Supporting Information

High-Affinity Copolymers Inhibit Digestive Enzymes by Surface Recognition

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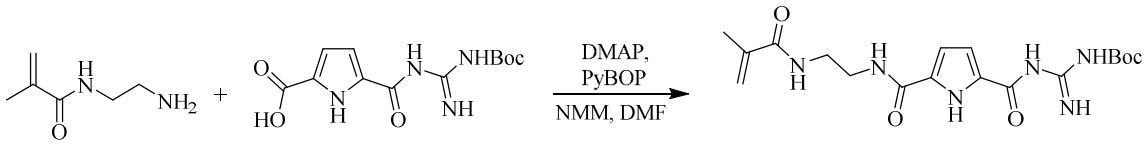
Synthetic Procedures

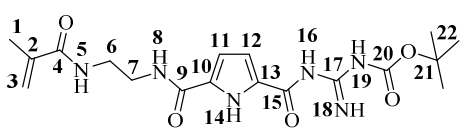
CBS Monomer 4:

5-*N*-Boc-guanidiniocarbonyl-2-*N*-(2-methacrylamidoethyl)-1*H*-pyrrole-2-carboxamide.



1 **2** **3**

Under argon 2.00 g (5.03 mmol, 1.3 eq.) **2** were treated with DMF (100 mL), *N*-methylmorpholine (1.66 mL, 1.53 g, 15.1 mmol, 3.0 eq.), DMAP (1.56 g, 12.8 mmol, 2.5 eq.) as well as PyBOP (2.88 g, 5.53 mmol, 1.1 eq.), and the mixture was stirred for 30 min. A suspension of the ethylene diamine monomer **1** (1.08 g, 5.38 mmol, 1.1 eq.) in DMF (5 – 10 mL) was added slowly. The resulting mixture was stirred under argon for 7 days. After dilution with water (500 mL) the product was extracted five times with chloroform (5x200 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. DMF was finally condensed off at 60 °C. The crude product was chromatographed twice over silica (EA/MeOH = 3:1/R_F = 0.8; then EA) to afford **3** as a colorless solid. Yield: 755 mg (1.86 mmol, 37 %). R_F = 0.13 (EA).

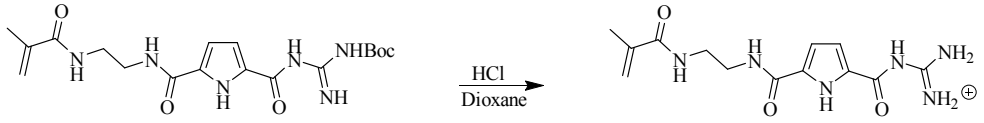


^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.51 (s, 9H, H-22), 1.96 (s, 3H, H-1), 3.48-3.72 (m, 4H, H-6, H-7), 5.35 (s, 1H, H-3a), 5.76 (s, 1H, H-3b), 6.64 (s, 1H, H-11, H-12), 6.78 (s, 1H, NH), 6.91 (s, 1H, H-11, H-12), 7.22 (sb, 1H, NH), 8.53 (sb, 2H, NH), 10.26 (sb, 1H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.7 (C-1), 28.2 (C-22), 40.4, 40.8 (C-6, C-7), 83.8 (C-21), 111.3, 114.5 (C-11, C-12), 120.7 (C-3), 132.5 (C-10, C-13), 139.5 (C-2), 153.6, 158.4, 161.7, 162.7 (C-9, C-15, C-17, C-20), 170.1 (C-4).

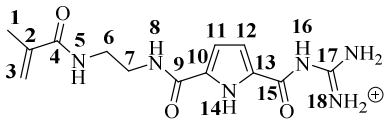
MS (**ESI**, pos., MeOH) for $C_{18}H_{26}N_6NaO_5$: calcd. 429.19; found 429.20.

5-Guanidiniocarbonyl-2-*N*-(2-methacrylamidoethyl)-1*H*-pyrrole-2-carboxamide Hydrochloride.

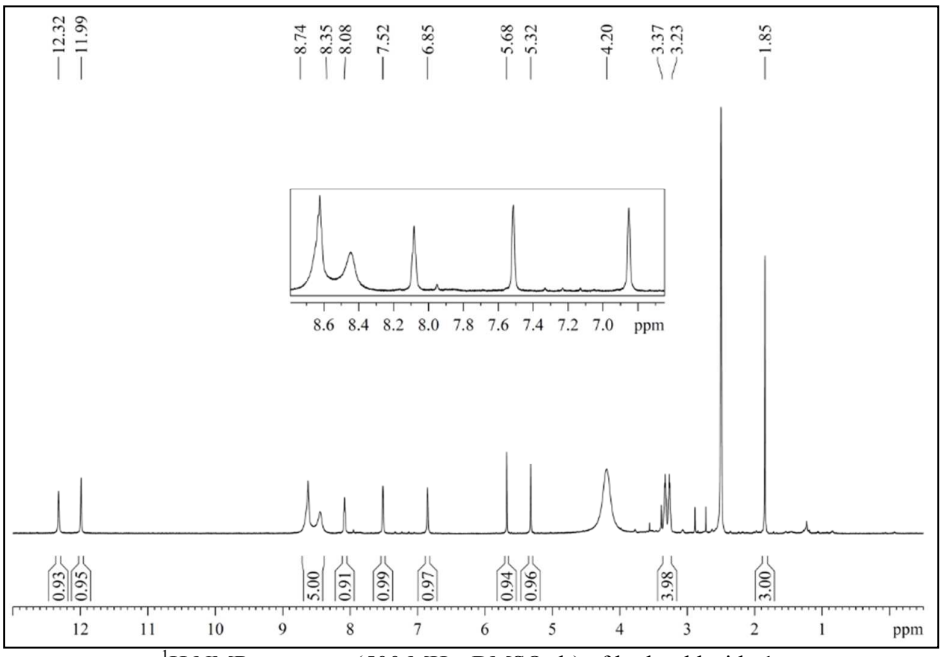


3 **4**

A solution of **3** (548 mg, 1.35 mmol) in Dioxane (40 mL) was treated dropwise with conc. HCl (11.5 mL). The mixture was stirred at room temperature for 24 h and subsequently evaporated to dryness to furnish **4** as a beige solid. Yield: 99%.

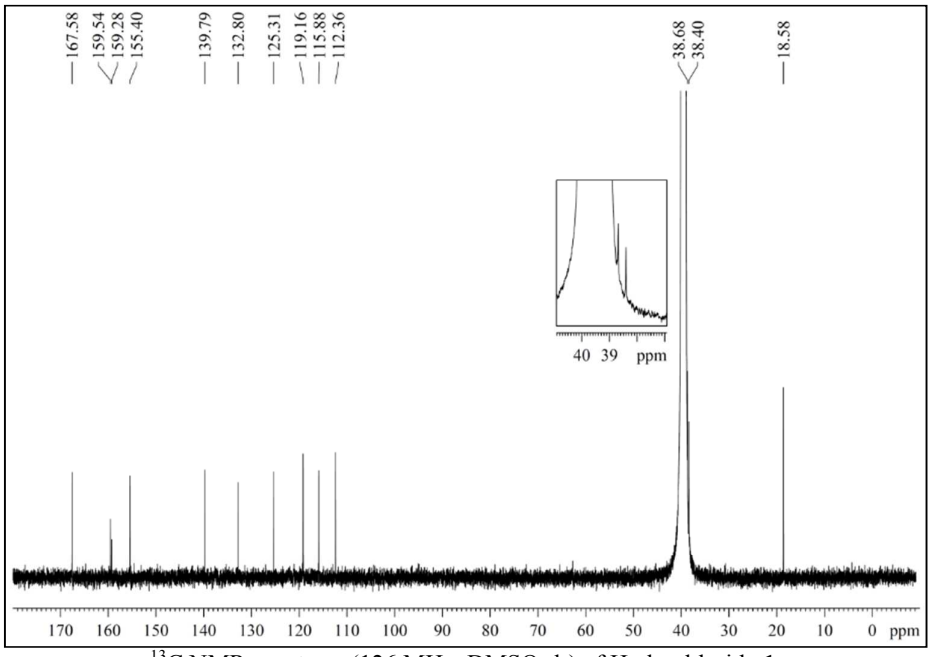


^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 1.85 (s, 3H, H-1), 3.23-3.37 (m, 4H, H-6, H-7), 5.32 (s, 1H, H-3a), 5.68 (s, 1H, H-3b), 1.85 (s, 3H, H-1), 3.23-3.37 (m, 4H, H-6, H-7), 5.32 (s, 1H, H-3a), 5.68 (s, 1H, H-3b), 6.85 (s, 1H, H-11, H-12), 7.52 (s, 1H, H-11, H-12), 8.08 (s, 1H, H-5, H-8), 8.35-8.74 (m, 5H, H-5, H-8, H-18), 11.99 (s, 1H, H-16), 12.33 (s, 1H, H-14).



^{1}H NMR spectrum (500 MHz, DMSO-d_6) of hydrochloride **1**.

^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 18.6 (C-1), 38.4, 38.7 (C-6, C-7), 112.4, 115.9 (C-11, C-12), 119.2 (C-3), 125.3, 132.8 (C-10, C-13), 139.8 (C-2), 155.4, 159.3, 159.5 (C-9, C-15, C-17), 167.6 (C-4).

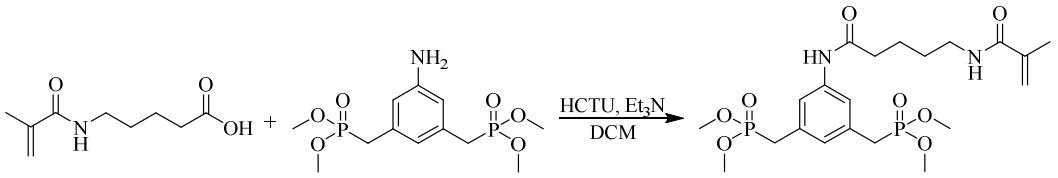


^{13}C NMR spectrum (126 MHz, DMSO-d_6) of Hydrochloride **1**.

MS (**ESI**, pos., MeOH) for $C_{13}H_{19}N_6O_3$: calcd. 307.15; found 307.14; for $C_{13}H_{18}N_6NaO_3$: calcd. 329.13; found 329.12.

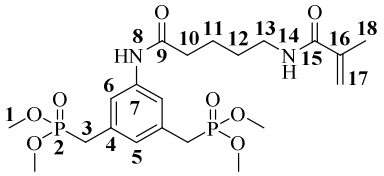
C_5-Bisphosphonate Monomer 8:

***N*-[3,5-bis(dimethoxyphosphorylmethyl)phenylaminocarbonyl-4-butyl]methacrylamide.**



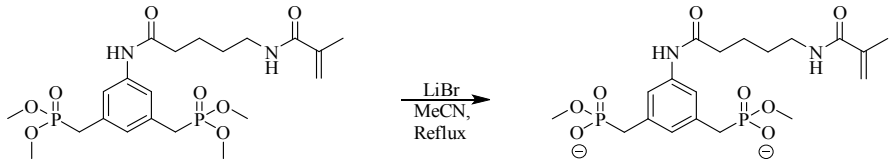
5 **6** **7**

The C_5-spacer-monomer **5** (274 mg, 1.48 mmol, 1.0 eq.), triethylamine (620 µL, 450 mg, 4.45 mmol, 3.0 eq.) and HCTU (808 mg, 1.95 mmol, 1.3 eq.) were successively added to dichloromethane (15 mL). The reaction mixture was treated dropwise at 0 °C with a solution of bisphosphonate **6** (600 mg, 1.78 mmol, 1.2 eq.) in dichloromethane (5 mL). The reaction mixture was degassed and stirred at room temperature for 14 h. Subsequently, it was washed several times with aq. NaOH (0.6 M), dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by chromatography over silica (DCM/MeOH = 24:1) to yield **7** as a yellow oil. Yield: 162 mg (0.32 mmol, 22 %). R_F = 0.32 (DCM/MeOH = 9:1).



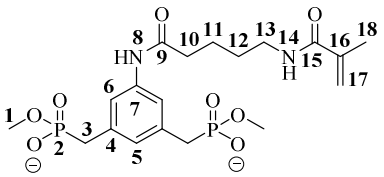
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.57-1.64 (m, 2H, H-12), 1.70-1.76 (m, 2H, H-11), 1.97 (s, 3H, H-18), 2.40 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-10), 3.10 (d, $^2J_{H,P}$ = 21.8 Hz, 4H, H-3), 3.33-3.39 (m, 2H, H-13), 3.69 (d, $^3J_{H,P}$ = 10.8 Hz, 12H, H-1), 5.34 (s, 1H, H-17a), 5.75 (s, 1H, H-17b), 6.44 (sb, 1H, H-14), 6.94 (s, 1H, H-5) 7.43 (s, 2H, H-6), 8.40 (s, 1H, H-8).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 18.9 (C-18), 22.7 (C-11), 29.0 (C-12), 32.7 (d, $^1J_{C,P}$ = 138 Hz, C-3), 36.7 (C-10), 38.8 (C-13), 53.1 (C-1), 119.8 (C-6), 120.1 (C-17), 126.5 (C-5), 132.3 (C-4), 139.2 (C-7), 139.9 (C-16), 169.1 (C-15), 172.0 (C-9).
^{31}P NMR (203 MHz, $CDCl_3$): δ [ppm] = 28.5 (P-2).
MS (**ESI**, pos., MeOH) for $C_{21}H_{34}N_2NaO_8P_2$: calcd. 527.17; found: 527.19.

Dilithium *N*-[3,5-bis(dimethoxyphosphorylmethyl)phenylamincarbonyl-4-butyl] methacrylamide.

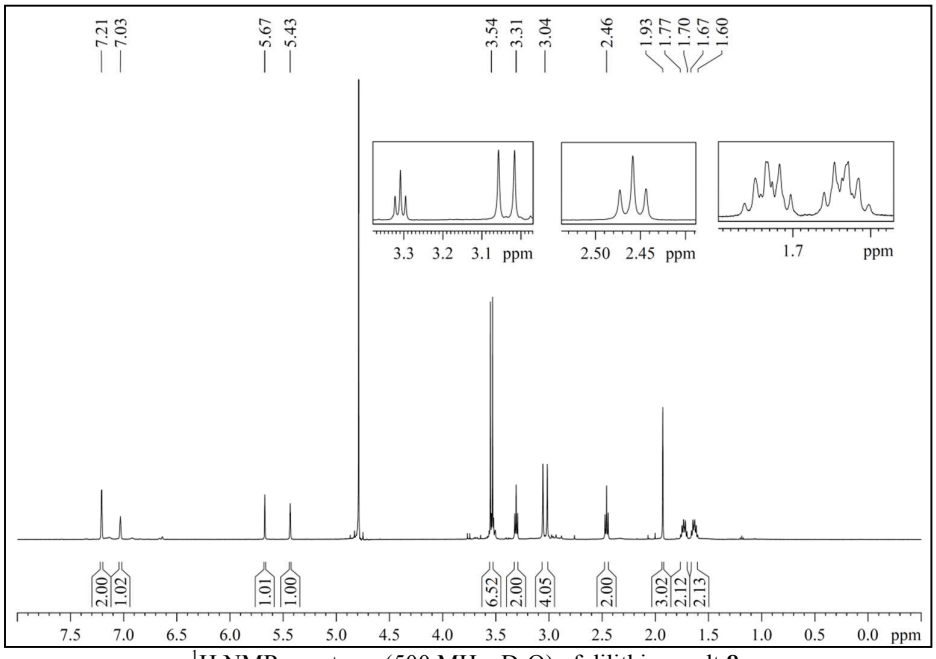


7 **8**

Tetramethyl bisphosphonate **7** (146 mg, 289 µmol, 1.0 eq.) was dissolved under argon in dry acetonitrile **(**5 mL) and treated with dry LiBr (70.4 mg, 810 µmol, 2.8 eq.). The reaction mixture was heated to reflux for 1 day and after cooling to room temperature, the solvent was centrifuged off. The solid residue was washed 4 times with dry acetonitrile (5 mL) and twice with diethyl ether (5 mL). The resulting beige solid **8** was dried under fine vacuum (0.001 mbar). Yield: 140 mg (287 µmol, 99 %).

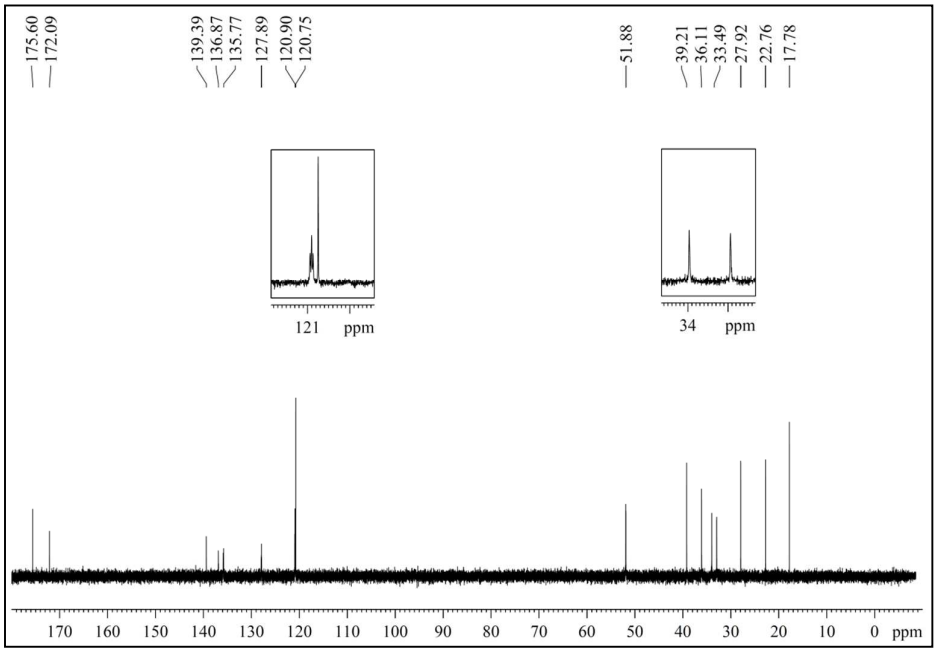


^{1}H NMR (500 MHz, D_2O): δ [ppm] = 1.60-1.67 (m, 2H, H-12), 1.70-1.77 (m, 2H, H-11), 1.93 (s, 3H, H-18), 2.46 (t, $^3J_{H,H}$ = 7.3 Hz, 2H, H-10), 3.04 (d, $^2J_{H,P}$ = 20.6 Hz, 4H, H-3), 3.31 (m, $^3J_{H,H}$ = 6.8 Hz, 2H, H-13), 3.54 (d, $^3J_{H,P}$ = 10.4 Hz, 6H, H-1), 5.43 (s, 1H, H-17a), 5.67 (s, 1H, H-17b), 7.03 (s, 1H, H-5), 7.21 (s, 2H, H-6).



^{1}H NMR spectrum (500 MHz, D_2O) of dilithium salt **8**.

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 17.8 (C-18), 22.8 (C-11), 27.9 (C-12), 33.5 (d, $^1J_{C,P}$ = 130 Hz, C-3), 36.1 (C-10), 39.1 (C-13), 51.9 (C-1), 120.8 (C-17), 120.9 (C-6), 127.9 (C-5), 135.8 (C-4), 136.9 (C-7), 139.4 (C-16), 172.1 (C-15), 175.6 (C-9).



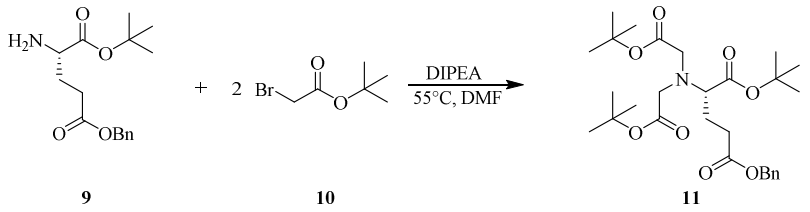
^{13}C NMR spectrum (126 MHz, D_2O) of dilithium salt **8**.

