Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information:

Reversible Switching of Substrate Activity of Poly-*N*-Isopropylacrylamide Peptide Conjugates

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General

Copper powder (*Aldrich*), $Cu(OTf)_2$ (*Aldrich*), 4,4'-di-*tert*-butyl-[2,2']bipyridine (*Aldrich*), *tert*-butyllithium (1.7 M in pentane, *Acros*), $LiAlH_4$ (*Merck*), chlorotimethylsilane (*Janssen Chimica*), NaI (*Fluka*), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI•HCl, *Fluka*), 1-hydroxybenzotriazole hydrate (HOBt, *Fluka*), *N*-methyl morpholine (*Merck*), trifluoroacetic acid (*Merck*), 1-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ, *Fluka*), TRIZMA® base (*Sigma*), $CaCl_2$•(H_2O) (*Fluka*) and chymotrypsin (91 U/mg, *Fluka*) were used as received. 1-Bromo-4-(1-bromoethyl)benzene,[1] 2,2,6,6-tetraethyl-4-methoxy-piperidine-1-oxyl[2,3] and {4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-phenyl}-methanol[4,5] were synthesized according to literature procedures. Oligopeptides and peptide-*p*-nitroanilides[6] are all purchasable and were synthesized applying standard solution phase Boc-strategy similar to the procedures described in this work. Styrene (*BASF)* and *n*-butyl acrylate (*Fluka*) were both distilled from CaH_2 under reduced pressure and *N*-isopropyl acrylamide (*Aldrich*) was recrystallized twice from *n*-hexane to remove the stabilizer. All monomers were stored at -30 °C under an argon atmosphere. Ultrapure water (18.2 MΩ) was produced using an *ELGA Maxima*. Benzene was distilled from Na, THF was distilled from K, dichloromethane was distilled from P_2O_5 and DMF and acetonitril were both destilled from CaH_2. All solvents for extraction and flash chromatography were distilled before use. All reactions with air or moisture sensitive starting materials, products, or intermediates were performed using standard Schlenk techniques.

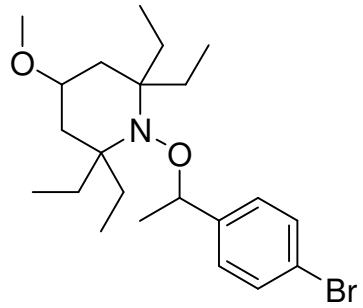
^{1}H-NMR (600 MHz, 500 MHz, 400 MHz, 300 MHz) and ^{13}C-NMR (150 MHz, 125 MHZ, 100 MHz, 75 MHz) spectra were recorded on a *Varian 600 unity plus*, a *Varian Inova 500*, a *Bruker AMX 400* or a *Bruker DPX 300* spectrometer. Chemical shifts δ in ppm are referenced to the solvent residual peak as an internal standard. TLC was carried out on *Merck* silica gel 60 F_{254} plates; detection by UV or dipping into a

solution of $Ce(SO_4)_2 \cdot H_2O$ (10 g), phosphormolybdic acid hydrate (25 g) and conc. H_2SO_4 (60 mL) in water (0.94 L), a solution of $NaHCO_3$ (5 g) and $KMnO_4$ (1.5 g) in water (400 mL) or a solution of ninhydrine (421 mg) and acetic acid (1.4 mL) in water (9.1 mL) and *n*-butanol (200 mL) followed by heating. Flash chromatography was carried out on *Merck* or *Fluka* silica gel 60 (40 – 63 μm) with an argon pressure of about 0.1-0.6 bar. IR spectra were recorded on a *Digilab FTS 4000* equipped with a *MKII Golden Gate Single Reflection ATR System* or a *Bruker IFS 28*. ESI-MS and HRMS were performed using a *Bruker MicroTof*. The mass spectra of the PNIPAM peptide conjugates were analysed using the simulation software *PolyCalc*.[7] Elemental analyses were performed on a *Vario EL III (Elementar)*. Melting points were determined with a *Stuart SMP10* and are uncorrected. UV/Vis spectra were recorded on a *Shimadzu UV-1601PC* or a *Shimadzu UV-2401PC* connected to a *MGW Lauda M3* thermostat. Data were analyzed with *Shimadzu UVPC* version 3.5 or version 3.9. Size exclusion chromatography (SEC) was carried out with degased THF as eluent at a flow rate of 1.0 ml/min at 25 °C on a system consisting of a *L6200A Intelligent Pump (Merck Hitachi)* or a *Knauer HPLC Pump 64*, a set of two *PLgel 5 μm MIXED-C* columms (300 × 7.5 mm, *Polymer Laboratories*; linear range of molecular weight: 200-2.000.000 g/mol) and a *Knauer Differential Refractometer* (λ = 950 ± 30 nm) detector. Data were analyzed with *PSS WinGPC Compact V.7.20* software (*Polymer Standards Service*) based upon calibration curves built upon 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 M-M-10) with peak molecular weights ranging from 200 to 1000000 g/mol. Multiple angle laser light scattering (MALLS) was performed with a *PSS SLD 7000* (LASER: P = 30 mW, λ = 660 nm; Angles: 35, 50, 75, 105, 130, 145 °) at a cell temperature of about 25 °C. The light scattering detector was used in combination with the GPC-apparatus described above and the concentration detector was thus a *Knauer Differential Refractometer* (λ = 950 ± 30 nm). The PNIPAM peptide conjugates were dissolved in THF at a concentration of 2 to 5 mmol/L (referring to $M_{n,\,theor.}$). The refractive index increments for the PNIPAM peptide conjugates were determined using a *PSS dn/dc 2010* Ablenkrefraktometer. The samples were dissolved in THF (1-8 g/L) and the measurements were performed at a temperature of 27 °C using a laser with a

wavelength of 620 nm. The LCST of the PNIPAM peptide conjugates were determined by ^{1}H-NMR spectroscopy[8] with a *Varian 600 unity plus*.

Procedures

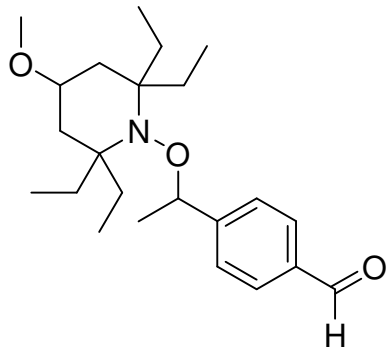
1-[1-(4-Bromo-phenyl)-ethoxy]-2,2,6,6-tetraethyl-4-methoxy-piperidine (3a)



1-Bromo-4-(1-bromoethyl)benzene (3.625 g, 13.73 mmol, 1.05 eq.), 2,2,6,6-tetraethyl-4-methoxy-piperidine-1-oxyl (**2b)** (3.170 g, 13.08 mmol, 1.00 eq.), copper powder (1.070 g, 16.84 mmol, 1.30 eq.), $Cu(OTf)_2$ (47 mg, 0.13 mmol, 0.01 eq.) and 4,4'-di-*tert*-butyl-[2,2']bipyridine (140 mg, 0.52 mmol, 0.04 eq.) were suspended in benzene (26 mL) and the reaction mixture was heated to 75 °C in a sealed tube for 18 h. Afterwards the mixture was filtered through a pad of silica gel and the solvents were removed under reduced pressure. Purification by flash chromatography (diethyl ether/pentane, 1:40→1:10) afforded the alkoxyamine **3a** as a light orange solid (4.980 g, 11.68 mmol, 89%).[9]

mp: 48 °C. IR (neat): 2965*s*, 2879*m*, 2816*w*, 1591*w*, 1463*s*, 1374*m*, 1332*w*, 1199*w*, 1152*m*, 1096*s*, 1067*m*, 1010*m*, 944*w*, 910*m*, 823*s*, 786*m*, 733*s* cm^{-1}. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.41 (*d*, ^3J = 8.4 Hz, 2 H, Ar-H); 7.13 (*d*, ^3J = 8.4 Hz, 2 H, Ar-H); 4.66 (*q*, ^3J = 6.6 Hz, 1 H, NOCH); 3.35 (*tt*, ^3J = 11.4 Hz, ^3J = 3.9 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 2.14-0.81 (*m*, 12 H, 6 CH_2); 1.38 (*d*, ^3J = 6.6 Hz, 3 H, $NOCCH_3$); 1.05 (*t*, ^3J = 7.4 Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.72 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.65 (*t*, ^3J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 145.5 (C), 131.0 (CH), 127.6 (CH), 120.4 (C), 81.8 (NOCH), 71.2 (MeOCH), 65.4 (C), 65.1 (C), 55.6 (OCH_3), 36.3 (CH_2), 35.8 (CH_2), 30.3 (CH_2), 29.5 (CH_2), 27.5 (CH_2), 27.2 (CH_2), 24.8 (NOC*C*H_3), 10.1 (CH_3), 9.9 (CH_3), 8.2 (CH_3), 7.9 (CH_3) ppm. ESI-MS: *m/z*: 448 ([M+Na]$^+$), 426 ([M+H]$^+$), 265 ([Nitroxide+Na]$^+$). HR-ESI-MS: calcd for $[C_{22}H_{37}BrNO_2]^+$ ([M+H]$^+$): 426.2002; found: 426.1997.

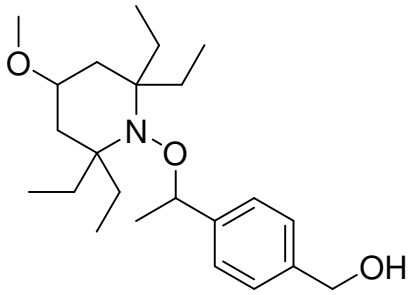
4-[1-(2,2,6,6-Tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzaldehyde



Alkoxyamine **3a** (4.980 g, 11.68 mmol, 1.00 eq.) was dissolved in THF (60 mL) and the solution was cooled to -78 °C. *tert*-buthyllithium (1.7 M in pentane; 14.1 mL, 23.97 mmol, 2.05 eq.) was added over a period of 30 min and the reaction mixture was stirred for another 30 min. Then DMF (5.4 mL, 70.08 mmol, 6.00 eq.) was added dropwise and the resulting suspension was stirred for 3 h at -78 °C. Afterwards the reaction mixture was allowed to warm to room temperature and was hydrolysed by adding NH_4Cl (aq. sat., 100 mL). The aqueous phase was extracted with diethyl ether (3 times 100 mL) and the combined organic layers were dried over $MgSO_4$. After purification by flash chromatography (diethyl ether/pentane, 1:10→1:3) the desired aldehyde was isolated as a light orange oil (4.280 g, 11.40 mmol, 98%).

IR (neat): 2964*s*, 2879*m*, 2817*w*, 2730*w*, 1704*s*, 1607*s*, 1578*w*, 1462*s*, 1377*m*, 1337*w*, 1304*w*, 1207*s*, 1154*m*, 1096*s*, 1058*s*, 990*m*, 942*w*, 910*w*, 830*s*, 766*s*, 623*w* cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): δ = 9.99 (*s*, 1 H, CHO); 7.82 (*d*, ^3J = 8.0 Hz, 2 H, Ar-H); 7.42 (*d*, ^3J = 8.0 Hz, 2 H, Ar-H); 4.77 (*q*, ^3J = 6.5 Hz, 1 H, NOCH); 3.36 (*tt*, ^3J = 11.5 Hz, ^3J = 3.5 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 2.13-0.88 (*m*, 12 H, 6 CH_2); 1.41 (*d*, ^3J = 6.5 Hz, 3 H, $NOCCH_3$); 1.06 (*t*, ^3J = 7.0 Hz, 3 H, CH_3); 0.93 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.71 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.64 (*t*, ^3J = 7.5 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 191.1 (CHO), 153.4 (C), 135.0 (C), 129.6 (CH), 126.3 (CH), 82.1 (NOCH), 71.0 (MeOCH), 65.4 (C), 65.0 (C), 55.6 (OCH_3), 36.1 (CH_2), 35.6 (CH_2), 30.2 (CH_2), 29.2 (CH_2), 27.4 (CH_2), 27.0 (CH_2), 24.7 ($NOCCH_3$), 10.1 (CH_3), 9.8 (CH_3), 8.1 (CH_3), 7.8 (CH_3) ppm. ESI-MS: *m/z*: 430 ($[M+MeOH+Na]^+$), 408 ($[M+MeOH+H]^+$), 398 ($[M+Na]^+$), 376 ($[M+H]^+$), 265 ($[Nitroxide+Na]^+$). HR-ESI-MS: calcd for $[C_{23}H_{37}NO_3Na]^+$ ($[M+Na]^+$): 398.2666; found: 398.2658. Elem. anal. in % calcd for $C_{23}H_{37}NO_3$: C 73.56, H 9.93, N 3.73; found: C 73.31, H 10.05, N 3.66.

