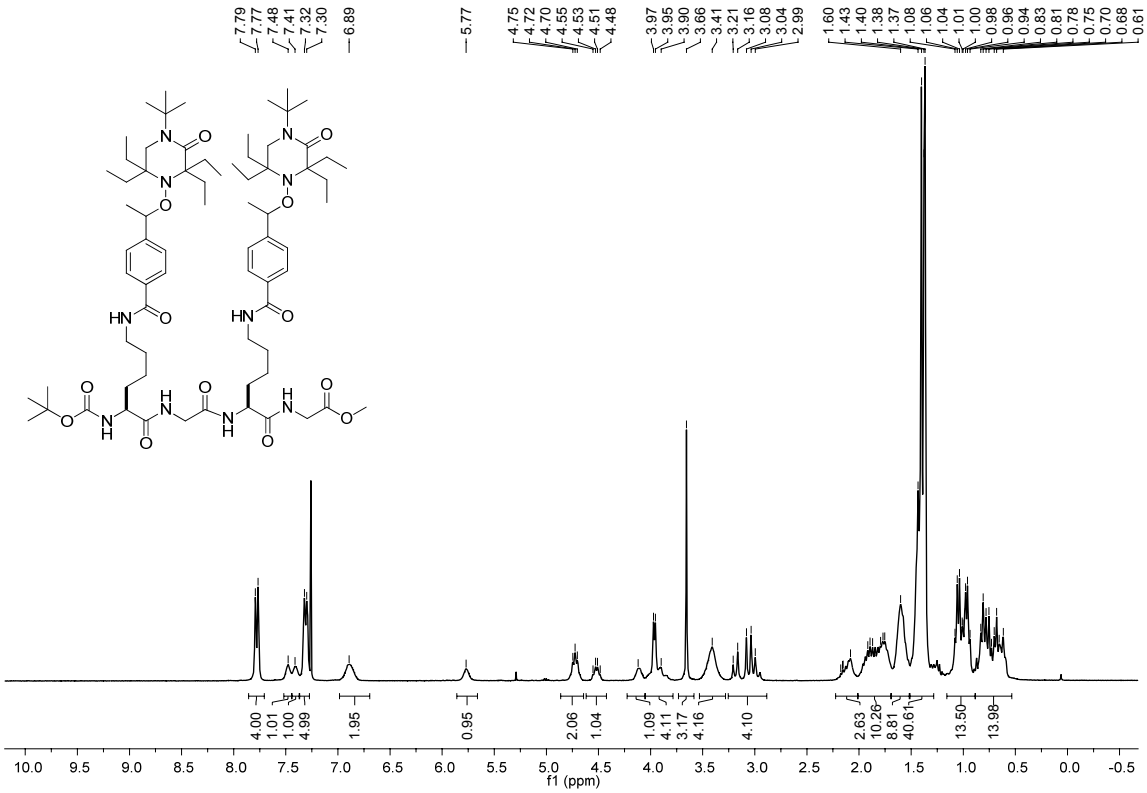
^{1}H-NMR spectrum of 36



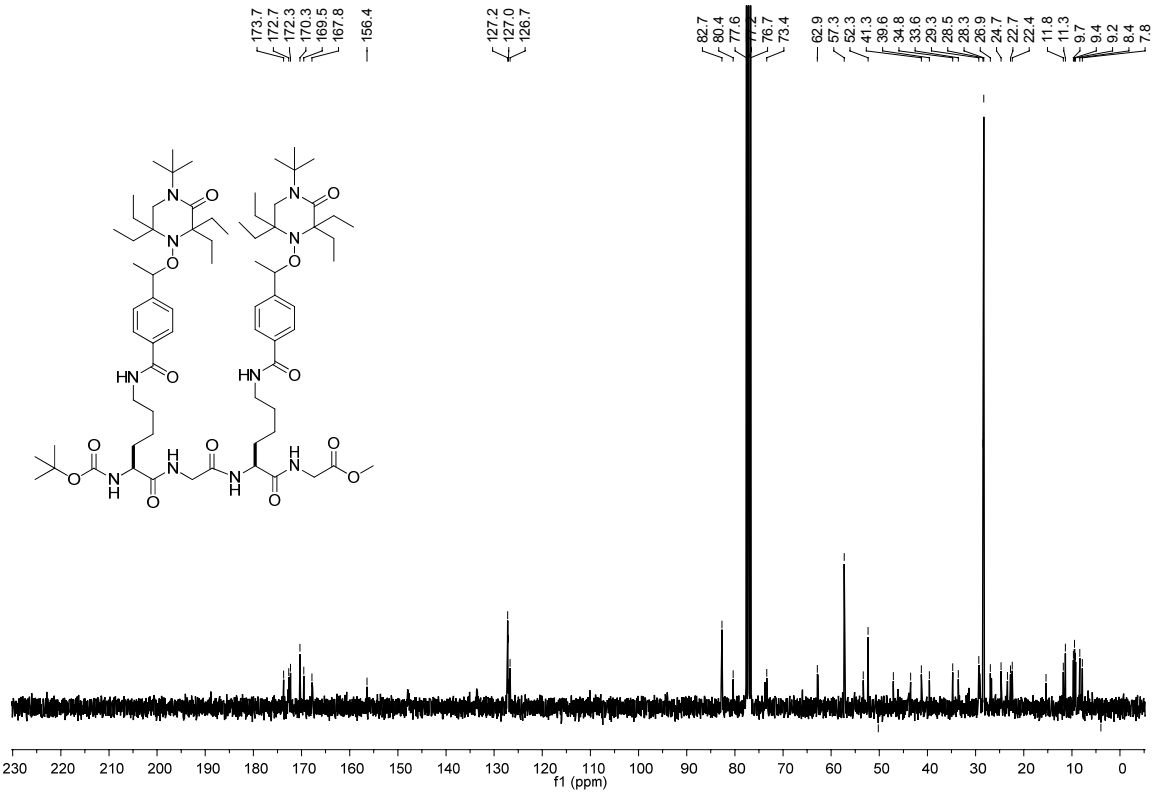
^{13}C-NMR spectrum of 36



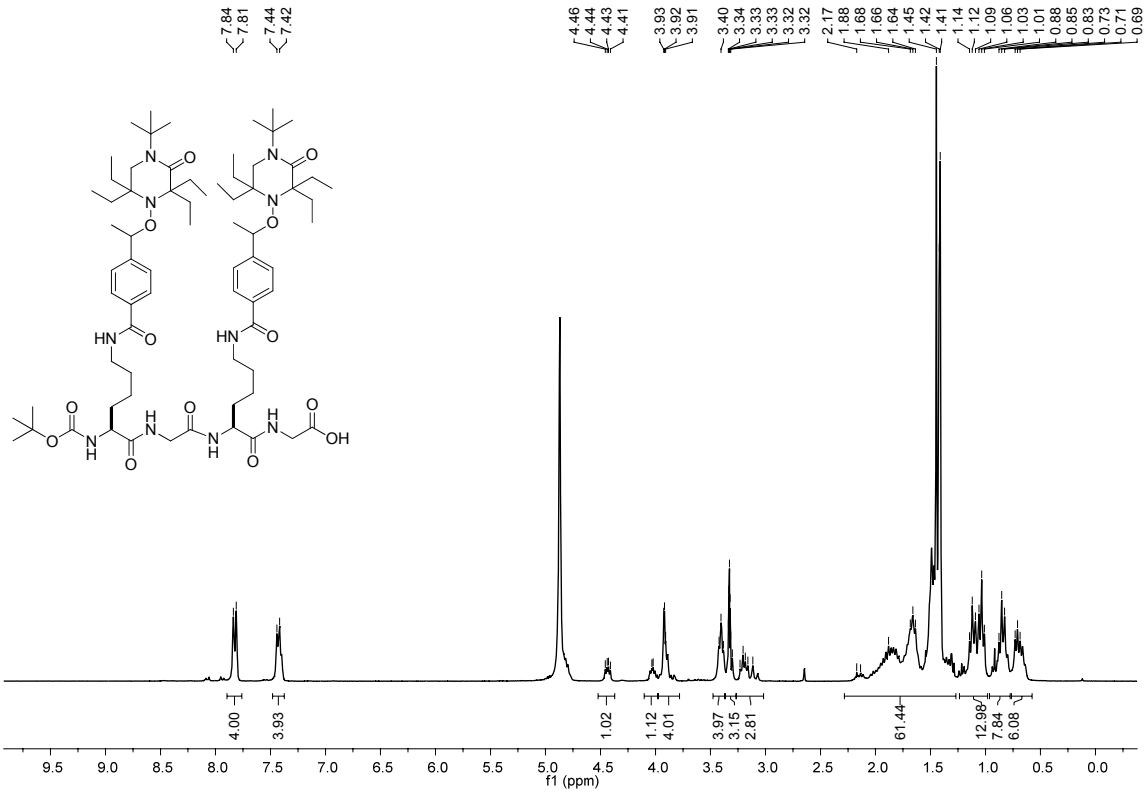
^{1}H-NMR spectrum of 17



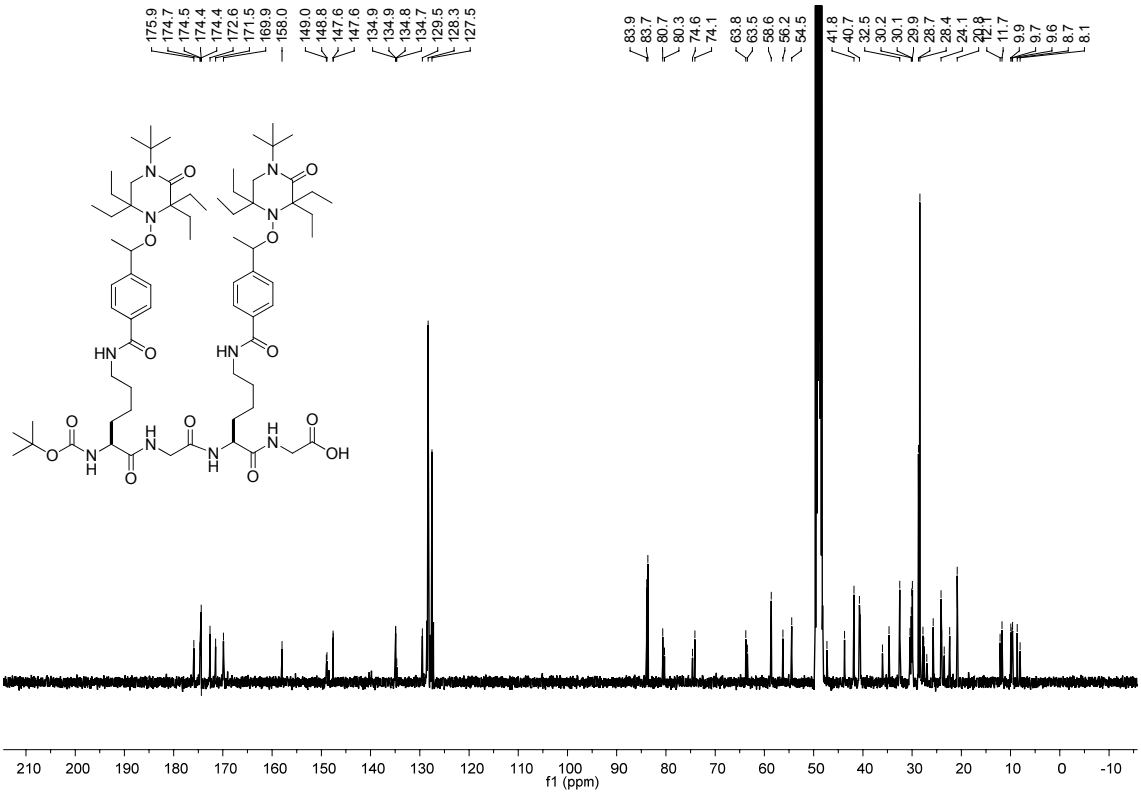
^{13}C-NMR spectrum of 17



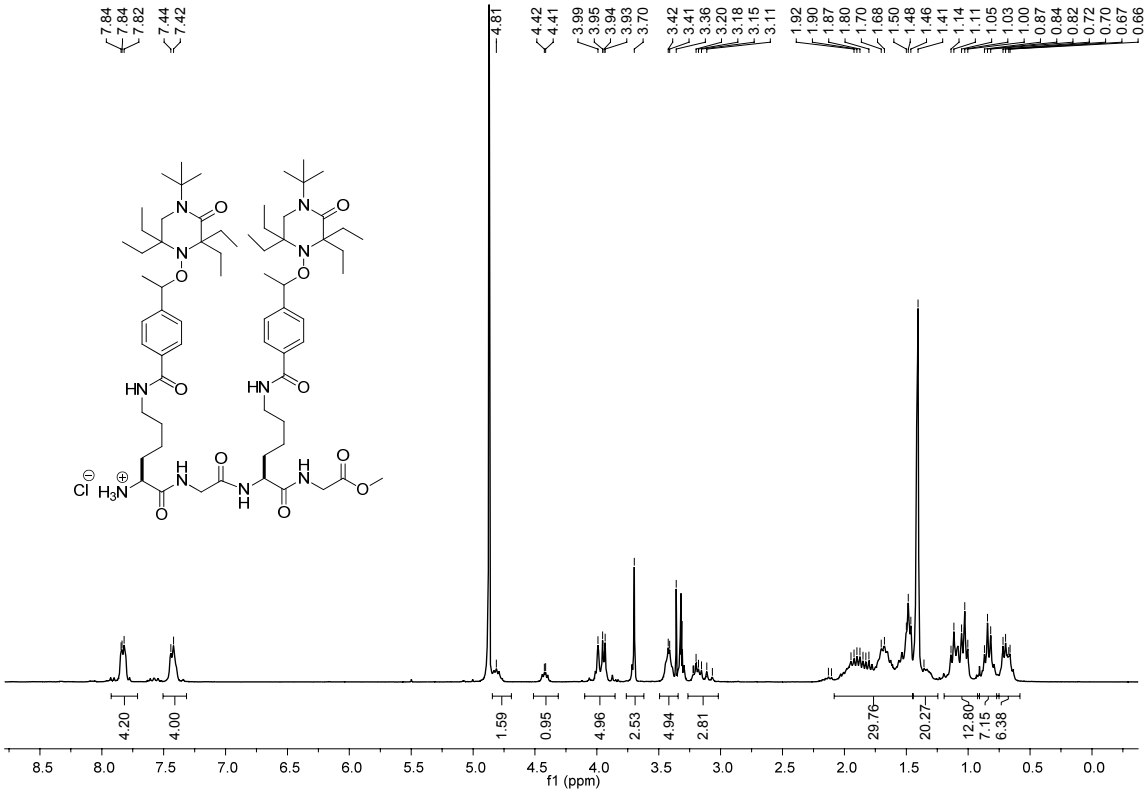
1H-NMR spectrum of 37



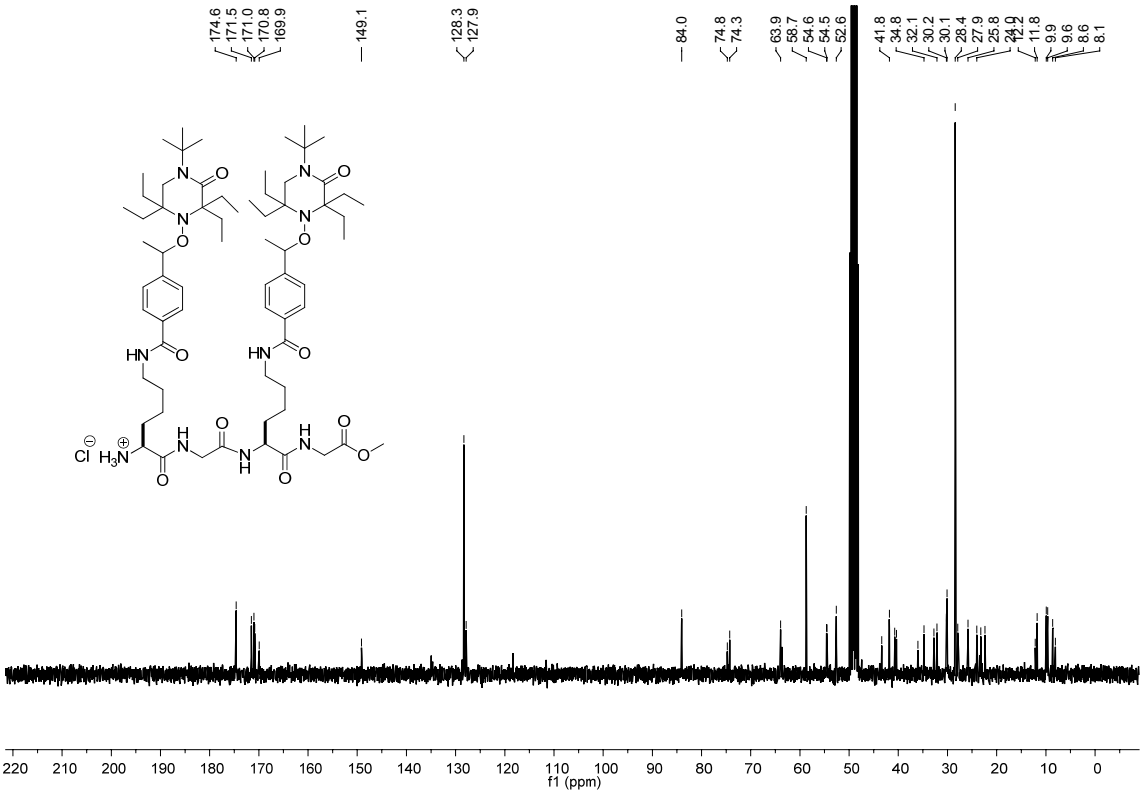