^{31}P NMR (203 MHz, D_2O): δ [ppm] = 23.8 (P-2).

MS (**ESI**, pos., MeOH) for $C_{19}H_{28}LiN_2Na_2O_8P_2$: calcd. 527.13; found 527.17.

NTA-Glu Monomer 14:

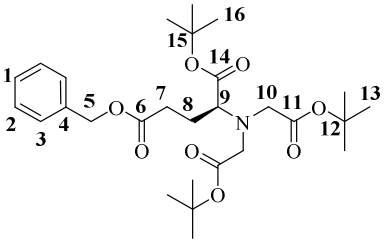
(2*S*)-2-(Bis-*tert*-butoxycarbonylmethyl)amino-2-*tert*-butoxycarbonyl-butanoic acid benzyl ester.



9 **10** **11**

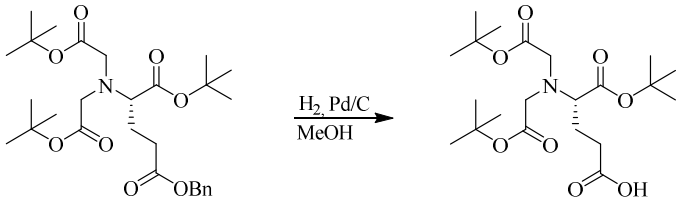
Under argon a solution of *γ*-benzyl *α*-*tert*-butyl L-glutamic acid **9** 1.00 g (3.03 mmol, 1.0 eq.) and DMF (25 mL) was treated with *tert*-butyl bromoacetate **10 (**1.79 mL, 2.36 g, 12.1 mmol, 4.0 eq.) as well as DIPEA (2.61 mL, 1.98 g, 15.3 mmol, 5.0 eq.). The reaction mixture was warmed to 55 °C and stirred for 18 h. After condensation of DMF (60 °C), the residue was suspended in ethyl acetate (15 mL) and filtered. The remaining solid was suspended several times in CH/EA (3:1) and likewise filtered. The combined phases were evaporated to dryness and the crude product was chromatographed over silica (CH/EE = 4:1) to yield **11** as a yellow oil. Yield: 1.44 g (2.76 mmol, 91 %). R_F = 0.6 (CH/EE = 4:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = **1.40-1.50** (m, 27H, H-13, H-16), **1.85-2.07** (m, 2H, H-8), **2.50-2.76** (m, 2H, H-7), **3.34-3.49** (m, 5H, H-9, H-10), **5.11** (s, 2H, H-5), **7.27-7.38** (m, 5H, H-1, H-2, H-3).



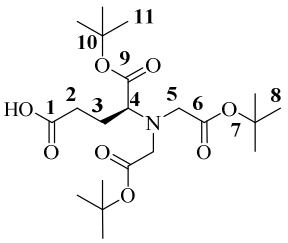
HRMS (**ESI**, pos., MeOH) for $C_{28}H_{44}NO_8$: calcd. 522.3061; found 522.3319; and for $C_{28}H_{43}NNaO_8$: calcd 544.2881; found 544.2941.

(2*S*)-2-(Bis-*tert*-butoxycarbonylmethyl)amino-2-*tert*-butoxycarbonyl-butanoic acid.



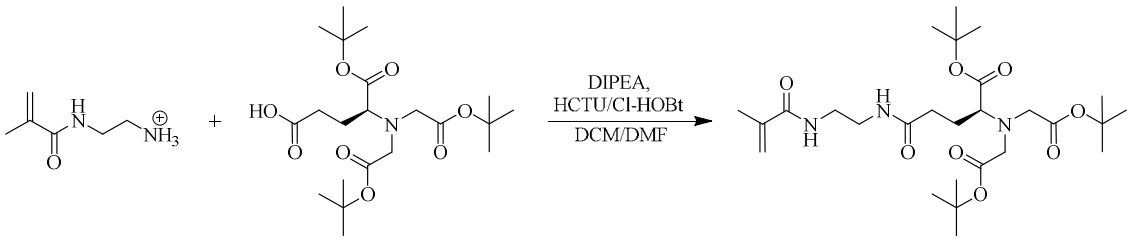
11 **12**

Benzyl ester **11** (1.40 g, 2.68 mmol) was dissolved in methanol (50 mL), treated with 10% Pd/C (0.2 g) and stirred at room temperature for 16h under hydrogen gas. The suspension was filtered over celite and evaporated to dryness to give a **12** as a yellow oil. Yield: 0.91 g (2.10 mmol, 78 %).



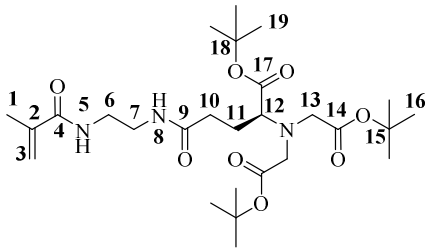
^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 1.49 (s, 9H, H-8), 1.50 (s, 18H, H-11), 1.93-2.10 (m, 2H, H-3), 2.64-2.77 (s, 2H, H-2), 3.42-3.47 (m, 1H, H-4), 3.52 (s, 4H, H-5).
^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 25.4 (C-3), 28.2 (C-8), 28.3 (C-11), 31.2 (C-2), 54.4 (C-5), 64.9 (C-4), 81.6, 82.0 (C-7, C-10), 170.5 (C-6), 171.3 (C-9), 176.3 (C-1).
HRMS (**ESI**, pos., MeOH) for $C_{21}H_{38}NO_8$: calcd. 432.2592; found 432.2676; and for $C_{21}H_{37}NNaO_8$: calcd. 454.2411; found 454.2490.

(2*S*)-2-(Bis-*tert*-butoxycarbonylmethyl)amino-(2-*tert*-butoxycarbonyl-butanoyl)amino-ethyl-methacrylamide.



1 **12** **13**

The Boc-protected NTA building block **12** (2.54 g, 5.89 mmol, 1.0 eq.) was dissolved in DCM/DMF (4:1, 125 mL). Under stirring DIPEA (6.17 mL, 4.58 g, 35.4 mmol, 6.0 eq.), HCTU (2.69 g, 6.49 mmol, 1.1 eq.) and Cl-HOBt (2.50 g, 14.7 mmol, 2.5 eq.) were slowly added. Subsequently the reaction mixture was treated dropwise with a solution of the ethylene diamine monomer hydrochloride **1** (1.78 g, 8.88 mmol, 1.5 eq.) in DCM/DMF (4:1, 25 mL). The suspension was stirred at room temperature for 3 days under argon. It then was evaporated to dryness und the residue was dissolved in dichloromethane (100 mL). The solution was washed twice with brine (100 mL) and dried over Na_2SO_4. After evaporation of the solvent the crude product was purified by chromatography over silica eluting with ethyl acetate, and afforded **13** a yellow solid. Yield: 1.98 g (3.66 mmol, 62 %). R_F = 0.3 (EA).

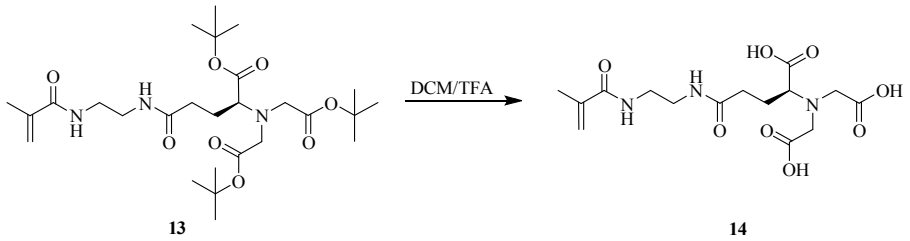


^{1}H NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.39 (sb, 27H, H-16, H-19), 1.73 (q, 2H, H-11), 1.84 (s, 3H, H-1), 2.09-2.25 (m, 2H, H-10), 3.11-3.15 (m, 4H, H-6, H-7), 3.23-3.44 (m, 5H, H-12, H-13), 5.31 (s, 1H, H-3a), 5.64 (s, 1H, H-3b), 7.83 (sb, 1H, NH), 7.90 (sb, 1H, NH).

^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 18.6 (C-1), 25.8 (C-11), 27.8, 27.8 (C-16, C-19), 31.6 (C-10), 38.2, 39,0 (C-6, C-7), 53.2 (C-13), 64.2 (C-12), 80.1, 80.1 (C-16, C-18) 119.1 (C-3), 139.9 (C-2), 167.6 (C-4), 171.3 (C-14), 172.1 (C-9).

HRMS (**ESI**, pos., MeOH) for $C_{27}H_{47}N_3NaO_8$: calcd. 564.3255; found 564.3364.

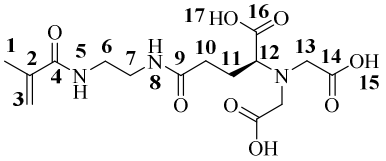
(2*S*)-4-Bis(carboxymethyl)amino-4-carboxy-butanoylaminoethyl-methacrylamide.

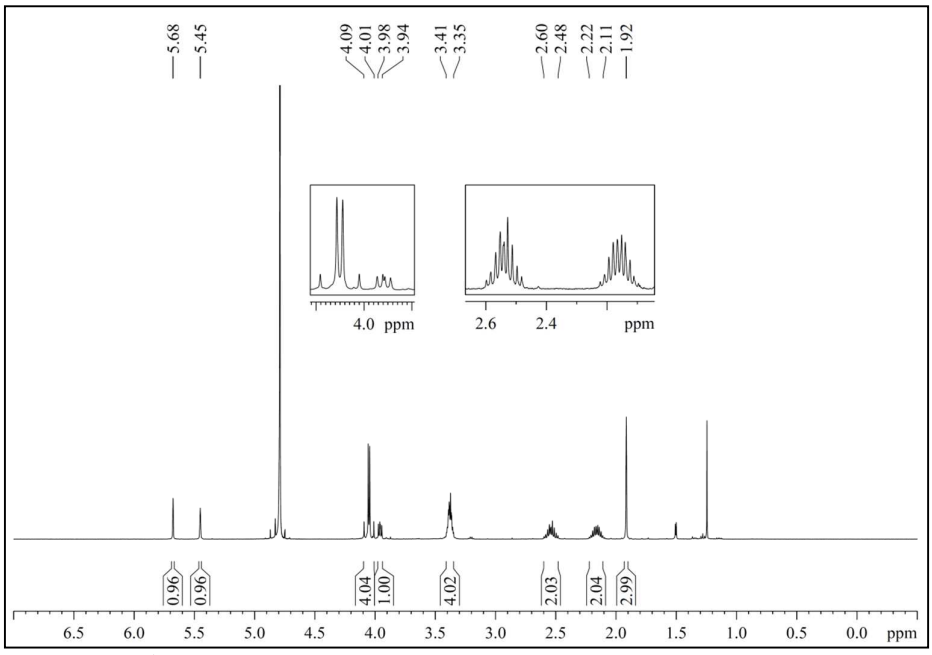


13 **14**

Tri-Boc-protected methacrylamide **13** (1.82 g, 3.35 mmol, 1.0 eq.) was dissolved in dichloromethane (50 mL) and treated dropwise at 0°C with TFA (8.0 mL, 11.9 g, 105 mmol, 31 eq.). The solution was stirred at room temperature for 16 h. Subsequently, the solvent was evaporated to dryness and excess TFA was removed with the aid of benzene to furnish **14** as a beige solid. Yield: 99%.

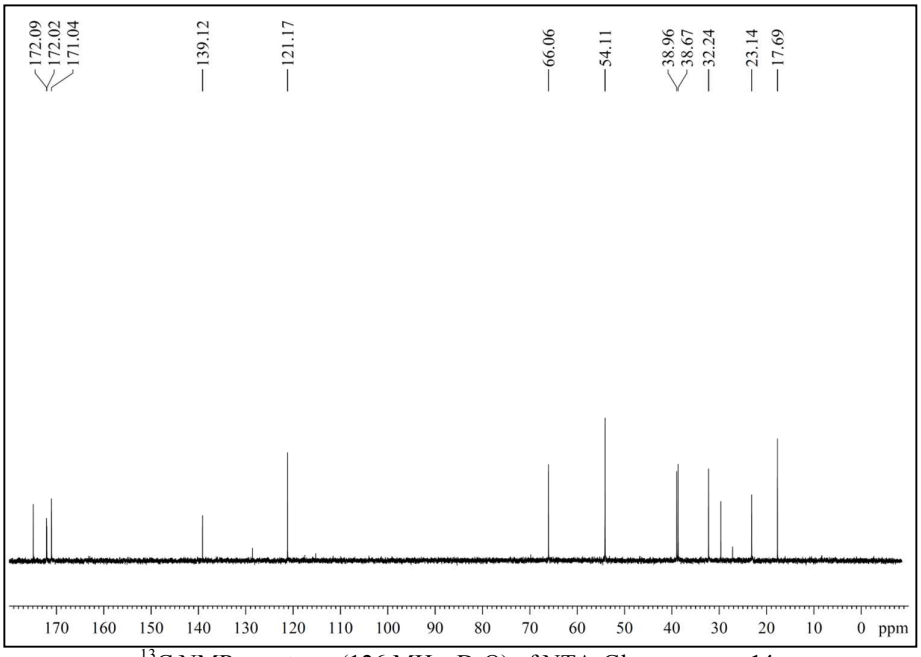
^{1}H NMR (500 MHz, D_2O): δ [ppm] = 1.92 (s, 3H, H-1), 2.11-2.22 (m, 2H, H-11), 2.48-2.60 (m, 2H, H-10), 3.35-3.41 (m, 4H, H-6, H-7), 3.94-3.98 (dd, 1H, H-12), 4.01-4.09 (2d, 4H, H-13), 5.45 (s, 1H, H-3a), 5.68 (s, 1H, H-3b).





^{1}H NMR spectrum (500 MHz, D_2O) of NTA-Glu monomer **14**.

^{13}C NMR (126 MHz, D_2O): δ [ppm] = 17.7 (C-1), 23.1 (C-11), 29.7 (C-10), 38.7 (C-7), 39.0 (C-6), 54.1 (C-13), 66.1 (C-12), 121.2 (C-3), 139.1 (C-2), 171.0 (C-14), 172.0 (C-16), 172.1 (C-4), 174.9 (C-9).

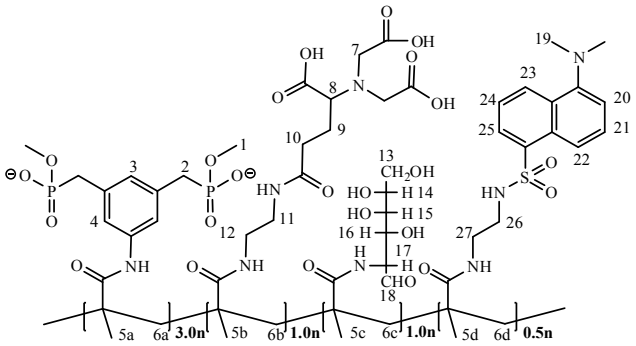


^{13}C NMR spectrum (126 MHz, D_2O) of NTA-Glu monomer **14.**

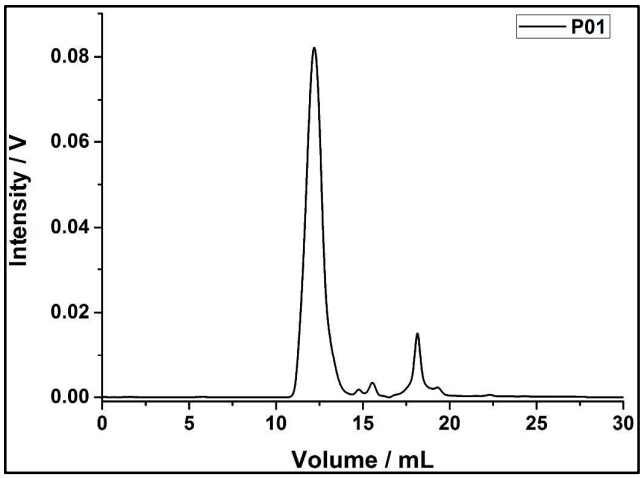
HRMS (**ESI**, pos., MeOH) for $C_{15}H_{24}N_3O_8$: calcd. 374.1558; found 374.1641.

Copolymers

P01:

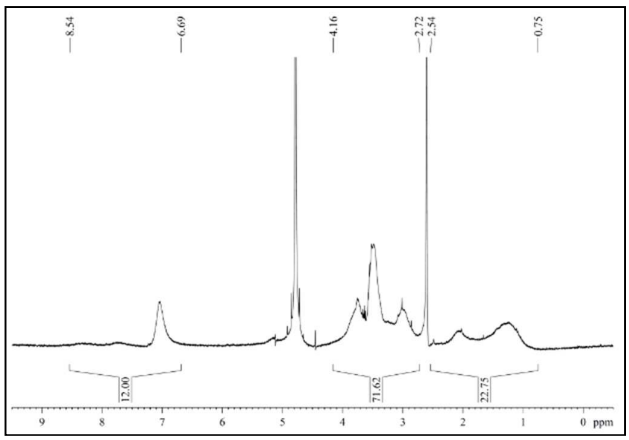


Yield: 49.6 mg (55%).



SEC Chromatogram of **P01**.

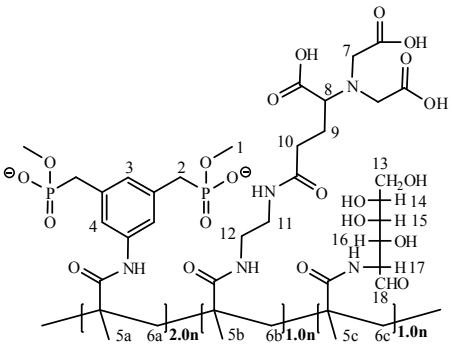
SEC (Pullulan standard): $\overline{M}_w$ = 259 000 g/mol, $\overline{M}_n$ = 135 000 g/mol, PDI = 1.9.



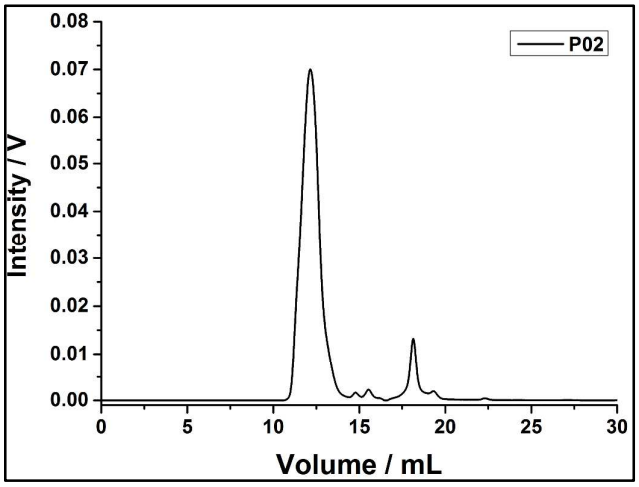
^{1}H NMR spectrum (300 MHz, D_2O) of P01.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.75-2.54 (m, 23.0H, H-5, H-6, H-9, H-10), 2.72-4.16 (m, 72.0H, H-1, H-2, H-7, H-8, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-19, H-26, H-27), 6.69-8.54 (m, 12.0H, H-3, H-4, H-20, H-21, H-22, H-23, H-24, H-25).

P02:

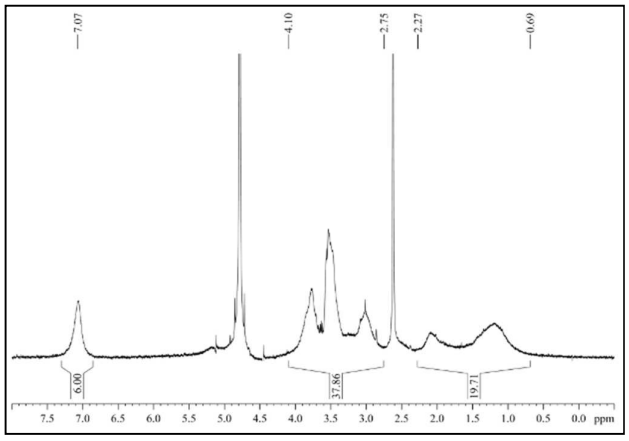


Yield: 34.2 mg (39 %).