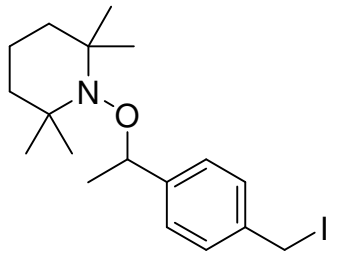
{4-[1-(2,2,6,6-Tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-phenyl}-methanol



4-[1-(2,2,6,6-Tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzaldehyde (4.280 g, 11.40 mmol) was dissolved in THF (45 mL) and $LiAlH_4$ (260 mg, 6.84 mmol, 2.4 eq.) was added at 0 °C. The suspension was stirred at room temperature for 3.5 h before the reaction was stopped by the addition of water (0.321 mL). After stirring of the reaction mixture for 5 min NaOH (15 % aq., 0.321 mL) was added and after another 5 min water (0.642 mL) was again dropped into the suspension. After 20 min a white percipitate had formed which was filtered off. Purification by flash chromatography (diethyl ether/pentane, 1:3→1:1) afforded the desired alcohol as a colourless oil (4.080 g, 10.81 mmol, 95%).

IR (neat): 3365*s*, 2963*s*, 2878*m*, 2817*w*, 1462*s*, 1375*m*, 1337*w*, 1282*w*, 1201*m*, 1152*m*, 1096*s*, 1057*s*, 1013*m*, 989*m*, 944*w*, 910*w*, 881*w*, 818*s*, 784*w*, 689*w*, 614*w* cm^{-1}. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.30-7.23 (*m*, 4 H, Ar-H); 4.71 (*q*, ^3J = 6.6 Hz, 1 H, NOCH); 4.65 (*s*, 2 H, OCH_2); 3.37 (*tt*, ^3J = 11.4 Hz, ^3J = 3.8 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 2.22 (*bs*, 1 H, OH); 2.20-0.88 (*m*, 12 H, 6 CH_2); 1.41 (*d*, ^3J = 6.6 Hz, 3 H, $NOCCH_3$); 1.07 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.94 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.73 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.67 (*t*, ^3J = 7.2 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 145.9 (C), 139.3 (C), 126.6 (CH), 125.9 (CH), 82.1 (NOCH), 71.3 (MeOCH), 65.3 (C), 65.0 (C + OCH_2), 55.5 (OCH_3), 36.3 (CH_2), 35.8 (CH_2), 30.3 (CH_2), 29.3 (CH_2), 27.5 (CH_2), 27.1 (CH_2), 24.9 ($NOCCH_3$), 10.1 (CH_3), 9.8 (CH_3), 8.2 (CH_3), 7.9 (CH_3) ppm. ESI-MS: *m/z*: 400 ($[M+Na]^+$), 378 ($[M+H]^+$), 265 ([Nitroxide+Na]$^+$). HR-ESI-MS: calcd for $[C_{23}H_{39}NO_3]^+$ ($[M+H]^+$): 378.3003; found: 378.3005. Elem. anal. in % calcd for $C_{23}H_{39}NO_3$: C 73.17, H 10.41, N 3.71; found: C 73.00, H 10.55, N 3.53.

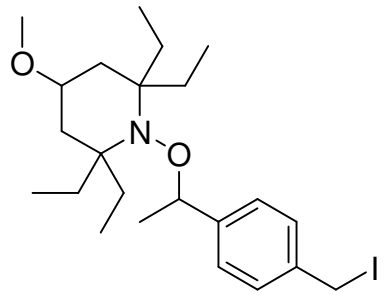
1-[1-(4-Iodomethyl-phenyl)-ethoxy]-2,2,6,6-tetramethyl-piperidine (4a)



{4-[1-(2,2,6,6-Tetramethyl-piperidin-1-yloxy)-ethyl]-phenyl}-methanol (1.826 g, 6.27 mmol, 1.00 eq.) and NaI (2.799 g, 18.80 mmol, 3.00 eq.) were dissolved in acetonitril (13 mL) and chlorotrimethylsilane (2.4 mL, 2.042 g, 18.80 mmol, 3.00 eq.) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and was then stirred for 4 h. The reaction was stopped by the addition of water (30 mL) and the aqueous layer was extracted with diethyl ether (3 times 30 mL). The combined organic phases were washed with $Na_2S_2O_3$ (aq. sat., 2 times 50 mL) and dried over $MgSO_4$. After removal of the solvent iodid **4a** (2.516 g, 6.27 mmol, 100%) was obtained as a yellow solid which was used for the following reaction without further purification.[10]

mp: 69-71 °C. IR (neat): 2972*m*, 2930*s*, 1509*w*, 1452*s*, 1375*w*, 1361*m*, 1282*w*, 1258*w*, 1241*w*, 1213*w*, 1182*w*, 1157*m*, 1132*m*, 1061*s*, 1019*w*, 990*w*, 957*w*, 934*s*, 882*w*, 840*s*, 791*w*, 751*w*, 699*w*, 581*s* cm^{-1}. ^{1}H-NMR (300 MHz; $CDCl_3$): δ = 7.29 (*d*, ^3J = 8.4 Hz, 2 H, Ar-H); 7.21 (*d*, ^3J = 8.4 Hz, 2 H, Ar-H); 4.73 (*q*, ^3J = 6.6 Hz, 1 H, NOCH); 4.43 (*s*, 2 H, CH_2I); 1.54-0.93 (*m*, 6 H, 3 CH_2); 1.43 (*d*, ^3J = 6.6 Hz, 3 H, $NOCCH_3$); 1.25 (*bs*, 3 H, CH_3); 1.13 (*bs*, 3 H, CH_3); 1.00 (*bs*, 3 H, CH_3); 0.64 (*bs*, 3 H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 145.6 (C), 137.6 (C), 128.4 (CH), 127.0 (CH), 82.6 (NOCH), 59.7 (C), 40.4 (CH_2), 34.4 (CH_3), 34.2 (CH_3), 23.3 ($NOCCH_3$), 20.3 (CH_3), 17.2 (CH_2), 6.0 (CH_2I) ppm. ESI-MS: *m/z*: 424 ($[M+Na]^+$), 402 ($[M+H]^+$), 179 ($[Nitroxide+Na]^+$). HR-ESI-MS: calcd for $[C_{18}H_{29}INO]^+$ ($[M+H]^+$): 402.1288; found: 402.1294.

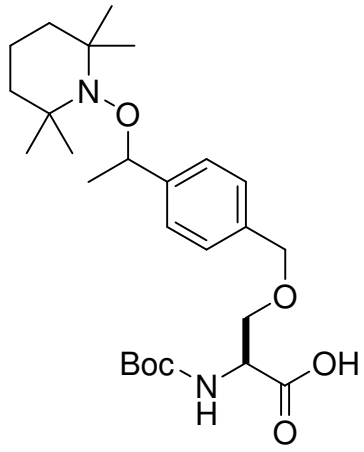
2,2,6,6-Tetraethyl-1-[1-(4-iodomethyl-phenyl)-ethoxy]-4-methoxy-piperidine (4b)



{4-[1-(2,2,6,6- Tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-phenyl}-methanol (4.080 g, 10.81 mmol, 1.00 eq.) and NaI (4.859 g, 32.42 mmol, 3.00 eq.) were dissolved in acetonitril (25 mL) and chlorotrimethylsilane (4.1 mL, 3.522 g, 32.42 mmol, 3.00 eq.) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and was then stirred for 2 h. The reaction was stopped by the addition of water (50 mL) and the aqueous layer was extracted with diethyl ether (3 times 50 mL). The combined organic phases were washed with $Na_2S_2O_3$ (aq. sat., 2 times 100 mL) and dried over $MgSO_4$. After removal of the solvent iodid **4b** (5.260 g, 10.79 mmol, 100%) was obtained as a yellow solid which was used for the following reaction without further purification.[10]

mp: 68-69 °C. IR (neat): 2965*s*, 2878*m*, 2816*w*, 1509*w*, 1462*s*, 1375*m*, 1337*w*, 1280*w*, 1200*w*, 1155*s*, 1097*s*, 1058*s*, 1015*w*, 990*m*, 944*w*, 910*w*, 882*w*, 840*s*, 785*w*, 755*w*, 689*w*, 582*s* cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): δ = 7.30 (*d*, ^3J = 8.0 Hz, 2 H, Ar-H); 7.17 (*d*, ^3J = 8.0 Hz, 2 H, Ar-H); 4.65 (*q*, ^3J = 6.5 Hz, 1 H, NOCH); 4.46 (*s*, 2 H, CH_2I); 3.35 (*tt*, ^3J = 11.5 Hz, ^3J = 3.8 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 2.12-0.80 (*m*, 12 H, 6 CH_2); 1.39 (*d*, ^3J = 6.5 Hz, 3 H, $NOCCH_3$); 1.05 (*t*, ^3J = 7.3 Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.72 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.62 (*t*, ^3J = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 146.2 (C), 137.5 (C), 128.4 (CH), 126.4 (CH), 81.9 (NOCH), 71.2 (MeOCH), 65.4 (C), 65.0 (C), 55.6 (OCH_3), 36.2 (CH_2), 35.8 (CH_2), 30.2 (CH_2), 29.4 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 24.7 ($NOCCH_3$), 10.2 (CH_3), 9.9 (CH_3), 8.2 (CH_3), 8.0 (CH_3), 6.0 (CH_2I) ppm. ESI-MS: *m/z*: 510 ($[M+Na]^+$), 488 ($[M+H]^+$), 265 ($[Nitroxide+Na]^+$). HR-ESI-MS: calcd for $[C_{23}H_{39}INO_2]^+$ ($[M+H]^+$): 488.2020; found: 488.2018.

