Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Peptide dendrimer enzyme models for ester hydrolysis and aldolization prepared by convergent thioether ligation

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Supporting Information

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Materials and Reagents

All reagents were purchased in the highest quality available either from Sigma Aldrich, Bachem, Acors Organics, Carl Roth GmbH or GE Healthcare. PyBOP, amino acids and their derivatives were purchased from Advanced ChemTech (Giessen, Germany) or Novabiochem. For SPPS amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Amb-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Dap(Fmoc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-DGlu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Pro-OH, Fmoc-DPro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH. Tenta Gel S RAM® resin (loading: 0.24 or 0.26 mmol·g^{-1}) was purchased from Rapp Polymere (Tübingen, Germany). Peptide dendrimer syntheses were performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper. Ligation reactions were done in solution using standard glass ware. All solvents used in reactions on solid phase and in solution were bought in p.a. quality and distilled prior to use. Analytical RP-HPLC was performed with a Waters Chromatography System (996 Photo diode array detector) using an Atlantis® column (dC18, 5 µm, 4.6 × 100 mm, flow rate 1.4 mL·min^{-1}) or a chiral Daicel Chiralpak AS® column (amylose tris[(S)-α-methylbenzylcarbamate] coated on a silica support, 10 µm, 4.6 × 250 mm, flow rate 2.0 mL·min^{-1}) for ee determination. Analytical RP-UPLC was performed with an Ultimate 3000 Rapid Separation LC System (DAD-3000RS diode array detector) using an Acclaim® RSLC 120 C18 column (2.2 µm, 120 Å, 3.0 × 50 mm, flow 1.2 ml·min^{-1}) from Dionex. Data recording and processing was either done with Waters Empower2 software (analytical RP-HPLC) or with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). Preparative RP-HPLC was performed with a Waters Prep LC4000 Chromatography System using a Delta-Pak® column (C18, 15 µm, pore size 300 Å, flow rate 80 mL·min^{-1}) or an Atlantis OBD column (dC18, 5 µm, 19 × 100 mm, flow rate 20 mL·min^{-1}). Compounds

were detected by UV absorption at 214 nm using a Waters 486 Tunable Absorbance Detector. The following elution solutions were used for all RP-HPLC: A mQ-deionized H_2O with 0.1% TFA; D mQ-deionized H_2O/HPLC-grade CH_3CN (40:60) with 0.1% TFA. MS spectra, recorded on either a Thermo Scientific LTQ OrbitrapXL, an AB Sciex QTrap or an AB Sciex API 2000 (LC-MS) and amino acid analysis were provided by the MS and protein analytical services of the Department of Chemistry and Biochemistry at the University of Berne. ^{1}H-NMR were recorded on a Bruker DRX500. Kinetic measurements were carried out using a CytoFluor Series 4000 multi-well plate reader from PerSeptive Biosystems. SDS-PAGE was done with equipment purchased from Bio-Rad.

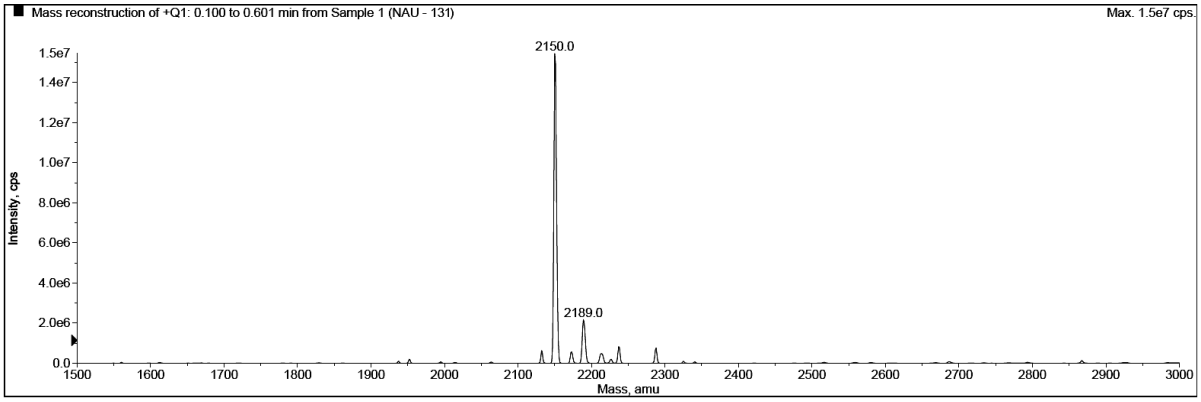
Dendrimer Spectra

Peptide Dendrimer G2C1

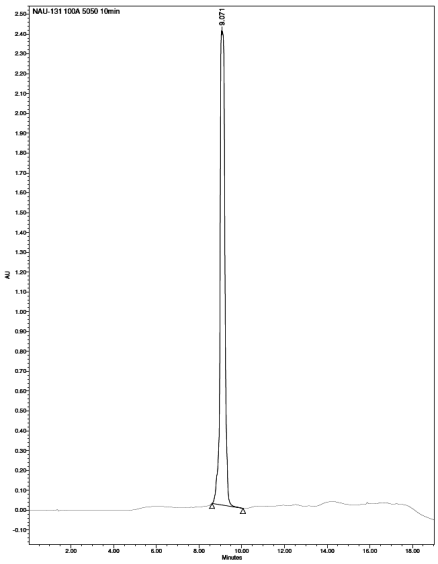
(ClAc-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2C1** was obtained as foamy colourless solid after preparative RP-HPLC (156.7 mg, 53.1 µmol, 44%). Analytical RP-HPLC: t_R = 9.071 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{80}H_{109}Cl_4N_{35}O_{28}$ found/calc. 2150.0/2150.8 $[M]^+$; 2189.0/2189.9 $[M+K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

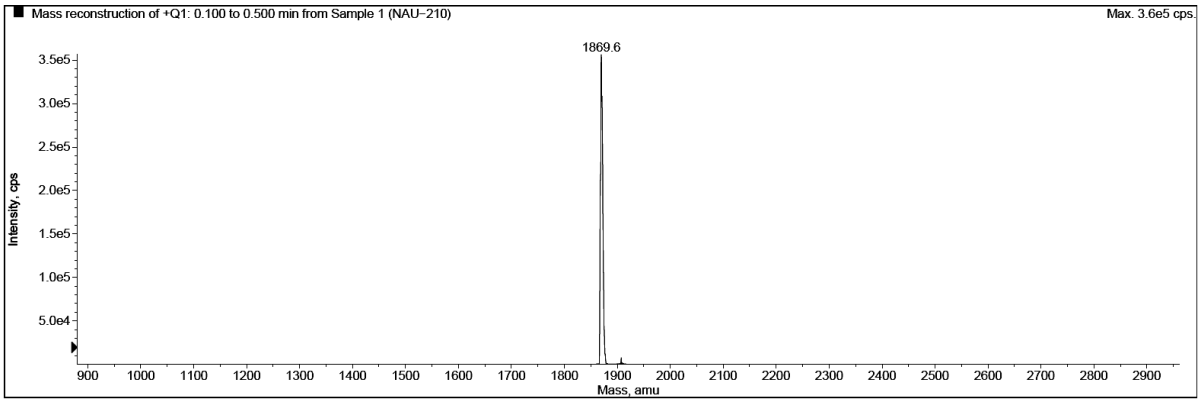


Peptide Dendrimer G2C2

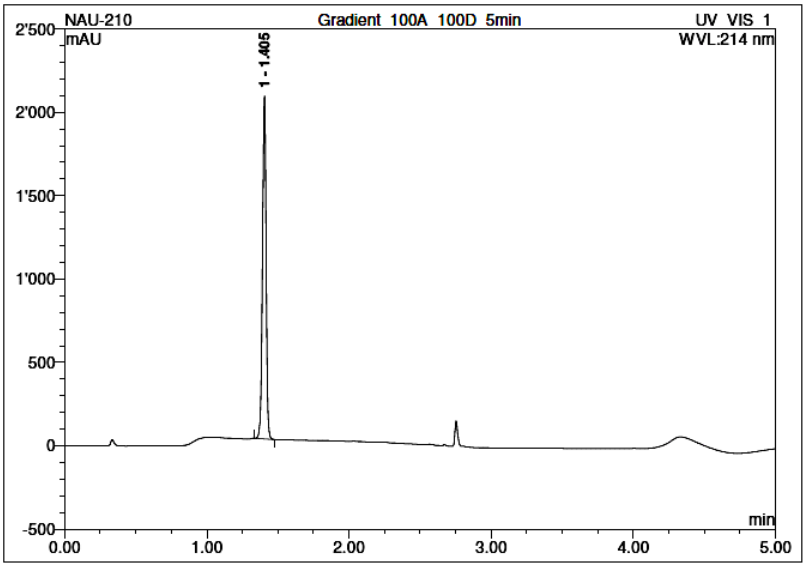
(ClAc-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2C2** was obtained as foamy colourless solid after preparative RP-HPLC (104.0 mg, 55.6 µmol, 46%). Analytical RP-UPLC: t_R = 1.405 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{73}H_{109}Cl_4N_{21}O_{28}$ found/calc. 1869.6/1870.6 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

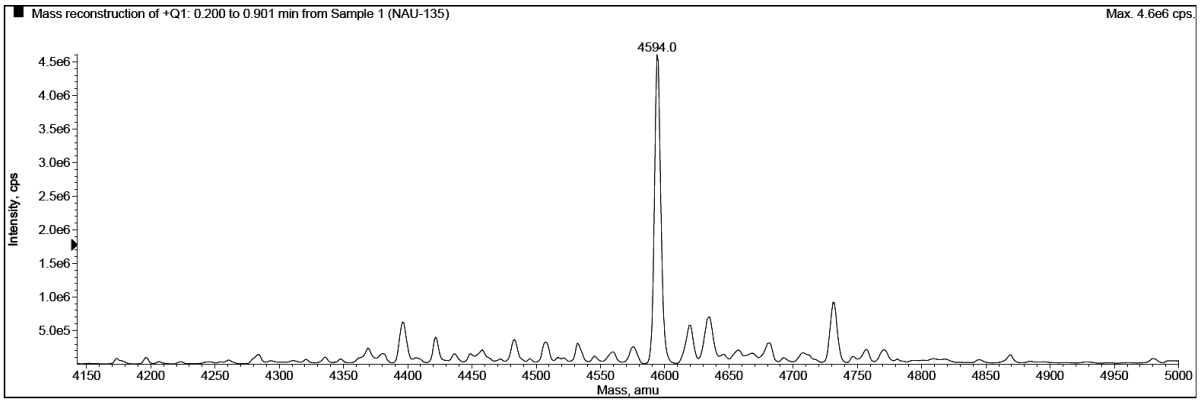


Peptide Dendrimer G3C1

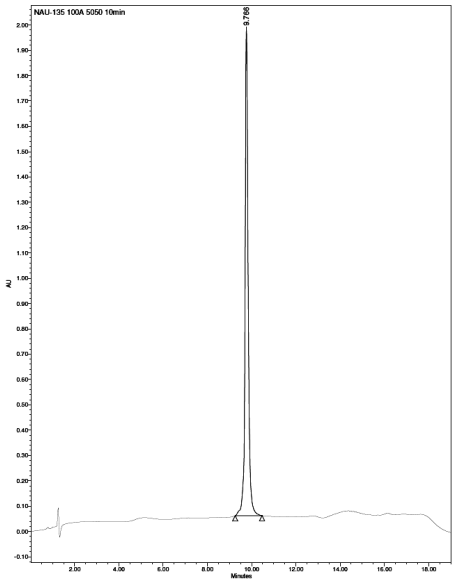
(ClAc-His-Ser)$_8$(*Dap*-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G3C1** was obtained as foamy yellowish solid after preparative RP-HPLC (116.2 mg, 18.4 µmol, 15%). Analytical RP-HPLC: t_R = 9.766 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{172}H_{233}Cl_8N_{75}O_{60}$ found/calc. 4594.0/4594.8 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

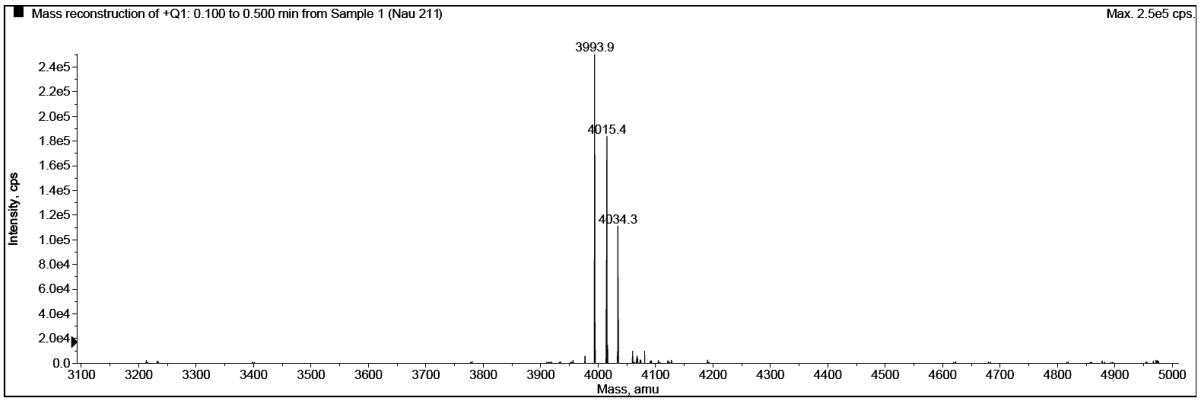


Peptide Dendrimer G3C2

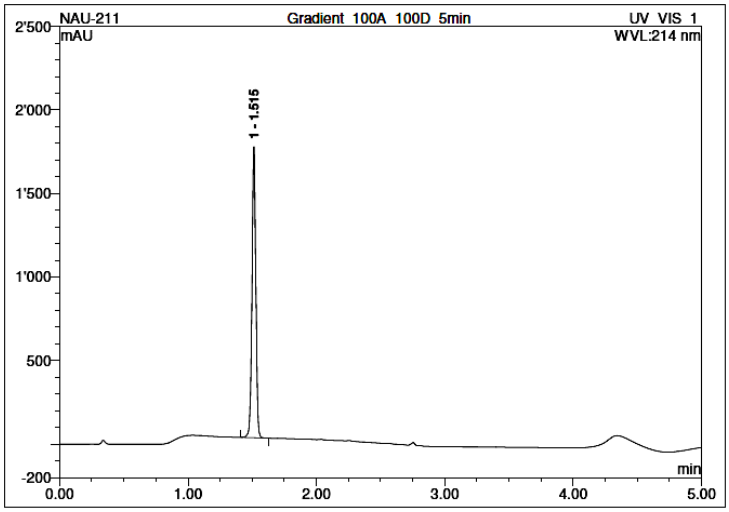
(ClAc-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G3C2** was obtained as foamy yellowish solid after preparative RP-HPLC (119.9 mg, 30.0 µmol, 25%). Analytical RP-UPLC: t_R = 1.515 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{157}H_{233}Cl_8N_{45}O_{60}$ found/calc. 3993.9/3994.4 $[M]^+$; 4015.4/4017.4 $[M+Na]^+$; 4034.3/4033.5 $[M+K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

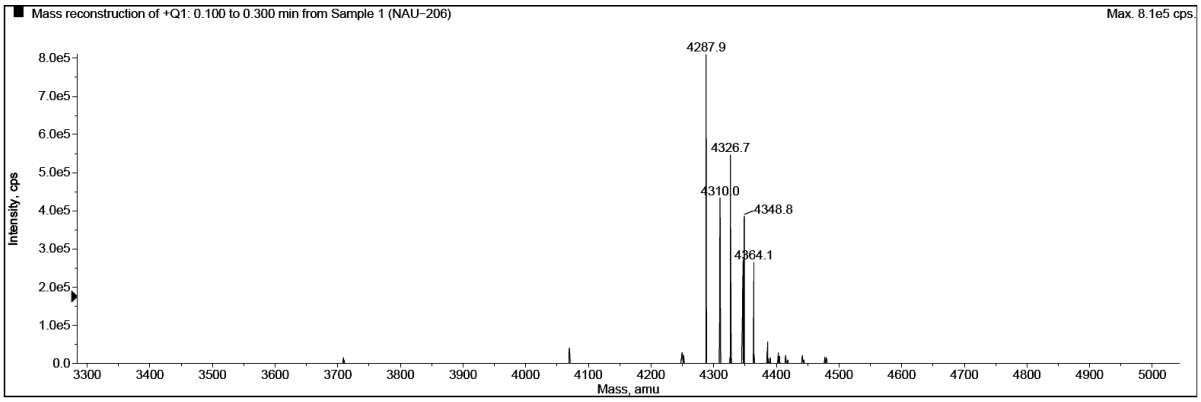


Peptide Dendrimer G3C3

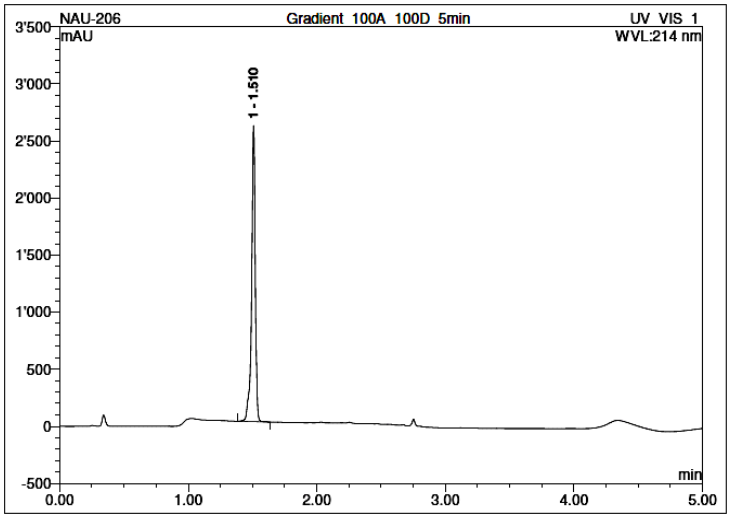
(ClAc-Pro-Ser)$_8$(*Lys*-Pro-Ser)$_4$(*Lys*-Pro-Ser)$_2$*Lys*-Pro-Ser-NH$_2$

From Tenta Gel S RAM® resin (800 mg, 0.24 mmol·g^{-1}), **G3C3** was obtained as foamy colourless solid after preparative RP-HPLC (149.7 mg, 34.9 µmol, 18%). Analytical RP-UPLC: t_R = 1.510 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{178}H_{275}Cl_8N_{45}O_{60}$ found/calc. 4287.9/4289.0 $[M]^+$; 4310.0/4312.0 $[M+Na]^+$; 4326.7/4328.1 $[M+K]^+$; 4348.8/4351.1 $[M+Na+K]^+$; 4364.1/4367.2 $[M+2K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