SEC chromatogram of **P02**.

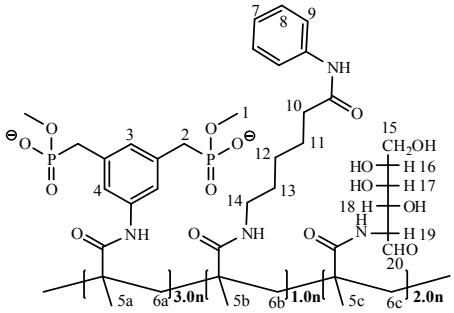
SEC (Pullulan-Standard): $\overline{M}_w$ = 296 000 g/mol, $\overline{M}_n$ = 139 000 g/mol, PDI = 2.1.



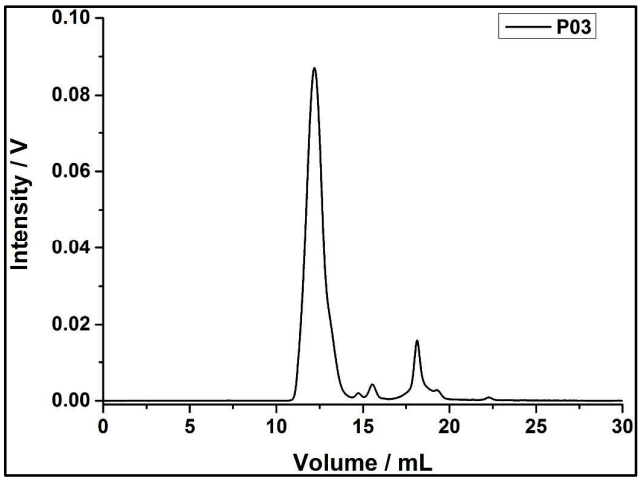
^{1}H NMR (300 MHz, D_2O) of **P02**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.69-2.27 (m, 20H, H-5, H-6, H-9, H-10), 2.75-4.10 (m, 38H, H-1, H-2, H-7, H-8, H-11, H-12, H-13, H-14, H-15, H-16, H-17), 7.07 (sb, 6H, H-3, H-4, H-20).

P03:

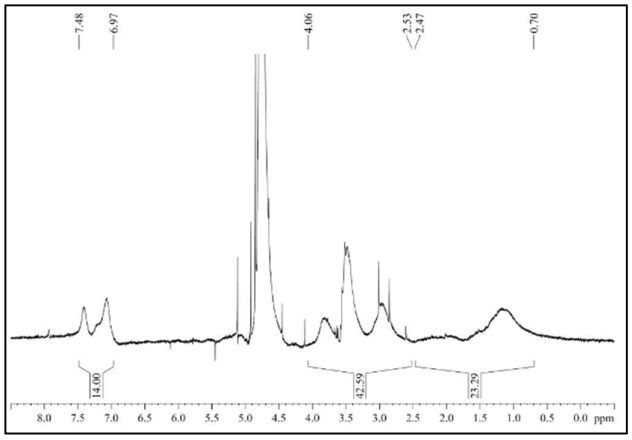


Yield: 46.1 mg (51 %).



SEC chromatogram of **P03**.

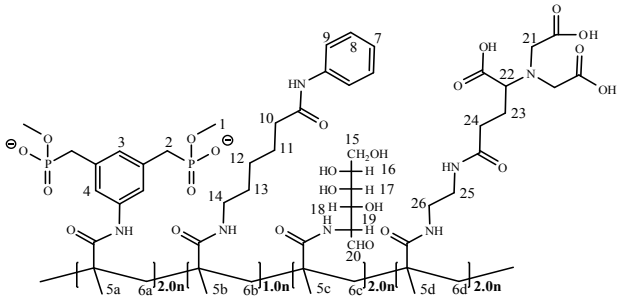
SEC (Pullulan-Standard): $\overline{M}_w$ = 248 000 g/mol, $\overline{M}_n$ = 122 000 g/mol, PDI = 2.0.



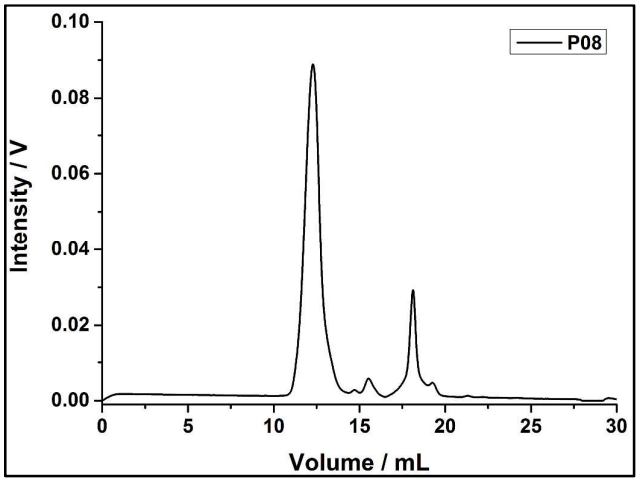
^{1}H NMR (300 MHz, D_2O) of **P03**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.70-2.47 (m, 23H, H-5, H-6, H-10, H-11, H-12,H-13), 2.53-4.06 (m, 43H, H-1, H-2, H-14, H-15, H-16, H-17, H-18, H-19), 6.97-7.48 (m, 14H, H-3, H-4, H-7, H-8, H-9).

P08:

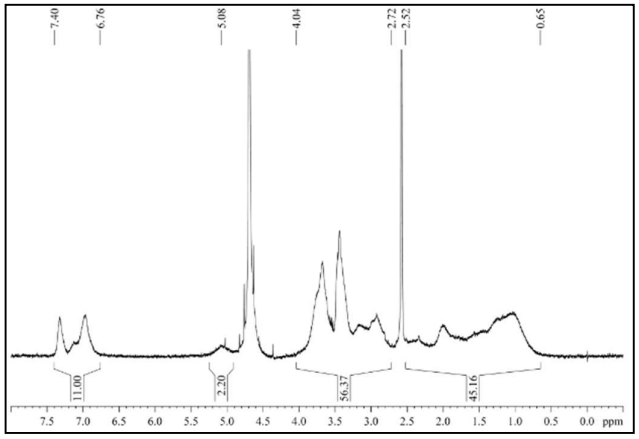


Yield: 43.7 mg (51 %).



SEC chromatogram of **P08**.

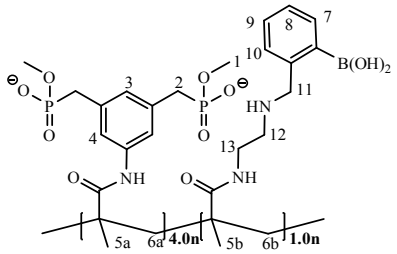
SEC (Pullulan-Standard): $\overline{M}_w$ = 255 000 g/mol, $\overline{M}_n$ = 122 000 g/mol; PDI = 2.1



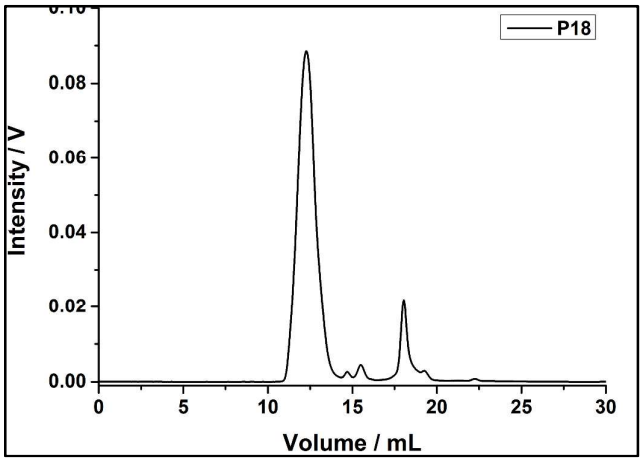
^{1}H NMR (300 MHz, D_2O) of **P08**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.65-2.52 (m, 45H, H-5, H-6, H-10, H-11, H-12, H-13, H-23, H-24), 2.72-4.04 (m, 56H, H-1, H-2, H-14, H-15, H-16, H-17, H-18, H-19, H-21, H-22, H-25, H-26), 5.08 (sb, 2H, H-20), 6.76-7.40 (m, 11H, H-3, H-4, H-7, H-8, H-9).

P18:

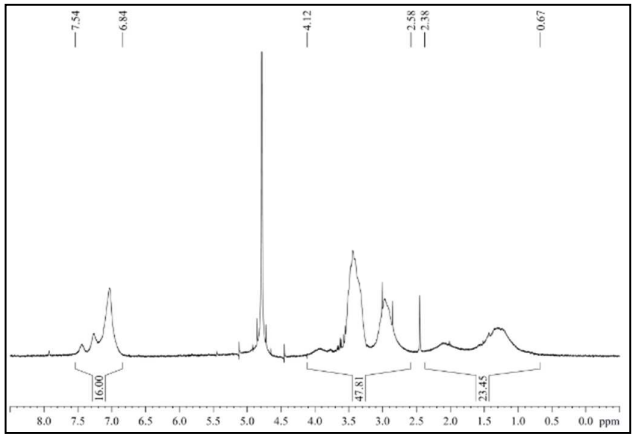


Yield: 49.9 mg (55 %).



SEC chromatogram of **P18**.

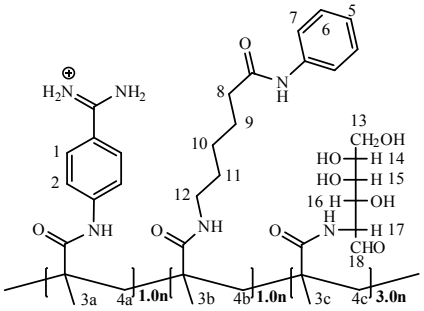
SEC (Pullulan-Standard): $\overline{M}_w$ = 232 000 g/mol, $\overline{M}_n$ = 116 000 g/mol; PDI = 2.0.



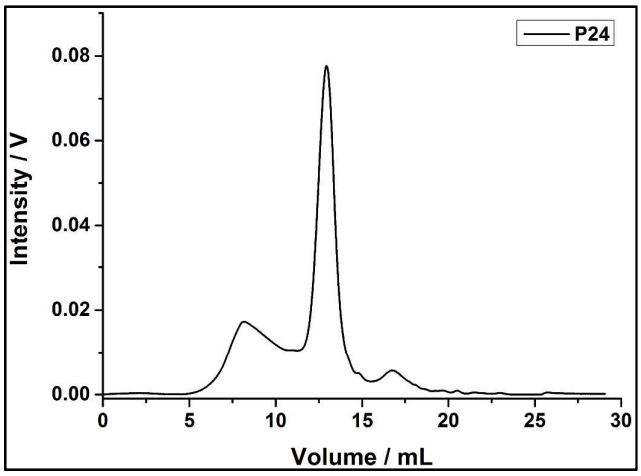
^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.67-2.38 (m, 23H, H-5, H-6), 2.58-4.12 (m, 48H, H-1, H-2, H-11, H-12, H-13), 6.84-7.54 (m, 16H, H-3, H-4, H-7, H-8, H-9, H-10).

^{1}H NMR (300 MHz, D_2O) of **P18**.

P24:

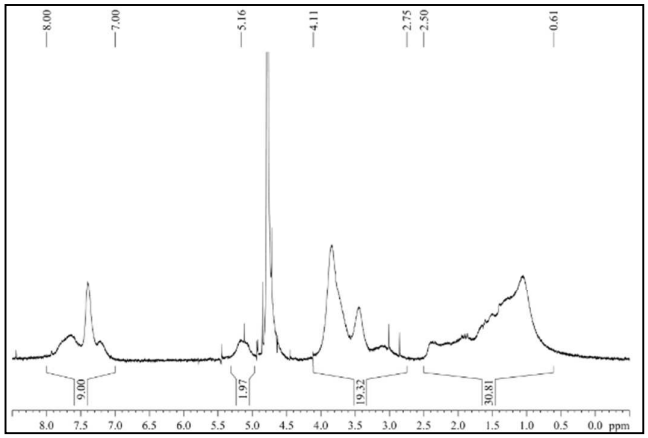


Yield: 59.1 mg (65 %).



SEC chromatogram of **P24**.

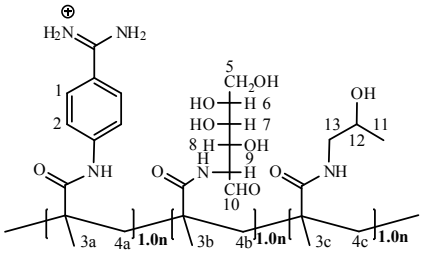
SEC (Pullulan-Standard): $\overline{M}_{w1}$ = 11 890 000 g/mol, $\overline{M}_{n1}$ = 4 432 000 g/mol; PDI_1 = 2.7; $\overline{M}_{w2}$ = 213 000 g/mol, $\overline{M}_{n2}$ = 107 000 g/mol; PDI_2 = 2.0.



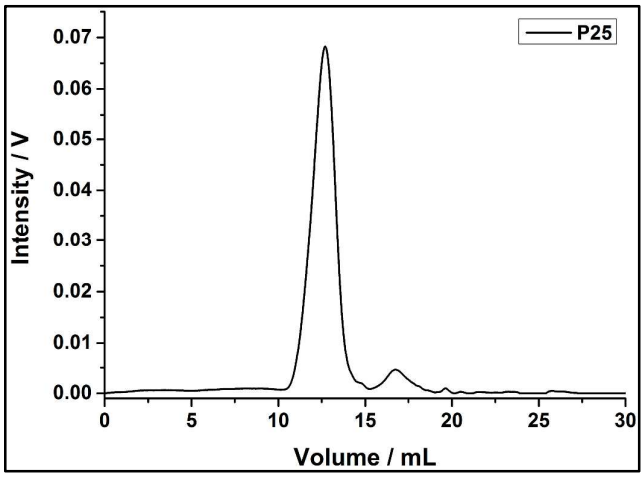
^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.61-2.50 (m, 31H, H-3, H-4, H-8, H-9, H-10, H-11), 2.75-4.11 (m, 19H, H-12, H-13, H-14, H-15, H-16, H-17), 5.16 (sb, 2H, H-18), 7.00-8.00 (m, 9H,
H-1, H-2, H-5, H-6, H-7).

^{1}H NMR (300 MHz, D_2O) of **P24**.

P25:

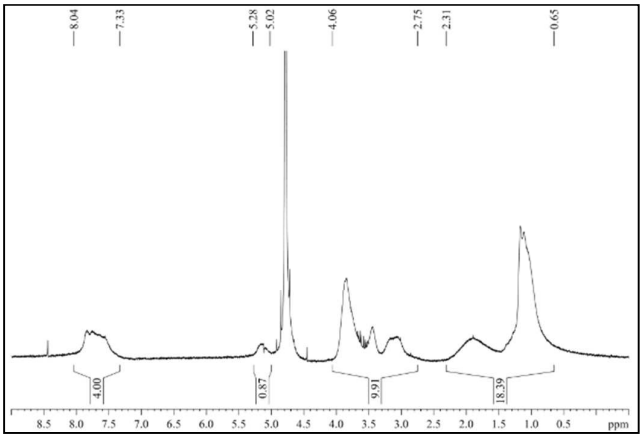


Yield: 53.7 mg (52 %).



SEC chromatogram of **P25**.

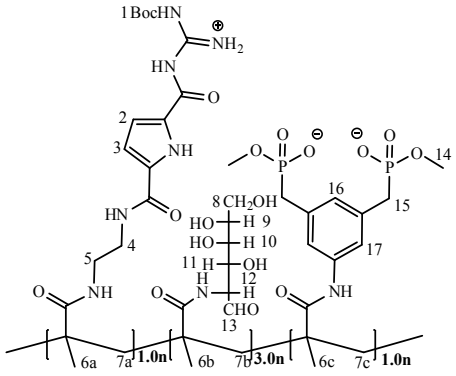
SEC (Pullulan-Standard): $\overline{M}_w$ = 323 000 g/mol, $\overline{M}_n$ = 142 000 g/mol; PDI = 2.3.



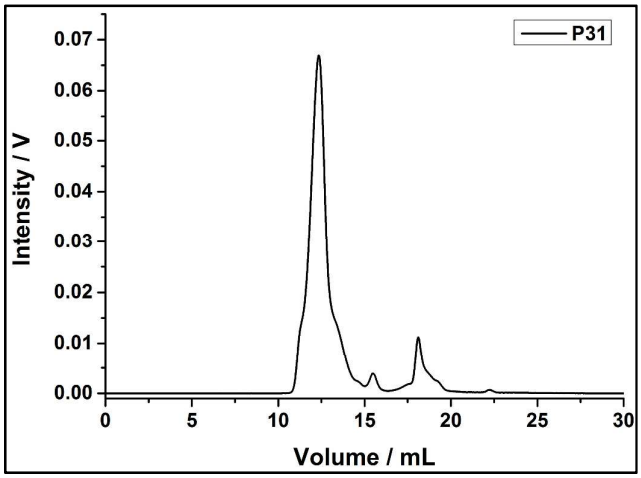
^{1}H NMR (300 MHz, D_2O) of **P25**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.65-2.31 (m, 18H, H-3, H-4, H-11), 2.75-4.06 (m, 10H, H-5, H-6, H-7, H-8, H-9, H-12, H-13), 5.28 (sb, 1H, H-10), 7.33-8.04 (m, 4H, H-1, H-2).

P31:

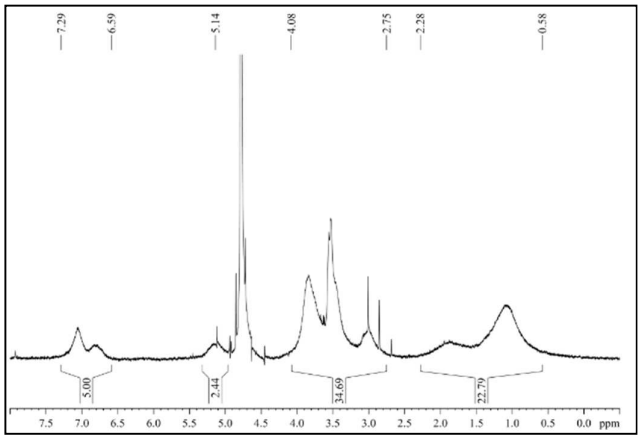


Yield: 39.2 mg (48 %).



SEC chromatogram of **P31**.

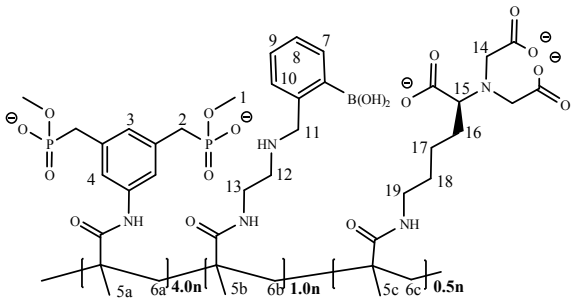
SEC (Pullulan-Standard): $\overline{M}_w$ = 245 000 g/mol, $\overline{M}_n$ = 87 000 g/mol; PDI = 2.8.