^{13}C-NMR spectrum of 37



^{1}H-NMR spectrum of 38



^{13}C-NMR spectrum of 38



Polymerization results

Polymerization of styrene, T = 125 °C, neat, sealed tube.

Entry	Initiator	Conc. (mol%)	Time (h)	Conv. (%)	$M_{n,th}$ (g/mol)	$M_{n,exp}$ (g/mol)	PDI
1	**1**	0,125	1	10	9300	10100	1,20
2	**1**	0,25	1	6	2900	2600	1,20
3	**1**	1,0	3	15	2200	2400	1,11
4	**1**	0,5	6	16	4000	5700	1,19
5	**1**	1,0	6	24	3000	3200	1,15
6	**1**	0,5	12	48	10600	9500	1,08
7	**1**	1,0	12	40	4800	5500	1,09
8	**1**	0,5	24	64	13800	11400	1,10
9	**1**	1,0	24	56	6400	5000	1,11
10	**9**	0,25	3	12	6100	7100	1,15
11	**9**	0,5	3	19	4900	5000	1,13
12	**9**	0,25	12	48	25500	22300	1,16
13	**9**	0,5	12	42	9800	8800	1,18
14	**9**	0,25	24	72	31000	25000	1,12
15	**9**	0,5	24	62	13900	11200	1,16
16	**11**	0,125	3	23	21500	18900	1,08
17	**11**	0,25	3	16	8700	8100	1,08
18	**11**	0,125	9	42	36900	32000	1,08
19	**11**	0,125	12	53	45800	29600	1,08
20	**11**	0,25	12	59	26500	24900	1,08
21	**11**	0,125	24	64	55200	31200	1,07
22	**11**	0,25	24	62	22600	22900	1,09
23	**13**	0,083	3	24	33300	21200	1,08
24	**13**	0,167	3	27	20000	17000	1,07
25	**13**	0,083	12	51	65600	95200	1,06
26	**13**	0,167	12	43	29800	33100	1,06
27	**14**	0,008	1	13	177300	131700	1,22
28	**14**	0,008	2	22	294200	297500	1,30
29	**14**	0,016	0,5	9	62000	64300	1,18
30	**14**	0,016	1	15	103700	87100	1,14
31	**14**	0,016	3	22	150600	70700	1,15
32	**14**	0,031	0,5	7	28800	30300	1,19
33	**14**	0,031	1	16	56400	37000	1,14

Polymerization of NIPAM, at 125 °C, 1.78 M in benzene, sealed tube.