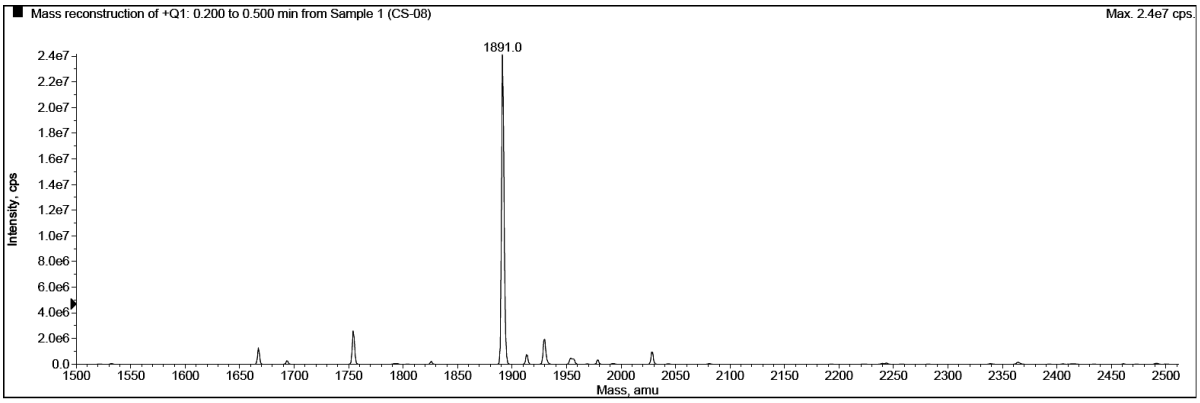


Peptide Dendrimer G2M1

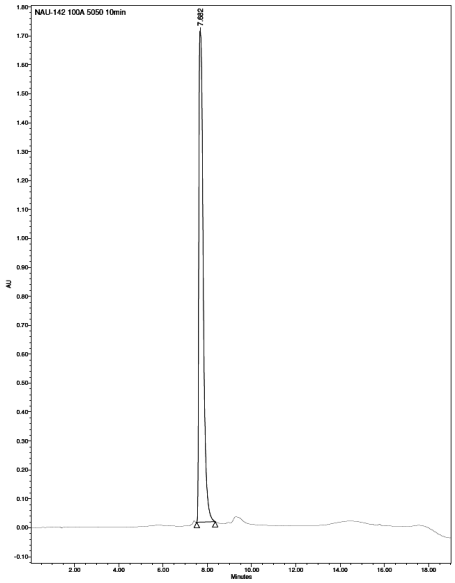
(Ac-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M1** was obtained as foamy colourless solid after preparative RP-HPLC (123.0 mg, 47.8 µmol, 40%). Analytical RP-HPLC: t_R = 7.682 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{74}H_{106}N_{32}O_{26}S$ found/calc. 1891.0/1891.9 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

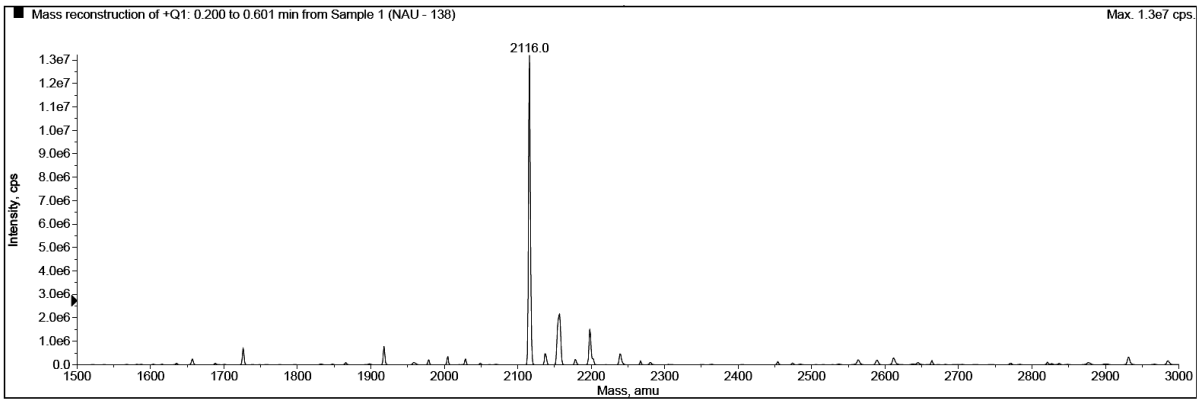


Peptide Dendrimer G2M2

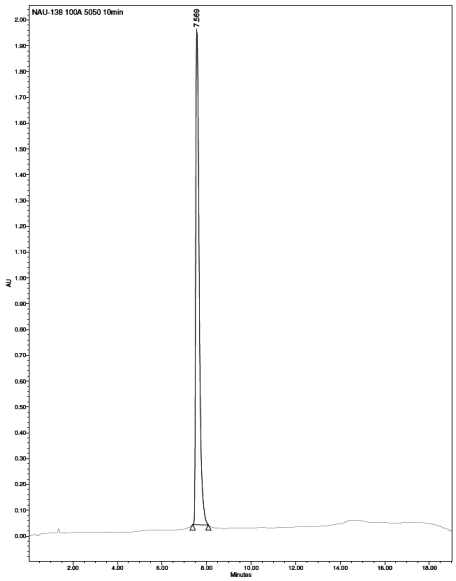
(Ac-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M2** was obtained as foamy colourless solid after preparative RP-HPLC (147.9 mg, 50.7 µmol, 42%). Analytical RP-HPLC: t_R = 7.569 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{83}H_{118}N_{36}O_{29}S$ found/calc. 2116.0/2116.1 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

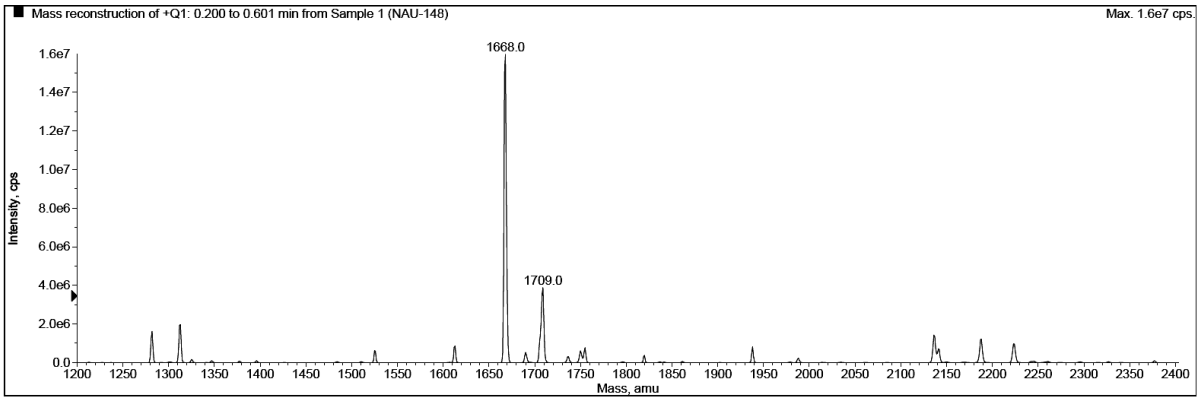


Peptide Dendrimer G2M3

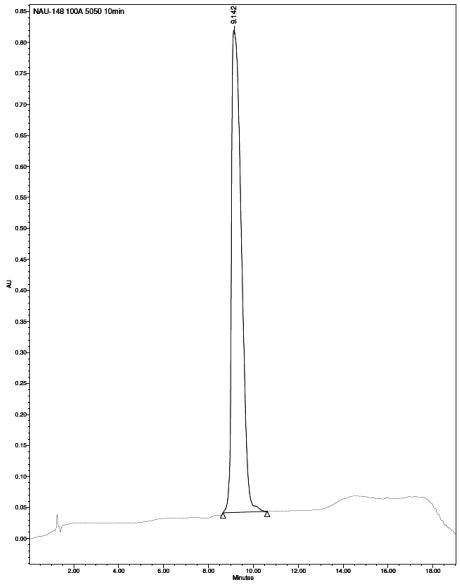
(Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M3** was obtained as foamy colourless solid after preparative RP-HPLC (118.8 mg, 56.0 µmol, 47%). Analytical RP-HPLC: t_R = 9.142 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{68}H_{110}N_{22}O_{25}S$ found/calc. 1668.0/1667.8 $[M]^+$; 1709.0/1708.8 $[M+2H+K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

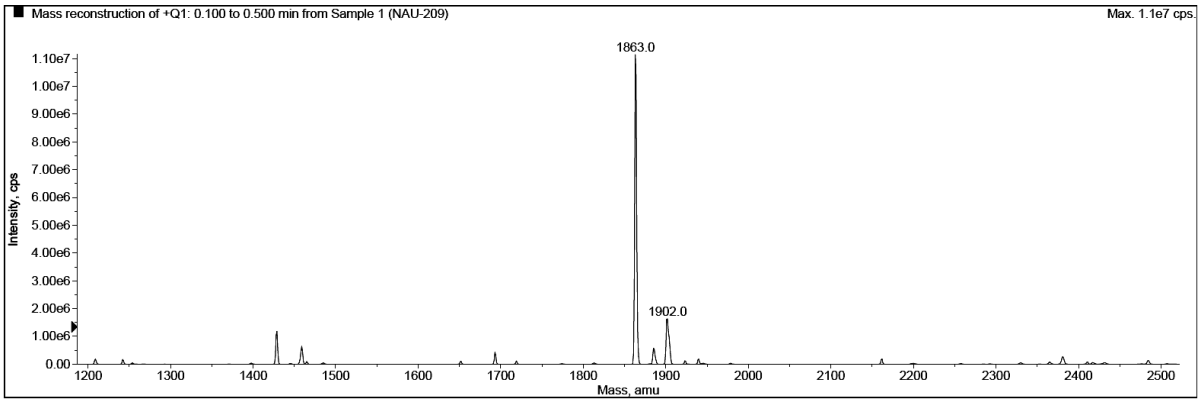


Peptide Dendrimer G2M4

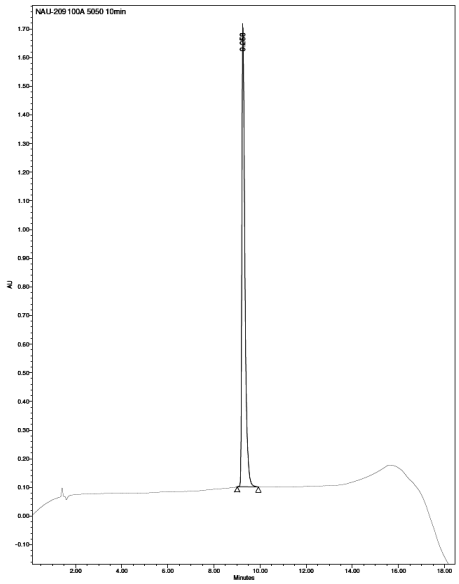
(Pro-Asp)$_4$(*Dap*-Pro-Asp)$_2$*Dap*-Pro-Asp-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M4** was obtained as foamy colourless solid after preparative RP-HPLC (110.5 mg, 59.3 µmol, 49%). Analytical RP-HPLC: t_R = 9.258 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{75}H_{110}N_{22}O_{32}S$ found/calc. 1863.0/1863.9 $[M]^+$; 1902.0/1903.0 $[M+K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

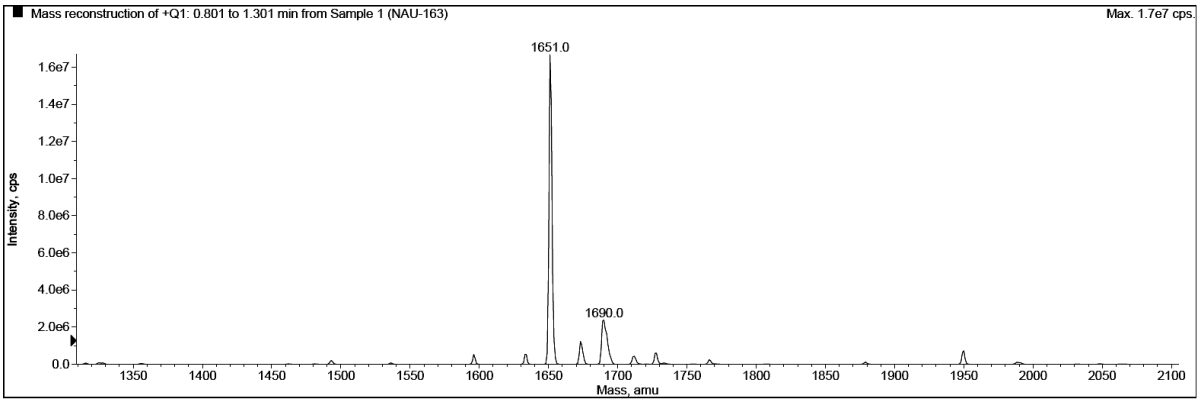


Peptide Dendrimer G2M5

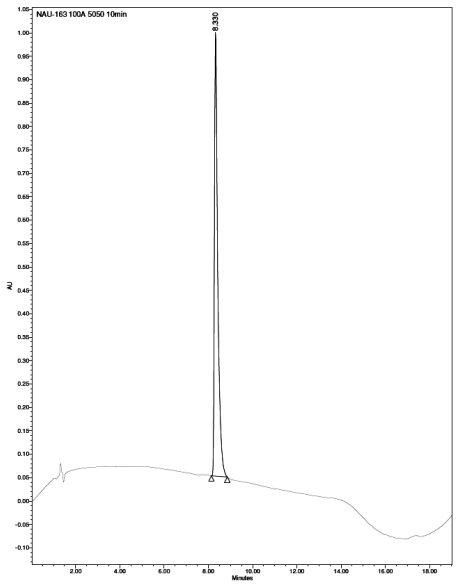
(Pro-Asp)$_4$(*Dap*-Pro-Asp)$_2$*Dap*-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M5** was obtained as foamy colourless solid after preparative RP-HPLC (109.0 mg, 66.0 µmol, 55%). Analytical RP-HPLC: t_R = 8.330 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{66}H_{98}N_{20}O_{28}S$ found/calc. 1651.0/1651.7 $[M]^+$; 1690.0/1690.8 $[M+K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

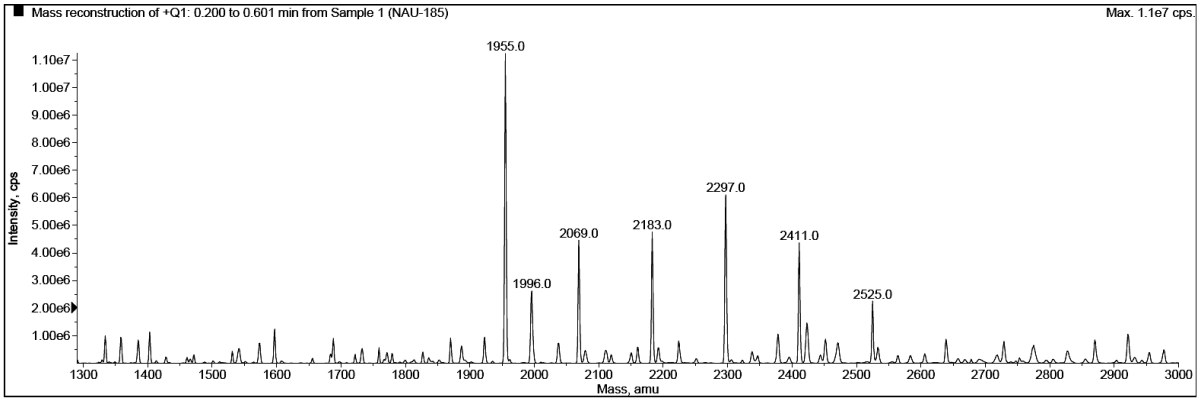


Peptide Dendrimer G2M6

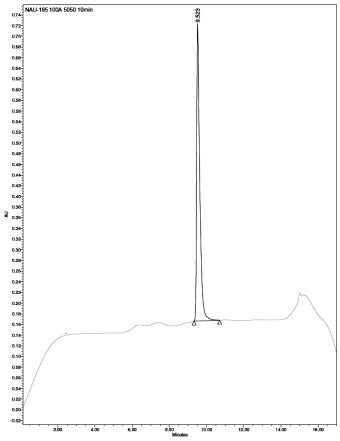
(Pro-Lys)$_4$(*Dap*-Pro-Lys)$_2$*Dap*-Pro-Lys-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M6** was obtained as foamy colourless solid after preparative RP-HPLC (195.9 mg, 71.2 µmol, 59%). Analytical RP-HPLC: t_R = 9.523 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{89}H_{159}N_{29}O_{18}S$ found/calc. 1955.0/1955.5 $[M]^+$; 1996.0/1994.6 $[M+K]^+$; 2069.0/2069.5 $[M+TFA]^+$; 2183.0/2183.5 $[M+2TFA]^+$; 2297.0/2297.5 $[M+3TFA]^+$; 2411.0/2411.5 $[M+4TFA]^+$; 2525.0/2525.5 $[M+5TFA]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

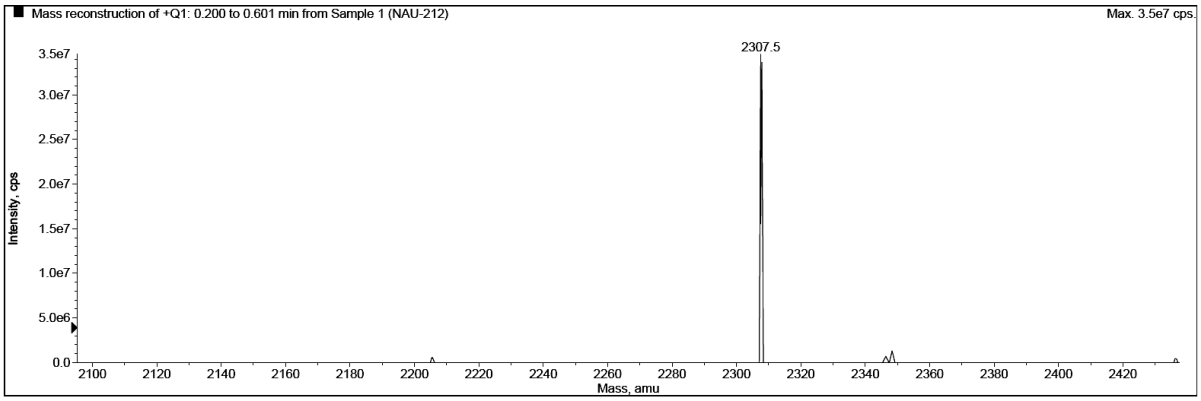


Peptide Dendrimer G2M7

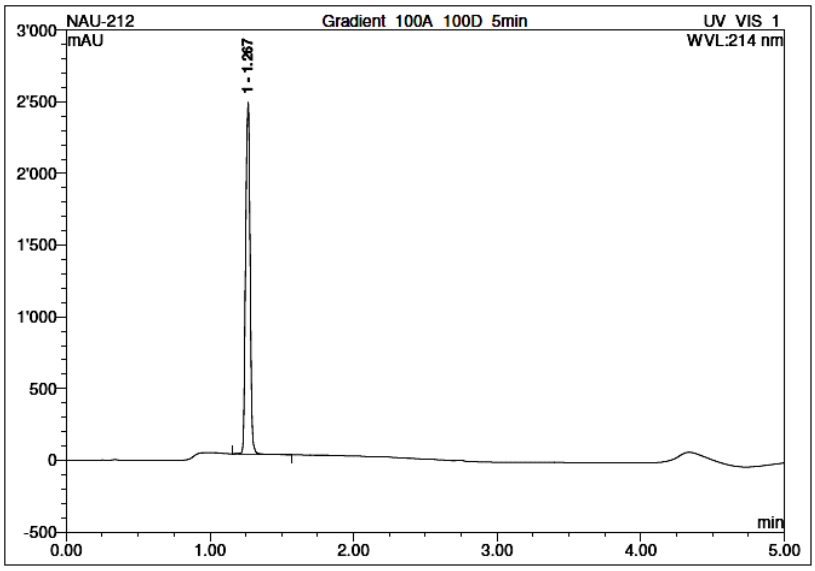
(DPro-Pro-Glu)$_4$(*Dap*-Pro-Asp)$_2$*Dap*-Pro-Asp-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G2M7** was obtained as foamy colourless solid after preparative RP-HPLC (149.9 mg, 54.2 µmol, 45%). Analytical RP-UPLC: t_R = 1.267 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{99}H_{146}N_{26}O_{36}S$ found/calc. 2307.5/2308.4 $[M]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