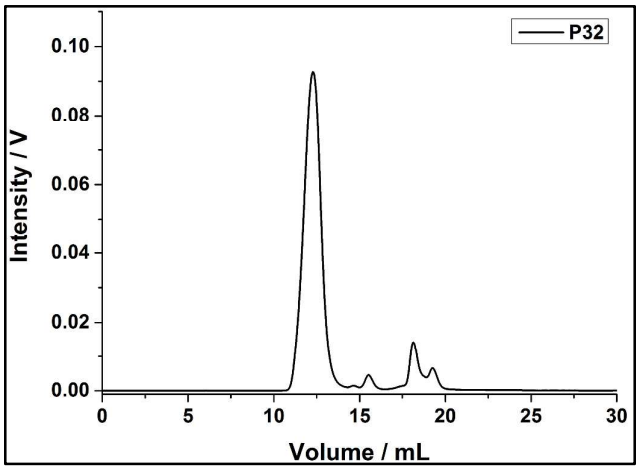
^{1}H NMR (300 MHz, D_2O) of **P31**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.58-2.28 (m, 23H, H-1, H-6, H-7), 2.75-4.08 (m, 35H, H-4, H-5, H-8, H-9, H-10, H-11, H-12, H-14, H-15), 5.14 (sb, 2H, H-13), 6.59-7.29 (m, 5H, H-2, H-3, H-16, H-17).

P32:

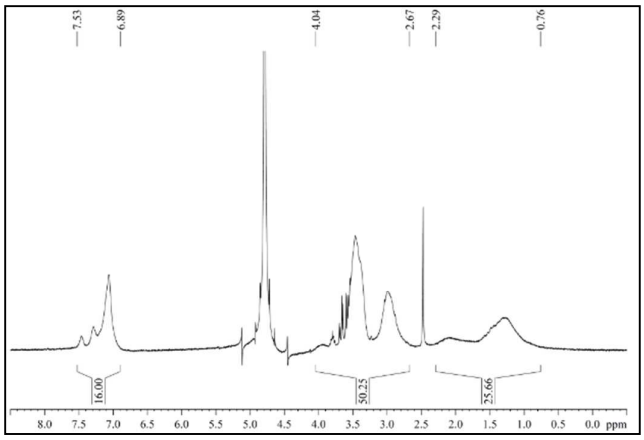


Yield: 37.1 mg (42 %).



SEC chromatogram of **P32**.

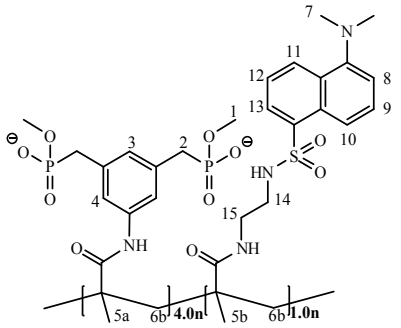
SEC (Pullulan-Standard): $\overline{M}_w$ = 256 000 g/mol, $\overline{M}_n$ = 134 000 g/mol; PDI = 1.9.



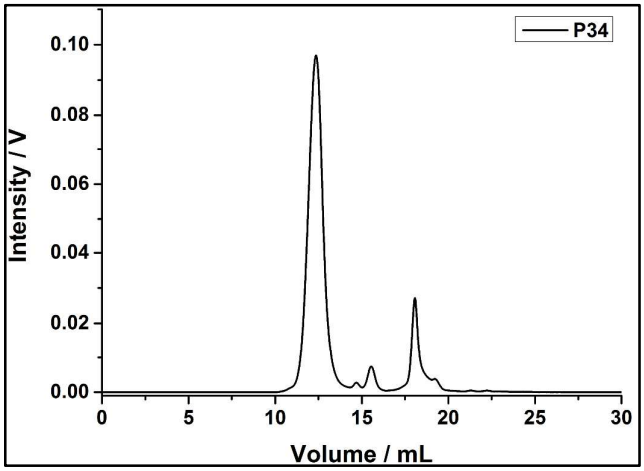
^{1}H NMR (300 MHz, D_2O) of **P32**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.76-2.29 (m, 25.5H, H-5, H-6, H-16, H-17, H-18), 2.67-4.04 (m, 50.5H, H-1, H-2, H-11, H-12, H-13, H-15, H-19), 6.89-7.53 (m, 16.0H, H-3, H-4, H-7, H-8, H-9, H-10).

P34:

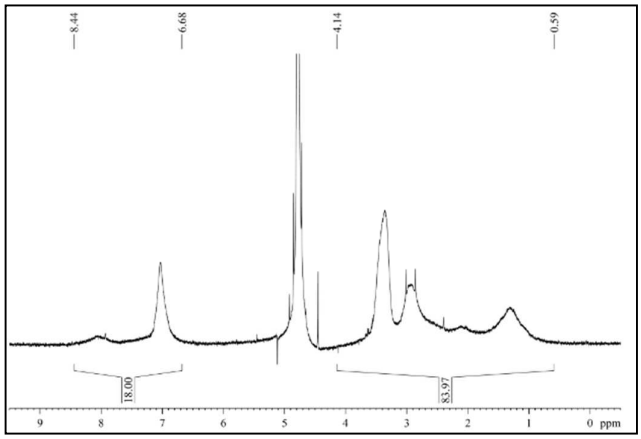


Yield: 41.7 mg (40 %).



SEC chromatogram of **P34**.

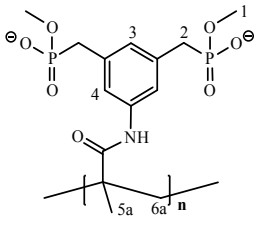
SEC (Pullulan-Standard): $\overline{M}_w$ = 224 000 g/mol, $\overline{M}_n$ = 120 000 g/mol; PDI = 1.9.



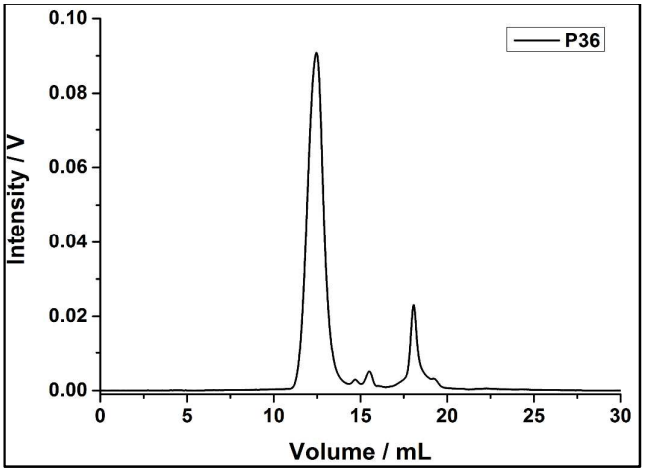
^{1}H NMR (300 MHz, D_2O) of **P34**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.59-4.14 (m, 84H, H-1, H-2, H-5, H-6, H-7, H-14, H-15), 6.68-8.44 (m, 18H, H-3, H-4, H-8, H-9, H-10, H-11, H-12, H-13).

P36:

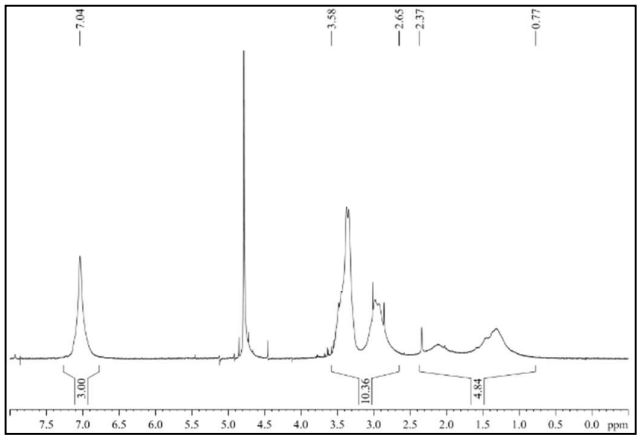


Yield: 37.4 mg (44 %).



SEC chromatogram of **P36**.

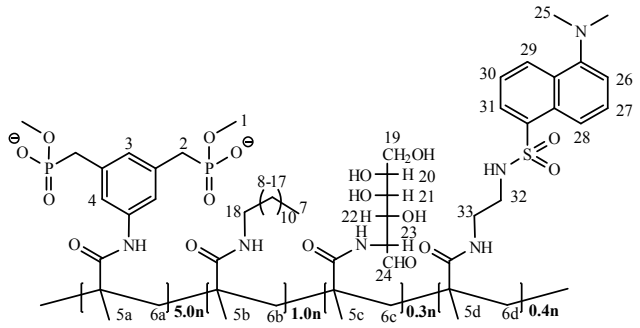
SEC (Pullulan-Standard): $\overline{M}_w$ = 182 000 g/mol, $\overline{M}_n$ = 103 000 g/mol; PDI = 1.8.



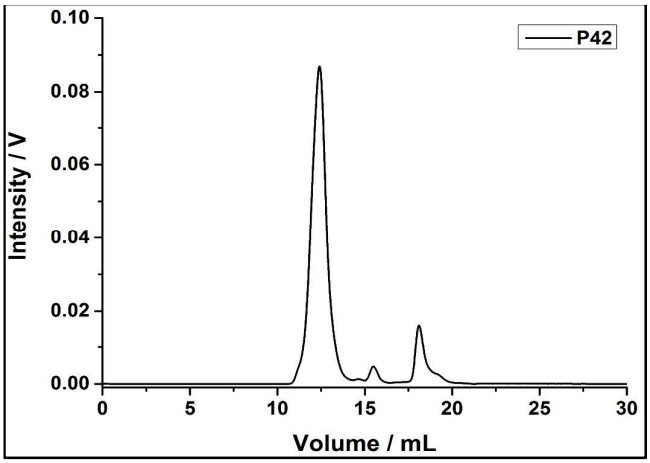
^{1}H NMR (300 MHz, D_2O) of **P36**.

^{1}H NMR (300 MHz, D_2O): δ [ppm] = 0.77-2.37 (m, 5H, H-5, H-6), 2.65-3.58 (m, 10H, H-1, H-2), 7.04 (sb, 3H, H-3, H-4).

P42:

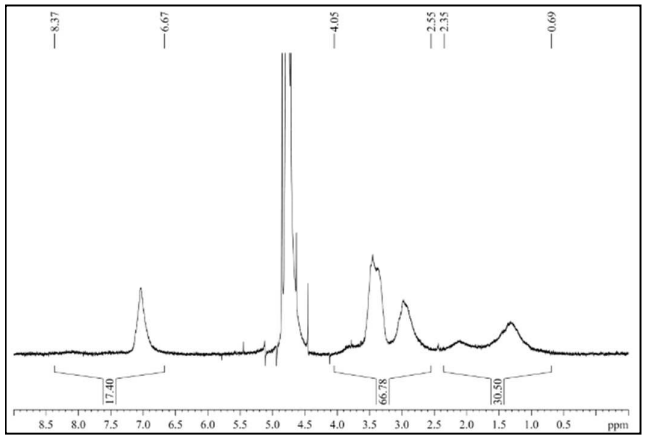


Yield: 41.2 mg (45 %).



SEC chromatogram of **P42**.

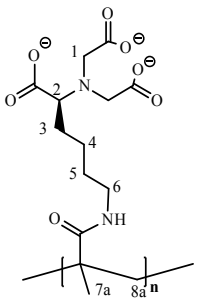
SEC (Pullulan-Standard): $\overline{M}_{w1}$ = 3 809 000 g/mol, $\overline{M}_{n1}$ = 3 247 000 g/mol; PDI_1 = 1.2.
$\overline{M}_{w1}$ = 190 000 g/mol, $\overline{M}_{n2}$ = 103 000 g/mol; PDI_2 = 1.8.

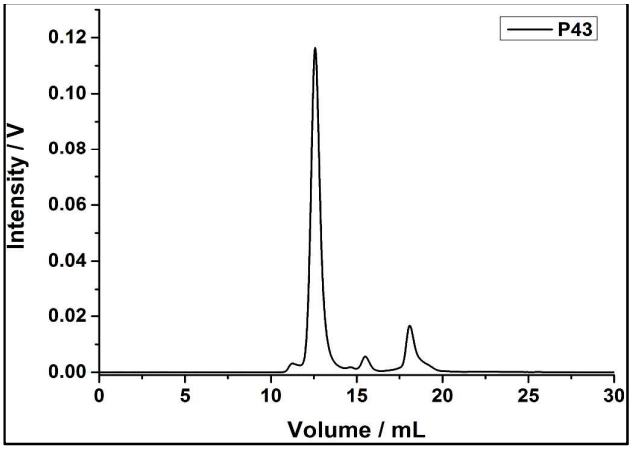


^{1}H NMR (300 MHz, D_2O) of **P42**.

^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.69-2.35 (m, 30.6H, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17), 2.55-4.05 (m, 66.9H, H-1, H-2, H-18, H-19,H-20, H-21, H-22, H-23, H-25, H-32, H-33), 6.67-8.37 (m, 17.4H, H-3, H-4, H-26, H-27, H-28, H-29, H-30, H-31).

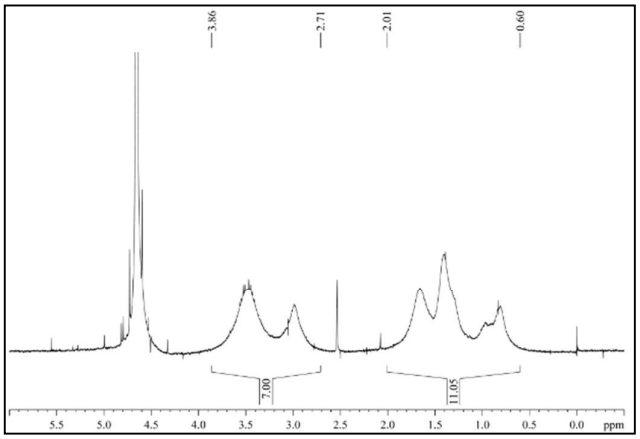
P43:





SEC chromatogram of **P43**.

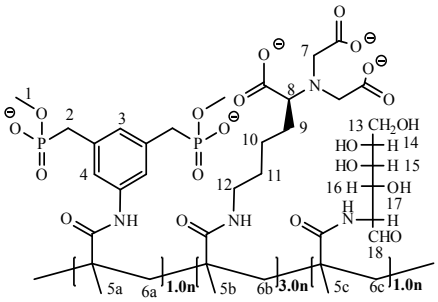
SEC (Pullulan-Standard): $\overline{M}_w$ = 202 000 g/mol, $\overline{M}_n$ = 112 000 g/mol; PDI = 1.8.

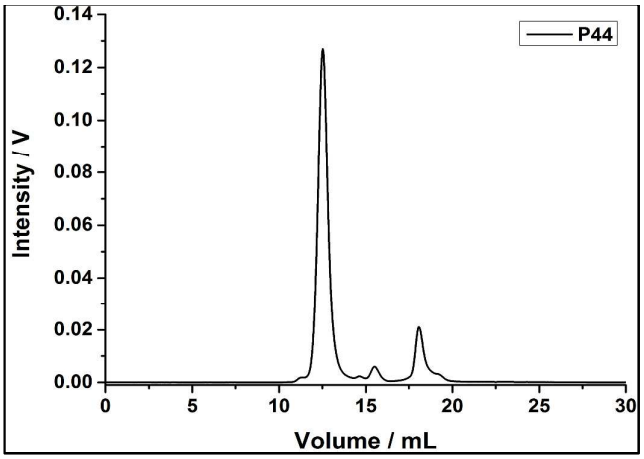


^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.60-2.01 (m, 11H, H-3, H-4, H-5, H-7, H-8), 2.71-3.86 (m, 7H, H-1, H-2, H-6).

^{1}H NMR (300 MHz, D_2O) of **P43**.

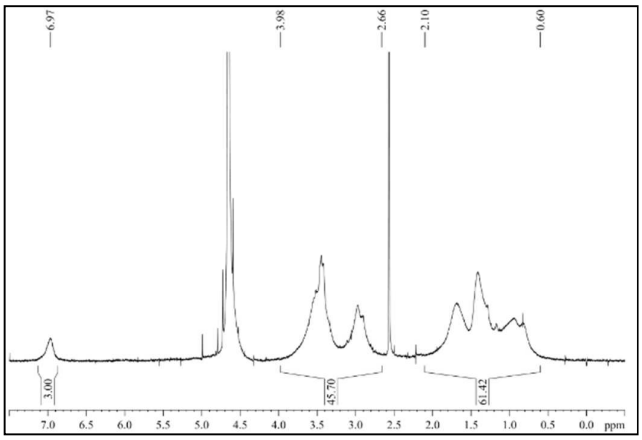
P44:





SEC chromatogram of **P44**.

SEC (Pullulan-Standard): $\overline{M}_w$ = 107 000 g/mol, $\overline{M}_n$ = 83 000 g/mol; PDI = 1.2.



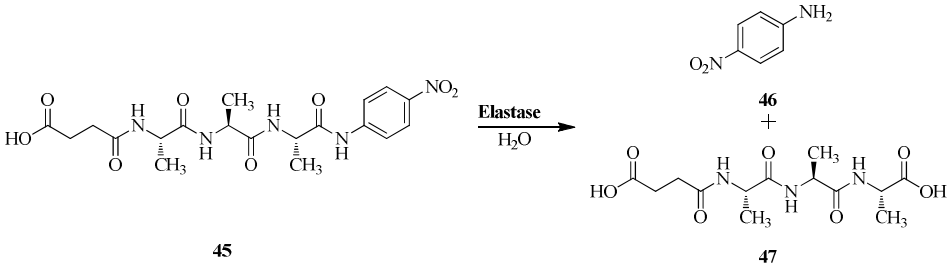
^{1}H NMR (300 MHz, D_2O):
δ [ppm] = 0.60-2.10 (m, 61H, H-5, H-6, H-9, H-10, H-11), 2.66-3.98 (m, 46H, H-1, H-2, H-7, H-8, H-12, H-13, H-14, H-15, H-16, H-17), 6.97 (sb, 3H, H-3, H-4).

^{1}H NMR (300 MHz, D_2O) of **P44**.

Enzyme assays

Elastase Assay[1]

This assay used Suc-$(Ala)_3$-pNA as an artificial substrate **45**.



45 **46** + **47**

Figure S1a. Hydrolytic cleavage of Suc-$(Ala)_3$-pNA **45** with concomitant release of *p*-nitroaniline **46**.

Enzyme and substrate solutions

Elastase buffer: 100 mM TRIZMA base, pH = 8.0.

Elastase solution: 100 µg Elastase (23.9 U/mg) were dissolved in 1.0 mL elastase buffer.

Substrate solution: 350 µg Suc-$(Ala)_3$-pNA were dissolved in 1.0 mL elastase buffer.

Procedure:

10 µL enzyme solution were treated with 10 µL polymer parent solution and incubated for 30 min. Subsequently, 60 µL buffer and 20 µL substrate solution were added and the reaction mixture was measured immediately.

Before each assay the 96-well microplate was shaken for 10 s. Photometric measurements then took place at 25 °C and 380 nm. They were conducted every 30 s for a total of 30 min; prior to each measurement the microplate was again shaken for 2 s.

Table S1. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Elastase	10 µL	$4.0 \cdot 10^{-6}$ mol/L	$4.0 \cdot 10^{-7}$ mol/L
Polymer or water	10 µL	20.0 mg/mL	2.0 mg/mL
30 min incubation at 25 °C			
Enzyme buffer	60 µL	-	-
Substrate	20 µL	$7.9 \cdot 10^{-4}$ mol/L	$1.6 \cdot 10^{-4}$ mol/L
Total volume	100 µL		

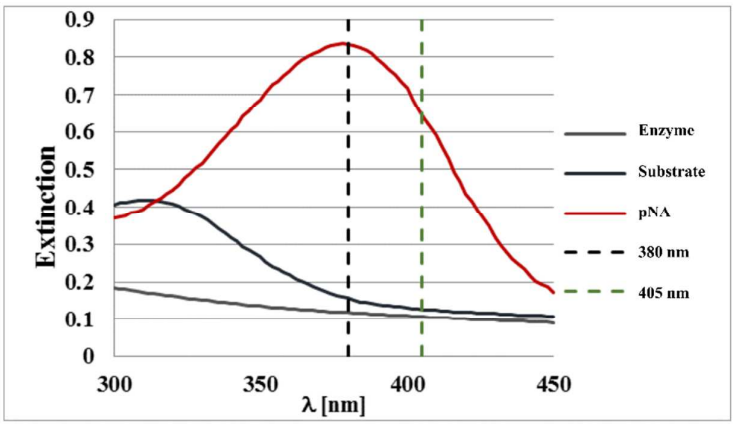


Figure S1b: Superimposed absorption spectra of enzyme, substrate and chromophore in the elastase assay.