(*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionic acid (5a)

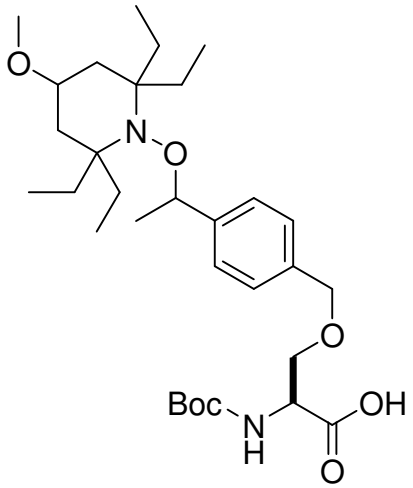


Boc-Ser-OH (307 mg, 1.50 mmol, 1.50 eq.) was dissolved in DMF (5 mL) and NaH (60 % suspension in mineral oil, 126 mg, 3.14 mmol, 3.14 eq.) was added at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and 30 min at room temperature. A solution of iodide **4a** (400 mg, 1.00 mmol, 1.00 eq.) in DMF (2 mL) was added dropwise at 0 °C and the suspension was stirred at room temperature for 2 h. Afterwards the reaction was stopped by careful addition of water (30 mL) and the aqueous phase was acidified with HCl (1 M aq.) until a pH value of 3 was reached. The aqueous layer was extracted with diethyl ether (2 times 30 mL) and ethyl acetate (2 times 30 mL) and the combined organic phases were dried over $MgSO_4$. Purification by flash chromatography (formic acid/diethyl ether/pentane, 1:300:600→1:500:500) afforded the desired unnatural amino acid **5a** as a colourless oil (244 mg, 0.51 mmol, 51%).[11]

Both diastereoisomers: IR (neat): 3317*s*, 2975*m*, 2931*s*, 2872*w*, 1745*w*, 1715*w*, 1663*s*, 1513*s*, 1454*m*, 1363*s*, 1282*w*, 1242*w*, 1209*w*, 1163*s*, 1110*w*, 1060*m*, 1020*w*, 989*w*, 935*m*, 849*w*, 822*m*, 790*w*, 700*w* cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.32-7.22 (*m*, 4 H, Ar-H); 5.46 (*bs*, 1 H, NH); 4.94-4.85 (*m*, 1 H, NOCH); 4.57-4.44 (*m*, 3 H, OCH_2-Ar + Ser-CH); 3.93-3.88 (*m*, 1 H, Ser-CH_2); 3.71 (*dd*, ^2J = 9.6 Hz, ^3J = 3.6 Hz, 1 H, Ser-CH_2); 1.61-0.68 (*m*, 9 H, 3 CH_2 + $NOCCH_3$); 1.45 (*s*, 9 H, Boc-H); 1.32 (bs, 3 H, CH_3); 1.18 (bs, 3 H, CH_3); 1.04 (bs, 3 H, CH_3); 0.68 (bs, 3 H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 174.1 (C), 173.9 (C), 155.7 (OOCN), 143.5 (C), 143.2 (C), 136.9 (C), 136.8 (C), 127.6 (CH), 127.5 (CH), 126.6 (CH), 126.5 (CH), 83.7 (NOCH), 83.6 (NOCH), 79.9 (Boc-C), 73.1 (OCH_2-Ar), 72.9 (OCH_2-Ar),

70.2 (Ser-CH_2), 70.0 (Ser-CH_2), 63.7 (C), 63.3 (C), 54.2 (Ser-CH), 38.9 (CH_2), 38.8 (CH_2), 32.1 (CH_3), 28.3 (Boc-CH_3), 23.6 (NOC*C*H_3), 23.5 (NOC*C*H_3), 20.7 (CH_3), 16.6 (CH_2), 16.5 (CH_2) ppm. ESI-MS: *m/z*: 501 ($[M+Na]^+$), 479 ($[M+H]^+$). HR-ESI-MS: calcd for $[C_{26}H_{43}N_2O_6]^+$ ($[M+H]^+$): 479.3316; found: 479.3317.

(*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionic acid (5b)

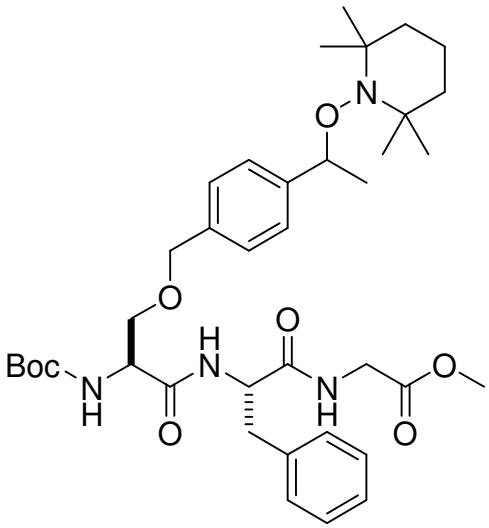


Boc-Ser-OH (1.263 mg, 6.15 mmol, 2.00 eq.) was dissolved in DMF (20 mL) and NaH (60 % suspension in mineral oil, 517 mg, 12.92 mmol, 4.20 eq.) was added at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and 30 min at room temperature. Iodide **4b** (1.500 g, 3.08 mmol, 1.00 eq.) was added at 0 °C and the suspension was stirred at room temperature for 3 h. Afterwards the reaction was stopped by careful addition of water (50 mL) and the aqueous phase was acidified with HCl (1 M aq.) until a pH value of 3 was reached. The aqueous layer was extracted with diethyl ether (2 times 70 mL) and ethyl acetate (2 times 70 mL) and the combined organic phases were dried over $MgSO_4$. Purification by flash chromatography (formic acid/diethyl ether/pentane, 1:250:750→1:500:500) afforded the desired unnatural amino acid **5b** as an orange oil (885 mg, 1.57 mmol, 51%).[11]

Both diastereoisomers: IR (neat): 3442*m*, 2974*s*, 2879*m*, 1718*s*, 1509*m*, 1463*m*, 1368*m*, 1283*w*, 1249*w*, 1163*s*, 1098*m*, 1061*m*, 1018*w*, 989*w*, 911*m*, 822*w*, 783*w*, 734*m*, 646*w* cm^{-1}. ^{1}H-NMR (400 MHz; $CDCl_3$): δ = 7.28-7.19 (*m*, 4 H, Ar-H); 5.40 (*bs*, 1 H, NH); 4.72-4.65 (*m*, 1 H, NOCH); 4.58-4.45 (*m*, 3 H, OCH_2-Ar + Ser-CH); 3.92-3.90 (*m*, 1 H, Ser-CH_2); 3.71-3.67 (*m*, 1 H, Ser-CH_2); 3.36 (*tt*, ^3J = 11.2 Hz, ^3J = 3.6 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 2.12-0.84 (*m*, 12 H, 6 CH_2); 1.45 (*s*,

9 H, Boc-H); 1.39 (*d*, ^3J = 6.4 Hz, 3 H, $NOCCH_3$); 1.05 (*t*, ^3J = 7.2 Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.4 Hz, 3 H, CH_3); 0.71 (*t*, ^3J = 7.0 Hz, 3 H, CH_3); 0.63 (*t*, ^3J = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 174.1 (C), 170.6 (C), 155.8 (OOCN), 155.7 (OOCN), 146.9 (C), 146.2 (C), 135.6 (C), 133.5 (C), 127.9 (CH), 127.2 (CH), 126.1 (CH), 126.0 (CH), 82.1 (NOCH), 82.0 (NOCH), 80.3 (Boc-C), 73.4 (OCH_2-Ar), 71.4 (MeOCH), 69.5 (Ser-CH_2), 65.4 (C), 65.1 (C), 55.6 (OCH_3), 53.7 (Ser-CH), 36.3 (CH_2), 35.8 (CH_2), 30.3 (CH_2), 29.4 (CH_2), 28.3 (Boc-CH_3), 27.5 (CH_2), 27.2 (CH_2), 24.9 ($NOCCH_3$), 10.2 (CH_3), 9.9 (CH_3), 8.3 (CH_3), 8.0 (CH_3) ppm. ESI-MS: *m/z*: 587 ($[M+Na]^+$), 565 ($[M+H]^+$). HR-ESI-MS: calcd for $[C_{31}H_{53}N_2O_7]^+$ ($[M+H]^+$): 565.3847; found: 565.3844.

[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-3-phenyl-propionylamino]-acetic acid methyl ester

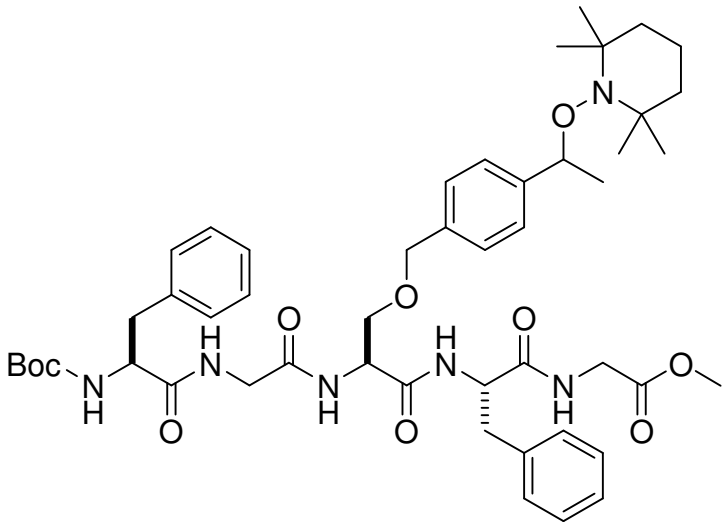


Amino acid **5a** (1.500 g, 3.13 mmol, 1.00 eq.), H-Phe-Gly-OMe (814 mg, 3.45 mmol, 1.10 eq.), HOBt•H_2O (527 mg, 3.45 mmol, 1.10 eq.) and 4-methylmorpholine (0.38 mL, 349 mg, 3.45 mmol, 1.10 eq.) were dissolved in dichloromethane (40 mL) and EDCI•HCl (661 mg, 3.45 mmol, 1.10 eq.) was added. The reaction mixture was stirred for 18 h at room temperature before it was diluted with dichloromethane (200 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 150 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (diethyl

ether/pentane, 1:1→diethyl ether). The desired tripeptide was isolated as a white foam (1.464 g, 2.10 mmol, 67%).

Both diastereoisomers: mp: 53-54 °C. IR (neat): 3296*s*, 2974*m*, 2931*s*, 1751*w*, 1649*s*, 1515*s*, 1454*w*, 1365*s*, 1242*w*, 1208*m*, 1170*s*, 1108*m*, 1062*w*, 1020*w*, 935*w*, 852*w*, 822*w*, 733*w*, 700*m* cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.31-7.13 (*m*, 9 H, Ar-H); 6.77 (*bs*, 1 H, Gly-NH); 6.68 (*d*, ^3J = 7.2 Hz, 1 H, Phe-NH); 5.30 (*bs*, 1 H, Ser-NH); 4.82-4.72 (*m*, 2 H, NOCH + Phe-CH); 4.50-4.42 (*m*, 2 H, OCH_2-Ar); 4.21-4.15 (*m*, 1 H, Ser-CH); 3.94-3.76 (*m*, 3 H, Gly-CH_2 + Ser-CH_2); 3.70 (*s*, 3 H, OCH_3); 3.58 (*dd*, ^2J = 9.2 Hz, ^3J = 6.2 Hz, 1 H, Ser-CH_2); 3.18 (*dd*, ^2J = 13.8 Hz, ^3J = 6.6 Hz, 1 H, Phe-CH_2); 3.08 (*dd*, ^2J = 14.0 Hz, ^3J = 6.5 Hz, 1 H, Phe-CH_2); 1.68-1.03 (*m*, 6 H, 3 CH_2); 1.46 (*d*, ^3J = 6.6 Hz, 3 H, NOCCH_3); 1.39 (*s*, 9 H, Boc-H); 1.28 (bs, 3 H, CH_3); 1.16 (bs, 3 H, CH_3); 1.03 (bs, 3 H, CH_3); 0.68 (bs, 3 H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 170.8 (C), 170.1 (C), 169.6 (C), 155.0 (OOCN), 145.8 (C), 136.1 (C), 135.5 (C), 129.3 (CH), 128.7 (CH), 127.5 (CH), 127.0 (CH), 126.7 (CH), 82.7 (NOCH), 80.6 (Boc-C), 73.4 (OCH_2-Ar), 69.5 (Ser-CH_2), 59.7 (C), 54.7 (Ser-CH), 53.7 (Phe-CH), 52.1 (OCH_3), 41.1 (Gly-CH_2), 40.3 (CH_2), 37.2 (Phe-CH_2), 34.5 (CH_3), 34.4 (CH_3), 28.2 (Boc-CH_3), 23.5 (NOC*C*H_3), 20.4 (CH_3), 20.3 (CH_3), 17.2 (CH_2) ppm. ESI-MS: *m/z*: 719 ($[M+Na]^+$), 697 ($[M+H]^+$), 360 ($[M+H+Na]^{2+}$). HR-ESI-MS: calcd for $[C_{38}H_{57}N_4O_8]^+$ ($[M+H]^+$): 697.4171; found: 697.4163.