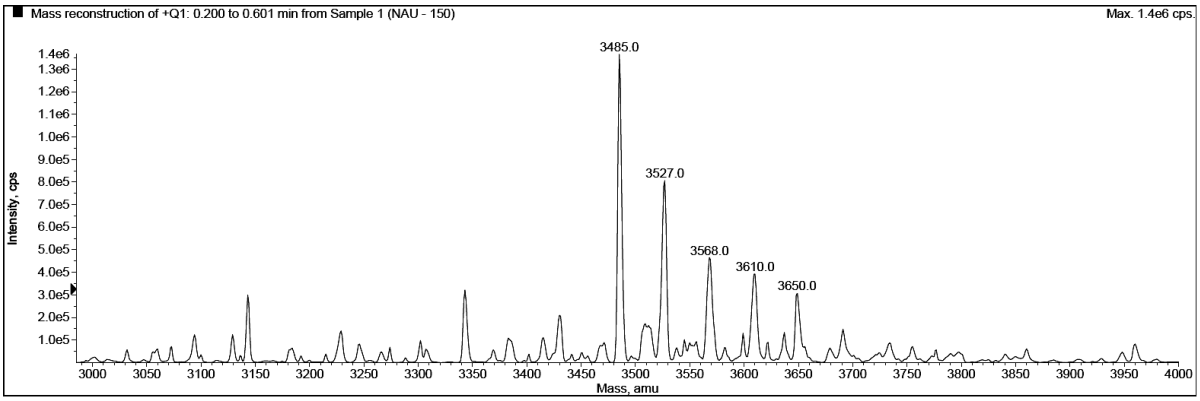


Peptide Dendrimer G3M1

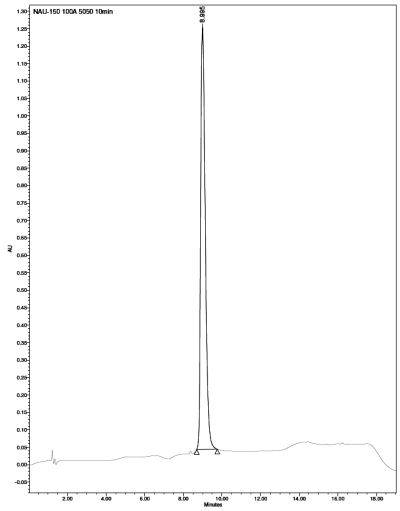
(Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-Cys-NH$_2$

From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), **G3M1** was obtained as foamy colourless solid after preparative RP-HPLC (149.0 mg, 42.8 µmol, 36%). Analytical RP-HPLC: t_R = 8.995 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{144}H_{230}N_{46}O_{53}S$ found/calc. 3485.0/3485.7 $[M]^+$; 3527.0/3526.8 $[M+2H+K]^+$; 3568.0/3567.9 $[M+4H+2K]^+$; 3610.0/3610.0 $[M+7H+3K]^+$; 3650.0/3650.2 $[M+8H+4K]^+$.

Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

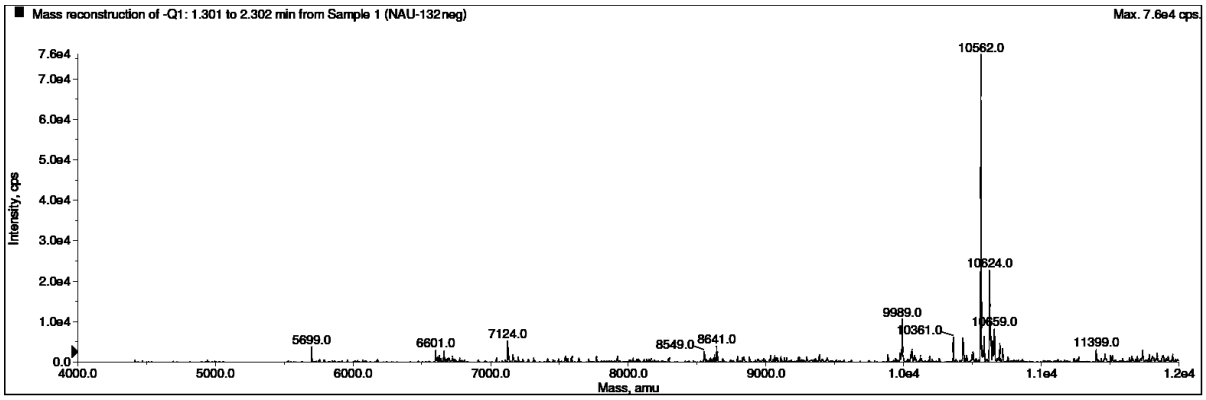


Peptide Dendrimer G4E

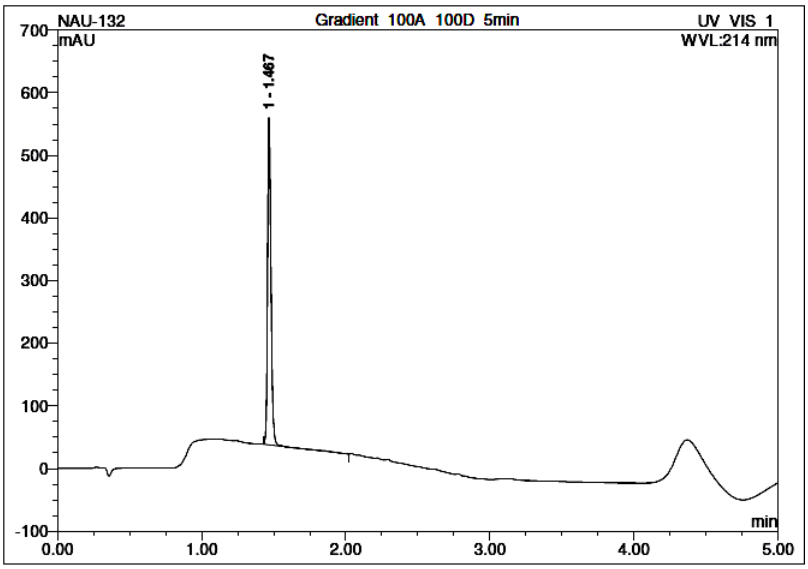
(Ac-Glu-Ala)$_{16}$(*Lys*-Amb-Tyr)$_8$(*Dap*-Cys(Asp)-x-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From starting materials **G2C1** and **B1G2** using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), **G4E** was obtained as colourless solid after preparative RP-HPLC (8.23 mg). Amino Acid Analysis: protein content 52.5% (4.32 mg, 0.40 µmol, yield 28%); found/calc. (%) Glu 17.6/18.8, Ala 17.3/18.8, Dap 11.8/8.2, Tyr 10.0/9.4, Amb 9.5/9.4, Lys 9.2/9.4, His 7.8/8.2, Ser 7.1/8.2, Asp 4.8/4.7, CM-Cys 4.8/4.7 (AMBA t_R = 7.03 min, 1552.4 pmol, Dap coeluted with Phe, 1941.7 pmol). Analytical RP-UPLC: t_R = 1.467 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI-): $C_{464}H_{629}N_{119}O_{160}S_4$ (4-fold ligation product) found/calc. 10562.0/10561.9 $[M]^-$; 10624.0/10624.0 $[M+Na+K]^-$. No incomplete ligation products observed (3-fold ligation product calc. 8459.1 $[M]^-$, 2-fold ligation product calc. 6356.3 $[M]^-$). D = $10.25 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 1.98 ± 0.08 nm.

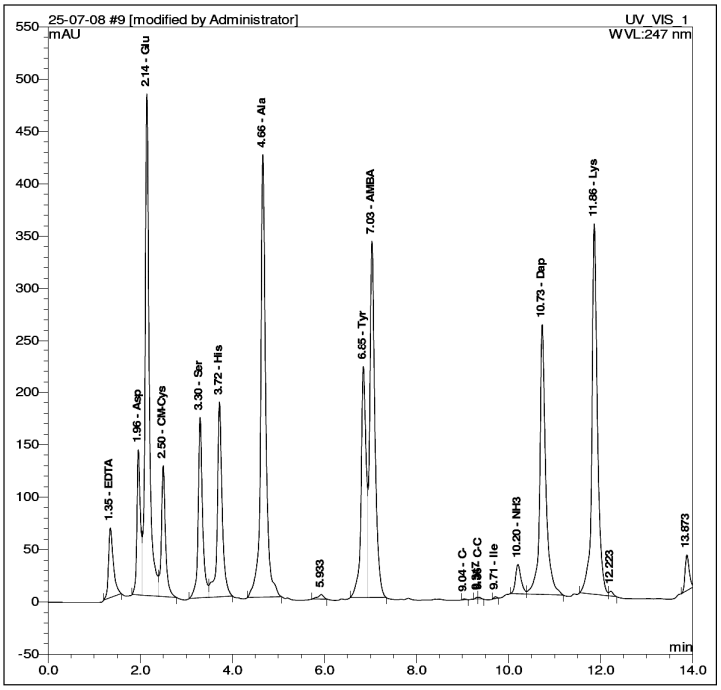
Mass spectrum, MS (ESI-):



Analytical RP-UPLC chromatogram:

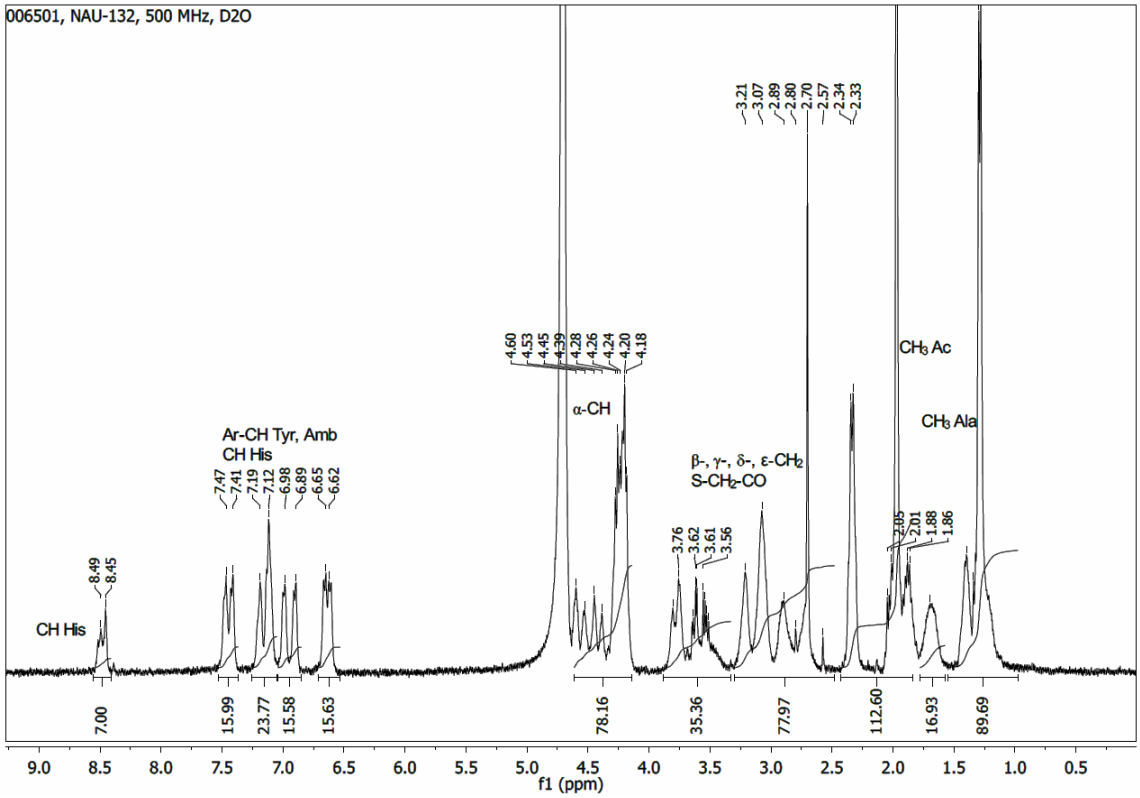


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,35	1,35	0,105	8,08	66,57		345,6	EDTA
1,96	1,96	0,083	12,47	138,31		792,4	Asp
2,14	2,15	0,083	50,36	479,81		2894,3	Glu
2,50	2,51	0,083	13,14	124,93		790,2	CM-Cys
3,30	3,30	0,090	19,23	172,02		1169,1	Ser
3,72	3,72	0,096	23,23	185,68		1274,4	His
4,66	4,67	0,100	54,36	423,60		2846,0	Ala
6,85	6,85	0,129	28,69	220,61		1639,0	Tyr
9,04	9,02	0,075	0,06	0,85		3,5	C-
9,36	9,32	n.a.	0,14	1,79		7,3	C-C
9,71	9,73	0,071	0,12	1,70		12,0	Ile
10,20	10,21	0,125	3,93	27,92		618,3	NH3
10,73	10,80	0,115	38,97	258,36		1941,7	Phe
11,86	11,88	0,112	49,75	354,49		1513,2	Lys
Total:			302,528	2456,635		15846,96	

^{1}H-NMR:

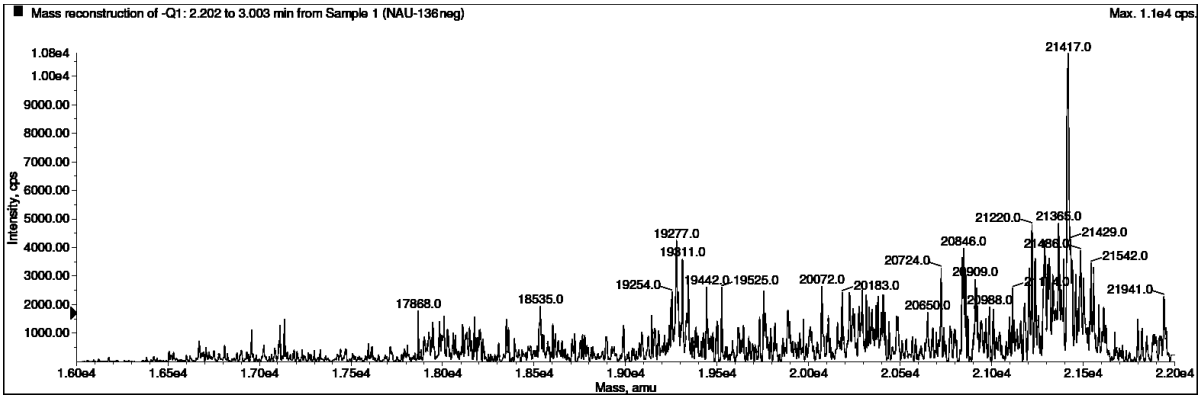

006501, NAU-132, 500 MHz, D2O
CH His
Ar-CH Tyr, Amb
CH His
α-CH
β-, γ-, δ-, ε-CH2
S-CH2-CO
CH3 Ac
CH3 Ala
f1 (ppm)

Peptide Dendrimer G5E

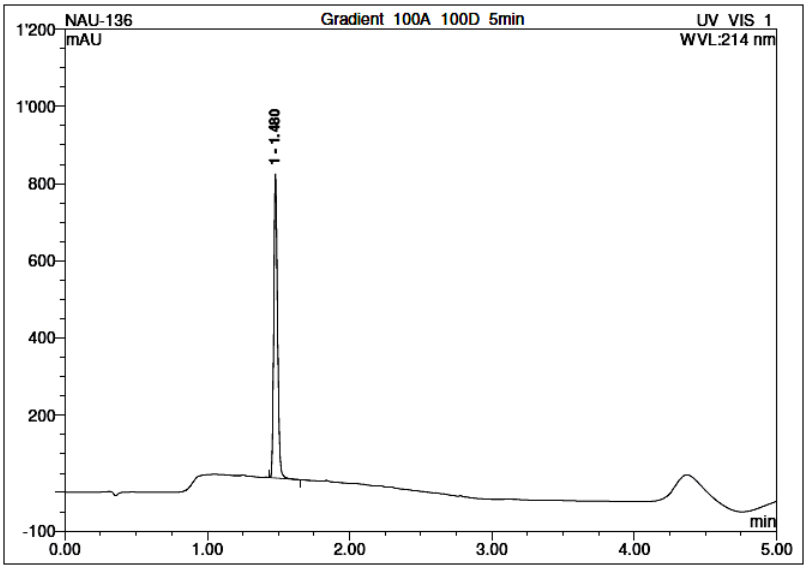
(Ac-Glu-Ala)$_{32}$(*Lys*-Amb-Tyr)$_{16}$(*Dap*-Cys(Asp)-x-His-Ser)$_8$(*Dap*-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From starting materials **G3C1** and **B1G2** using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), **G5E** was obtained as foamy colourless solid after preparative RP-HPLC (13.3 mg). Amino Acid Analysis: protein content 32.3% (4.29 mg, 0.19 µmol, yield 30%); found/calc. (%) Ala 19.2/18.5, Glu 15.2/18.5, Dap 13.1/8.7, Tyr 9.8/9.2, His 9.7/8.7, Lys 9.2/9.2, Amb 9.0/9.2, Ser 8.7/8.7, CM-Cys 4.2/4.6, Asp 1.9/4.6 (Dap coeluted with Phe, 1324.4 pmol). Analytical RP-UPLC: t_R = 1.480 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI-): $C_{940}H_{1273}N_{243}O_{324}S_8$ (8-fold ligation product, main peak) found/calc. 21417.0/21416.1 $[M-H]^-$. $C_{844}H_{1143}ClN_{222}O_{291}S_7$ (7-fold ligation product with one unreacted chloroacetyl group, peak height 33% of main peak) found/calc. 19311.0/19313.3 $[M-H]^-$, no 6-fold ligation product observed (calc. 17210.5 $[M-H]^-$). $D = 8.63 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 2.35 ± 0.02 nm.