Inhibition test and screening

A control measurement was conducted with the known elastase inhibitor diisopropylfluorophosphate (DIFP), followed by polymer screening with all polymer series.

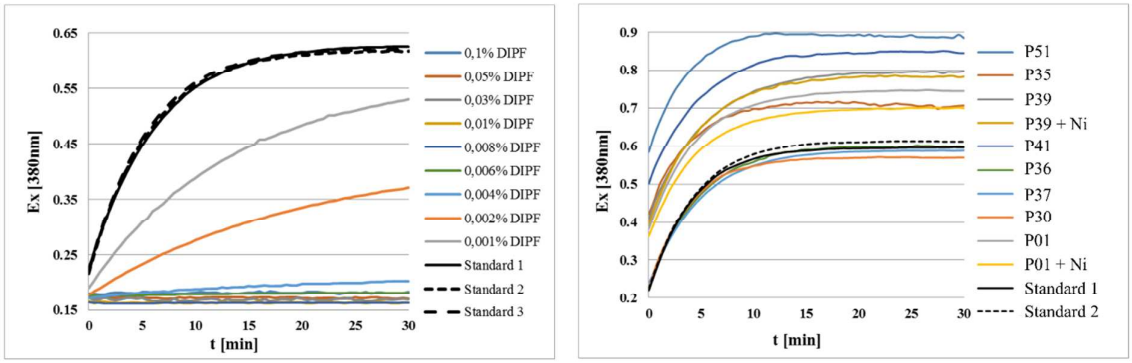


Figure S1c. Left: Elastase inhibition test with 0.1 - 0.001 % DIFP solution. Right: Screening of the first polymer series: none of the polymers altered the enzyme velocity of elastase. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour.

α-Chymotrypsin Assay[2]

This assay used Suc-Leu-Tyr-AMC as an artificial substrate **48**.

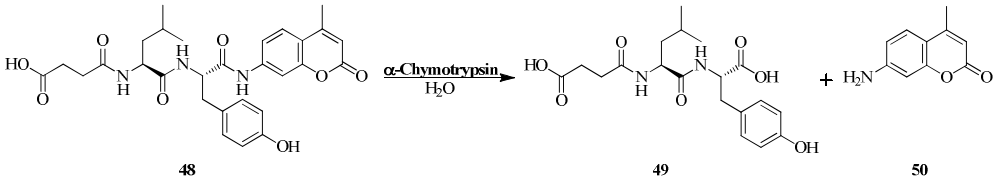


Figure S2a. Hydrolytic cleavage of Suc-Leu-Tyr-AMC **48** with concomitant release of AMP **50**.

Enzyme and substrate solutions

α-Chymotrypsin buffer:	50 mM TRIZMA base, 100 mM NaCl, 5 mM EDTA, pH = 8.0.
α-Chymotrypsin solution:	250 µg α-Chymotrypsin (≥40 U / mg) were dissolved in 1.0 mL α-chymotrypsin buffer.
Substrate stock solution:	275.8 µg Suc-Leu-Tyr-AMC were dissolved in 1.0 mL DMSO.
Substrate solution:	25 µL substrate stock solution, 475 µL DMSO and 500 µL α-chymotrypsin buffer represent the substrate solution.

Procedure:

10 µL enzyme solution were treated with 10 µL polymer stock solution. Subsequently, 160 µL buffer and 20 µL substrate solution were added and the reaction mixture was measured immediately.
Before each assay the 96-well microplate was shaken for 60 s. Photometric measurements then took place at 25 °C and 380 nm excitation as well as 444 nm emission. They were conducted every 60 s for a total of 30 min; prior to each measurement the microplate was again shaken for 20 s.

Table S2. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
α-Chymotrypsin	10 µL	10^{-5} mol/L	$5.0 \cdot 10^{-7}$ mol/L
Polymer or water	10 µL	20.0 mg/mL	1.0 mg/mL
No preincubation			
Enzyme buffer	160 µL	-	-
Substrate	20 µL	$12.5 \cdot 10^{-6}$ mol/L	$12.5 \cdot 10^{-7}$ mol/L
Total volume	200 µL		

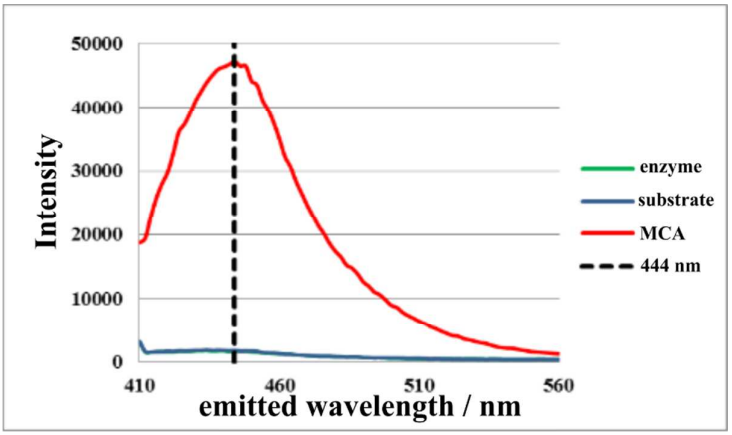


Figure S2b. Right: Superimposed absorption spectra of enzyme, substrate and chromophor in the α-chymotrypsin assay.

Inhibition test and screening

A control measurement was conducted with the known serine protease inhibitor aprotinin, followed by polymer screening with all polymer series.

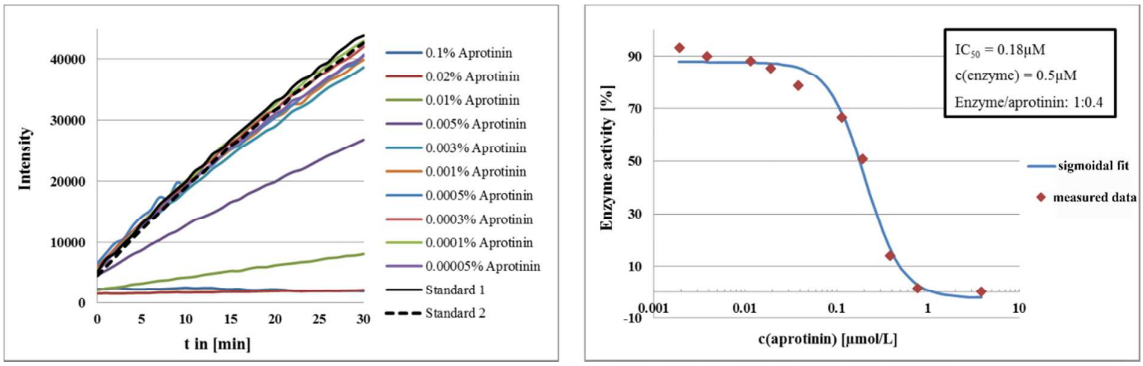


Figure S2c. Left: α-Chymotrypsin inhibition test with 0.1 - 0.00005 % aprotinin solution. Right: Logarithmic plot of the concentration dependent enzyme inhibition by aprotinin for the determination of its IC_{50} value (0.18 µM at 0.5 µM enzyme concentration).

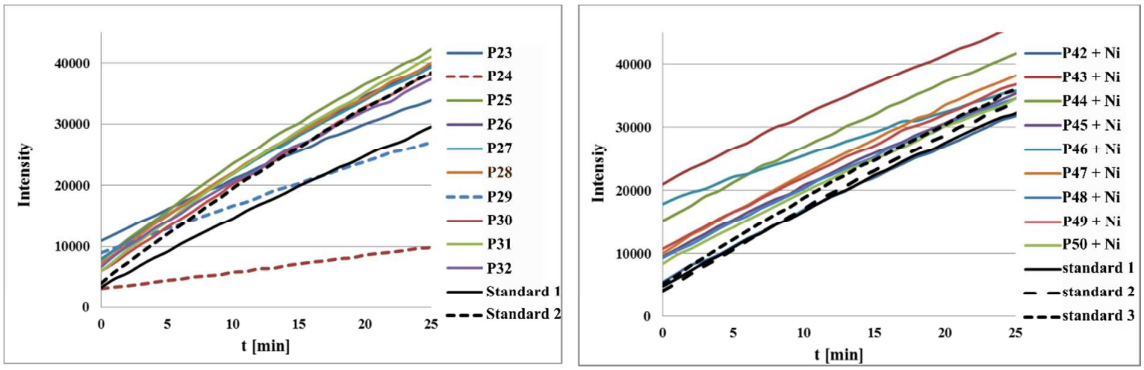


Figure S2d. Left: screening of the first polymer series: polymer **P29** only slightly altered the enzyme velocity of α-chymotrypsin, as opposed to P24, which turned out to be a strong inhibitor. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour. Right: screening of the second series of polymers. None of these showed enzyme inhibition properties.

Determination of the IC_{50} values for the best polymeric inhibitors:

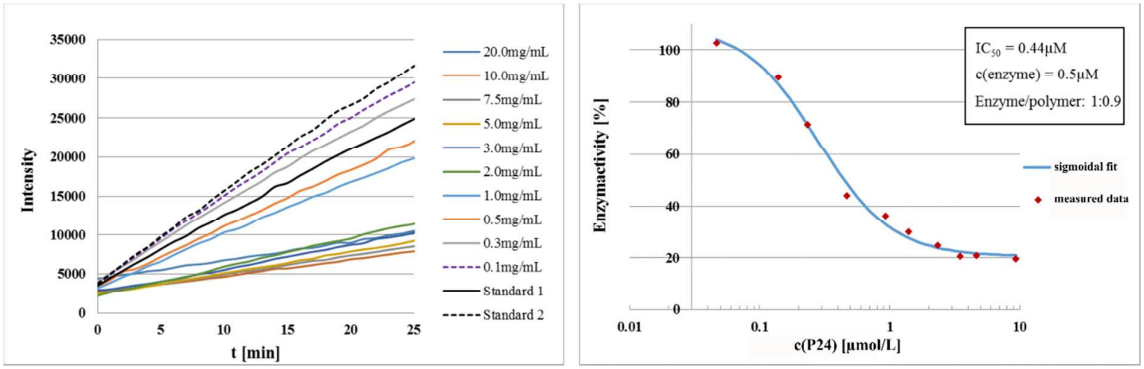
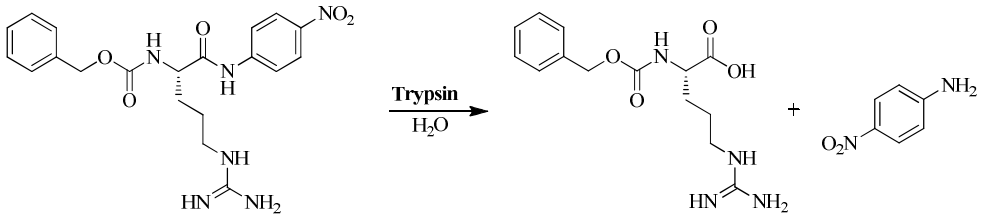


Figure S2e. Left: Inhibition of α-chymotrypsin depending on the concentration of **P24**. Polymer mass concentrations were between 20.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent α-chymotrypsin inhibition by **P24** for the determination of its IC_{50}-value (0.44 µM at 0.5 µM enzyme).

Trypsin Assay[3]

This assay used BAPNA as an artificial substrate **51**.



51 **52** **46**

Figure S3a. Hydrolytic cleavage of BAPNA **51** with concomitant release of *p*-nitroaniline **46**.

Enzyme and substrate solutions

Trypsin buffer:	75 mM TRIZMA base, 75 mM Na_2HPO_4, pH = 8.0.
Trypsin solution:	500-1000 µg Elastase (9000 U/mg) were dissolved in 1.0 mL HCl.
Substrate solution:	134-150 µg BAPNA • HCl were dissolved in 200 µL DMSO and 800 µL trypsin buffer.

Procedure:

5 µL enzyme solution were treated with 5 µL polymer parent solution and incubated for 30 min. Subsequently, 100 µL substrate solution were added and the reaction mixture was measured immediately. Before each assay the 96-well microplate was shaken for 10 s. Photometric measurements then took place at 25 °C and 405 nm. They were conducted every 30 s for a total of 30 min; prior to each measurement the microplate was again shaken for 4 s.

Table S3. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Trypsin	5 µL	2-4 • 10^{-5} mol/L	1-2 • 10^{-6} mol/L
Polymer or water	5 µL	20.0 mg/mL	0.9 mg/mL
30 min incubation at 25 °C			
Substrate	100 µL	3-4 • 10^{-4} mol/L	3 • 10^{-4} mol/L
Total volume	110 µL		

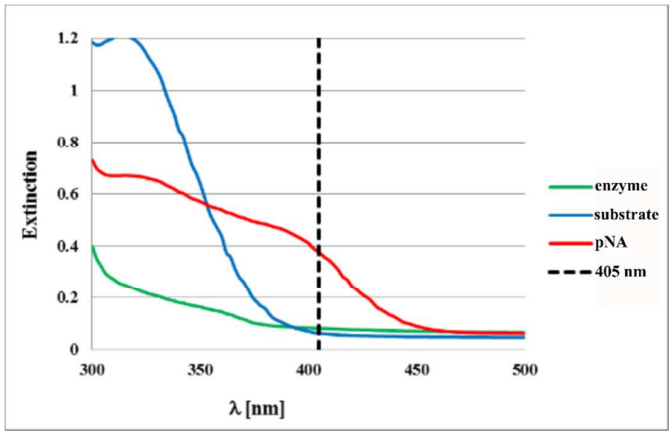


Figure S3b. Right: Superimposed absorption spectra of enzyme, substrate and chromophore in the trypsin assay.

Inhibition test and screening

A control measurement was conducted with the known trypsin inhibitor 4-aminobenzamidine (4-ABA), followed by polymer screening with all polymer series.

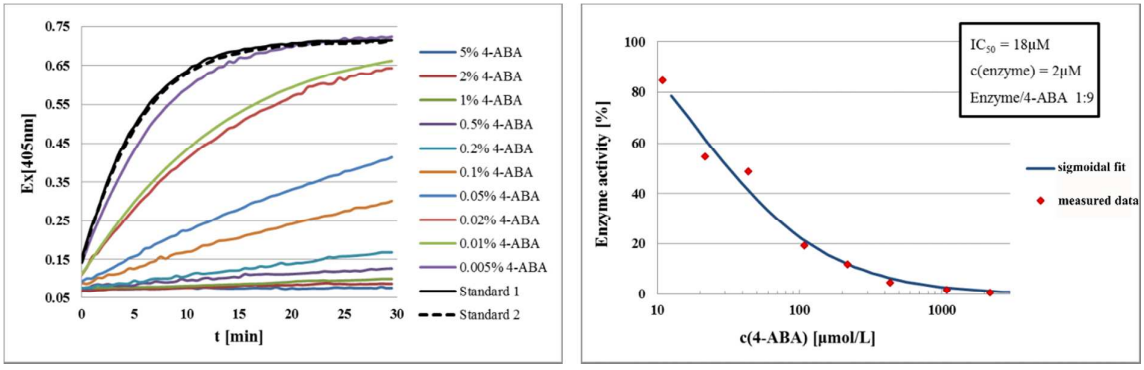


Figure S3c. Left: Trypsin inhibition test with 5 - 0.005 % 4-ABA solution. Right: Logarithmic plot of the concentration dependent enzyme inhibition by 4-ABA for the determination of its IC_{50} value (18 µM at 2 µM enzyme concentration).

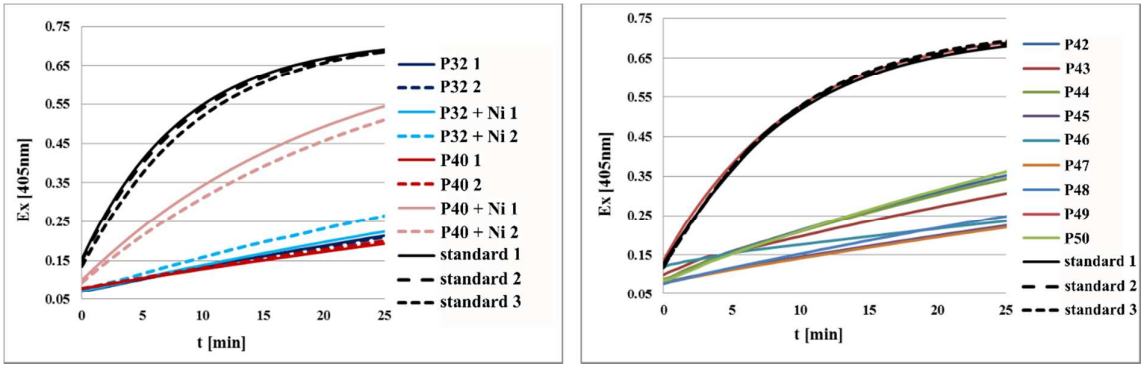


Figure S3d. Left: screening of the sixth polymer series: polymer P32 is a strong trypsin inhibitor. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour. Right: screening of the seventh series of polymers. All polymers showed potent enzyme inhibition properties.

Determination of the IC_{50} values for the best polymeric inhibitors:

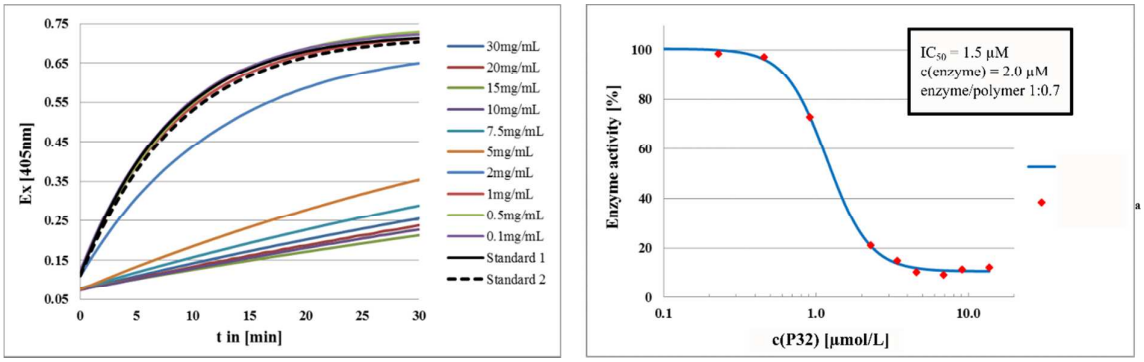


Figure S3e. Left: Inhibition of trypsin depending on the concentration of P32. Polymer mass concentrations were between 30.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent trypsin inhibition by P32 for the determination of its IC_{50}-value (1.3 µM at 2.0 µM enzyme).

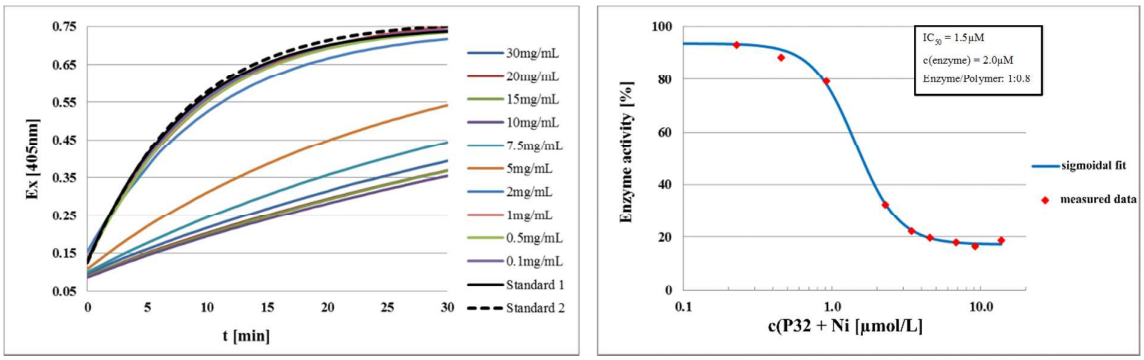


Figure S3f. Left: Inhibition of trypsin depending on the concentration of P32+Ni. Polymer mass concentrations were between 30.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent trypsin inhibition by P32+Ni for the determination of its IC_{50}-value (1.5 µM at 2.0 µM enzyme).