[(*S*)-2-((*S*)-2-[2-((*S*)-2-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-acetylamino]-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-3-phenyl-propionylamino]-acetic acid methyl ester (6)

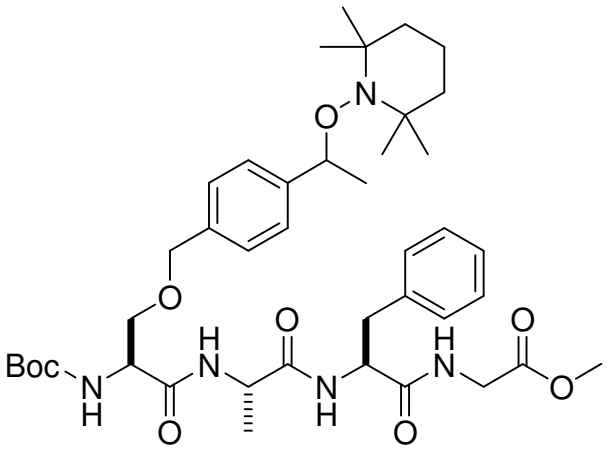


[(S)-2-((S)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-3-phenyl-propionylamino]-acetic acid methyl ester (329 mg, 0.47 mmol, 1.00 eq.) was dissolved in dichloromethane (10 mL) and treated with TFA (3 mL). After stirring for 3 h the reaction mixture was diluted with dichloromethane (50 mL), washed with $NaHCO_3$ (aq. sat., 3 times 50 mL) and dried over $MgSO_4$. After removal of the solvent *in vacuo* a colourless residue (282 mg) was obtained. The residue, Boc-Phe-Gly-OH (160 mg, 0.50 mmol, 1.05 eq.), HOBt•H_2O (83 mg, 0.54 mmol, 1.15 eq.) and 4-methylmorpholine (0.06 mL, 55 mg, 0.54 mmol, 1.15 eq.) were dissolved in dichloromethane (4 mL) and EDCI•HCl (104 mg, 0.54 mmol, 1.15 eq.) was added. The reaction mixture was stirred for 1 h at room temperature before it was diluted with dichloromethane (30 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 30 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate/dichloromethane, 1:1→ ethyl acetate). The desired pentapeptide **6** was isolated as a white solid (380 mg, 0.42 mmol, 89%).

Both diastereoisomers: mp: 166-168 °C. IR (neat): 3285*s*, 2975*w*, 2930*m*, 1751*w*, 1700*m*, 1633*s*, 1522*s*, 1440*m*, 1365*w*, 1215*m*, 1172*m*, 1111*w*, 1060*w*, 935*w*, 823*w*, 737*w*, 700*m* cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.45 (*d*, ^3J = 6.3 Hz, 1 H, Gly-NH); 7.28-7.12 (*m*, 17 H, 3 NH, Ar-H); 5.75 (*d*, ^3J = 6.6 Hz, 1 H, Boc-NH); 4.86 (*q*,

^3J = 5.1 Hz, 1 H, Phe-CH); 4.73 (*q*, ^3J = 6.6 Hz, 1 H, NOCH); 4.50 (*bs*, 2 H, Boc-Phe-CH + Ser-CH); 4.39 (*d*, ^2J = 15.6 Hz, 1 H, OCH_2-Ar); 4.36 (*d*, ^2J = 15.6 Hz, 1 H, OCH_2-Ar); 4.11 (*dd*, ^2J = 18.0 Hz, ^3J = 5.7 Hz, 1 H, Gly-CH_2); 3.98-3.71 (*m*, 4 H, Gly-CH_2 + Ser-CH_2); 3.73 (*s*, 3 H, OCH_3); 3.57 (*dd*, ^2J = 9.9 Hz, ^3J = 5.4 Hz, 1 H, Ser-CH_2); 3.35-3.22 (*m*, 2 H, Phe-CH_2); 3.10 (*dd*, ^2J = 13.8 Hz, ^3J = 9.3 Hz, 1 H, Phe-CH_2); 2.92 (*dd*, ^2J = 13.5 Hz, ^3J = 5.7 Hz, 1 H, Boc-Phe-CH_2); 1.70-1.02 (*m*, 6 H, 3 CH_2); 1.44 (*d*, ^3J = 6.6 Hz, 3 H, $NOCCH_3$); 1.35 (*s*, 9 H, Boc-H); 1.26 (*bs*, 3 H, CH_3); 1.15 (*bs*, 3 H, CH_3); 1.02 (*bs*, 3 H, CH_3); 0.66 (*bs*, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 173.1 (C), 171.4 (C), 170.6 (C), 169.6 (C), 169.6 (C), 155.9 (C), 145.5 (C), 137.0 (C), 136.9 (C), 135.6 (C), 129.3 (CH), 129.2 (CH), 128.4 (CH), 128.3 (CH), 127.4 (CH), 126.6 (CH), 126.6 (CH), 82.7 (NOCH), 80.0 (Boc-C), 73.1 (OCH_2-Ar), 68.9 (Ser-CH_2), 59.6 (C), 55.2 (Boc-Phe-CH), 54.5 (Phe-CH), 53.9 (Ser-CH), 52.2 (OCH_3), 43.9 (Gly-CH_2), 41.1 (Gly-CH_2), 40.3 (CH_2), 37.7 (Phe-CH_2), 37.5 (Phe-CH_2), 34.4 (CH_3), 34.1 (CH_3), 28.2 (Boc-CH_3), 23.5 (NOC*C*H_3), 20.3 (CH_3), 17.1 (CH_2) ppm. ESI-MS: *m/z*: 923 ($[M+Na]^+$), 902 ($[M+H]^+$), 473 ($[M+2Na]^{2+}$), 462 ($[M+H+Na]^{2+}$). HR-ESI-MS: calcd for $[C_{49}H_{69}N_6O_{10}]^+$ ($[M+H]^+$): 901.5070; found: 901.5070.

{(*S*)-2-[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-propionylamino]-3-phenyl-propionylamino}-acetic acid methyl ester

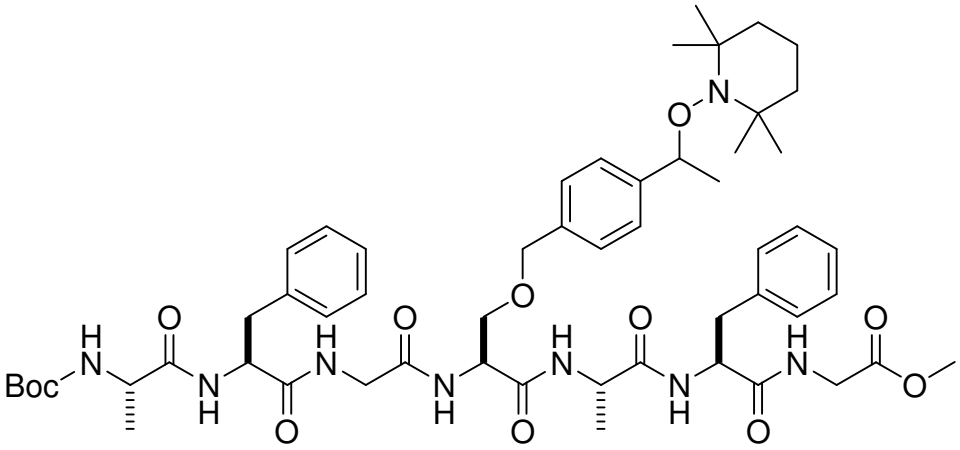


Amino acid **5a** (833 mg, 1.74 mmol, 1.00 eq.), HCl•H-Ala-Phe-Gly-OMe (535 mg, 1.74 mmol, 1.00 eq.), HOBt•H_2O (293 mg, 1.92 mmol, 1.10 eq.) and 4-methylmorpholine (0.40 mL, 370 mg, 3.65 mmol, 2.10 eq.) were dissolved in

dichloromethane (8 mL) and EDCI•HCl (368 mg, 1.92 mmol, 1.10 eq.) was added. The reaction mixture was stirred for 18 h at room temperature before it was diluted with dichloromethane (50 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 40 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate). The desired tetrapeptide was isolated as a white solid (1.209 g, 1.57 mmol, 90%).

Both diastereoisomers: mp: 155-157 °C. IR (neat): 3279*s*, 3085*w*, 3975*m*, 2931*m*, 1761*m*, 1672*m*, 1638*s*, 1535*s*, 1452*w*, 1365*m*, 1246*w*, 1208*m*, 1179*m*, 1061*w*, 935*w*, 734*w*, 700*w* cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): δ = 7.30-7.16 (*m*, 9 H, Ar-H); 6.89-6.88 (*m*, 2 H, Gly-NH + Phe-NH); 6.81 (*d*, ^3J = 5.0 Hz, 1 H, Ala-NH); 5.42 (*bs*, 1 H, Ser-NH); 4.80-4.76 (*m*, 1 H, Phe-CH); 4.77 (*q*, ^3J = 6.5 Hz, 1 H, NOCH); 4.52 (*s*, 2 H, OCH_2-Ar); 4.32-4.25 (*m*, 2 H, Ala-CH + Ser-CH); 4.13 (*dd*, ^2J = 18.0 Hz, ^3J = 6.0 Hz, 1 H, Gly-CH_2); 3.87 (*dd*, ^2J = 18.0 Hz, ^3J = 4.5 Hz, 1 H, Gly-CH_2); 3.80 (*dd*, ^2J = 9.5 Hz, ^3J = 4.5 Hz, 1 H, Ser-CH_2); 3.72 (*s*, 3 H, OCH_3); 3.60-3.56 (*m*, 1 H, Ser-CH_2); 3.31-3.28 (*m*, 1 H, Phe-CH_2); 2.96 (*dd*, ^2J = 14.5 Hz, ^3J = 9.0 Hz, 1 H, Phe-CH_2); 1.55-1.25 (*m*, 6 H, 3 CH_2); 1.45 (*d*, ^2J = 6.5 Hz, 3 H, $NOCCH_3$); 1.43 (*s*, 9 H, Boc-H); 1.27 (*bs*, 3 H, CH_3); 1.22 (*d*, ^3J = 7.5 Hz, 3 H, Ala-CH_3); 1.15 (*bs*, 3 H, CH_3); 1.01 (*bs*, 3 H, CH_3); 0.64 (*bs*, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.8 (C), 171.1 (C), 171.0 (C), 170.0 (C), 145.8 (C), 136.9 (C), 135.5 (C), 129.0 (CH), 128.5 (CH), 127.6 (CH), 126.8 (CH), 126.7 (CH), 82.7 (NOCH), 80.6 (Boc-C), 73.5 (OCH_2-Ar), 69.4 (Ser-CH_2), 59.6 (C), 54.4 (Ser-CH), 53.7 (Phe-CH), 52.2 (OCH_3), 50.1 (Ala-CH), 41.1 (Gly-CH_2), 40.6 (CH_2), 37.3 (Phe-CH_2), 34.4 (CH_3), 34.2 (CH_3), 28.2 (Boc-CH_3), 23.5 (NOC*C*H_3), 20.3 (CH_3), 17.4 (Ala-CH_3), 17.2 (CH_2) ppm. ESI-MS: *m/z*: 790 ($[M+Na]^+$), 768 ($[M+H]^+$), 396 ($[M+2Na]^{2+}$). HR-ESI-MS: calcd for $[C_{41}H_{62}N_5O_9Na]^{2+}$ ($[M+H+Na]^{2+}$): 395.7217; found: 395.7222.