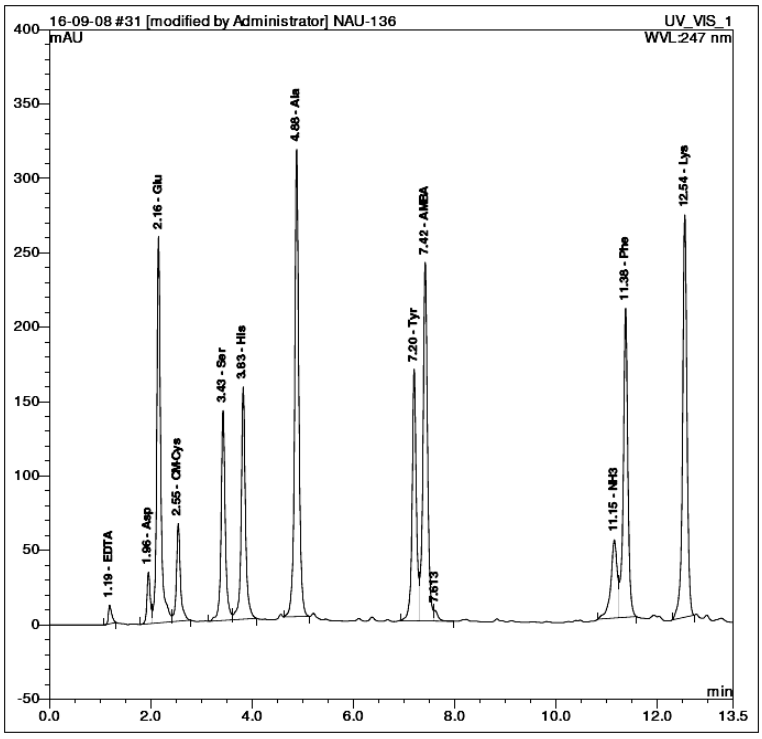
Mass spectrum, MS (ESI-):



Analytical RP-UPLC chromatogram:

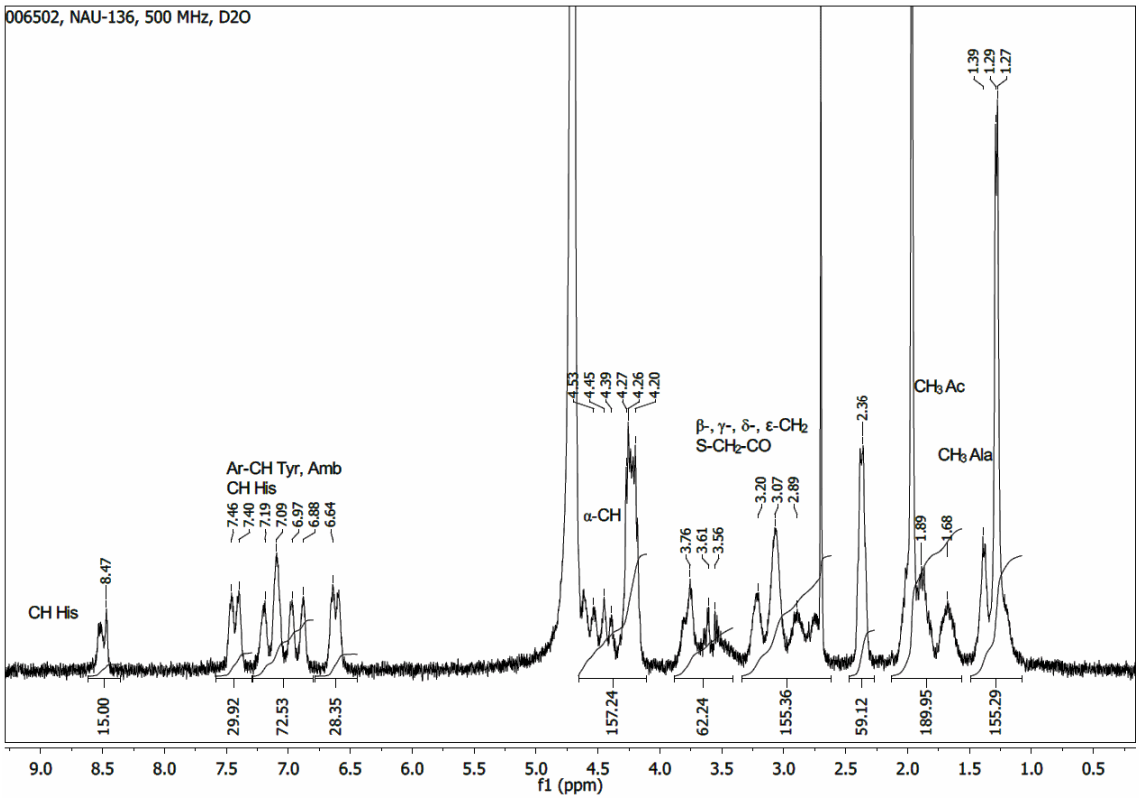


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,19	1,20	0,068	0,93	12,44		405,8	EDTA
1,96	1,96	0,066	2,60	34,73		195,7	Asp
2,16	2,16	0,072	23,34	259,76		1541,2	Glu
2,55	2,55	0,073	6,03	65,63		422,2	CM-Cys
3,43	3,43	0,077	13,41	141,19		879,5	Ser
3,83	3,83	0,077	15,06	156,13		975,8	His
4,88	4,88	0,080	29,88	314,07		1940,5	Ala
7,20	7,20	0,083	16,41	168,98		989,3	Tyr
7,42	7,42	0,090	25,38	241,01		906,8	AMBA
11,15	11,15	0,136	7,81	52,47		1112,5	NH3
11,38	11,38	0,088	21,68	207,72		1324,4	Phe
12,54	12,54	0,086	27,26	270,46		933,8	Lys
Total:			189,773	1924,580		11627,32	

^{1}H-NMR:

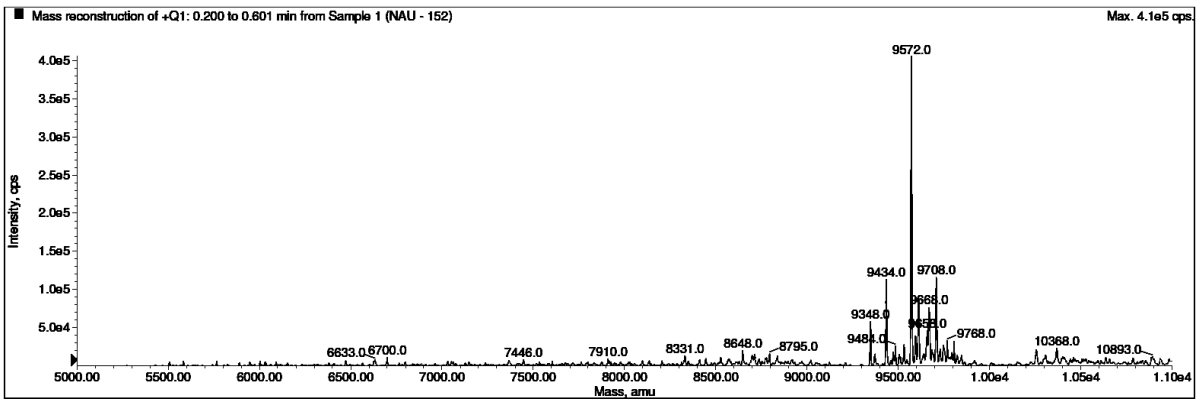

006502, NAU-136, 500 MHz, D2O
CH His
Ar-CH Tyr, Amb
CH His
α-CH
β-, γ-, δ-, ε-CH2
S-CH2-CO
CH3 Ac
CH3 Ala
f1 (ppm)

Peptide Dendrimer G4H1

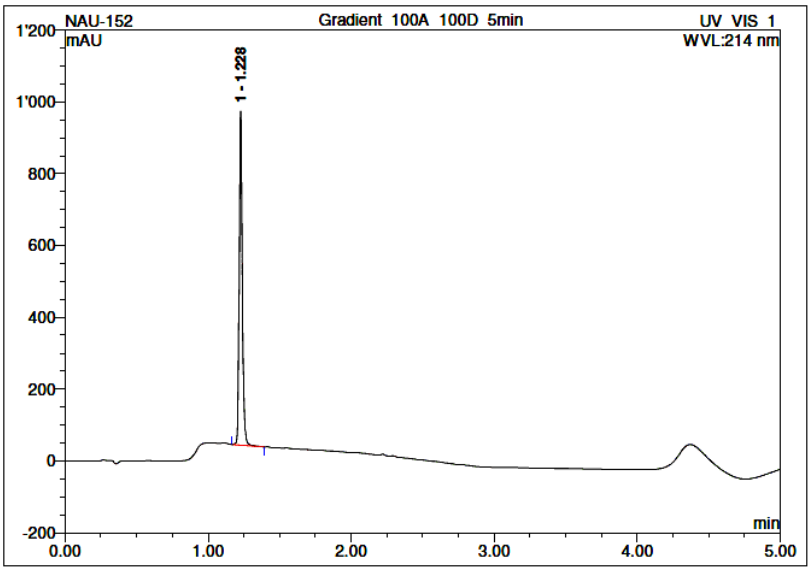
(Ac-His-Ser)$_{16}$(*Dap*-His-Ser)$_8$(*Dap*-Cys-x-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From starting materials **G2C1** and **G2M1** using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), **G4H1** was obtained as foamy colourless solid after preparative RP-HPLC (4.02 mg). Amino Acid Analysis: protein content 46.6% (1.87 mg, 0.17 µmol, yield 12%); found/calc. (%) His 47.8/38.3, Ser 31.5/38.3, Dap 16.3/18.5, CM-Cys 4.4/4.9. Analytical RP-UPLC: t_R = 1.228 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{376}H_{529}N_{163}O_{132}S_4$ (4-fold ligation product) found/calc. 9572.0/9572.5 $[M]^+$. No incomplete ligation products observed (3-fold ligation product calc. 7717.1 $[M]^+$, 2-fold ligation product calc. 5861.6 $[M]^+$). $D = 7.70 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; $R_h = 2.63 \pm 0.06$ nm.

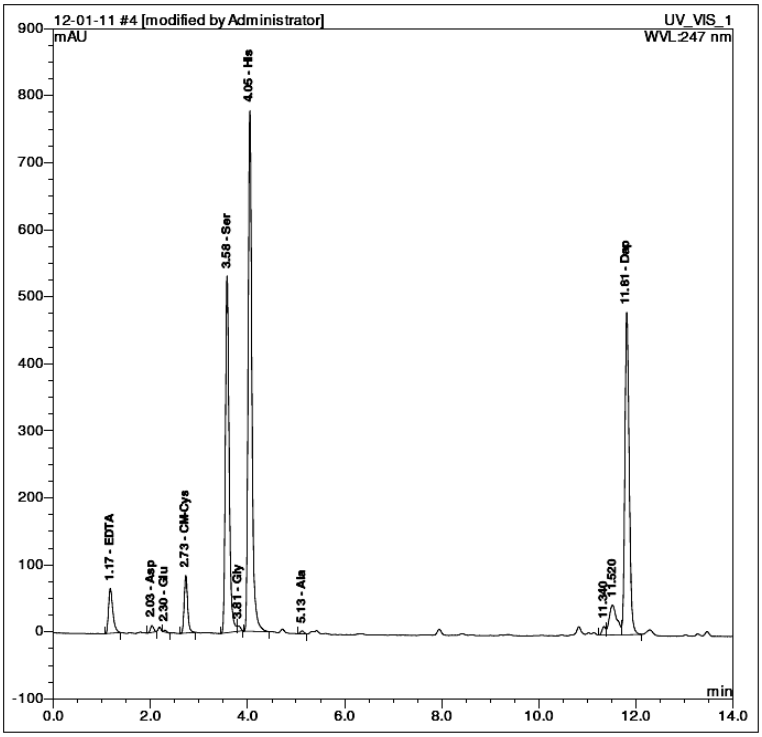
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

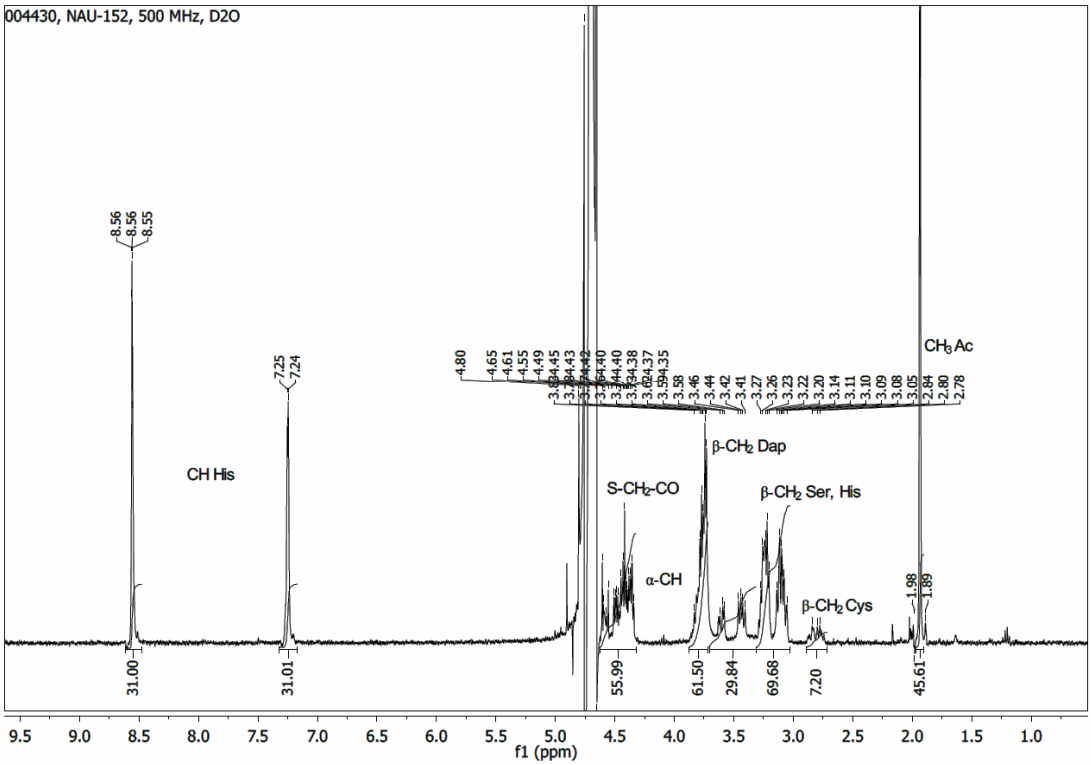


Amino Acid Analysis:

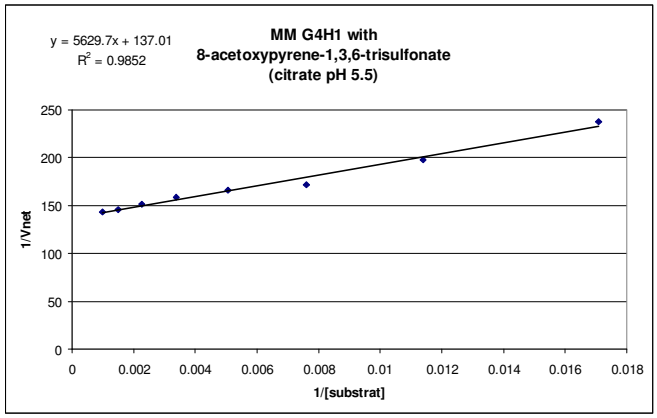


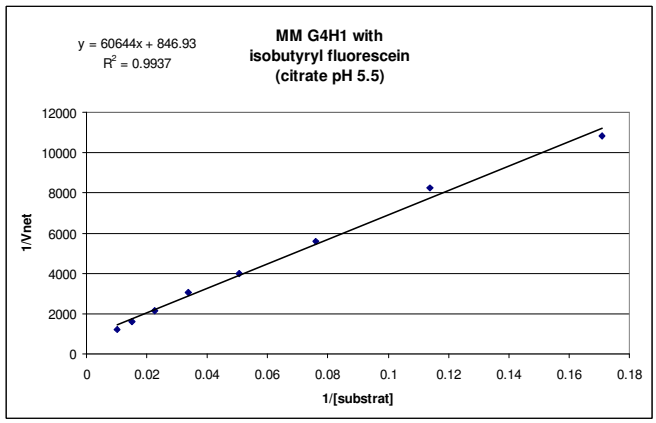
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,17	1,17	0,092	6,80	67,03		527,9	EDTA
2,03	2,07	0,074	0,76	10,08		53,6	Asp
2,30	2,30	0,056	0,18	3,08		17,5	Glu
2,73	2,73	0,073	6,95	85,87		483,0	CM-Cys
3,58	3,59	0,080	47,82	532,12		3414,5	Ser
3,81	3,84	n.a.	0,58	7,58		48,9	Gly
4,05	4,06	0,078	69,23	776,90		5188,9	His
5,13	5,15	0,081	0,40	4,71		30,0	Ala
11,81	11,77	0,094	49,62	480,82		1768,3	Dap
Total:						11532,76	

^{1}H-NMR:



Representative Michaelis-Menten plots for determination of k_{cat} and K_M:



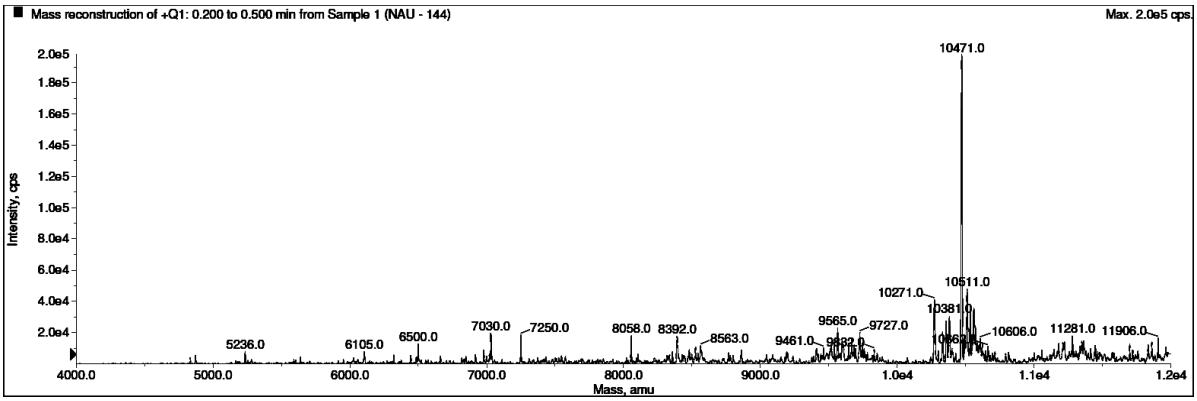


Peptide Dendrimer G4H2

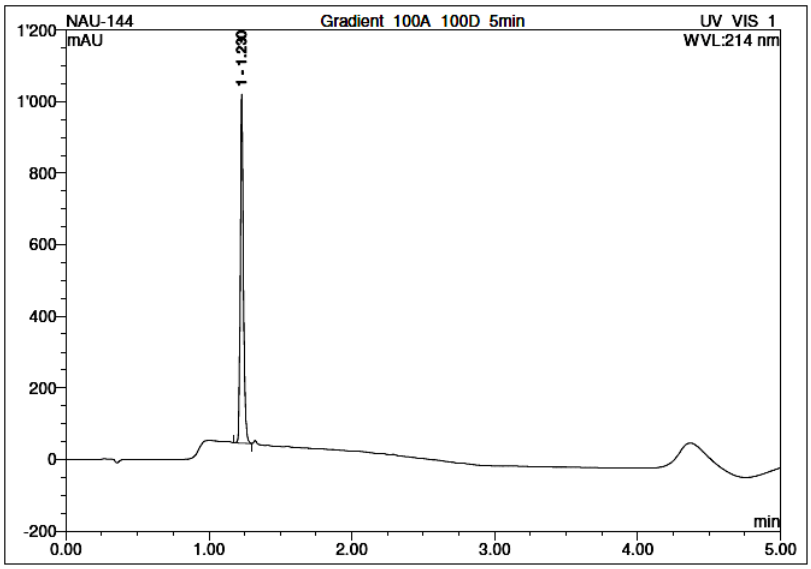
(Ac-His-Ser)$_{16}$(*Dap*-His-Ser)$_8$(*Dap*-His-Ser-Cys-x-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From starting materials **G2C1** and **G2M2** using the general procedure described above (solvent: 0.2 M phosphate buffer pH 8.5), **G4H2** was obtained as foamy colourless solid after preparative RP-HPLC (2.24 mg). Amino Acid Analysis: protein content 59.7% (1.34 mg, 0.11 µmol, yield 8%); found/calc. (%) His 48.8/39.3, Ser 32.1/39.3, Dap 14.7/16.9, CM-Cys 4.3/4.5. Analytical RP-UPLC: t_R = 1.230 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{412}H_{577}N_{179}O_{144}S_4$ (4-fold ligation product, main peak) found/calc. 10471.0/10470.4 $[M+H]^+$; 10511.0/10510.5 $[M+2H+K]^+$. $C_{329}H_{460}ClN_{143}O_{115}S_3$ (3-fold ligation product with one unreacted chloroacetyl group, peak height 9% of main peak) found/calc. 8392.0/8390.7 $[M+H]^+$, no 2-fold ligation product observed (calc. 6310.1 $[M]^+$). $D = 6.94 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; $R_h = 2.92 \pm 0.09$ nm.