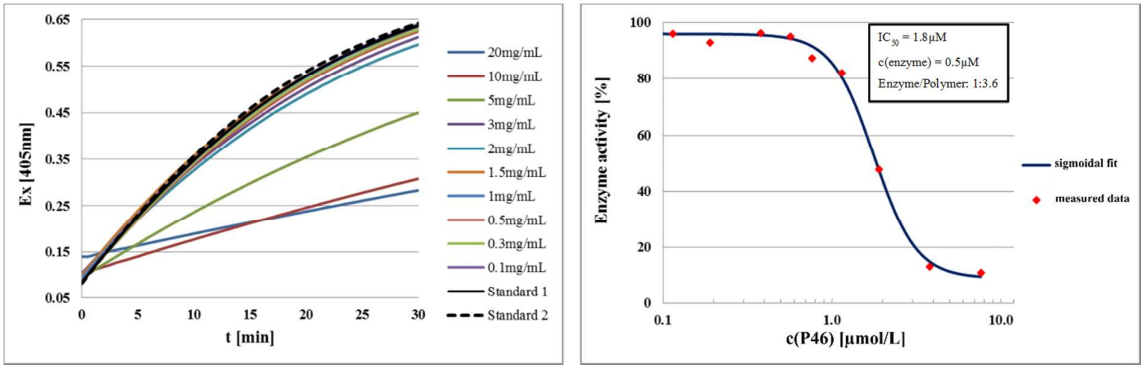
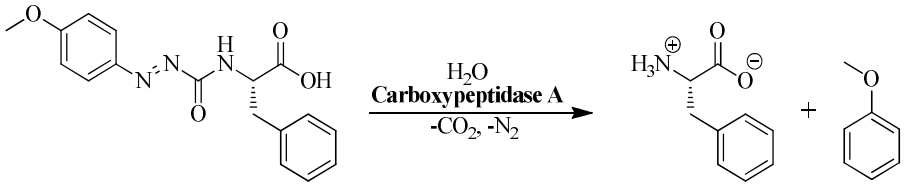


Figure S3g. Left: Inhibition of trypsin depending on the concentration of P46. Polymer mass concentrations were between 20.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent trypsin inhibition by P46 for the determination of its IC_{50}-value (1.8 µM at 0.5 µM enzyme).

Carboxypeptidase A Assay[4]

This assay used *N*-(4-methoxyphenylazoformyl)-Phe-OH as an artificial substrate **53**.



53 **54** **55**

Figure S4a. Hydrolytic cleavage of *N*-(4-methoxyphenylazoformyl)-Phe-OH **53** with concomitant release of Phe **54** and anisole **55**.

The assay kit *Sigma Aldrich* [CS1130] was used for all inhibition measurements.

Enzyme and substrate solutions

Carboxypeptidase A buffer: according to assay kit; pH = 8.0.
Carboxypeptidase A soln: according to assay kit.
Substrate stock solution: according to assay kit.
Substrate solution: 2 µL substrate stock solution, 800 µL doubly distilled water und 200 µL enzyme buffer represent the substrate solution.

Procedure:

89 µL (screening: 97 µL) doubly distilled water were treated with 1 µL enzyme solution, followed by 10 µL (screening: 2 µL) polymer parent solution. Subsequently, 100 µL substrate solution were added and the reaction mixture was measured immediately.

Before each assay the 96-well microplate was shaken for 2 s. Photometric measurements then took place at 25 °C and 350 nm. They were conducted every 30 s for a total of 30 min; prior to each measurement the microplate was again shaken for 5 s.

Table S4. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Carboxypep. A	1 µL	unknown	unknown
Polymer/water	2 or 10 µL	20.0 mg/mL	0.2 or 1.0 mg/mL
Water (dd)	97 or 89 µL	-	.
No preincubation			
Substrate	100 µL	unknown	unknown
Total volume	200 µL		

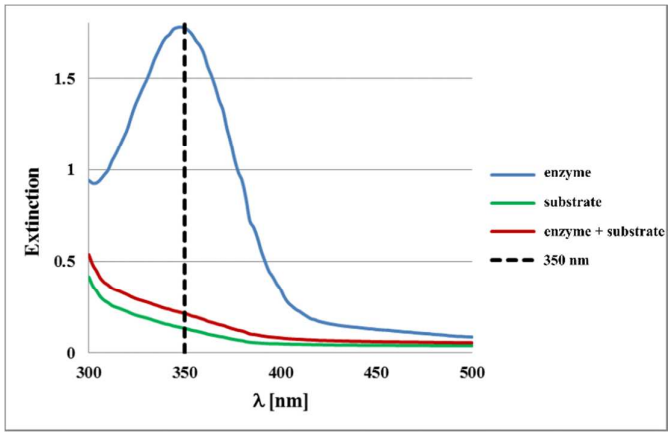


Figure S4b. Right: Superimposed absorption spectra of enzyme, substrate and chromophore in the Carboxypeptidase A assay.

Inhibition test and screening

A control measurement was conducted with un unknown inhibitor extracted from the potato tuber which was part of the assay kit, followed by polymer screening with all polymer series.

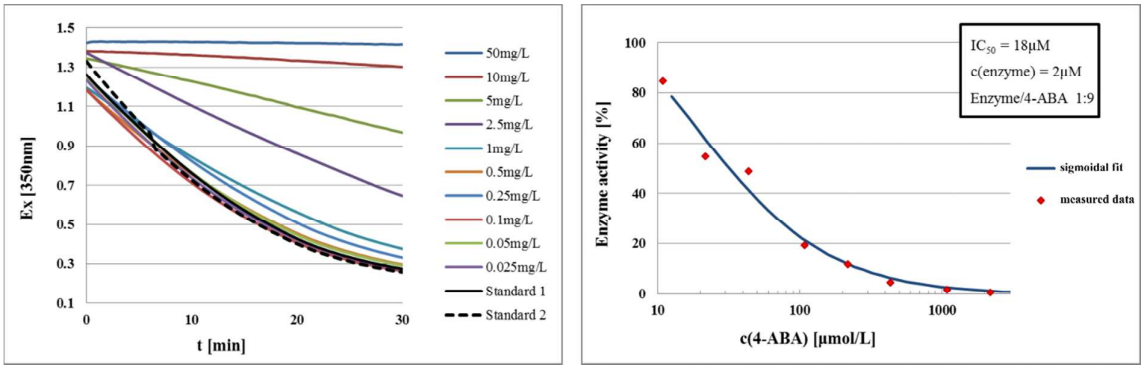


Figure S4c. Left: Carboxypeptidase inhibition test with unknown inhibitor of 50 - 0.025 mg/mL mass concentration. Right: Logarithmic plot of the concentration dependent enzyme inhibition by the unknown inhibitor for the determination of its IC_{50} value (2.2 mg/L at an unknown enzyme concentration).

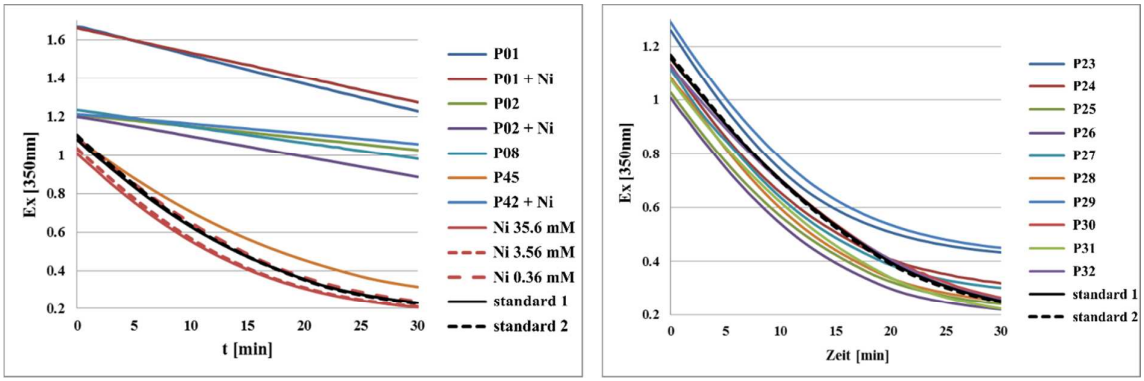


Figure S4d. Screening of the first polymer series with and without Ni content. Polymers P01, P02 and P08 turned out to be potent carboxypeptidase A inhibitors, whereas P43 was inactive. Ni^{2+} ions were shown to have no effect on enzyme activity. Right: Screening of the fourth polymer series. None of these inhibited Carboxypeptidase A.

Determination of the IC_{50} values for the best polymeric inhibitors:

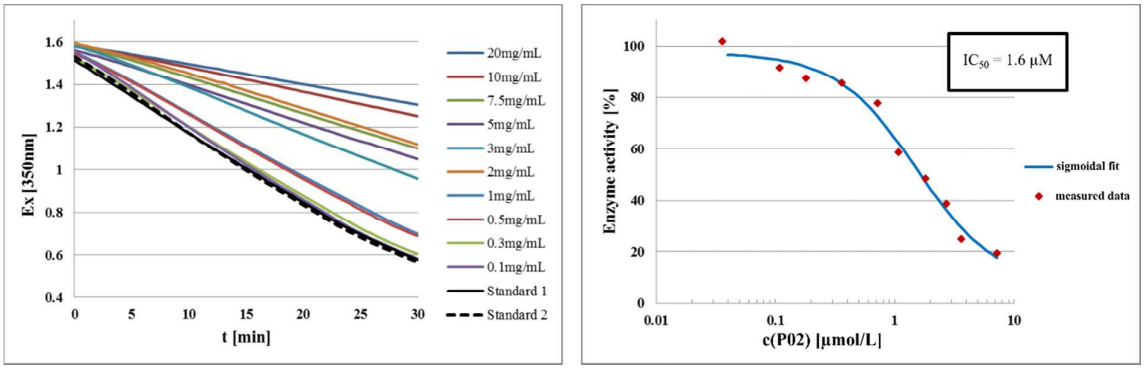


Figure S4e. Left: Inhibition of Carboxypeptidase A depending on the concentration of P02. Polymer mass concentrations were between 20.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent trypsin inhibition by P02 for the determination of its IC_{50}-value (1.6 µM at an unknown enzyme concentration).

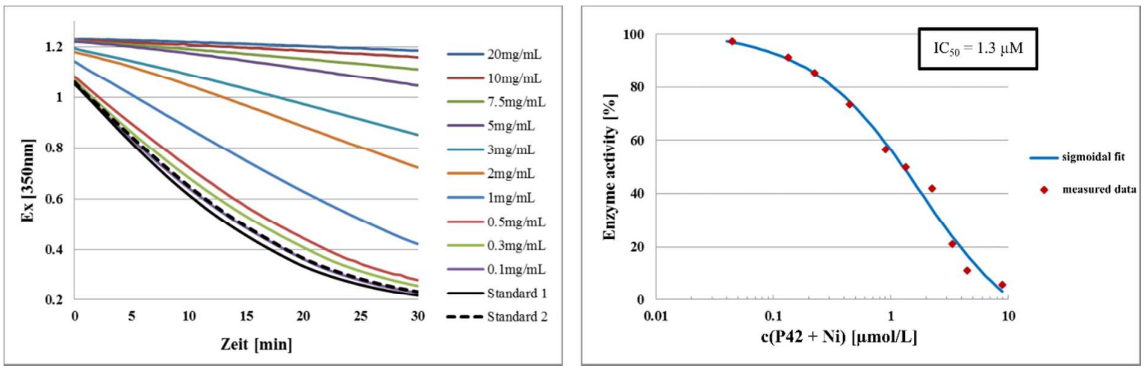


Figure S4f. Left: Inhibition of Carboxypeptidase A depending on the concentration of polymer P42+Ni. Polymer mass concentrations were between 20.0 - 0.1 mg/mL. Right: Logarithmic plot of the concentration-dependent trypsin inhibition by P42+Ni for the determination of its IC_{50}-value (1.3 µM at an unknown enzyme concentration).

Kallikrein Assay[5]

This assay used Bz-Phe-Val-Arg-pNA as an artificial substrate **56**.

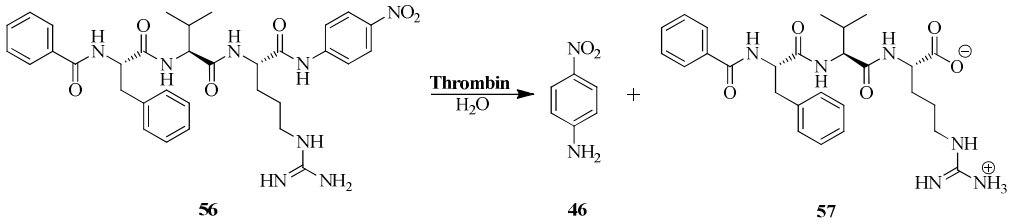


Figure S5a. Hydrolytic cleavage of Bz-Phe-Val-Arg-pNA **56** with concomitant release of *p*-nitroaniline **46**.

Enzyme and substrate solutions

Kallikrein buffer:	20 mM TRIZMA base, 100 mM NaCl, pH = 7.8.
Kallikrein solution:	1.0 mg Kallikrein (40.0 U/mg) were dissolved in 1 mL Kallikrein buffer.
Substrate stock solution:	6.8 mg Bz-Phe-Val-Arg-pNA • HCl were dissolved in 1.0 mM DMSO.
Substrate solution:	250 µL substrate stock solution and 750 µL Kallikrein buffer represent the substrate solution.

Procedure:

5 µL enzyme solution were treated with 5 µL polymer parent solution. Subsequently, 90 µL buffer as well as 10 µL substrate solution were added and the reaction mixture was measured immediately. Before each assay the 96-well microplate was shaken for 15 min. Photometric measurements then took place at 25 °C and 380 nm. They were conducted every min for a total of 60 min; prior to each measurement the microplate was again shaken for 30 s.

Table S5. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Kallikrein	5 µL	$12 \cdot 10^{-6}$ mol/L	$5 \cdot 10^{-7}$ mol/L
Polymer or water	5 µL	20.0 mg/mL	0.9 mg/mL
Buffer	90 µL	-	-
No preincubation			
Substrate	10 µL	$2.5 \cdot 10^{-3}$ mol/L	$2.3 \cdot 10^{-4}$ mol/L
Total volume	110 µL		

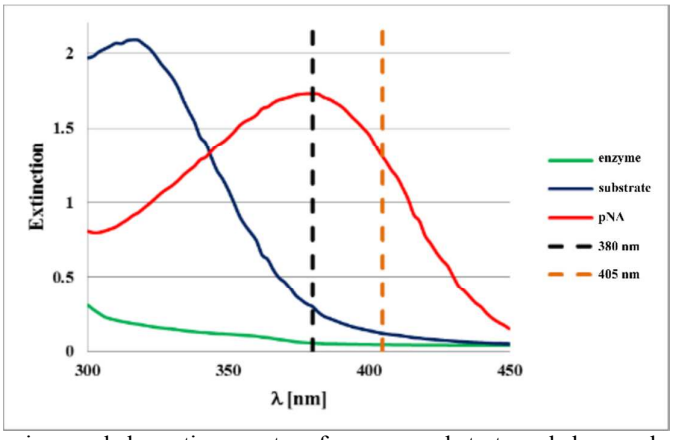


Figure S5b. Right: Superimposed absorption spectra of enzyme, substrate and chromophore in the trypsin assay.

Inhibition test and screening

A control measurement was conducted with the serine protease inhibitor aprotinin, followed by polymer screening with all polymer series.

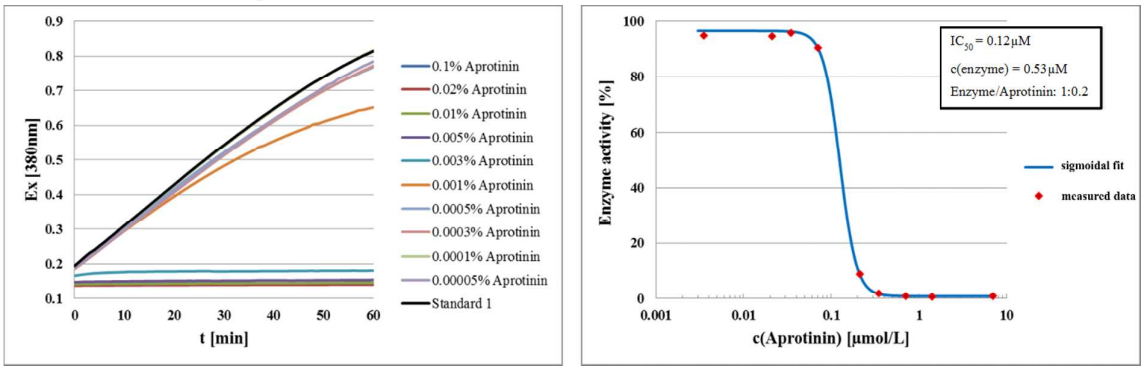


Figure S5c. Left: Kallikrein inhibition test with 0.1 - $5 \cdot 10^{-5}$ % aprotinin solution. Right: Logarithmic plot of the concentration dependent enzyme inhibition by aprotinin for the determination of its IC_{50} value (0.12 µM at 0.53 µM enzyme concentration).

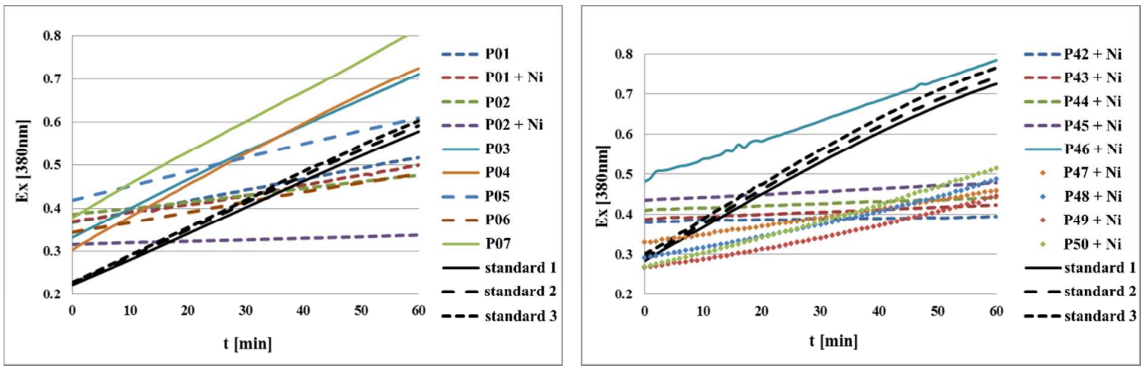


Figure S5d. Left: screening of the first polymer series: polymer **P01** and **P02** are strong Kallikrein inhibitors, in the absence and also in the presence of Ni^{2+} ions. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour. Right: screening of the eighth series of polymers. Polymers **P42-P45** are potent Kallikrein inhibitors, further improved by the presence of Ni^{2+} ions.