Boc-Ala-Phe-Gly-Ser(O-{4-[1-(2,2,6,6-Tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-phenyl}-methyl)-Ala-Phe-Gly-OMe (7)

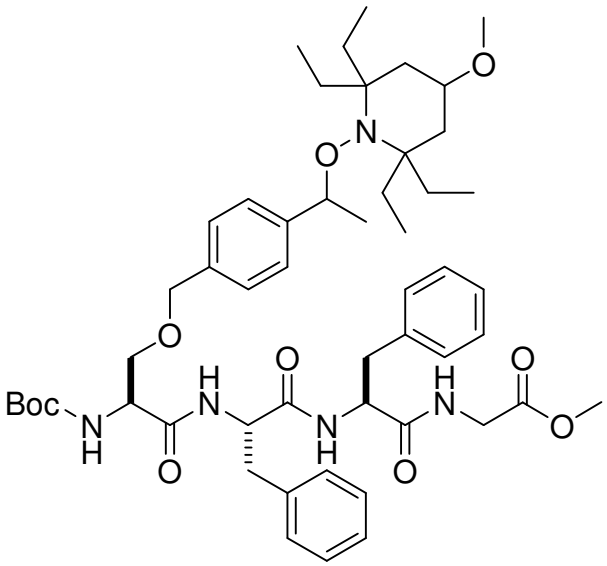


{(S)-2-[(S)-2-((S)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetramethyl-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-propionylamino]-3-phenyl-propionylamino}-acetic acid methyl ester (813 mg, 1.06 mmol, 1.00 eq.) was dissolved in dichloromethane (2.5 mL) and treated with TFA (2.5 mL). After stirring for 1 h the reaction mixture was diluted with dichloromethane (40 mL), washed with $NaHCO_3$ (aq. sat., 3 times 40 mL) and dried over $MgSO_4$. After removal of the solvent *in vacuo* a colourless oil (700 mg) was obtained. The oil, Boc-Ala-Phe-Gly-OH (433 mg, 1.10 mmol, 1.05 eq.), HOBt•H_2O (176 mg, 1.15 mmol, 1.10 eq.) and 4-methylmorpholine (0.13 mL, 117 mg, 1.15 mmol, 1.10 eq.) were dissolved in dichloromethane (5 mL) and EDCI•HCl (221 mg, 1.15 mmol, 1.10 eq.) was added. The reaction mixture was stirred for 1 h at room temperature before it was diluted with dichloromethane (50 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 30 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (methanol/dichloromethane, 3:100→5:100). The desired heptapeptide **7** was isolated as a white solid (898 mg, 0.86 mmol, 82%).

Both diastereoisomers: mp: Decomp. above 200 °C. IR (KBr): 3294*s*, 3029*w*, 2976*m*, 1747*w*, 1634*s*, 1523*s*, 1451*w*, 1366*m*, 1244*w*, 1215*m*, 1168*m*, 1063*w*, 1023*w*, 700*m* cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-D_6): δ = 8.39 (*t*, ^3J = 6.0 Hz, 1 H, Gly-NH); 8.25 (*t*, ^3J = 5.4 Hz, 1 H, Gly-NH); 8.14 (*d*, ^3J = 7.8 Hz, 1 H, Ala-NH); 8.11 (*d*, ^3J = 7.8 Hz, 1 H, Ser-NH); 7.88 (*d*, ^3J = 8.4 Hz, 1 H, Phe-NH); 7.84 (*d*, ^3J = 8.4 Hz, 1 H, Phe-NH); 7.27-7.13 (*m*, 14 H, Ar-H); 6.88 (*d*, ^3J = 7.2 Hz, 1 H, Ala-NH); 4.73 (*q*, ^3J = 6.6 Hz,

1 H, NOCH); 4.57 (*dt*, ^3J = 7.8 Hz, ^3J = 5.4 Hz, 1 H, Ser-CH); 4.55-4.50 (*m*, 2 H, Phe-CH); 4.47 (*d*, ^2J = 12.0 Hz, 1 H, OCH_2-Ar); 4.43 (*d*, ^2J = 12.0 Hz, 1 H, OCH_2-Ar); 4.25 (*m*, 1 H, Ala-CH); 3.92-3.87 (*m*, 1 H, Ala-CH); 3.84 (*m*, 2 H, Gly-CH_2); 3.76 (*d*, ^3J = 5.4 Hz, 2 H, Gly-CH_2); 3.61 (*s*, 3 H, OCH_3); 3.60-3.55 (*m*, 2 H, Ser-CH_2); 3.04-2.99 (*m*, 2 H, Phe-CH_2); 2.82 (*dd*, ^2J = 13.8 Hz, ^3J = 9.6 Hz, 1 H, Phe-CH_2); 2.76 (*dd*, ^2J = 13.8 Hz, ^3J = 9.0 Hz, 1 H, Phe-CH_2); 1.51-1.21 (*m*, 6 H, 3 CH_2); 1.38 (*d*, ^3J = 6.6 Hz, 3 H, NOCCH_3); 1.35 (*s*, 9 H, Boc-H); 1.24 (*bs*, 3 H, CH_3); 1.15 (*d*, ^3J = 7.2 Hz, 3 H, Ala-CH_3); 1.10 (*bs*, 3 H, CH_3); 1.06 (*d*, ^3J = 7.2 Hz, 3 H, Ala-CH_3); 0.96 (*bs*, 3 H, CH_3); 0.60 (*bs*, 3 H, CH_3) ppm. ^{13}C-NMR (150 MHz, DMSO-D_6): δ = 171.6 (C), 171.2 (C), 170.1 (C), 169.0 (C), 168.6 (C), 144.3 (C), 137.6 (C), 137.5 (C), 136.7 (C), 129.2 (CH), 129.1 (CH), 128.0 (CH), 127.9 (CH), 127.3 (CH), 126.2 (CH), 82.1 (NOCH), 78.1 (Boc-C), 72.0 (OCH_2-Ar), 69.9 (Ser-CH_2), 59.2 (C), 59.1 (C), 53.5 (2 Phe-CH), 52.5 (Ser-CH), 51.7 (OCH_3), 49.9 (Ala-CH), 48.3 (Ala-CH), 41.9 (Gly-CH_2), 40.6 (Gly-CH_2), 37.6 (Phe-CH_2), 37.5 (Phe-CH_2), 34.1 (CH_3), 33.9 (CH_3), 28.1 (Boc-CH_3), 23.2 (NOC*C*H_3), 20.4 (CH_3), 20.1 (CH_3), 18.1 (Ala-CH_3), 18.0 (Ala-CH_3), 16.7 (CH_2) ppm. ESI-MS: *m/z*: 1066 ($[M+Na]^+$), 1044 ($[M+H]^+$), 544 ($[M+2Na]^{2+}$), 533 ($[M+H+Na]^{2+}$). HR-ESI-MS: calcd for $[C_{55}H_{78}N_8O_{12}Na]^+$ ($[M+Na]^+$): 1065.5631; found: 1065.5625.

{(*S*)-2-[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-{4-[1-(2,2,6,6-tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzyloxy}-propionylamino)-3-phenyl-propionylamino]-3-phenyl-propionylamino}-acetic acid methyl ester (8)

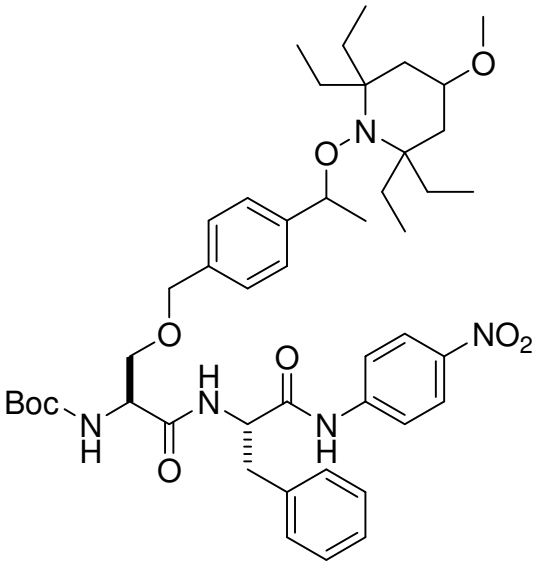


Amino acid **5b** (585 mg, 1.04 mmol, 1.00 eq.) and H-Phe-Phe-Gly-OMe (477 mg, 1.24 mmol, 1.20 eq.) were dissolved in dichloromethane (4 mL) and IIDQ (629 mg, 2.07 mmol, 2.00 eq.) was added. The reaction mixture was stirred for 15 h at room temperature before it was diluted with dichloromethane (50 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 40 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate/pentane, 1/4→ ethyl acetate). The desired tetrapeptide **8** (n = 0) was isolated as a white solid (756 mg, 0.81 mmol, 78%).

Both diastereoisomers: mp: 126-128 °C. IR (neat): 3272*s*, 2973*s*, 1762*m*, 1673*m*, 1639*s*, 1536*s*, 1455*w*, 1367*w*, 1206*m*, 1099*m*, 1059*w*, 911*w*, 735*m*, 698*w* cm^{-1}. ^{1}H-NMR (600 MHz; $CDCl_3$): δ = 7.27-7.00 (*m*, 14 H, Ar-H); 6.82 (*bs*, 1 H, NH); 6.73 (*bs*, 1 H, NH); 6.62 (*bs*, 1 H, NH); 5.30 (*bs*, 1 H, Ser-NH); 4.82-4.78 (*m*, 1 H, Phe-CH); 4.69 (*q*, ^3J = 6.5 Hz, 1 H, NOCH); 4.50 (*m*, 1 H, Phe-CH); 4.48 (*s*, 2 H, OCH_2-Ar); 4.18-4.10 (*m*, 2 H, Ser-CH + Gly-CH_2); 3.83 (*dd*, ^2J = 18.0 Hz, ^3J = 4.5 Hz, 1 H, Gly-CH_2); 3.74-3.70 (*m*, 1 H, Ser-CH_2); 3.73 (*s*, 3 H, $COOCH_3$); 3.49-3.46 (*dd*, ^2J = 9.0 Hz, ^3J = 7.0 Hz, 1 H, Ser-CH_2); 3.35 (*tt*, ^3J = 11.5 Hz, ^3J = 3.5 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 3.21-3.19 (*m*, 1 H, Phe-CH_2); 3.01-2.90 (*m*, 3 H, Phe-CH_2); 2.13-0.84 (*m*, 15 H, 6 CH_2 + $NOCCH_3$); 1.49 (*s*, 9 H, Boc-H); 1.05 (*t*, ^3J = 7.0

Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.69 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.63 (*t*, ^3J = 7.0 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.3 (C), 170.8 (C), 170.5 (C), 170.0 (C), 155.8 (OOCN), 146.5 (C), 136.8 (C), 135.8 (C), 135.4 (C), 129.1 (CH), 129.0 (CH), 128.9 (CH), 128.6 (CH), 127.4 (CH), 127.2 (CH), 126.8 (CH), 126.1 (CH), 82.0 (NOCH), 80.6 (Boc-C), 73.2 (OCH_2-Ar), 71.2 (MeOCH), 69.3 (Ser-CH_2), 65.4 (C), 65.0 (C), 55.7 (OCH_3), 55.1 (Ser-CH), 54.4 (Phe-CH), 53.7 (Phe-CH), 52.2 (OCH_3), 41.1 (Gly-CH_2), 37.1 (CH_2), 36.9 (CH_2), 36.3 (Phe-CH_2), 35.8 (Phe-CH_2), 30.3 (CH_2), 29.4 (CH_2), 28.2 (Boc-CH_3), 27.4 (CH_2), 27.1 (CH_2), 25.0 (NOC*C*H_3), 10.2 (CH_3), 9.9 (CH_3), 8.3 (CH_3), 8.0 (CH_3) ppm. ESI-MS: *m/z*: 953 ($[M+Na]^+$), 931 ($[M+H]^+$), 477 ($[M+H+Na]^{2+}$). HR-ESI-MS: calcd for $[C_{52}H_{75}N_5O_{10}Na]^+$ ($[M+Na]^+$): 952.5406; found: 952.5394.