Entry	Initiator	Conc. (mol%)	TIme (h)	Conv. (%)	$M_{n,th}$ (g/mol)	$M_{n,exp}$ (g/mol)	PDI
1	**16**	0,5	6	24	6100	10000	1,21
2	**16**	1,0	6	15	2500	6900	1,17
3	**16**	0,5	12	33	8100	11400	1,19
4	**16**	1,0	12	38	5000	7400	1,18
5	**16**	0,5	24	56	13500	20200	1,12
6	**16**	1,0	24	76	9300	12600	1,20
7	**17**	0,25	3	19	10000	9400	1,25
8	**17**	0,5	3	16	5000	5900	1,20
9	**17**	0,25	6	27	13700	12200	1,24
10	**17**	0,5	6	21	6200	8200	1,18
11	**17**	0,25	12	43	20900	20700	1,14
12	**17**	0,5	12	37	9700	10300	1,17
13	**17**	0,25	24	64	30200	26500	1,14
14	**17**	0,5	24	71	17400	19500	1,14
15	**18**	0,125	3	21	21800	17500	1,16
16	**18**	0,25	3	25	13600	12100	1,17
17	**18**	0,125	6	31	30500	28500	1,10
18	**18**	0,25	6	29	15700	21200	1,10
19	**18**	0,125	12	44	42700	37600	1,13
20	**18**	0,25	12	46	23100	23800	1,11
21	**18**	0,125	24	57	54300	45500	1,09
22	**18**	0,25	24	74	35700	33600	1,11
23	**19**	0,063	1	23	44300	47900	1,22
24	**19**	0,125	1	33	35500	36600	1,24
25	**19**	0,063	2	33	64500	49800	1,20
26	**19**	0,125	2	44	40000	39000	1,18
27	**19**	0,063	6	50	96400	53600	1,19
28	**19**	0,125	6	71	70000	43400	1,20
29	**19**	0,063	12	73	138200	98300	1,52
30	**19**	0,125	12	78	76400	43400	1,20
31	**19**	0,063	24	85	160100	149300	2,40
32	**19**	0,125	24	87	84500	85900	1,78

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.

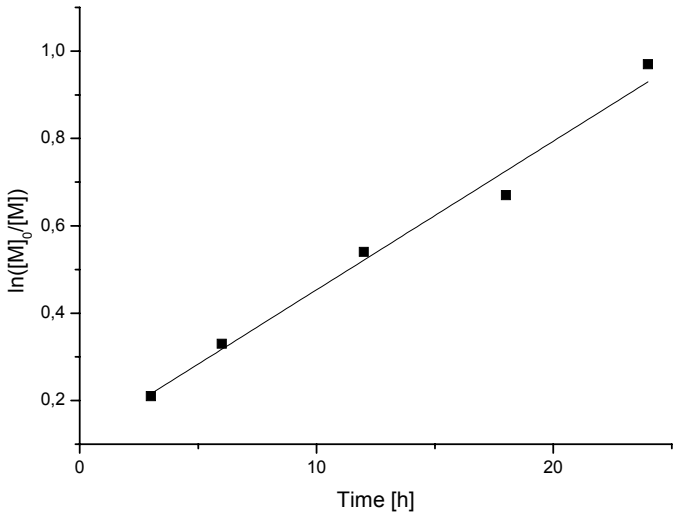


Figure 1: Monomer conversion vs. time (styrene, initiator **9**, 0.5 mol%, 125 °C).

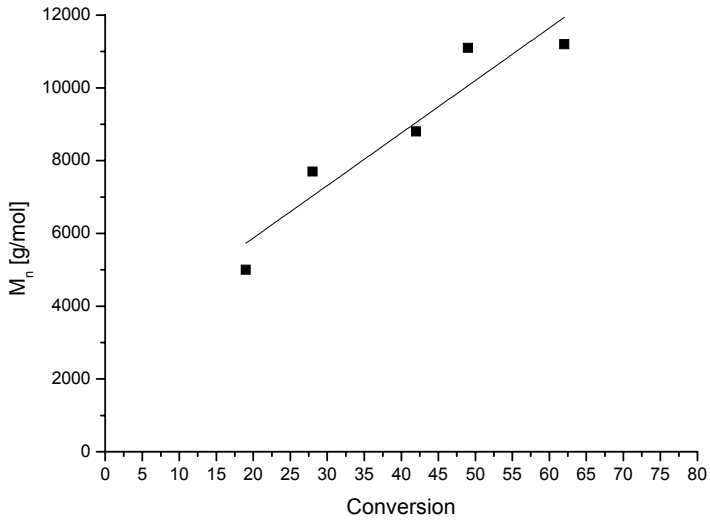


Figure 2: $M_{n,exp.}$ vs. monomer conversion (styrene, initiator **9**, 0.5 mol%, 125 °C).

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.