Determination of the IC_{50} values for the best polymeric inhibitors:

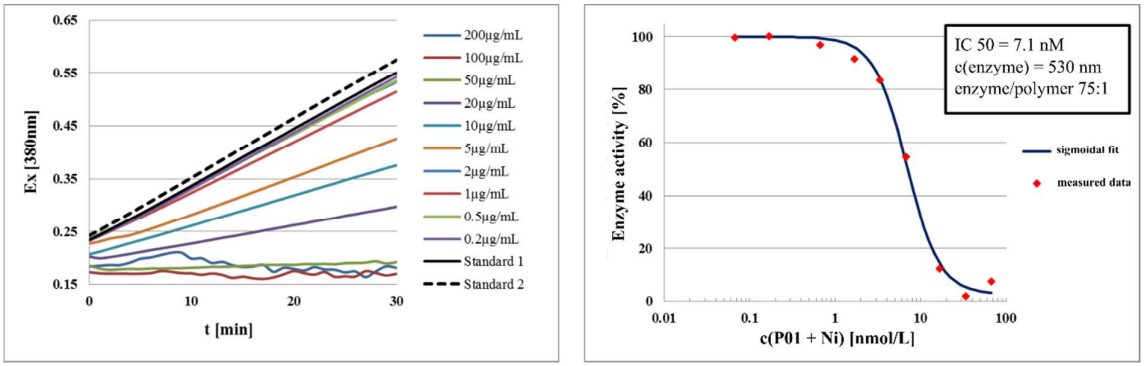


Figure S5e. Left: Inhibition of kallikrein depending on the concentration of P01+Ni. Polymer mass concentrations were between 200.0 - 0.2 µg/mL. Right: Logarithmic plot of the concentration-dependent kallikrein inhibition by P01+Ni for the determination of its IC_{50}-value (7.1 nM at 530 nM enzyme).

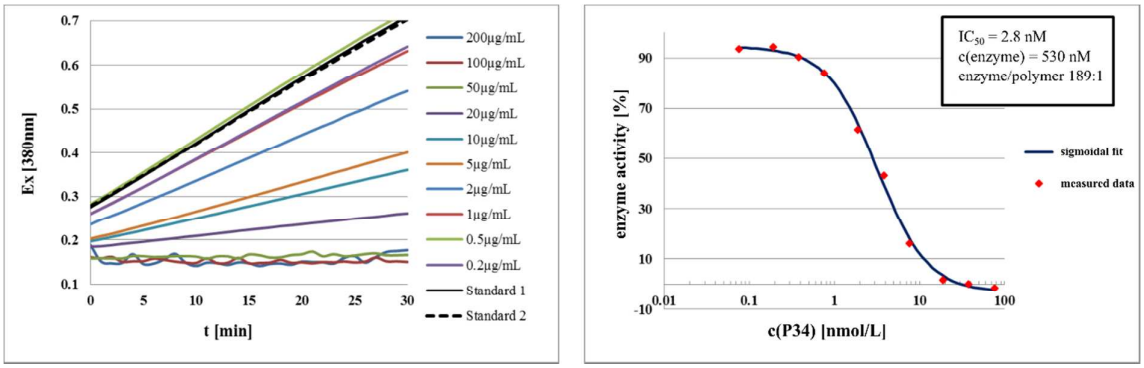


Figure S5f. Left: Inhibition of kallikrein depending on the concentration of **P34**. Polymer mass concentrations were between 200 - 0.2 µg/mL. Right: Logarithmic plot of the concentration-dependent kallikrein inhibition by **P34** for the determination of its IC_{50}-value (2.8 nM at 530 nM enzyme).

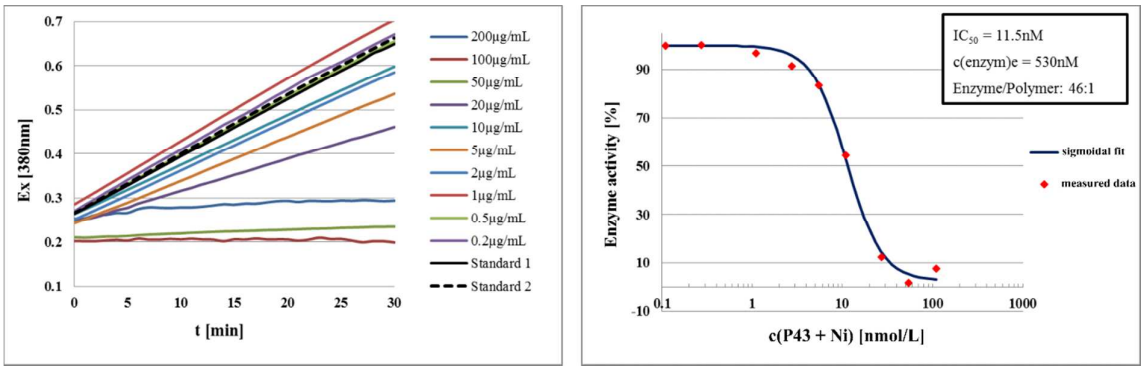


Figure S5g. Left: Inhibition of kallikrein depending on the concentration of polymer **P43**+Ni. Polymer mass concentrations were between 200 - 0.2 µg/mL. Right: Logarithmic plot of the concentration-dependent kallikrein inhibition by **P43**+Ni for the determination of its IC_{50}-value (12 nM at 530 nM enzyme).

Thrombin Assay[5]

This assay used Bz-Phe-Val-Arg-pNA as an artificial substrate **56**.

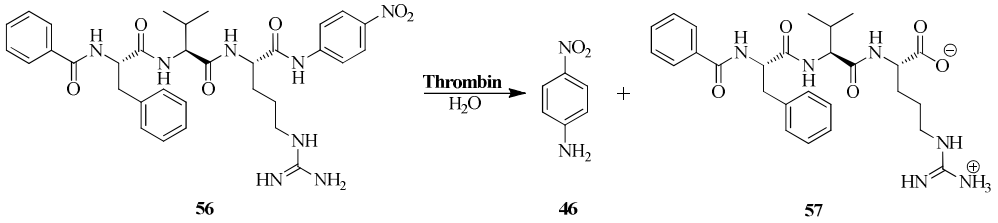


Figure S6a. Hydrolytic cleavage of Bz-Phe-Val-Arg-pNA **56** with concomitant release of *p*-nitroaniline **46**.

Enzyme and substrate solutions

Thrombin buffer:	50 mM HEPES, 200 mM NaCl, pH = 7.5.
Thrombin stock solution:	100 U (1 U = 0.324 µg) thrombin were dissolved in 1.0 mL 0.1% albumin solution (32.4 mg/L; 900 nM).
Thrombin solution:	100 µL thrombin stock solution were dissolved in 900 µL thrombin buffer.
Substrate solution:	2.7 mg Bz-Phe-Val-Arg-pNA • HCl were dissolved in 1.0 mM DMSO.

Procedure:

10 µL enzyme solution were treated with 5 µL polymer parent solution. Subsequently, 80 µL buffer as well as 5 µL substrate solution were added and the reaction mixture was measured immediately.
Before each assay the 96-well microplate was shaken for 10 min. Photometric measurements then took place at 25 °C and 380 nm. They were conducted every min for a total of 60 min; prior to each measurement the microplate was again shaken for 30 s.

Table S6. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Kallikrein	10 µL	$9 \cdot 10^{-8}$ mol/L	$9 \cdot 10^{-9}$ mol/L
Polymer or water	5 µL	1.0 mg/mL	0.9 mg/mL
Buffer	80 µL	-	-
No preincubation			
Substrate	5 µL	$4 \cdot 10^{-3}$ mol/L	$2 \cdot 10^{-4}$ mol/L
Total volume	100 µL		

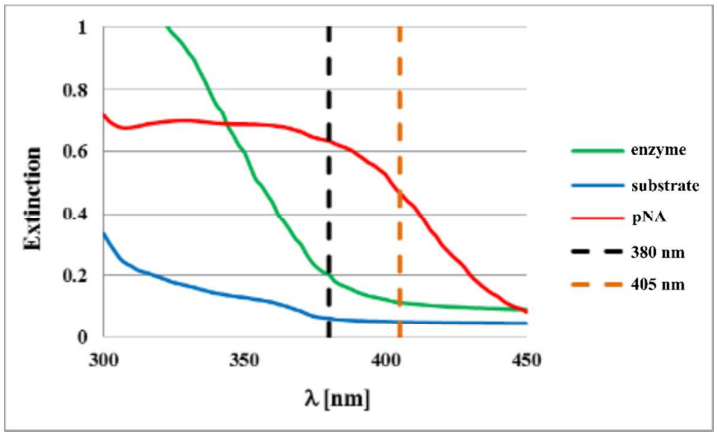


Figure S6b. Right: Superimposed absorption spectra of enzyme, substrate and chromophore in the trypsin assay.

Inhibition test and screening

A control measurement was conducted with the known thrombin inhibitor argatroban, followed by polymer screening with all polymer series.

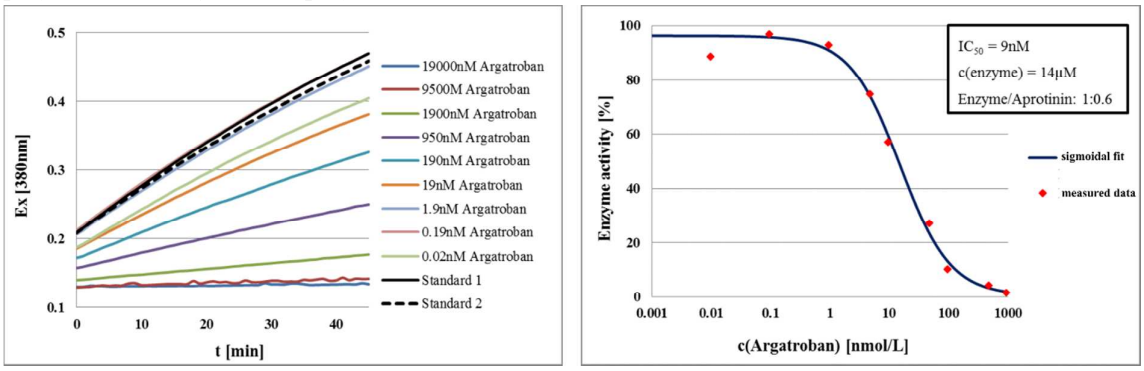


Figure S6c. Left: Thrombin inhibition test with 0.02 - $2 \cdot 10^{-2}$ nM argatroban solution. Right: Logarithmic plot of the concentration dependent enzyme inhibition by aprotinin for the determination of its IC_{50} value (9 nM at 14 nM enzyme concentration).

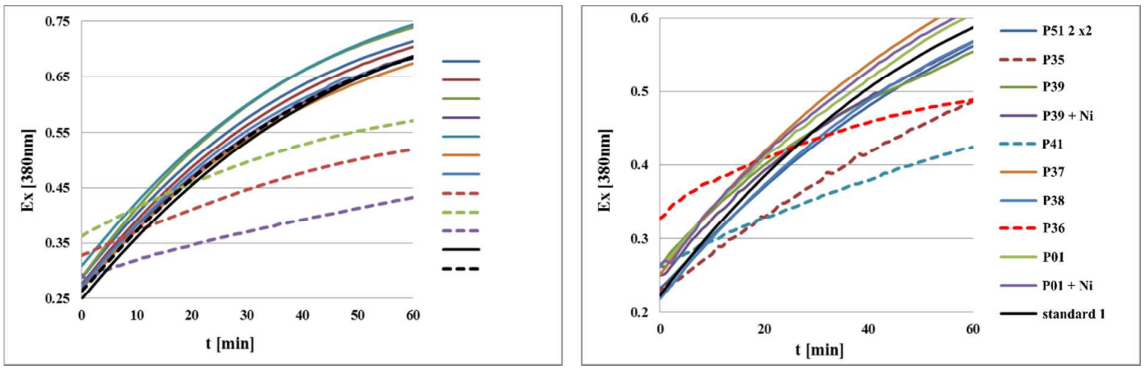


Figure S6d. Left: screening of the fourth polymer series: polymer **P32** and **P34** are thrombin inhibitors, in the absence and also in the presence of Ni^{2+} ions. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour. Right: screening of the fifth series of polymers. Polymers P35, P41 and P36 are thrombin inhibitors.

Determination of the IC_{50} values for the best polymeric inhibitors:

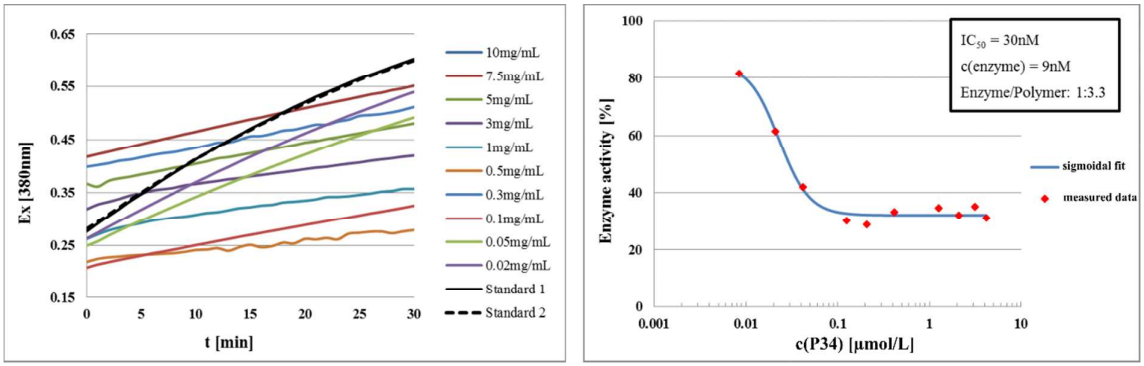


Figure S6e. Left: Inhibition of thrombin depending on the concentration of **P34**. Polymer mass concentrations were between 10.0 - 0.02 mg/mL. Right: Logarithmic plot of the concentration-dependent thrombin inhibition by **P34** for the determination of its IC_{50}-value (30 nM at 9 nM enzyme).

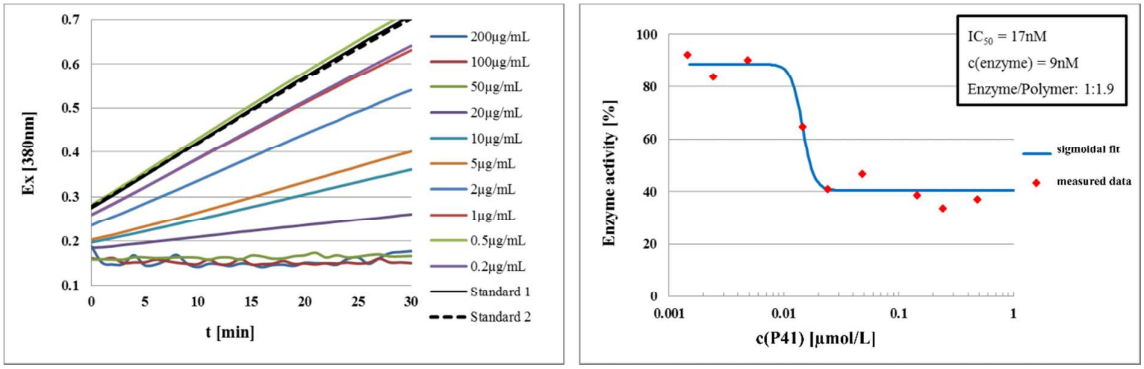


Figure S6f. Left: Inhibition of thrombin depending on the concentration of **P41**. Polymer mass concentrations were between 3 – 0.003 mg/mL. Right: Logarithmic plot of the concentration-dependent thrombin inhibition by **P41** for the determination of its IC_{50}-value (17 nM at 9 nM enzyme).

Cathepsin D Assay[6]

This assay used the intramolecularly self-quenched peptide MCA-Gly-Lys-Pro-lle-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-D-Arg-NH_2 as an artificial substrate **58**.

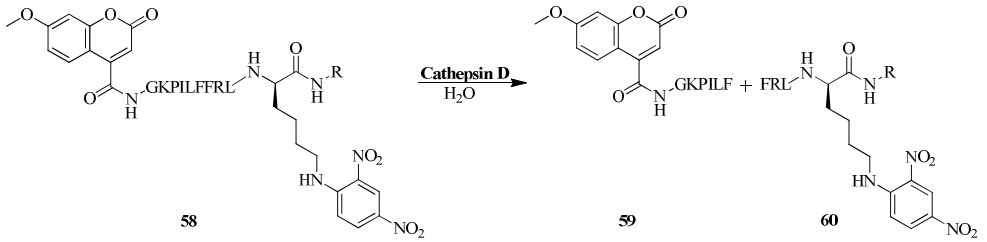


Figure S7a. Hydrolytic cleavage of MCA-Gly-Lys-Pro-lle-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-D-Arg-NH_2 **58** with concomitant release of fluorescent coumarine peptide **59** and dinitrophenyl peptide **60**.

The assay kit *Sigma Aldrich* [CS0800] was used for all measurements.

Enzyme and substrate solutions

Dilution buffer	975 µL assay buffer were diluted with 25 µL 0.1 % albumin solution.
Cathepsin D stock solution:	Cathepsin D (5U) was dissolved in 0.5 mL doubly distilled water (0.7 mg/mL).
Cathepsin D solution:	10 µL Cathepsin D stock solution were diluted with 90 µL dilution buffer. 10 µL of this solution were diluted again with 290 µL dilution buffer (2.3 µg/mL).
Substrate solution:	A solution of MCA-Gly-Lys-Pro-lle-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-D-Arg-NH_2 in DMSO was part of the assay-kit.

Table S7. Survey of all final concentrations within the elastase assay.

Solutions	Volume	Concentration	Assay Conc.
Cathepsin D	5 µL	$5 \cdot 10^{-8}$ mol/L	$3 \cdot 10^{-9}$ mol/L
Polymer or water	5 µL	1.0 mg/mL	48 µg/mL
Buffer	20 µL	-	-
Db. dist. water	73 µM	-	-
10 min preincubation under shaking at 37°C			
Substrate	2 µL	$1 \cdot 10^{-3}$ mol/L	$5 \cdot 10^{-5}$ mol/L
Total volume	105 µL		

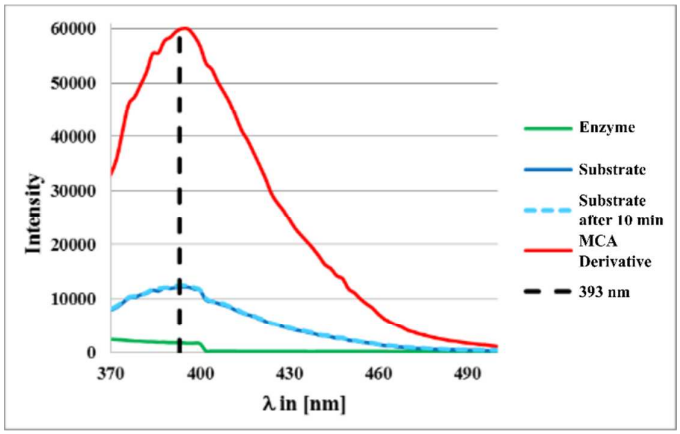


Figure S7b. Right: Superimposed absorption spectra of enzyme, substrate and chromophore in the trypsin assay.

Procedure:

50 µL enzyme solution were treated with 5 µL polymer parent solution. Subsequently, 73 µL doubly distilled water and 20 µL assay buffer were added and the reaction mixture was incubated at 37 °C for 10 min. After addition of 2 µL substrate solution, the reaction mixture was measured immediately. Before each assay the 96-well microplate was shaken for 30 s. Photometric measurements then took place at 37 °C and 328 nm excitation as well as 393 nm emission. They were conducted every 30 s for a total of 30 min; prior to each measurement the microplate was again shaken for 5 s.

Inhibition test and screening

A control measurement was conducted with the known cathepsin D inhibitor pepstatin A, followed by polymer screening with all polymer series. Already the lowest pepstatin A concentration produced full inhibition of cathepsin D.

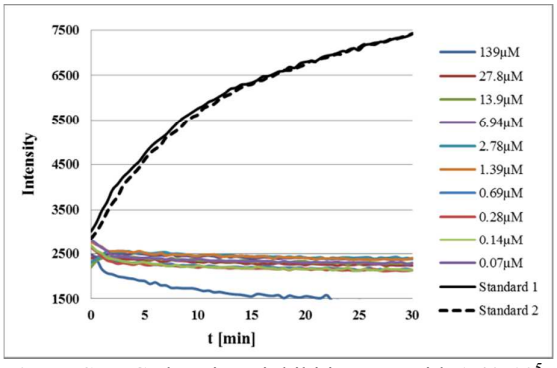


Figure S7c. Cathepsin D inhibition test with $1.40 \cdot 10^5$ nM - $7 \cdot 10^2$ nM pepstatin A solution.