((*S*)-1-[(*S*)-1-(4-Nitro-phenylcarbamoyl)-2-phenyl-ethylcarbamoyl]-2-{4-[1-(2,2,6,6-tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzyloxy}-ethyl)-carbamic acid *tert*-butyl ester (12, n=0)

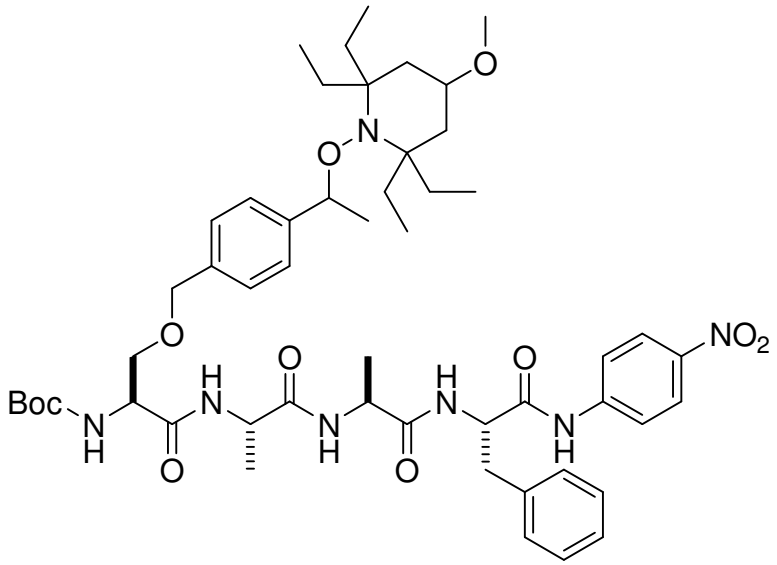


Amino acid **5b** (190 mg, 0.34 mmol, 1.00 eq.) and H-Phe-*p*-nitroanilide (96 mg, 0.34 mmol, 1.00 eq.) were dissolved in dichloromethane (1.5 mL) and IIDQ (112 mg, 0.37 mmol, 1.10 eq.) was added. The reaction mixture was stirred for 5 h at room temperature before it was diluted with dichloromethane (20 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 10 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (ethyl acetate/pentane, 1/3→1/1). The

desired alkoxyamine **12** (n = 0) was isolated as a white solid (164 mg, 0.20 mmol, 59%).

Both diastereoisomers: mp: 84-86 °C. IR (neat): 3295*s*, 2973*s*, 1659*s*, 1617*w*, 1597*w*, 1514*s*, 1368*w*, 1342*s*, 1302*w*, 1255*m*, 1165*m*, 1098*m*, 911*w*, 856*m*, 734*m*, 699*w* cm^{-1}. ^{1}H-NMR (500 MHz; $CDCl_3$): δ = 8.84 (*bs*, 1 H, Ar-NH); 8.16 (*d*, ^3J = 9.0 Hz, 2 H, Ar-H); 7.87 (*d*, ^3J = 9.0 Hz, 2 H, Ar-H); 7.27-7.20 (*m*, 7 H, Ar-H); 7.12-7.09 (*m*, 2 H, Ar-H); 6.60 (*bs*, 1 H, Phe-NH); 5.35 (*bs*, 1 H, Ser-NH); 4.92-4.88 (*m*, 1 H, Phe-CH); 4.69 (*q*, ^3J = 6.5 Hz, 1 H, NOCH); 4.52-4.46 (*m*, 2 H, OCH_2-Ar); 4.16-4.13 (*m*, 1 H, Ser-CH); 3.81 (*dd*, ^2J = 9.5 Hz, ^3J = 4.5 Hz, 1H, Ser-CH_2); 3.71-3.68 (*m*, 1 H, Ser-CH_2); 3.49-3.45 (*m*, 1 H, Phe-CH_2); 3.34 (*tt*, ^3J = 11.5 Hz, ^3J = 3.8 Hz, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 3.05-3.00 (*m*, 1 H, Phe-CH_2); 2.12-0.84 (*m*, 12 H, 6 CH_2); 1.38 (*d*, ^3J = 6.5 Hz, 3 H, NOCCH_3); 1.26 (*s*, 9 H, Boc-H); 1.05 (*t*, ^3J = 7.0 Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.5 Hz, 3 H, CH_3); 0.70-0.62 (*m*, 6 H, 2•CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.0 (C), 169.6 (C), 156.4 (OOCN), 146.7 (C), 146.7 (C), 143.7 (C), 143.6 (C), 135.6 (C), 135.6 (C), 135.2 (C), 129.2 (CH), 129.0 (CH), 127.5 (CH), 127.5 (CH), 127.4 (CH), 127.4 (CH), 126.1 (CH), 126.1 (CH), 124.7 (CH), 119.5 (CH), 82.0 (NOCH), 81.4 (Boc-C), 73.5 (OCH_2-Ar), 73.5 (OCH_2-Ar), 71.2 (MeOCH), 68.8 (Ser-CH_2), 68.7 (Ser-CH_2), 65.4 (C), 65.4 (C), 65.0 (C), 56.0 (Ser-CH), 55.7 (OCH_3), 53.8 (Phe-CH), 36.6 (Phe-CH_2), 36.2 (CH_2), 35.8 (CH_2), 30.2 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 28.0 (Boc-CH_3), 27.4 (CH_2), 27.2 (CH_2), 25.0 (NOC*C*H_3), 25.0 (NOC*C*H_3), 10.2 (CH_3), 9.9 (CH_3), 8.2 (CH_3), 8.0 (CH_3) ppm. ESI-MS: *m/z*: 854 ($[M+Na]^+$), 832 ($[M+H]^+$). HR-ESI-MS: calcd for $[C_{46}H_{66}N_5O_9]^+$ ($[M+H]^+$): 832.4855; found: 832.4839.

((*S*)-1-((*S*)-1-{(*S*)-1-[(*S*)-1-(4-Nitro-phenylcarbamoyl)-2-phenyl-ethylcarbamoyl]-ethylcarbamoyl}-ethylcarbamoyl)-2-{4-[1-(2,2,6,6-tetraethyl-4-methoxy-piperidin-1-yloxy)-ethyl]-benzyloxy}-ethyl)-carbamic acid *tert*-butyl ester (14, n=0)



Amino acid **5b** (740 mg, 1.31 mmol, 1.00 eq.), H-Ala-Ala-Phe-*p*-nitroanilide (588 mg, 1.38 mmol, 1.05 eq.), HOBt•H_2O (211 mg, 1.38 mmol, 1.05 eq.) and 4-methylmorpholine (0.15 mL, 139 mg, 1.38 mmol, 1.05 eq.) were dissolved in dichloromethane (10 mL) and EDCI•HCl (264 mg, 1.38 mmol, 1.05 eq.) was added. The reaction mixture was stirred for 5 h at room temperature before it was diluted with dichloromethane (50 mL). The solution was washed with HCl (0.1 M aq.), $NaHCO_3$ (aq. sat.) and NaCl (aq. sat.) (2 times 50 mL each) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by flash chromatography (dichloromethane). The desired alkoxyamine **14** (n = 0) was isolated as a white solid (1.080 g, 1.11 mmol, 85%).

Both diastereoisomers: mp: Decomp. above 170 °C. IR (neat): 3280*s*, 3090*w*, 2974*m*, 2932*w*, 2878*w*, 1635*s*, 1513*s*, 1453*w*, 1343*m*, 1253*w*, 1166*m*, 1098*m*, 1060*w*, 911*w*, 733*w*, 698*w* cm^{-1}. ^{1}H-NMR (600 MHz; $CDCl_3$): δ = 9.00 (*bs*, 1 H, Ar-NH); 8.20 (*d*, ^3J = 9.0 Hz, 2 H, Ar-H); 8.08 (*d*, ^3J = 9.0 Hz, 2 H, Ar-H); 7.47 (*d*, ^3J = 5.4 Hz, 1 H, Ala-NH); 7.30-7.15 (*m*, 10 H, Ar-H + Phe-NH); 6.54 (*bs*, 1 H, Ala-NH); 5.45 (*bs*, 1 H, Ser-NH); 4.82 (*ddd*, ^3J = 11.7 Hz, ^3J = 8.4 Hz, ^3J = 3.3 Hz, 1 H, Phe-CH); 4.68 (*q*, ^3J = 6.6 Hz, 1 H, NOCH); 4.53-4.48 (*m*, 2 H, OCH_2-Ar); 4.20-4.12 (*m*, 2 H, 2 Ala-CH); 4.08-4.05 (*m*, 1 H, Ser-CH); 3.73-3.69 (*m*, 2H, Ser-CH_2); 3.61 (*dd*, ^2J = 14.4 Hz,

^3J = 3.6 Hz, 1 H, Phe-CH_2); 3.36-3.31 (*m*, 1 H, MeOCH); 3.31 (*s*, 3 H, OCH_3); 3.02 (*dd*, ^2J = 13.8 Hz, ^3J = 12.0 Hz, 1 H, Phe-CH_2); 2.12-0.78 (*m*, 12 H, 6 CH_2); 1.49 (*s*, 9 H, Boc-H); 1.47 (*d*, ^3J = 7.8 Hz, 3 H, Ala-CH_3); 1.38 (*d*, ^3J = 7.0 Hz, 3 H, $NOCCH_3$); 1.20 (*d*, ^3J = 7.2 Hz, 3 H, Ala-CH_3); 1.05 (*t*, ^3J = 6.6 Hz, 3 H, CH_3); 0.92 (*t*, ^3J = 7.2 Hz, 3 H, CH_3); 0.69 (*t*, ^3J = 6.6 Hz, 3 H, CH_3); 0.62 (*t*, ^3J = 6.6 Hz, 3 H, CH_3) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 173.8 (C), 172.5 (C), 172.2 (C), 170.4 (C), 156.8 (C), 146.9 (C), 144.5 (C), 143.3 (C), 137.8 (C), 134.8 (C), 129.2 (CH), 128.3 (CH), 127.6 (CH), 127.6 (CH), 126.5 (CH), 126.3 (CH), 124.7 (CH), 119.5 (CH), 81.9 (NOCH), 80.6 (Boc-C), 73.6 (OCH_2-Ar); 71.1 (MeOCH), 68.1 (Ser-CH_2), 65.4 (C), 65.0 (C), 57.1 (Ser-CH), 55.7 (OCH_3), 55.0 (Phe-CH), 51.6 (Ala-CH), 51.2 (Ala-CH), 36.8 (Phe-CH_2), 36.2 (CH_2), 35.8 (CH_2), 30.2 (CH_2), 29.4 (CH_2), 28.2 (Boc-CH_3), 27.4 (CH_2), 27.1 (CH_2), 24.9 (NOCCH_3), 17.3 (Ala-CH_3), 16.2 (Ala-CH_3), 10.2 (CH_3), 9.9 (CH_3), 8.2 (CH_3), 8.0 (CH_3) ppm. ESI-MS: *m/z*: 997 ($[M+Na]^+$), 975 ($[M+H]^+$), 499 ($[M+H+Na]^{2+}$). HR-ESI-MS: calcd for $[C_{52}H_{75}N_7O_{11}Na]^+$ ($[M+Na]^+$): 996.5417; found: 996.5415. Elem. anal. in % calcd for $C_{52}H_{75}N_7O_{11}$: C 64.11, H 7.76, N 10.06; found: C 63.96, H 7.89, N 9.92.