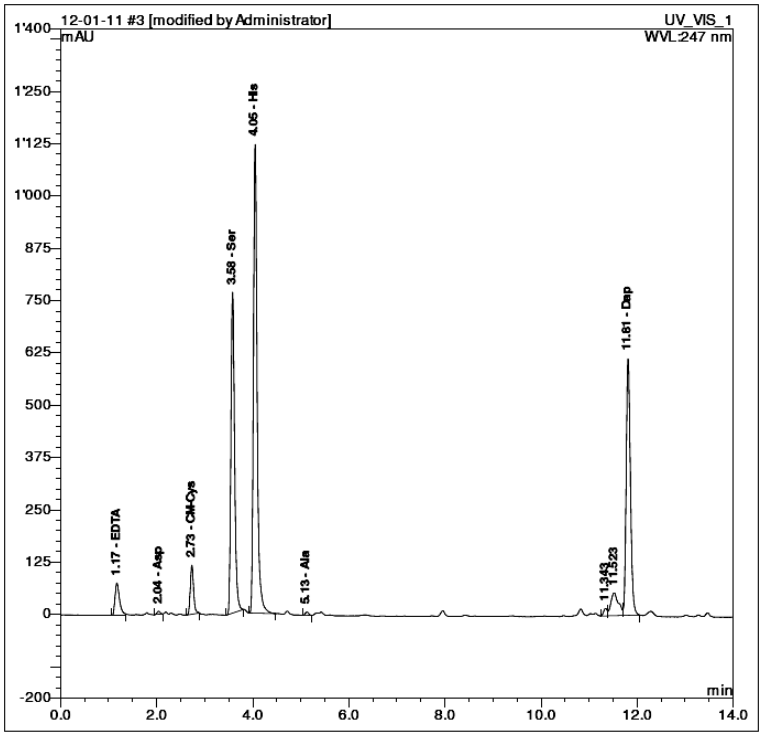
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

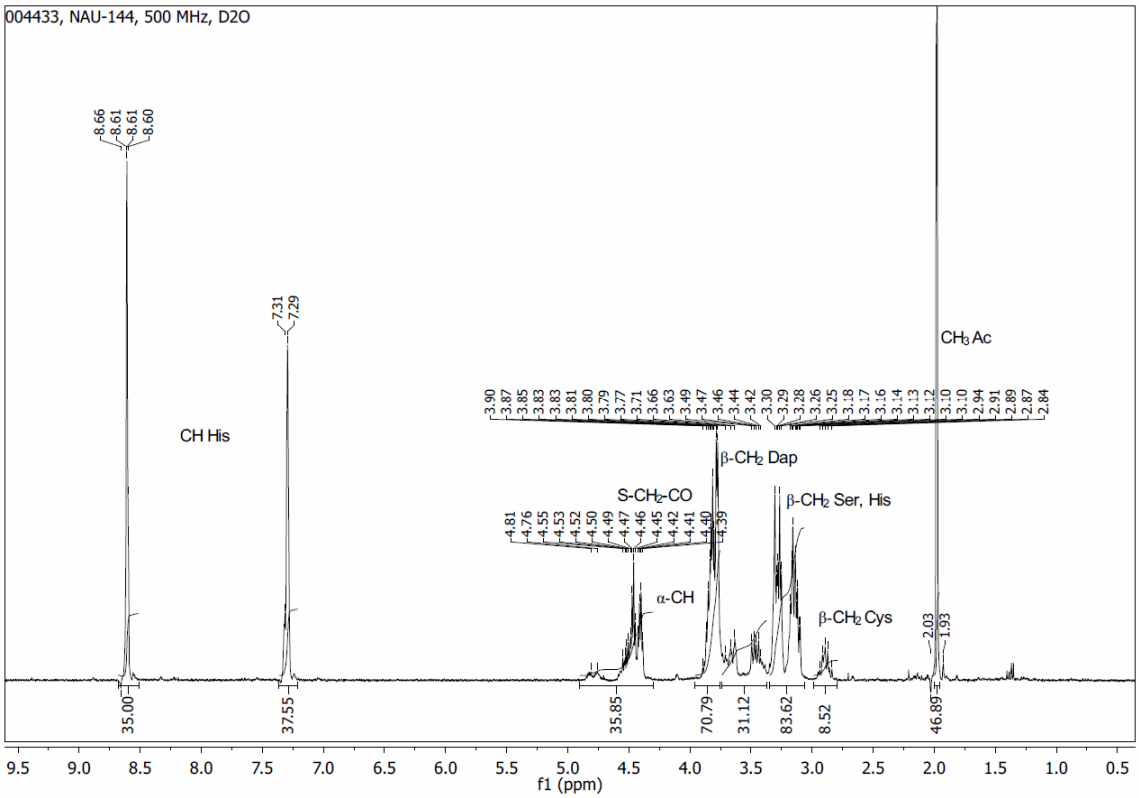


Amino Acid Analysis:

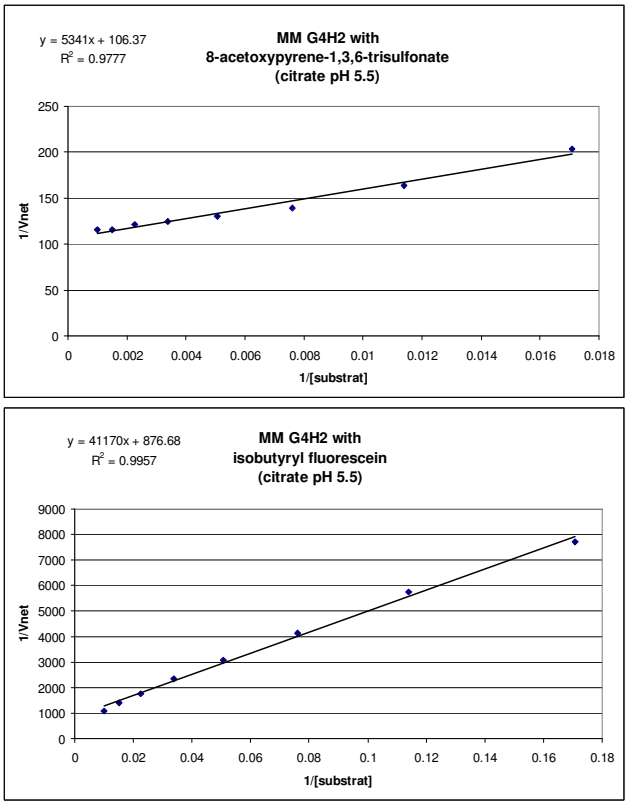


RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,17	1,17	0,097	8,41	77,42		609,7	EDTA
2,04	2,07	0,073	0,48	6,93		36,9	Asp
2,73	2,73	0,072	9,21	117,09		658,6	CM-Cys
3,58	3,59	0,080	67,61	767,23		4923,1	Ser
4,05	4,06	0,077	99,37	1120,17		7481,6	His
5,13	5,15	0,079	0,63	7,69		49,0	Ala
11,81	11,77	0,093	62,49	613,08		2254,8	Dap
Total:						16013,72	

^{1}H-NMR:



Representative Michaelis-Menten plots for determination of k_{cat} and K_M:

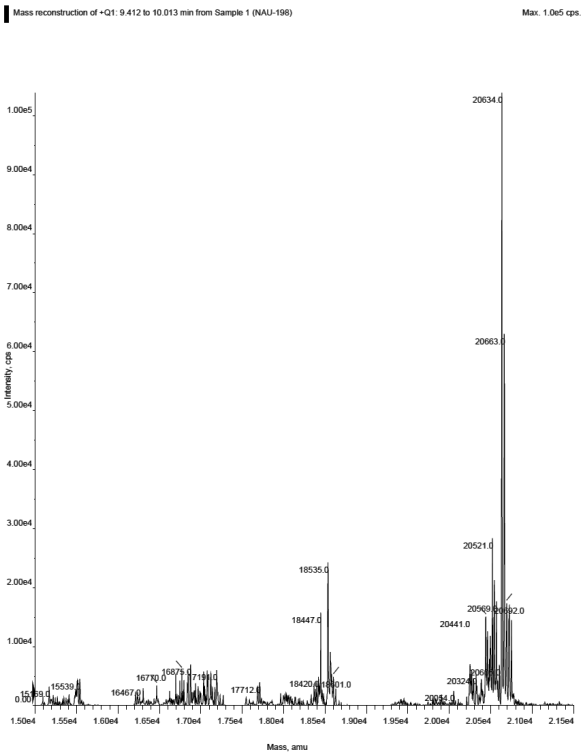


Peptide Dendrimer G5H

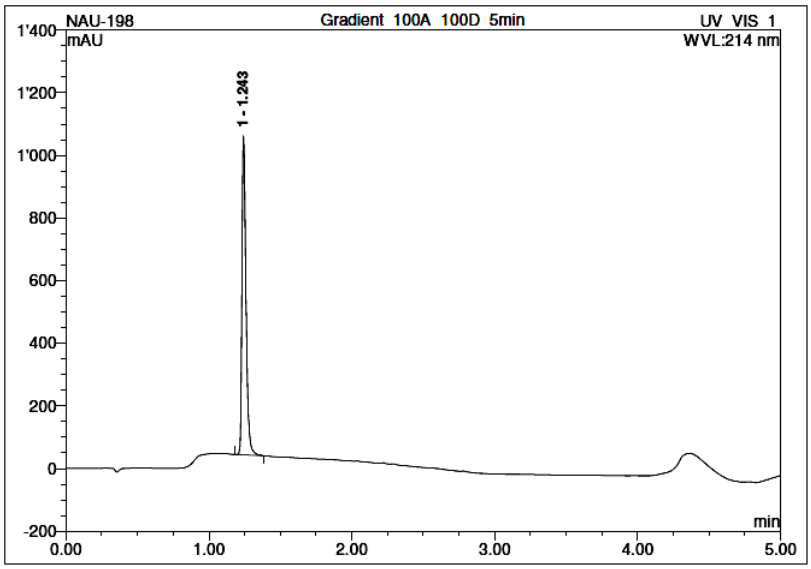
(Ac-His-Ser)$_{32}$(*Dap*-His-Ser)$_{16}$(*Dap*-His-Ser-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G2M2** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G5H** was obtained as foamy colourless solid after preparative RP-HPLC (13.7 mg). Amino Acid Analysis: protein content 64.1% (8.75 mg, 0.38 µmol, yield 50%); found/calc. (%) His 37.2/30.9, Ser 31.8/39.2, Dap 15.6/17.1, Pro 10.9/8.3, CM-Cys 4.5/4.4. Analytical RP-UPLC: t_R = 1.243 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (LC-MS+): $C_{821}H_{1169}N_{333}O_{292}S_8$ (8-fold ligation product, main peak) found/calc. 20634.0/20633.6 $[M+2H]^+$. $C_{738}H_{1053}N_{297}O_{264}S_7$ (7-fold ligation product with one hydroxyacetyl group, peak height 23% of main peak) found/calc. 18535.0/18534.5 $[M+H]^+$, no 6-fold ligation product observed (calc. 16655.3 $[M]^+$). $D = 8.07 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 2.51 ± 0.07 nm.

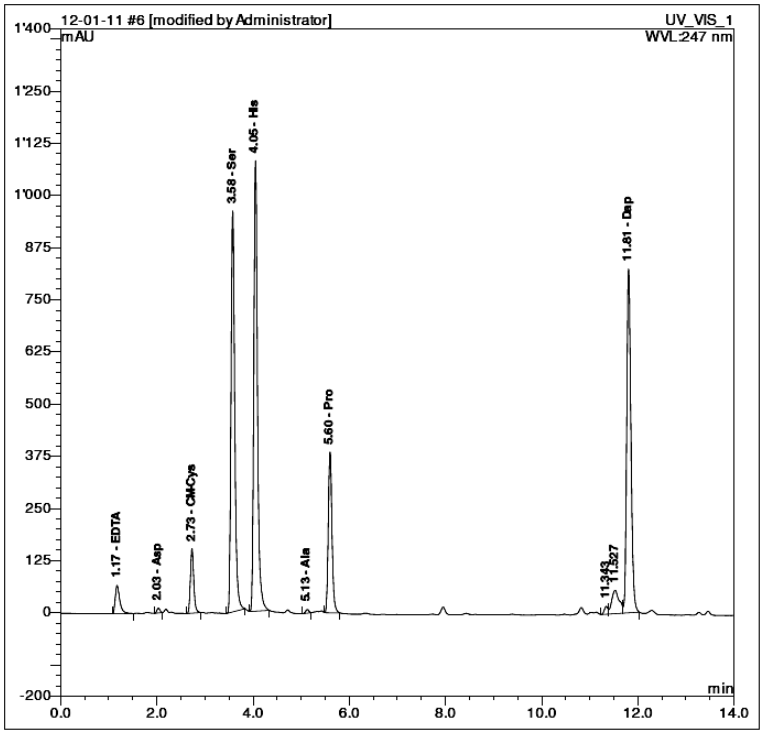
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

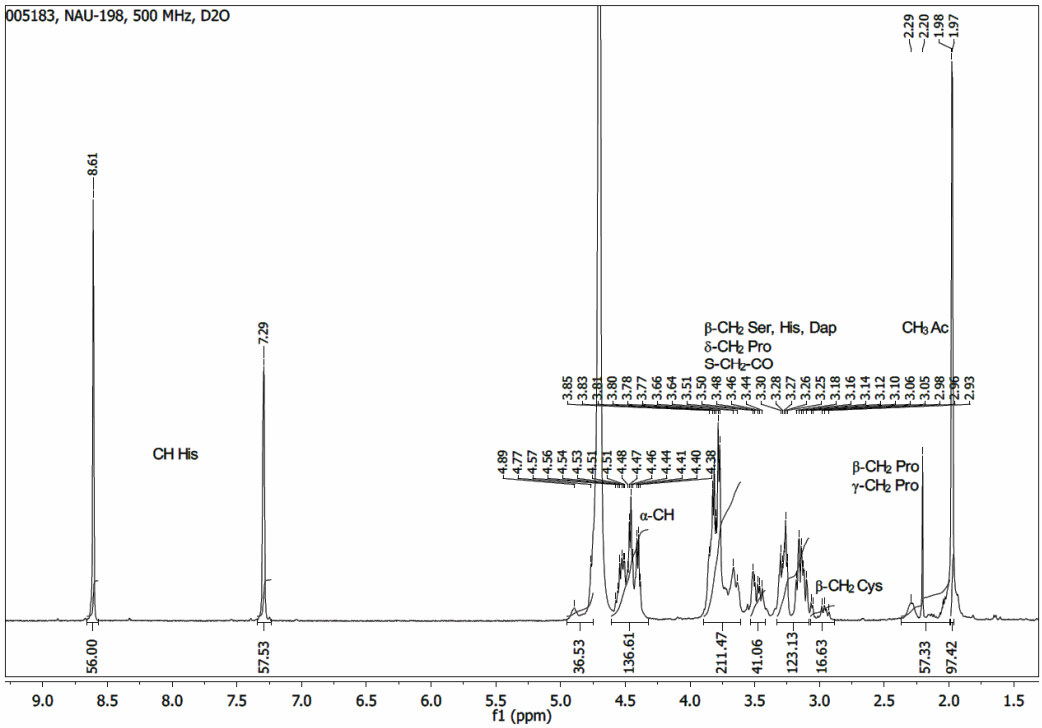


Amino Acid Analysis:

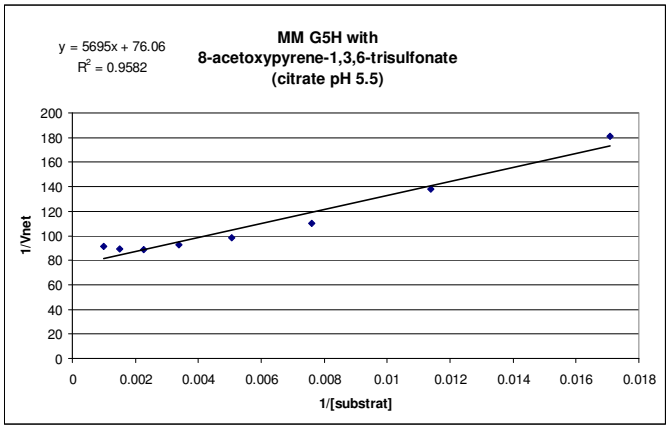


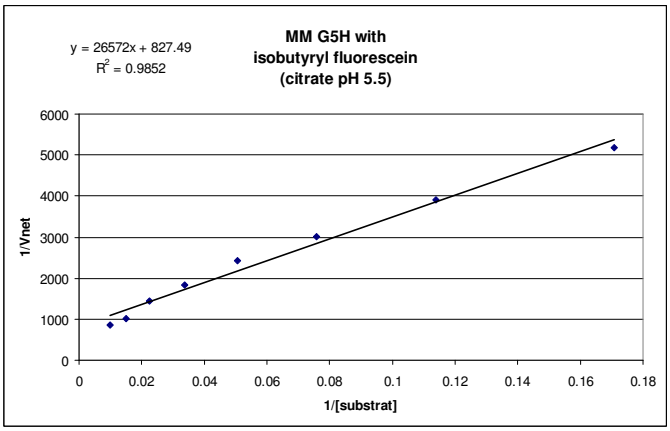
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,17	1,17	0,095	7,30	67,68		533,1	EDTA
2,03	2,07	0,070	0,82	11,25		59,9	Asp
2,73	2,73	0,073	12,46	154,82		870,9	CM-Cys
3,58	3,59	0,081	86,15	961,32		6168,4	Ser
4,05	4,06	0,078	95,58	1079,17		7207,8	His
5,13	5,15	0,073	0,65	8,58		54,7	Ala
5,60	5,64	0,082	34,41	385,45		2109,4	Pro
11,81	11,77	0,092	83,53	824,23		3031,3	Dap
Total:						20035,41	

^{1}H-NMR:



Representative Michaelis-Menten plots for determination of k_{cat} and K_M:



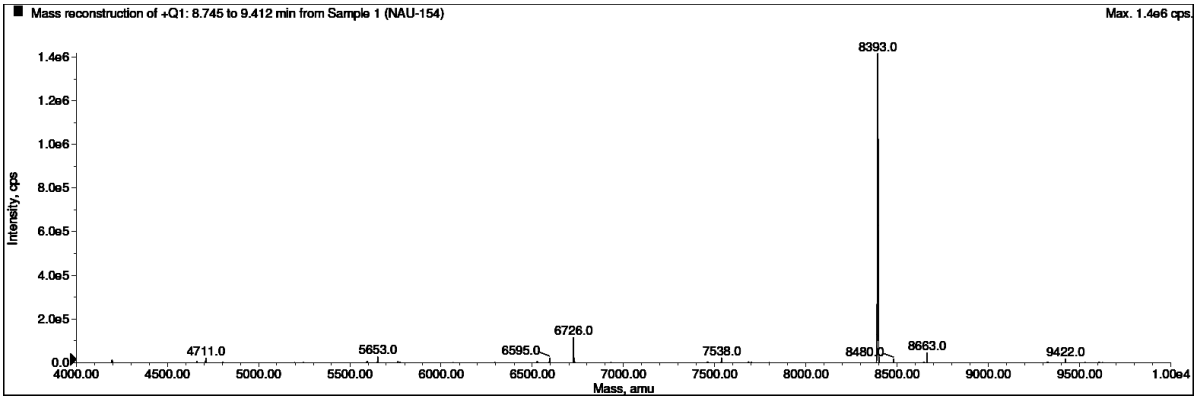


Peptide Dendrimer G4P

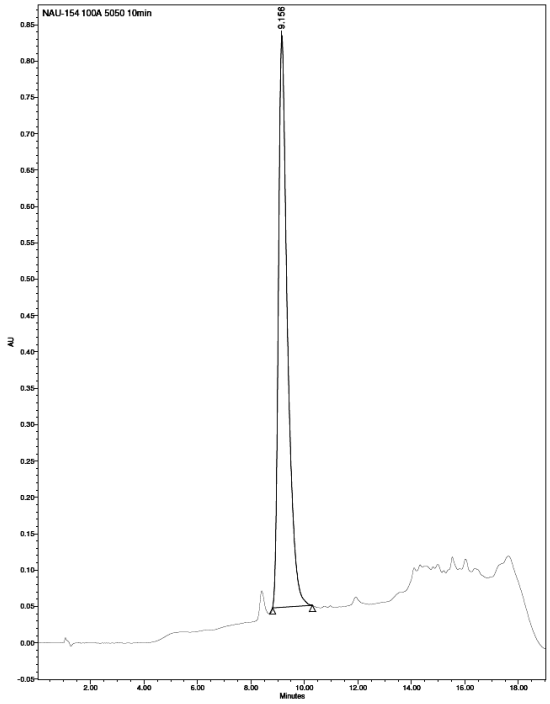
(Pro-Ser)$_{16}$(*Dap*-Pro-Ser)$_8$(*Dap*-Pro-Ser-Cys-x-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G2C2** and **G2M3** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G4P** was obtained as foamy colourless solid after preparative RP-HPLC (2.05 mg). Amino Acid Analysis: protein content 65.8% (1.35 mg, 0.15 µmol, yield 55%); found/calc. (%) Pro 44.0/39.3, Ser 33.8/39.3, Dap 16.9/16.9, CM-Cys 5.4/4.5. Analytical RP-HPLC: t_R = 9.156 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (LC-MS+): $C_{345}H_{545}N_{109}O_{128}S_4$ (4-fold ligation product) found/calc. 8393.0/8395.9 $[M]^+$. No incomplete ligation products observed (3-fold ligation product calc. 6856.0 $[M]^+$, 2-fold ligation product calc. 5316.2 $[M]^+$). D = $9.72 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 2.09 ± 0.07 nm.

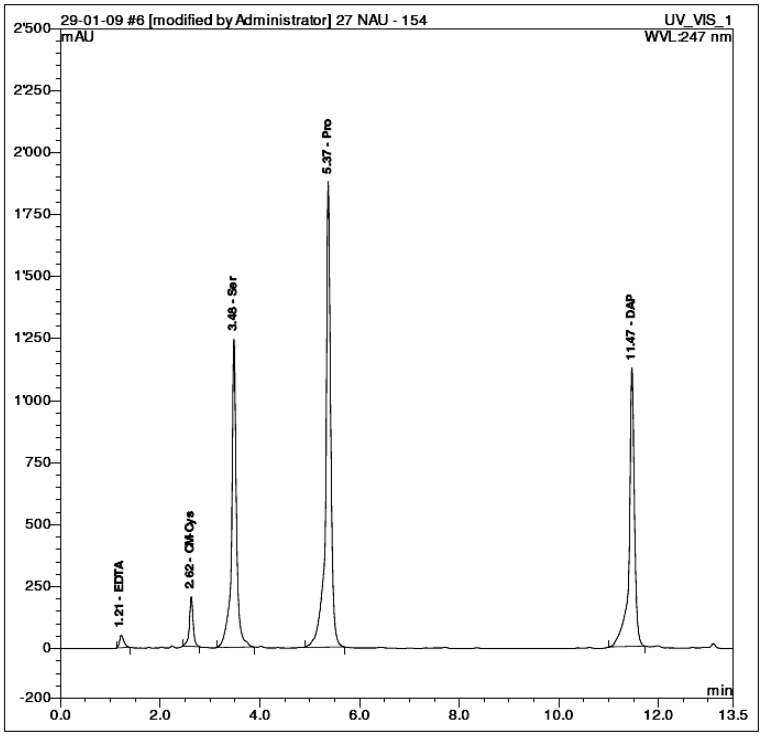
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

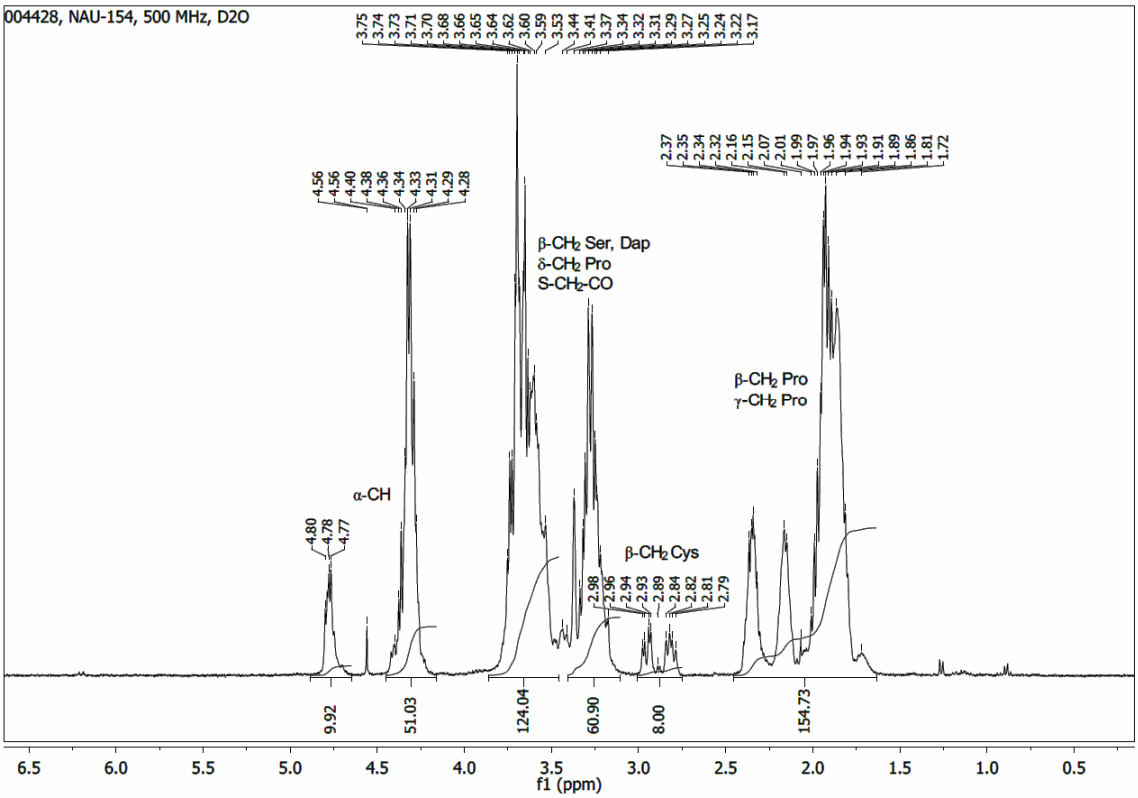


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,21	1,21	0,091	5,13	50,45		364,7	EDTA
2,62	2,63	0,071	17,44	201,02		1376,2	CM-Cys
3,48	3,50	0,082	139,73	1243,76		8666,3	Ser
5,37	5,41	0,087	220,32	1878,58		11291,6	Pro
11,47	11,49	0,093	140,81	1124,07		4329,8	DAP
Total:			523,414	4497,873		26028,54	

^{1}H-NMR:

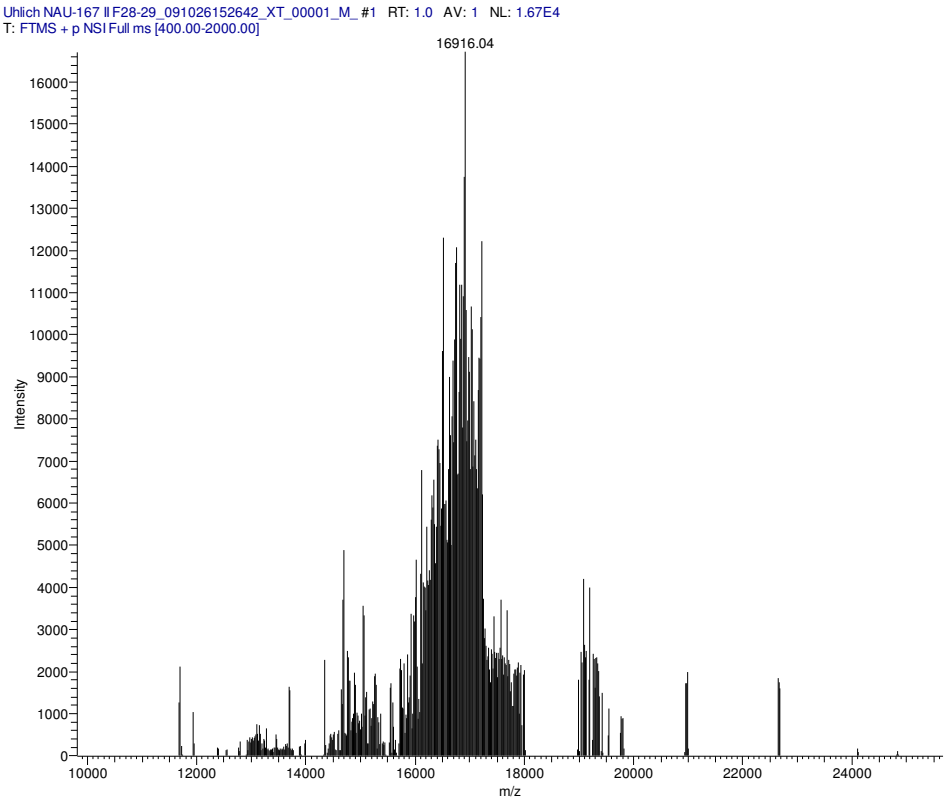


Peptide Dendrimer G5P1

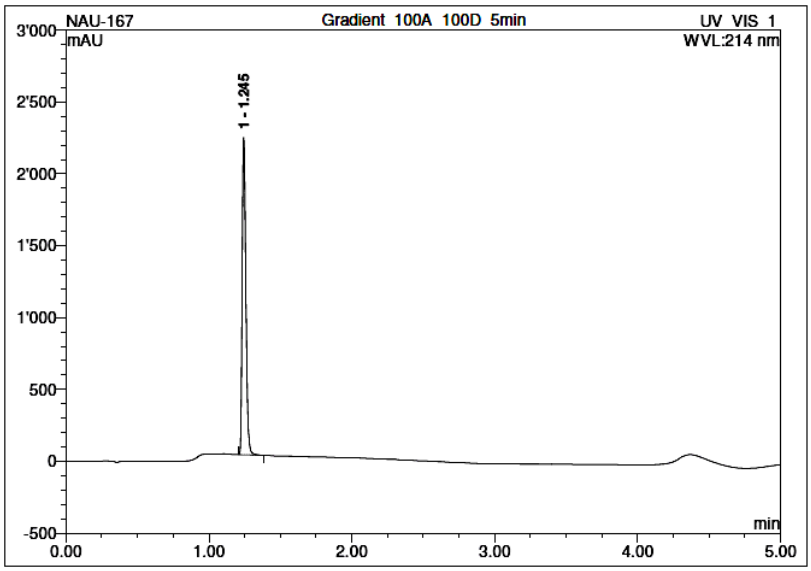
(Pro-Asp)$_{32}$(*Dap*-Pro-Asp)$_{16}$(*Dap*-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G2M5** using the general procedure described above (solvent: 0.1 M phosphate buffer pH 8.5, 20 eq KI), **G5P1** was obtained as foamy colourless solid after preparative RP-HPLC (10.3 mg). Amino Acid Analysis: protein content 76.5% (7.86 mg, 0.43 µmol, yield 57%); found/calc. (%) Pro 39.4/38.2, Asp 30.7/29.1, Dap 19.0/18.8, Ser 6.3/9.1, CM-Cys 4.7/4.8. Analytical RP-UPLC: t_R = 1.245 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{685}H_{1009}N_{205}O_{284}S_8$ (8-fold ligation product) found/calc. 16916.0/16916.1 $[M]^+$. No incomplete ligation products observed (7-fold ligation product calc. 15392.3 $[M]^+$, 6-fold ligation product calc. 13868.6 $[M]^+$). D = $7.36 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 2.76 ± 0.05 nm.

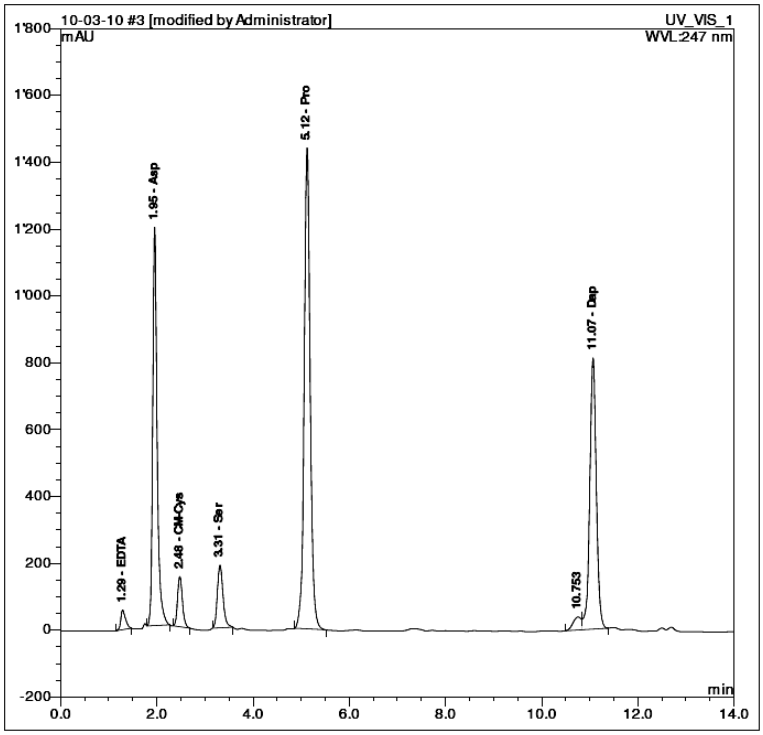
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

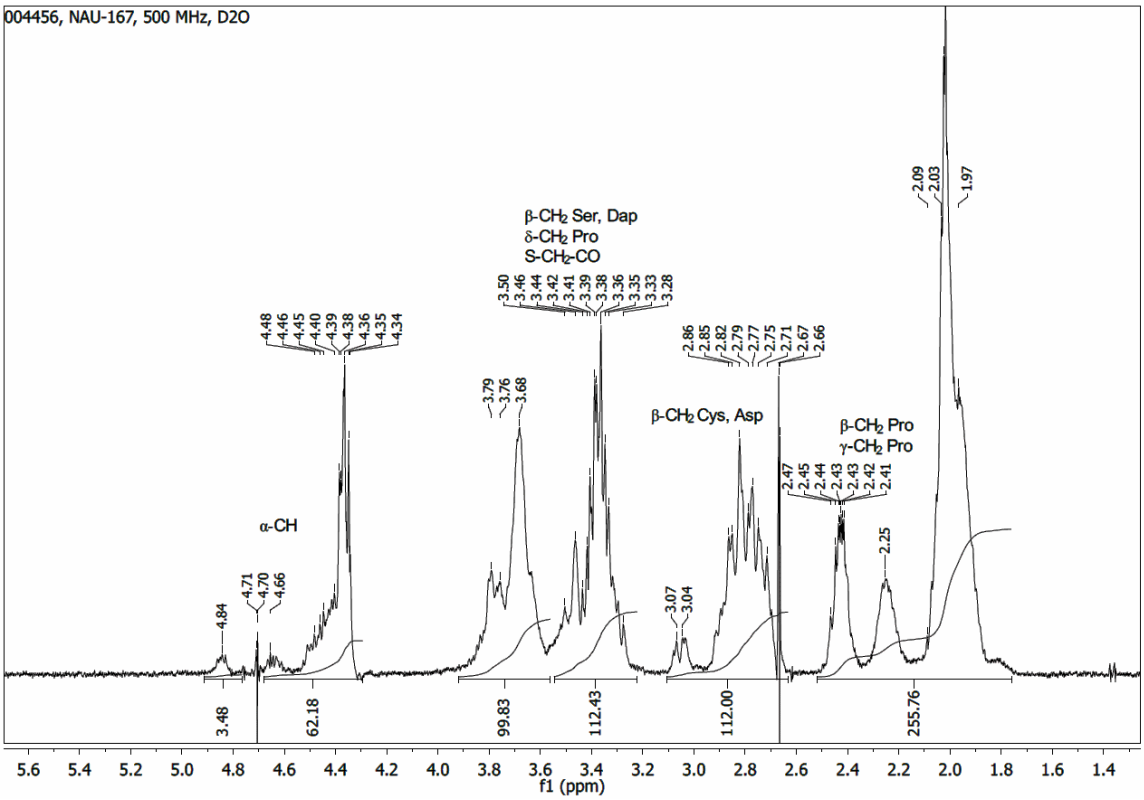


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,29	1,25	0,105	6,73	58,72		216,5	EDTA
1,95	1,95	0,090	125,82	1191,46		8419,4	Asp
2,48	2,48	0,108	17,67	150,15		1287,3	CM-Cys
3,31	3,31	0,124	25,58	187,14		1732,4	Ser
5,12	5,15	0,128	207,01	1438,38		10804,3	Pro
11,07	11,07	0,143	129,03	811,67		5208,7	Dap
Total:			511,837	3837,522		27668,43	