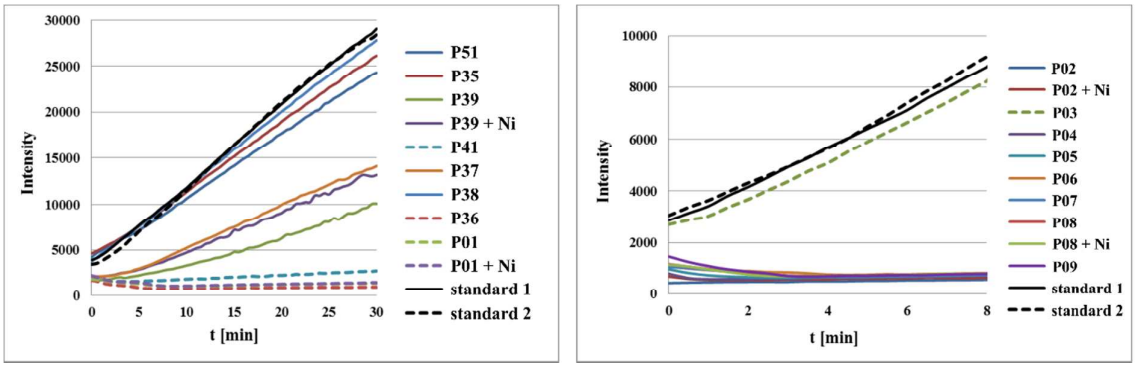


Figure S7d. Left: screening of the first polymer series: polymers P41, P36 and P01 are strong Cathepsin D inhibitors. On the contrary, P39 and P37 showed only moderate inhibition. Enzyme velocity was determined from the linear part of the kinetic curves (< first 5 min) with Michelis Menten behaviour. Right: screening of the second series of polymers: all polymers except P03 are potent Cathepsin D inhibitors.

Determination of the IC_{50} values for the best polymeric inhibitors:

Due to the large number of similarly potent Cathepsin D inhibing polymers, and the limited availability of the assay kit, only one representative copolymer was used for the IC_{50} determination.

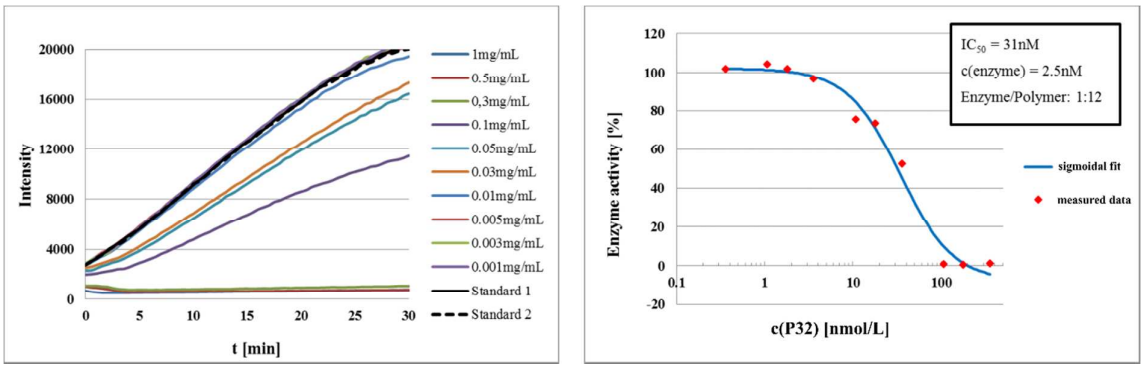


Figure S7e. Left: Inhibition of cathepsin D depending on the concentration of **P32**. Polymer mass concentrations were between 1 - 0.001 mg/mL. Right: Logarithmic plot of the concentration-dependent cathepsin D inhibition by **P32** for the determination of its IC_{50}-value (31 nM at 2.5 nM enzyme).

Isothermal Microcalorimetry

On the example of trypsin, protein affinities and thermodynamic parameters were determined by ITC titrations.

A) ITC-Titration of the NTA monomer **3** (1 mM) compared to its corresponding NTA homopolymer „**P42**“ (1 mM in monomer units) complexed by trypsin (0.1 mM) in 75 mM TRIZMA base and 75 mM Na_2HPO_4 (pH = 8.0).

	K_a [M^{-1}]	n	ΔH [kcal/mol]	TΔS [kcal/mol]	ΔG [kcal/mol]
Monomer	n.d.	n.d.	n.d.	n.d.	n.d.
P42	11600 ± 2000	0.5 ± 0.1	-7.28 ± 1.84	-1.74	-5.54

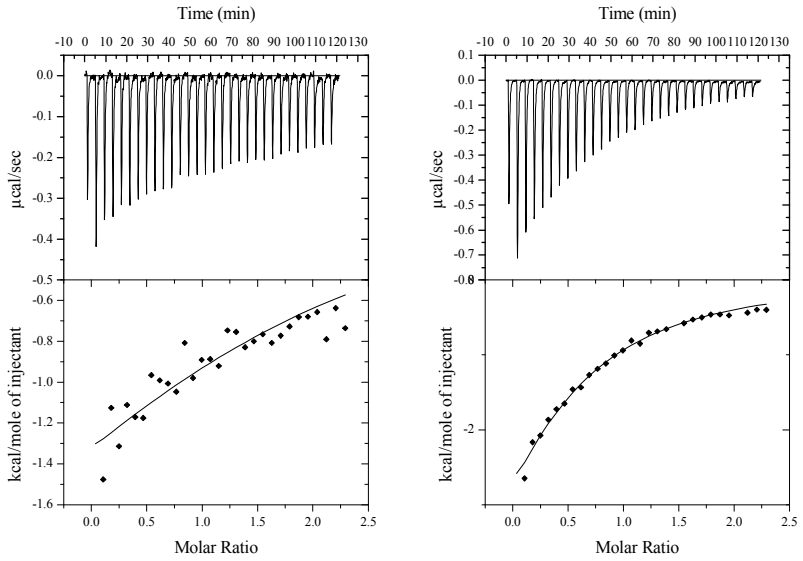


Figure S8a. Only minimal attraction occurs between monomers and trypsin, and no binding curve is produced. On the opposite, a well-defined binding isotherm indicates specific exothermic complex formation between NTA homopolymer IS1 and trypsin. The 2:1 stoichiometry indicates that each linear copolymer can accommodate two protein molecules.

B) ITC titration of the NTA homopolymer **P42** (1 mM in momomer units) loaded with 50 mol-% Ni^{2+} ions (0.5 mM) complexed by trypsin (0.1 mM) in 75 mM TRIZMA base and 75 mM Na_2HPO_4 (pH = 8.0).

	K_a [M^{-1}]	n	ΔH [kcal/mol]	TΔS [kcal/mol]	ΔG [kcal/mol]
P42+Ni	n.d.	n.d.	n.d.	n.d.	n.d.

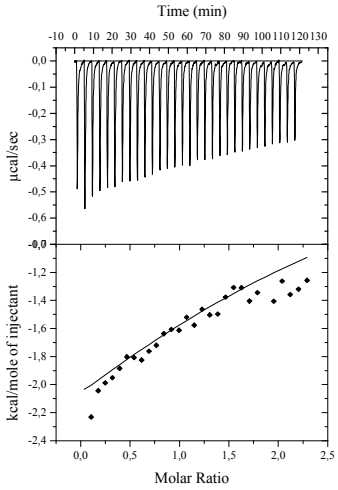


Figure S8b. In the presence of Ni^{2+} ions, the homopolymer with its NTA unit forms strong Ni^{2+} chelate complexes. Surprisingly, the affinity towards trypsin is lost. This rules out, that histidine complexation is essential for trypsin binding. On the other hand, it points to electrostatic attraction of the free NTA head to lysines/arginines on the protein surface, which is blocked after Ni^{2+} complexation.

C) ITC-Titration of the NTA homopolymer **P42** (1 mM in monomer units) versus trypsin (0.1 mM) in 75 mM TRIZMA base and 75 mM Na_2HPO_4 (pH = 8.0). In the next step, the protein-polymer complex is titrated back with $NiSO_4$-Lösung (1 mM).

	K_a [M^{-1}]	n	ΔH [kcal/mol]	TΔS [kcal/mol]	ΔG [kcal/mol]
P42	11400 ± 1400	0.5 ± 0.1	-7.32 ± 1.58	-1.77	-5.54

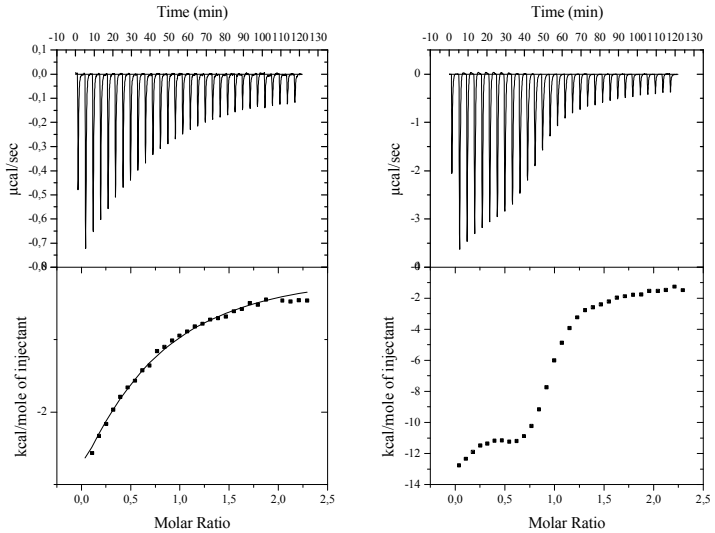


Figure S8c. Left: compexation of trypsin with NTA homopolymer P42. Right: subsequent back titration of the polymer-trypsin complex with Ni^{2+} ions. Both events are exothermic: specific complexation of lysine residues on the protein surface by the free NTA heads, and the subsequent superior Ni^{2+} complexation of the NTA units, leading to release of uncomplexed trypsin.

Enzyme Kinetics

On the example of trypsin, enzyme kinetics were determined by a Lineweaver-Burk plot. Inhibition was examined by **P52**, a good trypsin inhibitor.

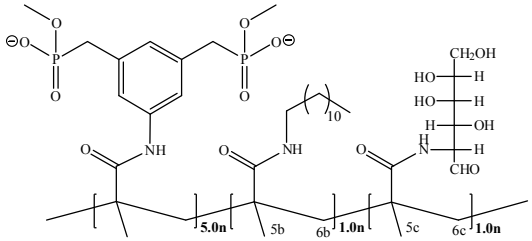


Figure S9a. Structure of **P52**, containing 5 eq. bisphosphonate monomers, 1 eq. dodecylamide monomers and 1 eq. glucoseamide monomer.

The enzyme assay was identical to the above-delineated trypsin procedure. All measurements were carried out in triplicate under steady-state conditions.

Calibration:

Since during steady-state the reaction velocity „r“ of trypsin degradation is proportional to the increase in released chromophor (pNA), its absorption intensity was calibrated for solutions of known concentration.

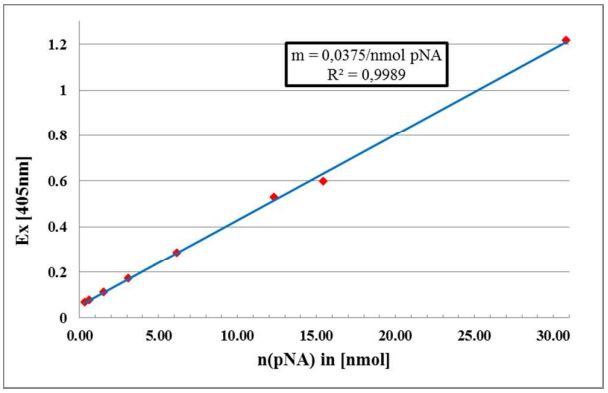


Figure S9b. Linear increase in chromophor extinction with increasing concentration of pNA.

Thus, an increase in extinction by 0.0375 corresponds to the release of 1 nmol pNA. With equations (1) and (2), the reaction velocity can be calculated:

$$\Delta Ex \sim dc(pNA) \sim dc(substrate) \quad (1)$$

$$dc(substrate)/dt = r \quad (2)$$

Three different enzyme activities were measured: without inhibitor as well as with 2.5 µM and 5.0 µM **P52**. In order to probe, if the inhibitor operates in a competitive fashion with reference to the substrate, the enzymatic activity was determined in dependence of increasing substrate concentrations. Graphical evaluation of the resulting enzyme velocities in a doubly reciprocal plot „r^{-1}“ versus „c^{-1}“ produced the first graph for the free enzyme.

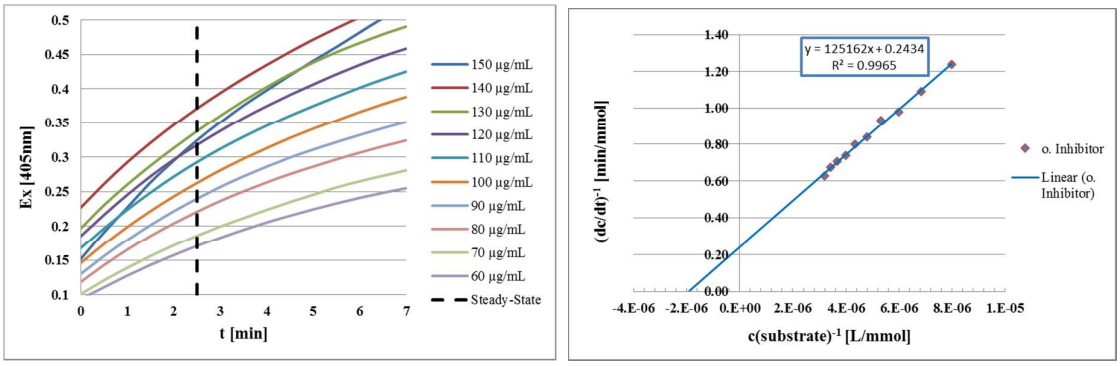


Figure S9c. Enzymatic pNA hydrolysis by trypsin at variable substrate concentrations. Right: Resulting doubly reciprocal Lineweaver-Burk plot for the free enzyme.

The same series of measurements were subsequently carried out in the presence of 2.6 µM and 5.0 µM **P52** (4.2 g/L and 8.0 g/L stock solutions).

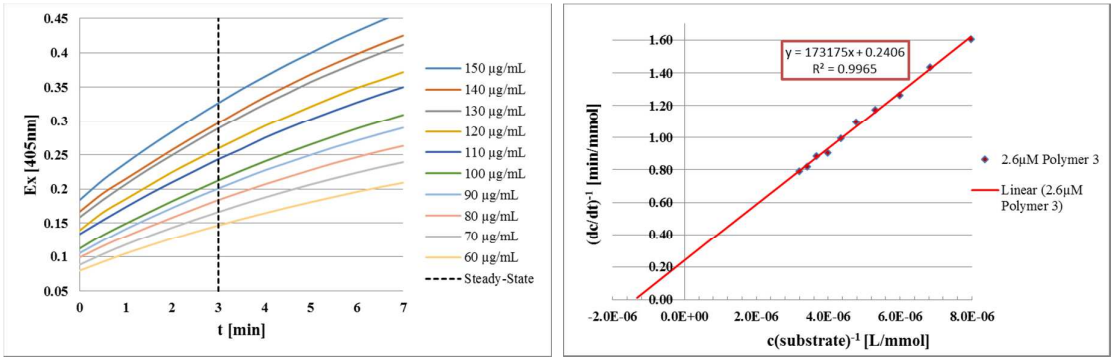


Figure S9d. Enzymatic pNA hydrolysis by trypsin at variable substrate concentrations in the presence of 2.5 µM inhibitor **P52**. Right: Resulting doubly reciprocal Lineweaver-Burk plot for the free enzyme.

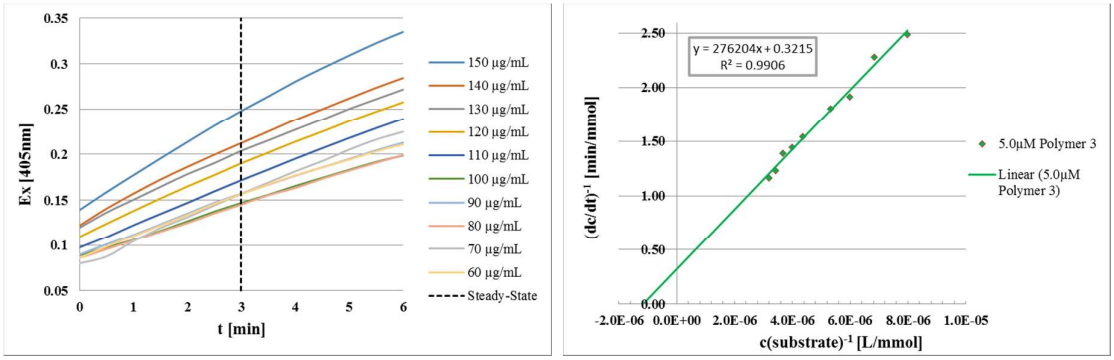


Figure S9e. Enzymatic pNA hydrolysis by trypsin at variable substrate concentrations in the presence of 5.0 µM inhibitor **P52**. Right: Resulting doubly reciprocal Lineweaver-Burk plot for the free enzyme.

Final combination of all three Lineweaver-Burk plots into one graph reveals a common intersection with the ordinate, clearly indicating a competitive mechanism with reference to the substrate for trypsin inhibition by the polymer. In this case, the polymer competes with the substrate to be bound on the protein surface. Most likely, this is achieved, when the polymer sterically blocks the entrance to the active site.

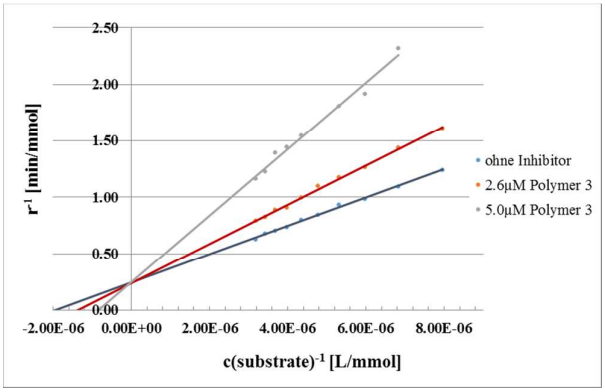


Figure S9f. Combined Lineweaver-Burk plots for the inhibition of trypsin by polymer **P52** at varying substrate concentrations. A common intersection on the ordinate testifies of a competitive inhibition mechanism.

References

[1] Elastase Assay: J. Bieth, B. Spiess, C. G. Wermuth, *Biochem. Med.*, **1974**, *11*, 350 - 357.

[2] α-Chymotrypsin Assay: R. L. Stein, F. Melandri, L. Dick, *Biochemistry*, **1996**, *35*, 3899 – 3908.

[3] Trypsin Assay: B. F. Erlanger, N. Kokowsky, W. Cohen, *Arch. Biochem. Biophys*, **1961**, *95*, 271 - 278.

[4] Carboxypeptidase A Assay: Assay: W. L. Mock, Y. Liu, D. J. Stanford, *Anal. Biochem.*, **1996**, *239*, 218 - 222.

[5] Kallikrein and Thrombin Assay: L. Svendsen, B. Blomback, M. Blombxck, P. I. Olsson, *Thromb res*, **1972**, *1*, 267 - 278.

[6] Cathepsin D Assay: Y. Yasuda, T. Kageyama, A. Akamine, M. Shibata, E. Kominami, Y. Uchiyama, K. Yamamoto, *J. Biochem.*, **1999**, *125*, 1137 - 1143.