General procedure for the nitroxide mediated polymerization of styrene or *n*-butyl acrylate using peptide initiators.

The peptide initiator (1.0 or 0.5 mol-%) was suspended in styrene or *n*-butyl acrylate (400 mg, 3.84 mmol or 3.12 mmol resp., 100 eq. or 200 eq. resp.) and the suspension was degassed in three freeze-thaw cycles. The reaction mixture was sealed under argon and heated to 105 °C or 125 °C for 8-24 h. The polymerization was stopped upon cooling to room temperature and the polymer was dissolved in dichloromethane. The solution was poured into a petri dish and residual monomer was removed in a vacuum drying cabinet at elevated temperature (60 °C) for 15 h. Conversion was determined gravimetrically, molecular weight and polydispersity index were determined by size exclusion chromatography (SEC) (table S1).

General procedure for the polymerization of *N*-isopropyl acrylamide.

In a Schlenk-tube the peptide initiator (1.0 or 0.5 mol-%) and *N*-isopropyl acrylamide (201 mg, 1.78 mmol) were dissolved in benzene (1.0 mL). The solution was degassed in three freeze-thaw cycles. The polymerization was carried out under argon at 125 °C

for 8-40 h. The resulting mixture was cooled to room temperature, dissolved in a small amount of acetone and precipitated from Et_2O. The polymer was filtered and dried on air. Conversion was evaluated by ^{1}H-NMR spectroscopy comparing the vinylic monomer resonance signal at δ = 5.31 (*dd*, 1 H, ^2J = 10.1 Hz, $^3J_{cis}$ = 2.2 Hz, CH=CH_{cis}H$_{trans}$) ppm as well as the *iso*-propylic monomer signal at δ = 4.26-4.09 (*m*, 1 H, CH(CH$_3$)$_2$) with the *iso*-propylic resonance of the polymer at δ = 4.32-4.01 (*br m*, 1 H, CH(CH$_3$)$_2$) ppm, additionally the resonance of the methyl groups of the monomer (δ = 1.01 (*d*, 6 H, ^3J = 6.6 Hz, CH(CH_3)$_2$) ppm) and the polymer (δ = 1.45-1.02 (*br m*, 6 H, CH(CH_3)$_2$) ppm) were set into relation. The molecular weight was determined by MALLS and mass spectrometry (ESI),[7] the polydispersity index was determined by size exclusion chromatography (SEC) and mass spectrometry (ESI) (table S1).[7]

Entry	Initiator	Ini. Conc.	Monomer	Polymer	Temp.	Time	Conv.	$M_{n,theor.}$	M_n (SEC)	PDI (SEC)	M_n (ESI)	PDI (ESI)	dn/dc	M_w (MALLS)
		(mol-%)			[°C]	[h]	[%]	[g/mol]	[g/mol]		[g/mol]		[mL/g]	[g/mol]
1	**6** (n=0)	**1.0**	styrene	**6**	125	24	72	8400	6100	1.15	n.d.	n.d.	n.d.	n.d.
2	**6** (n=0)	**0.5**	styrene	**6**	105	24	45	10300	9900	1.18	n.d.	n.d.	n.d.	n.d.
2	**6** (n=0)	**0.5**	styrene	**6**	125	24	76	16700	11700	1.15	n.d.	n.d.	n.d.	n.d.
3	**7** (n=0)	**0.5**	styrene	**7**	105	24	41	9500	9500	1.20	n.d.	n.d.	n.d.	n.d.
3	**7** (n=0)	**0.5**	styrene	**7**	125	18	65	14600	12200	1.16	n.d.	n.d.	n.d.	n.d.
4	**8**	**0.5**	styrene	**9**	105	24	80	17600	16700	1.10	n.d.	n.d.	n.d.	n.d.
5	**8**	**1.0**	*n*-BA	**10**	125	8	80	11200	14900	1.13	n.d.	n.d.	n.d.	n.d.
6	**8**	**1.0**	NIPAM	**11**	125	8	42	5700	4400	1.26	6500	1.16	0.0935	7900
7	**8**	**0.5**	NIPAM	**11**	125	20	71	17000	4600	1.36	14700	1.08	0.0935	10800
8	**12** (n=0)	**0.5**	NIPAM	**12**	125	36	72	17100	4500	1.38	15800	1.04	0.0945	12700
9	**14** (n=0)	**0.5**	NIPAM	**14**	125	40	54	13200	4400	1.38	13500	1.06	0.1017	10900

Table S1. Results of the polymerizations.

Proof of control/livingness of styrene polymerizations using initiator 8.

Following the procedure described above styrene was polymerized at 105 °C for 2-16 h using 0.5 mol-% of **8**. Conversion was determined gravimetrically, molecular weight and polydispersity index were determined by size exclusion chromatography (SEC) (figures S1 and S2).

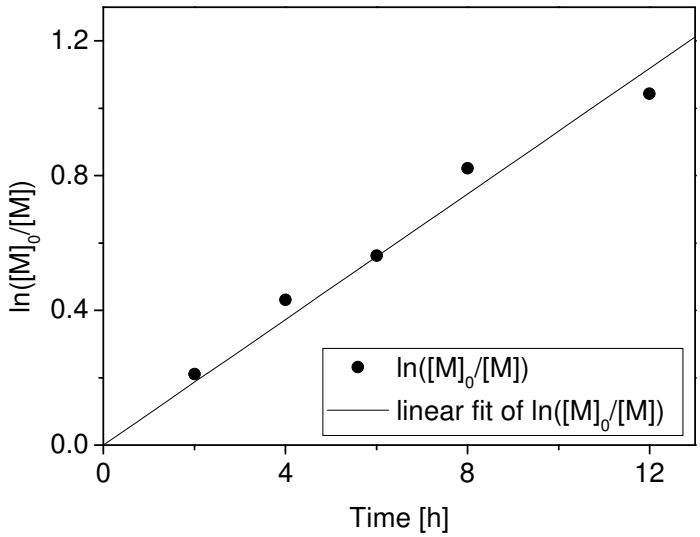


Figure S1. Monomer conversion vs time (styrene, bulk, 105 °C, 0.5 mol-% **8**).

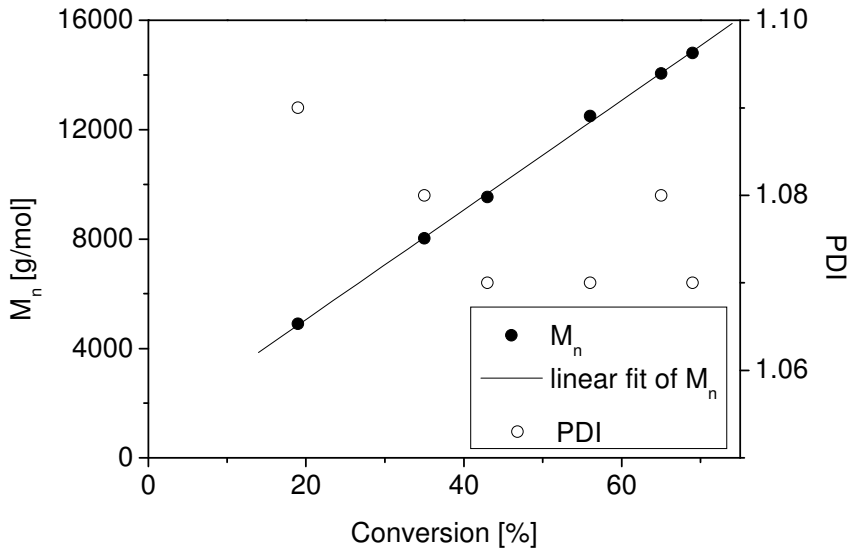


Figure S2. Molecular weight and PDI vs monomer conversion (styrene, bulk, 105 °C, 0.5 mol-% **8**).

Proof of control/livingness of *n*-butyl acrylate polymerizations using initiator 8.

Following the procedure described above *n*-butyl acrylate was polymerized at 125 °C for 1-7 h using 0.5 mol-% of **8**. Conversion was determined gravimetrically,

molecular weight and polydispersity index were determined by size exclusion chromatography (SEC) (figures S3 and S4).

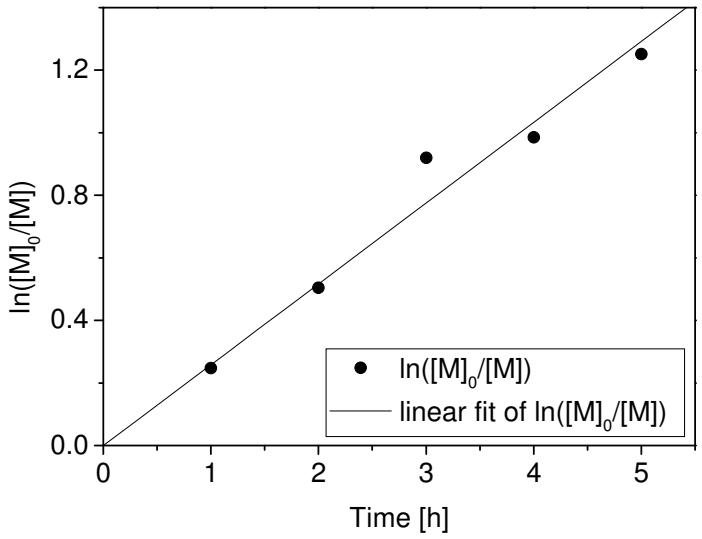


Figure S3. Monomer conversion vs time (*n*-butyl acrylate, bulk, 125 °C, 0.5 mol-% **8**).

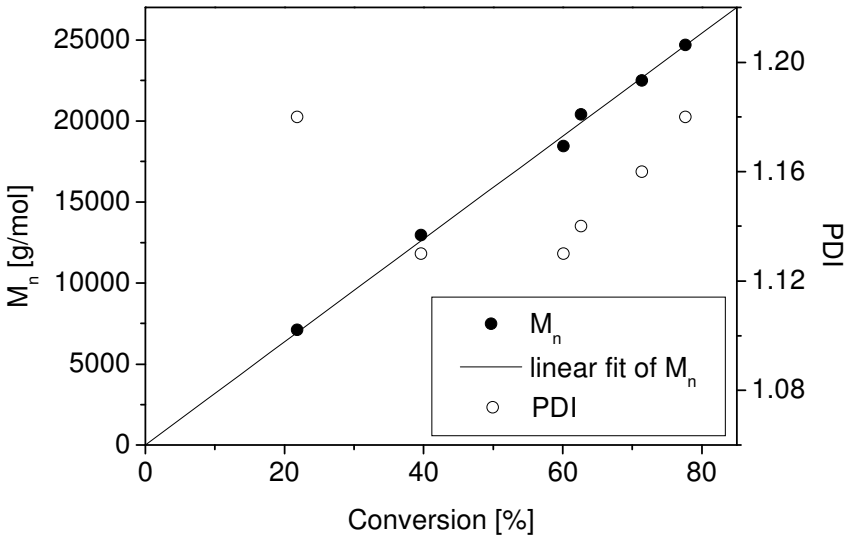


Figure S4. Molecular weight and PDI vs monomer conversion (*n*-butyl acrylate, bulk, 125 °C, 0.5 mol-% **8**).

Determination of the LCSTs of 11, 12 and 14 (n≠0) using ^{1}H-NMR spectroscopy.

The samples were prepared in D_2O at a concentration of 3 mg/mL. Spectra were collected between 25 °C and 40 °C with intervals of 1 °C. The intensities of the signals of the methyl groups of PNIPAM (δ = 1.45-1.02 (*bs*, 6 H, CH(CH_3)$_2$) ppm) were measured in relation to the solvent residual peak at 4.79 ppm and then normalized. For every conjugate the LCST was determined at a normalized intensity of 0.5 (figure S5).[12] For **11** the LCST was found to equal 31 °C, for **12** and **14** the LCST was 29 °C.