^{1}H-NMR:

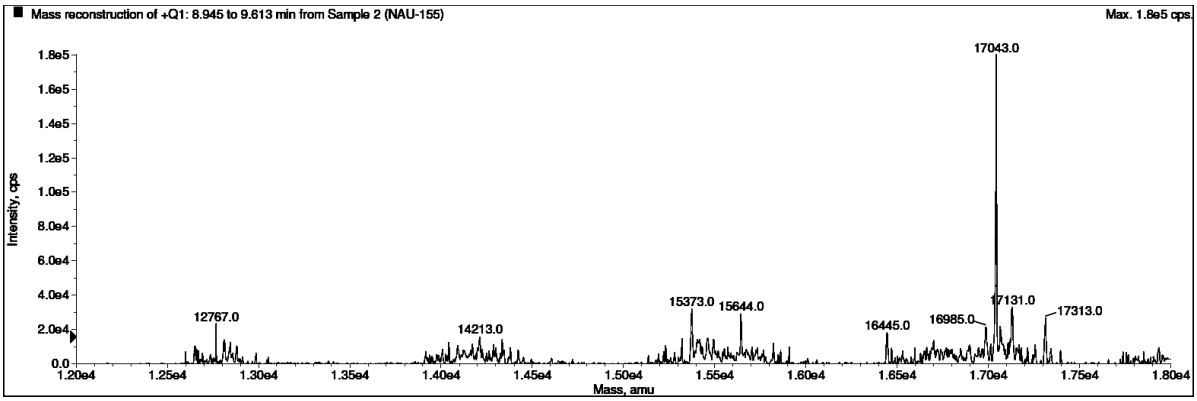

004456, NAU-167, 500 MHz, D2O
β-CH2 Ser, Dap
δ-CH2 Pro
S-CH2-CO
α-CH
β-CH2 Cys, Asp
β-CH2 Pro
γ-CH2 Pro
f1 (ppm)

Peptide Dendrimer G5P2

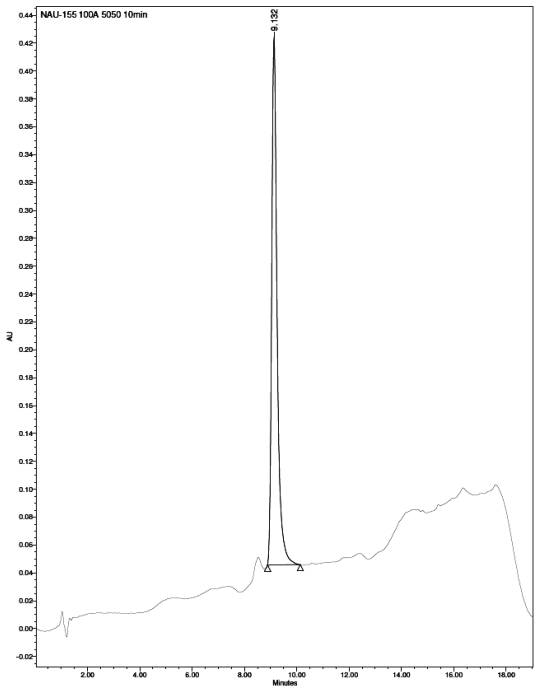
(Pro-Ser)$_{32}$(*Dap*-Pro-Ser)$_{16}$(*Dap*-Pro-Ser-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G2M3** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G5P2** was obtained as foamy colourless solid after preparative RP-HPLC (1.48 mg). Amino Acid Analysis: protein content 62.4% (0.92 mg, 0.05 µmol, yield 40%); found/calc. (%) Pro 44.1/39.2, Ser 33.3/39.2, Dap 17.2/17.1, CM-Cys 5.4/4.4. Analytical RP-HPLC: t_R = 9.132 min (A/D 100/0 to 50/50 in 10 min, λ = 214 nm). MS (ESI+): $C_{701}H_{1105}N_{221}O_{260}S_8$ (8-fold ligation product) found/calc. 17043.0/17045.1 $[M]^+$. No incomplete ligation products observed (7-fold ligation product calc. 15505.2 $[M]^+$, 6-fold ligation product calc. 13965.4 $[M]^+$). D = $5.93 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 3.42 ± 0.04 nm.

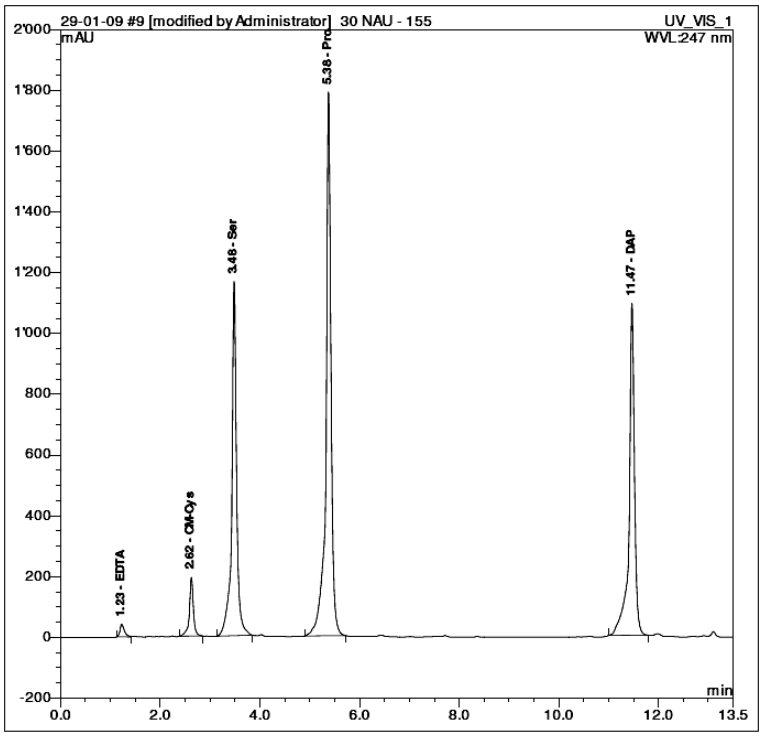
Mass spectrum, MS (ESI+):



Analytical RP-HPLC chromatogram:

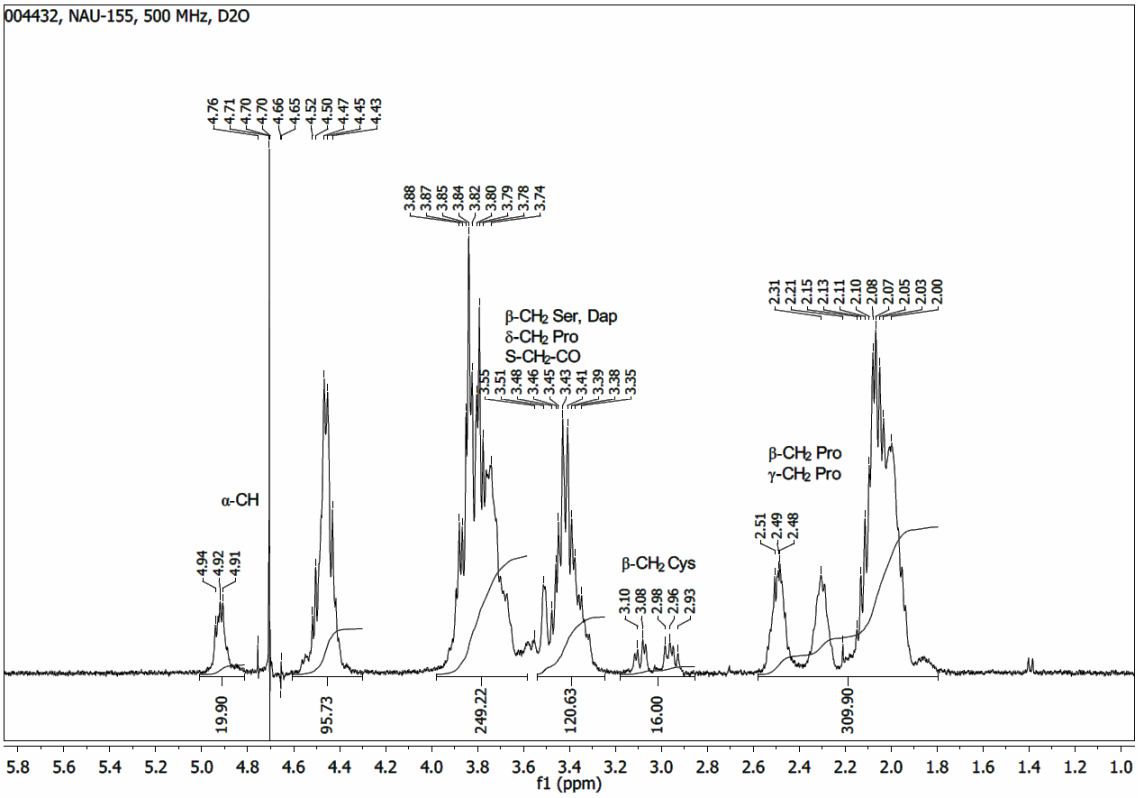


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,23	1,21	0,090	4,41	41,71		301,5	EDTA
2,62	2,63	0,073	17,97	192,86		1320,3	CM-Cys
3,48	3,50	0,082	129,94	1165,48		8120,9	Ser
5,38	5,41	0,087	210,46	1789,89		10758,5	Pro
11,47	11,49	0,094	138,43	1091,98		4206,2	DAP
Total:			501,211	4281,917		24707,35	

^{1}H-NMR:

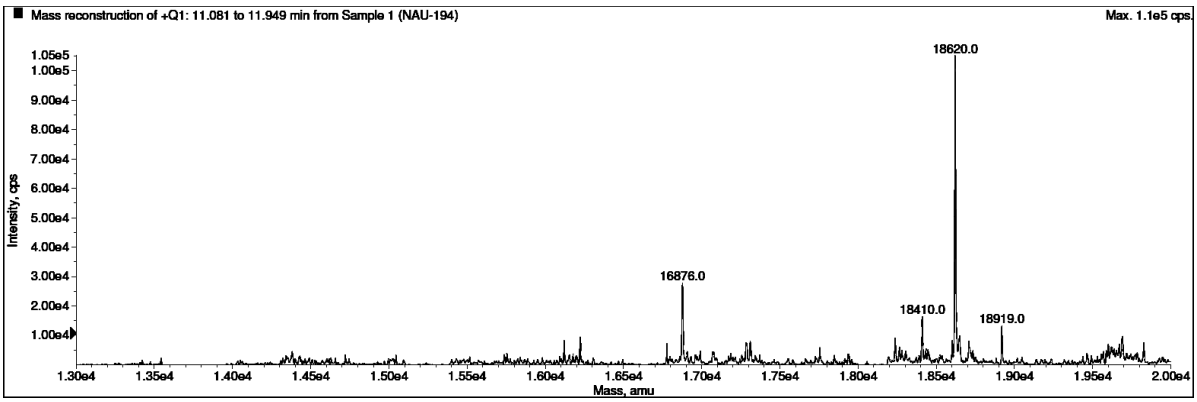

004432, NAU-155, 500 MHz, D2O
α-CH
β-CH2 Ser, Dap
δ-CH2 Pro
S-CH2-CO
β-CH2 Cys
β-CH2 Pro
γ-CH2 Pro
19.90
95.73
249.22
120.63
16.00
309.90
f1 (ppm)

Peptide Dendrimer G5P3

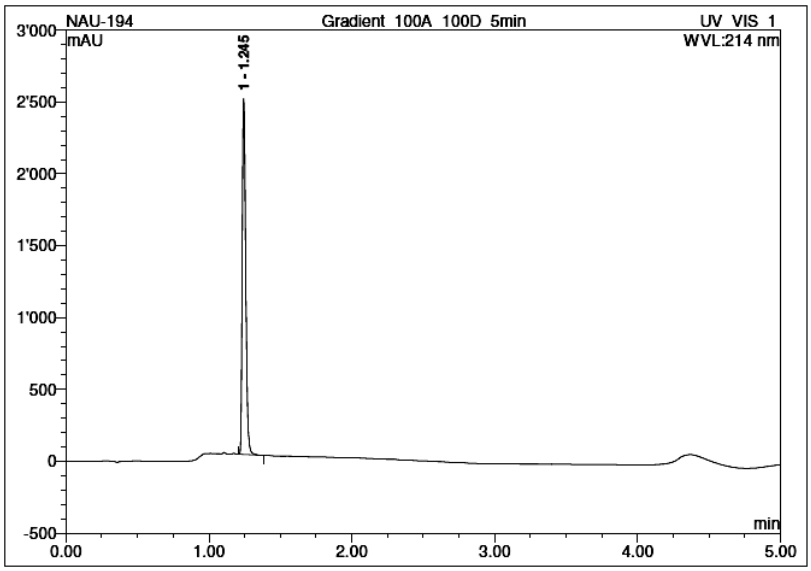
(Pro-Asp)$_{32}$(*Dap*-Pro-Asp)$_{16}$(*Dap*-Pro-Asp-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G2M4** using the general procedure described above (solvent: 0.5 M $NaHCO_3$ buffer pH 8.0, 20 eq KI), **G5P3** was obtained as foamy colourless solid after preparative RP-HPLC (14.7 mg). Amino Acid Analysis: protein content 63.6% (9.33 mg, 0.46 µmol, yield 62%); found/calc. (%) Pro 40.4/39.2, Asp 31.9/30.9, Dap 17.1/17.1, Ser 6.6/8.3, CM-Cys 4.1/4.4. Analytical RP-UPLC: t_R = 1.245 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (LC-MS+): $C_{757}H_{1105}N_{221}O_{316}S_8$ (8-fold ligation product, main peak) found/calc. 18620.0/18619.7 $[M+6H]^+$. $C_{682}H_{996}IN_{199}O_{284}S_7$ (7-fold ligation product with one unreacted iodoactyl group, peak height 26% of main peak) found/calc. 16876.0/16877.7 $[M]^+$, no 6-fold ligation product observed (calc. 15141.9). D = $6.50 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 3.12 ± 0.04 nm.

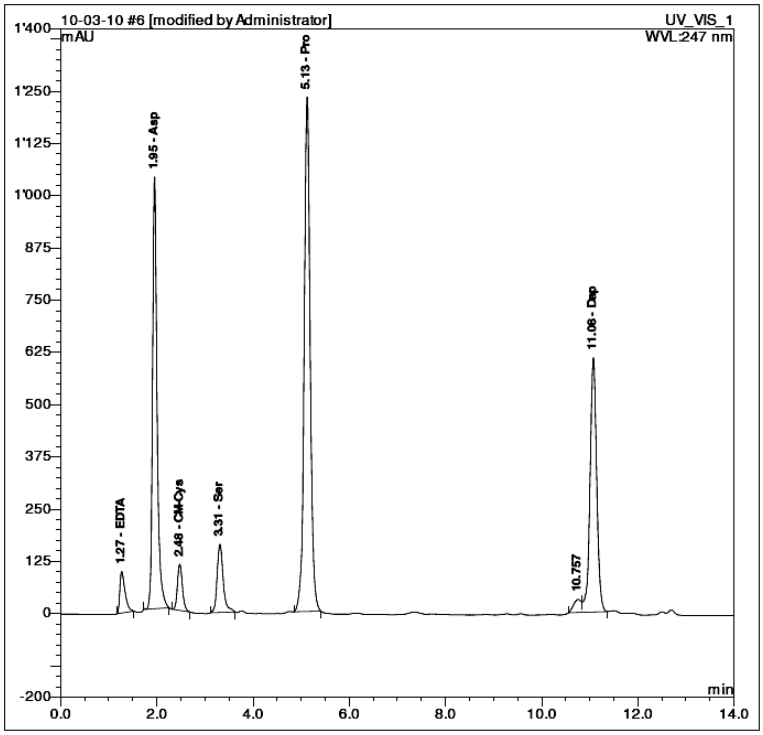
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