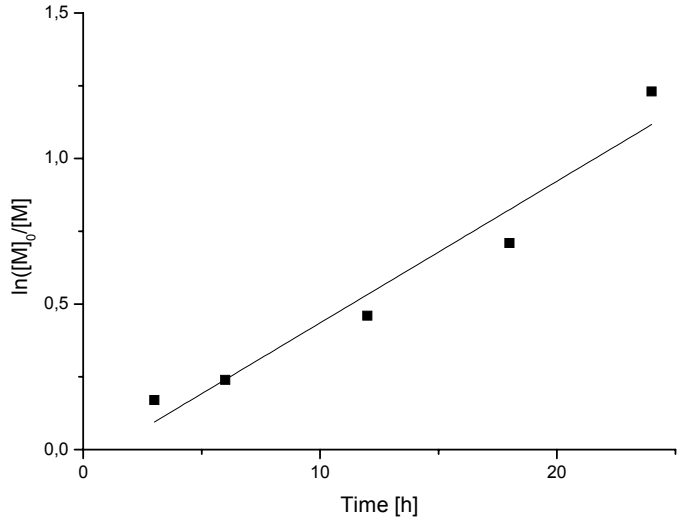


Figure 3: Monomer conversion vs. time (NIPAM, initiator **17**, 0.5 mol%, 125 °C).

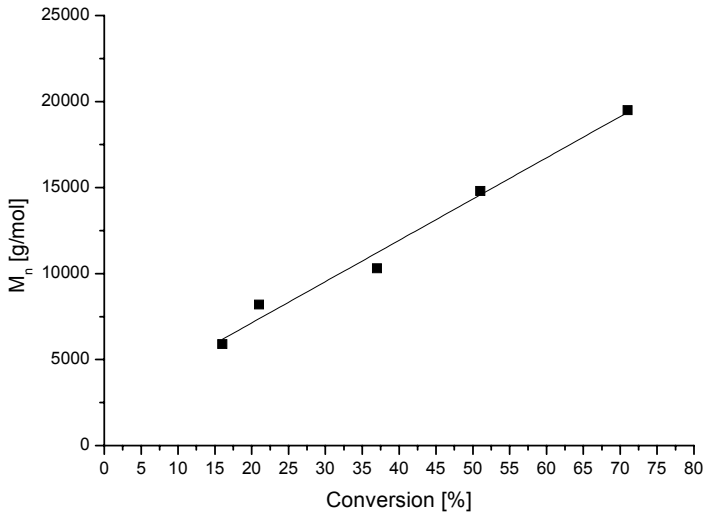


Figure 4: Molecular weight vs. conversion (NIPAM, initiator **17**, 0.5 mol%, 125 °C).

DLS-data of peptide-PNIPAM conjugates

Temperature [°C]	n =1		n = 2		n= 4		n = 8	
	$V_{(max)}$ [nm]	$n_{(max)}$ [nm]	$V_{(max)}$ [nm]	$n_{(max)}$ [nm]	$V_{(max)}$ [nm]	$n_{(max)}$ [nm]	$V_{(max)}$ [nm]	$n_{(max)}$ [nm]
10	-	-	4.50	3.93	6.43	5.23	11.83	8.88
12	-	-	4.28	3.68	5.98	4.88	12.10	9.36
14	-	-	4.42	3.86	55.08	42.88	12.19	9.60
16	3.07	2.31	4.28	3.68	79.02	63.25	11.87	9.08
18	3.44	2.71	4.32	3.77	109.4	90.73	11.71	8.87
20	3.63	2.97	320.6	252.8	148.8	125.5	11.72	8.83
21	3.66	3.15	488.1	426.6	-	-	11.85	9.02
22	3.91	3.48	569.9	494.9	183.9	157.7	12.36	9.13
23	89.5	69.5	644.3	594.6	-	-	54.69	40.62
24	111.3	89.4	672.3	625.8	207.2	172.0	83.60	66.76
25	168.0	130.1	701.7	649.0	-	-	107.5	88.49
26	234.6	185.2	738.0	676.2	213.7	181.8	116.6	98.15
27	346.0	233.2	750.6	687.6	-	-	-	-
28	437.7	260.9	802.2	711.4	218.6	176.3	130.0	110.0
29	497.4	265.8	814.2	727.6	-	-	-	-
30	503.7	386.8	843.9	757.8	212.8	175.4	128.8	108.1
32	536.9	366.0	899.2	737.4	212.1	176.2	125.2	106.4
34	708.1	338.9	905.3	768.2	216.2	173.5	124.7	106.0

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

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