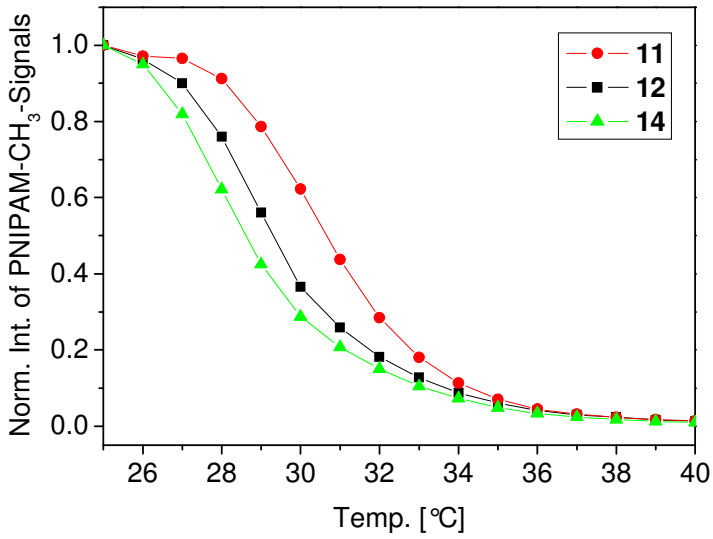


Figure S5. Plot of the normalized intensities of the PNIPAM-CH_3-^{1}H-NMR-signals versus temperature for the PNIPAM peptide conjugates **11**, **12** and **14**.

Enzymatic hydrolysis of conjugate 12 at 22 °C.

Conjugate **12** was dissolved in Tris-HCl-buffer (0.1 mol/L, 0.01 mol/L $CaCl_2$, pH 7.8) at a concentration of 1 mg/mL. 3 mL of this solution were treated with 0.1 mL of a solution of chymotrypsin (3.3 mg/mL in buffer (see above), 300 U/mL) while the cuvette was kept at 22 °C by a thermostat. The release of *p*-nitroaniline via enzymatic hydrolysis was monitored by UV-Vis absorbtion spectroscopy (figure S6). After 400 min the degradation was complete. The previously colourless solution had turned yellow.

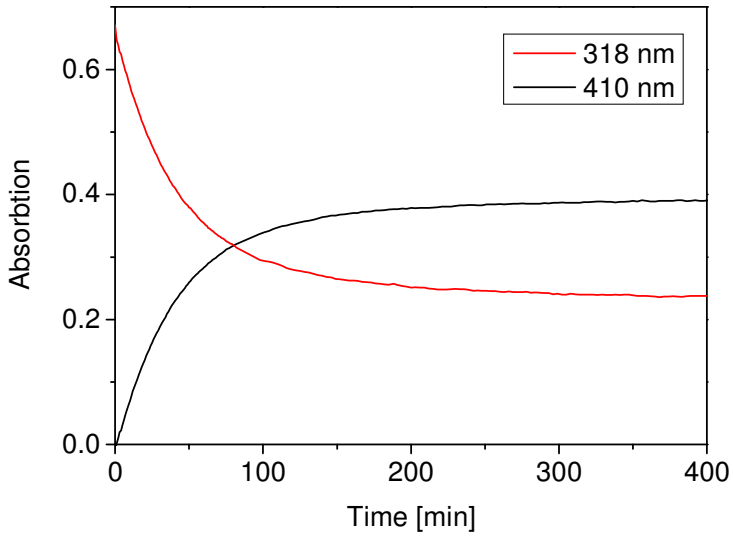


Figure S6. Enzymatic hydrolysis of conjugate **12** at 22 °C – plot of absorbtion versus time.

Enzymatic hydrolysis of conjugate 12 at 37 °C.

Conjugate **12** was dissolved in Tris-HCl-buffer (0.1 mol/L, 0.01 mol/L $CaCl_2$, pH 7.8) at a concentration of 1 mg/mL. 3 mL of this solution were heated in a cuvette to 37 °C whereupon the conjugate percipitated. 0.1 mL of a solution of chymotrypsin (3.3 mg/mL in buffer (see above), 300 U/mL) was added and the cuvette was kept at 37 °C by a thermostat. After 24 h the cuvette was heated to 80 °C for 10 min to denaturate the enzyme and was then cooled to 22 °C. A clear colourless solution was again obtained. UV/Vis absorbtion spectra before and after the procedure showed no significant differences.

Enzymatic hydrolysis of conjugate 14 at 22 °C.

Conjugate **14** was dissolved in Tris-HCl-buffer (0.1 mol/L, 0.01 mol/L $CaCl_2$, pH 7.8) at a concentration of 1 mg/mL. 3 mL of this solution were treated with 0.05 mL of a solution of chymotrypsin (132 mg/L in buffer (see above), 12 U/mL) while the cuvette was kept at 22 °C by a thermostat. The release of *p*-nitroaniline via enzymatic hydrolysis was monitored by UV-Vis absorbtion spectroscopy (figure S7). After 20 min the degradation was complete. The previously colourless solution had turned yellow. Obviously, conjugate **14** is degraded faster than conjugate **12**. It is well

known that the amino acid sequence has a great influence on the substrate activity.[13] Therefore we will not draw any conclusions regarding the effect of the polymer on the kinetics of the enzymatic reaction.

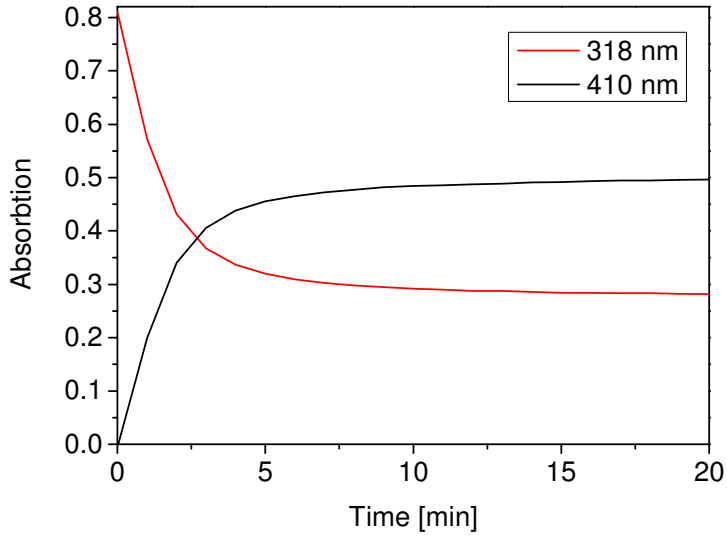


Figure S7. Enzymatic hydrolysis of conjugate **14** at 22 °C – plot of absorbtion versus time.

Enzymatic hydrolysis of conjugate 14 at 37 °C.

Conjugate **14** was dissolved in Tris-HCl-buffer (0.1 mol/L, 0.01 mol/L $CaCl_2$, pH 7.8) at a concentration of 1 mg/mL. 3 mL of this solution were heated in a cuvette to 37 °C whereupon the conjugate percipitated. 0.05 mL of a solution of chymotrypsin (132 mg/L in buffer (see above), 12 U/mL) was added and the cuvette was kept at 37 °C by a thermostat. After 4 h the cuvette was heated to 80 °C for 10 min to denaturate the enzyme and was then cooled to 22 °C. A clear colourless solution was again obtained. UV/Vis absorbtion spectra before and after the procedure showed no significant differences.

Reversible switching of substrate activity using conjugate 14.

Conjugate **14** was dissolved in Tris-HCl-buffer (0.1 mol/L, 0.01 mol/L $CaCl_2$, pH 7.8) at a concentration of 1 mg/mL. 3 mL of this solution were treated with 0.01 mL of a solution of chymotrypsin (132 mg/L in buffer (see above), 12 U/mL) while the cuvette was kept at 22 °C by a thermostat. The release of *p*-nitroaniline via enzymatic

hydrolysis was monitored by UV-Vis absorbtion spectroscopy (figure S8). After 10 min the cuvette was heated up to 37 °C and kept at this temperature for 30 min before it was heated to 80 °C for 15 min to denaturate the enzyme. The vessel was then cooled back to 22 °C and 0.05 mL of the same enzyme solution were added. After 15 min the degradation was complete. The previously colourless solution had turned yellow.

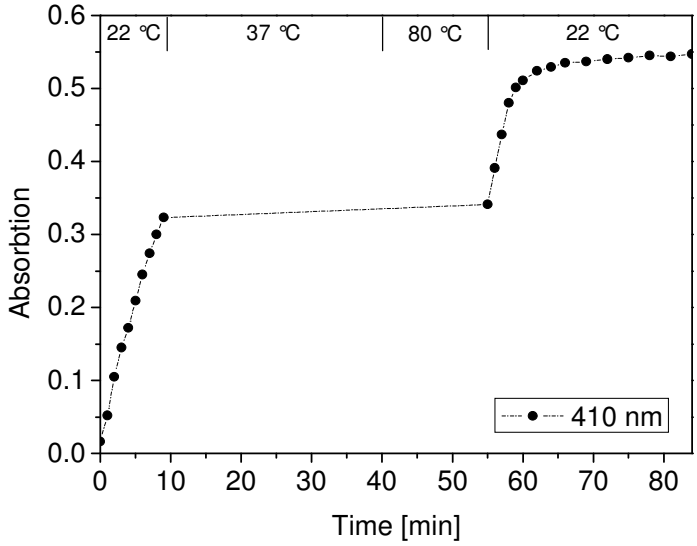


Figure S8. Enzymatic hydrolysis of conjugate 14 at 22 °C interrupted upon temperature increase.– plot of absorbtion versus time.

1 K. Schwetlick et al., *Organikium, 19. edition*, Johann Ambrosius Barth Leipzig, 1993, p. 176.

2 T. Schulte, K. O. Siegenthaler, H. Luftmann, M. Letzel, A. Studer, *Macromolecules*, 2005, **38**, 6833.

3 A. J. Herrera, A. Studer, *Synthesis*, 2005, 1389.

4 M. A. A. Ghani, D. Abdallah, P. M. Kazmaier, B. Keoshkerian, E. Buncel, *Can. J. Chem.*, 2004, **82(9)**, 1403.

5 M. Stasiak, C. Röben, N. Rosenberger, F. Schleth, A. Studer, A. Greiner, J. H. Wendorff, *Polymer*, 2007, **48**, 5208.

6 D. T. S. Rijkers, H. P. H. M. Adams, H. C. Hemker, G. I. Tesser, *Tetrahedron*, 1995, **51(41)**, 11235.

7 S. Kehr, H. Luftmann, *e-Polymers*, 2007, no. 010.

8 A. Larsson, D. Kuckling, M. Schönhoff, *Coll. Surf. A*, 2001, **190**, 185.

9 K. Matyjaszewski, B. E. Woodworth, X. Zhang, S. G. Gaynor, Z. Metzner, *Macromolecules*, 1998, **31**, 5955.

10 G. M. Day, O. T. Howell, M. R. Metzler, P. D. Woodgate, *Aust. J. Chem.*, 1997, **50(4)**, 425.

11 P. D. Greenspan, K. L. Clark, R. A. Tommasi et al., *J. Med. Chem.*, 2001, **44(26)**, 4524.

12 J. E. Chung, M. Yokoyama, T. Aoyagi, Y. Sakurai, T. Okano, *J. Control. Release*, 1998, **53**, 119.

13 E. G. DelMar, C. Largman, J. W. Broderick, M. C. Geokas, *Anal. Biochem.* 1979, **99**, 316.