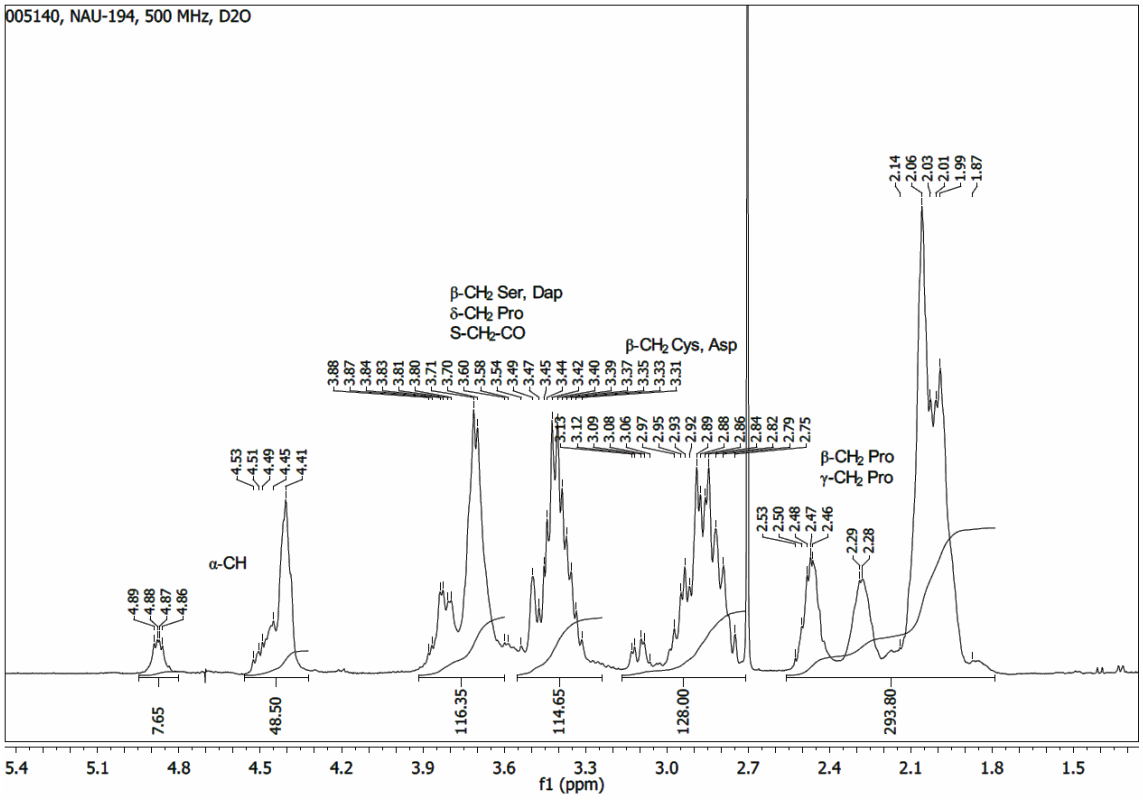


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,27	1,25	0,113	12,40	99,21		365,7	EDTA
1,95	1,95	0,092	111,47	1033,47		7302,9	Asp
2,48	2,48	0,110	13,20	110,19		944,7	CM-Cys
3,31	3,31	0,127	23,89	163,31		1511,8	Ser
5,13	5,15	0,129	177,38	1231,46		9250,0	Pro
11,08	11,07	0,144	97,82	608,95		3907,8	Dap
Total:			436,168	3246,587		23282,87	

^{1}H-NMR:

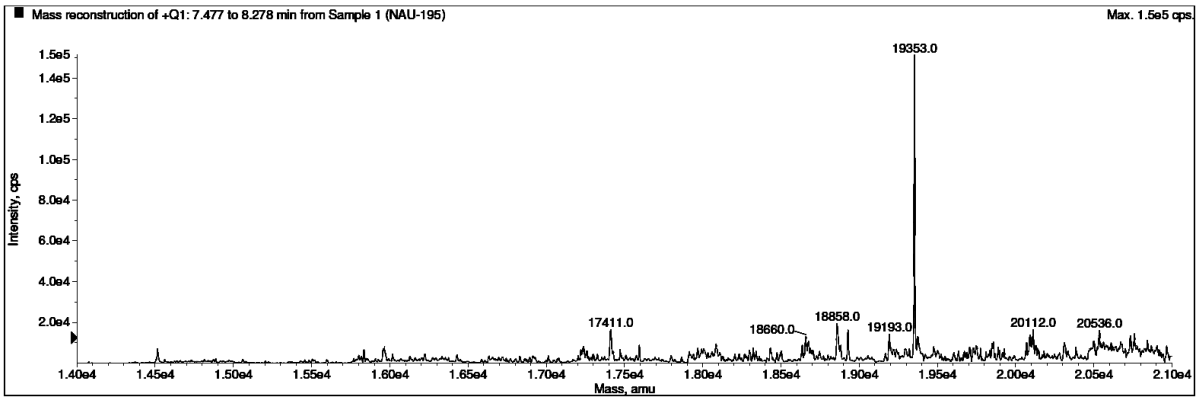

005140, NAU-194, 500 MHz, D2O
β-CH2 Ser, Dap
δ-CH2 Pro
S-CH2-CO
β-CH2 Cys, Asp
β-CH2 Pro
γ-CH2 Pro
α-CH
7.65
48.50
116.35
114.65
128.00
293.80
f1 (ppm)

Peptide Dendrimer G5P4

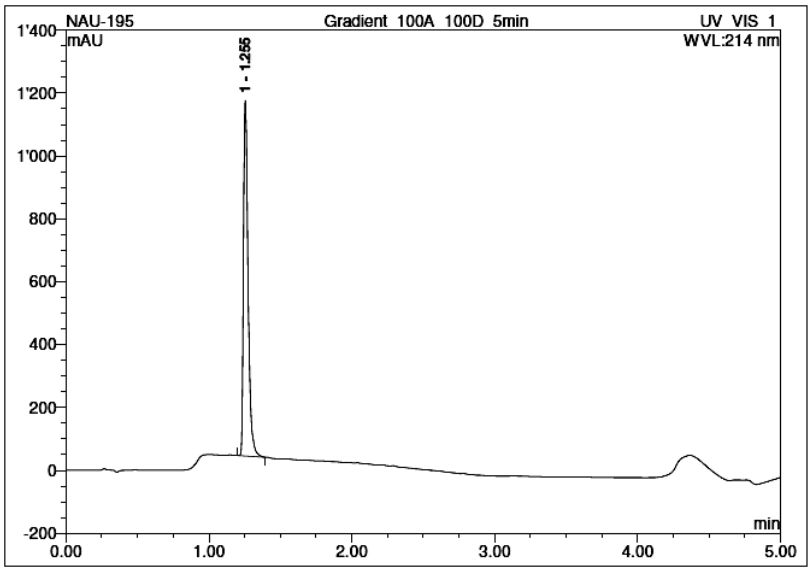
(Pro-Lys)$_{32}$(*Dap*-Pro-Lys)$_{16}$(*Dap*-Pro-Lys-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G2M6** using the general procedure described above (solvent: 0.5 M $NaHCO_3$ buffer pH 8.0, 20 eq KI), **G5P4** was obtained as crystalline colourless solid after preparative RP-HPLC (16.4 mg). Amino Acid Analysis: protein content 82.3% (13.5 mg, 0.58 µmol, yield 77%); found/calc. (%) Pro 40.8/39.2, Lys 31.5/30.9, Dap 17.8/17.1, Ser 6.4/8.3, CM-Cys 3.5/4.4. Analytical RP-UPLC: t_R = 1.255 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (LC-MS+): $C_{869}H_{1497}N_{277}O_{204}S_8$ (8-fold ligation product, main peak) found/calc. 19353.0/19352.4 $[M+6H]^+$. $C_{780}H_{1340}N_{248}O_{187}S_7$ (7-fold ligation product with one hydroxyacetyl group, peak height 10% of main peak) found/calc. 17411.0/17411.0 $[M+2H]^+$, no 6-fold ligation product observed (calc. 15471.5 $[M]^+$). $D = 4.27 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 4.74 ± 0.15 nm.

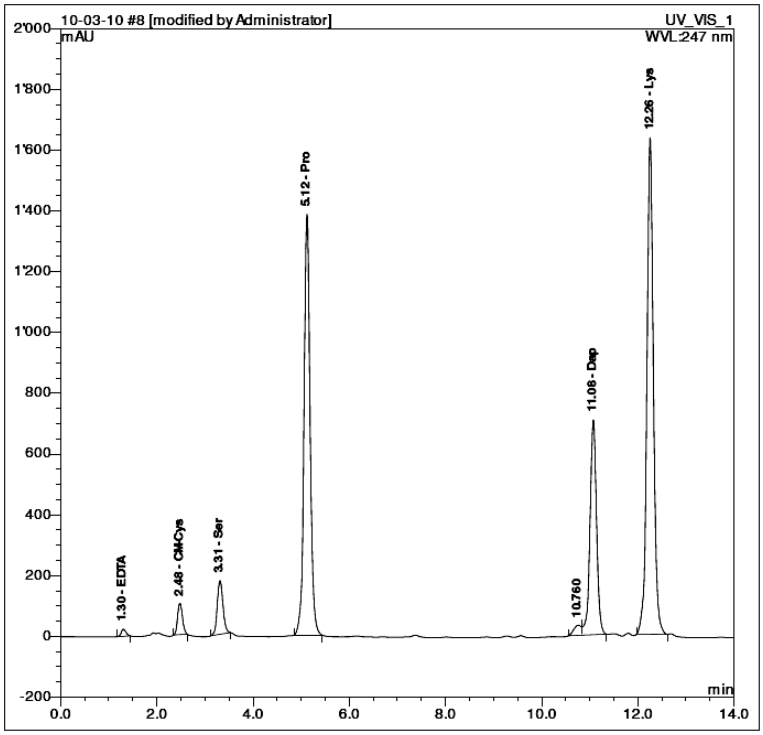
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

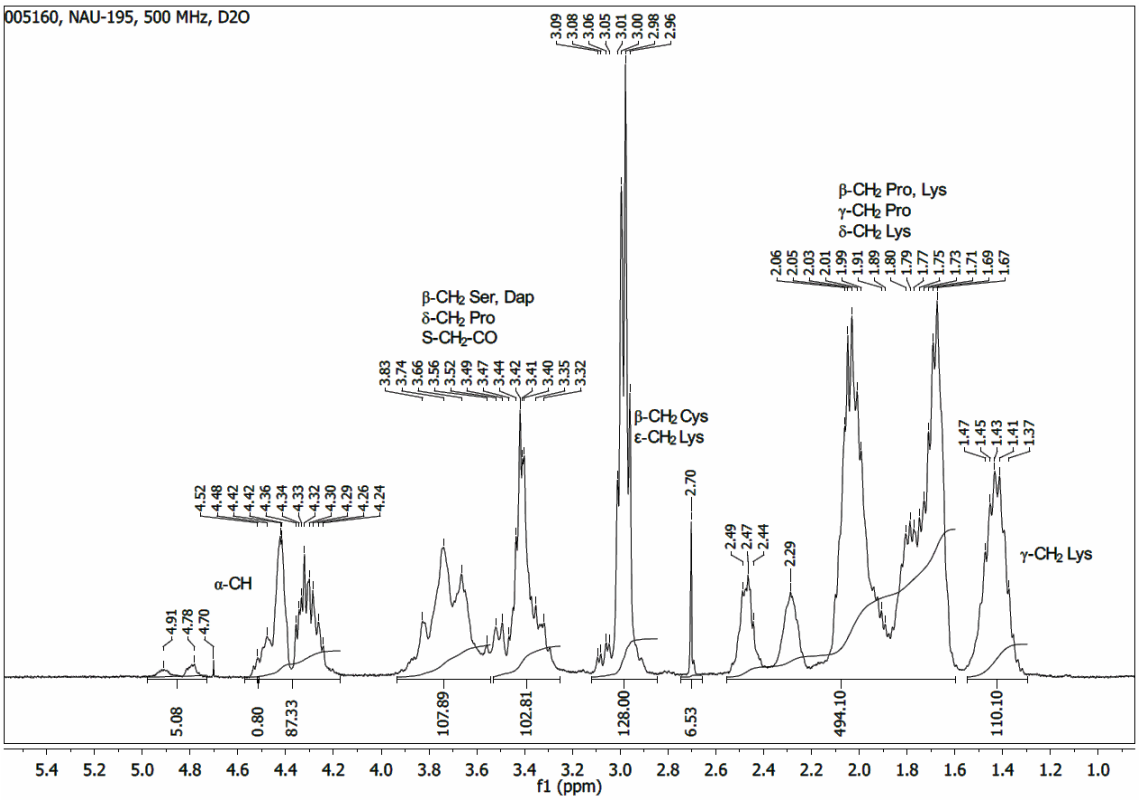


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,30	1,25	0,105	2,65	23,48		86,5	EDTA
2,48	2,48	0,111	12,09	102,85		881,8	CM-Cys
3,31	3,31	0,126	24,30	176,90		1637,5	Ser
5,12	5,15	0,131	201,70	1384,30		10398,0	Pro
11,08	11,07	0,142	111,38	707,13		4537,9	Dap
12,26	12,29	0,140	255,27	1634,17		8016,5	Lys
Total:			607,403	4028,835		25558,22	

^{1}H-NMR:

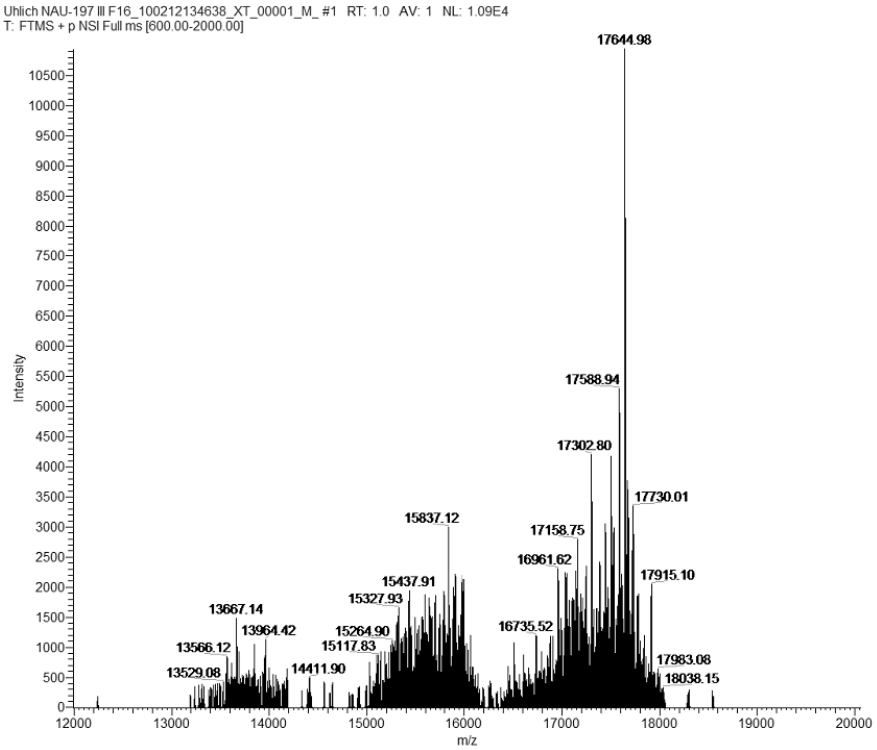

005160, NAU-195, 500 MHz, D2O
β-CH2 Pro, Lys
γ-CH2 Pro
δ-CH2 Lys
β-CH2 Ser, Dap
δ-CH2 Pro
S-CH2-CO
β-CH2 Cys
ε-CH2 Lys
α-CH
γ-CH2 Lys
f1 (ppm)

Peptide Dendrimer G5P5

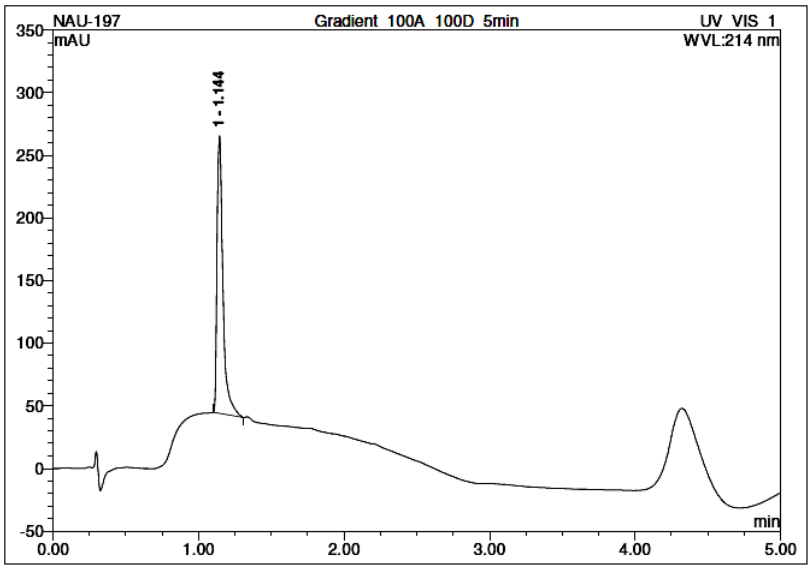
(Pro-Ser)$_{32}$(*Dap*-Pro-Ser)$_{16}$(*Dap*-Pro-Ser-Cys-x-His-Ser)$_8$(*Dap*-His-Ser)$_4$(*Dap*-His-Ser)$_2$*Dap*-His-Ser-NH$_2$

From starting materials **G3C1** and **G2M3** using the general procedure described above (solvent: 0.5 M $NaHCO_3$ buffer pH 8.0/CH_3CN (2/1 v/v), 20 eq KI), **G5P5** was obtained as foamy colourless solid after preparative RP-HPLC (2.77 mg,). Amino Acid Analysis: protein content 52.2% (1.45 mg, 0.07 µmol, yield 11%); found/calc. (%) Pro 37.5/30.9, Ser 31.2/39.2, His 10.7/8.3, Dap 16.0/17.1, CM-Cys 4.6/4.4. Analytical RP-UPLC: t_R = 1.144 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (ESI+): $C_{716}H_{1105}N_{251}O_{260}S_8$ (8-fold ligation product, main peak) found/calc. 17645.0/17645.5 $[M]^+$. $C_{648}H_{997}N_{229}O_{236}S_7$ (7-fold ligation product with one hydroxyacetyl group, peak height 19% of main peak) found/calc. 15995.0/15995.7 $[M]^+$, no 6-fold ligation product observed (calc. 14565.8 $[M]^+$). $D = 5.57 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; $R_h = 3.64 \pm 0.10$ nm.

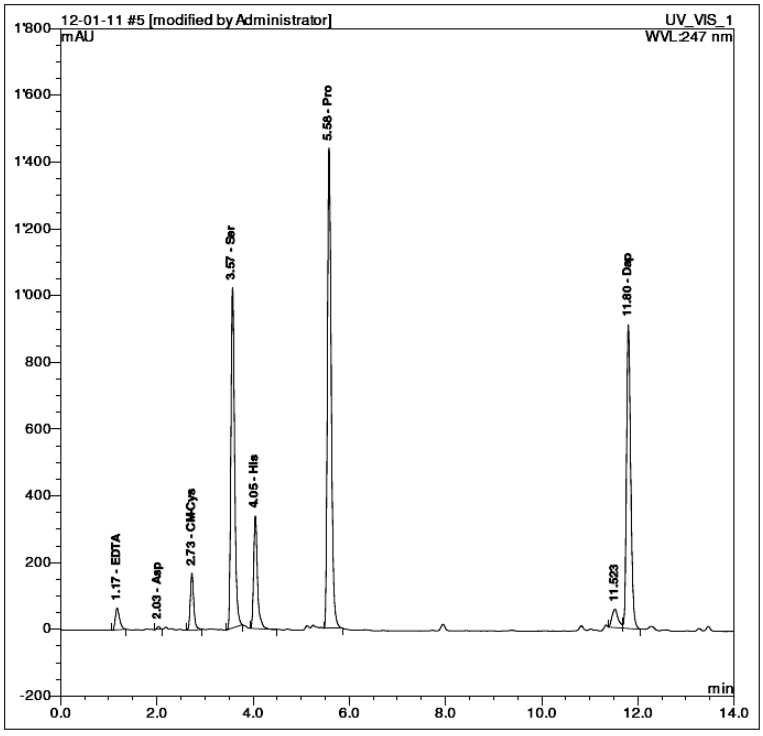
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

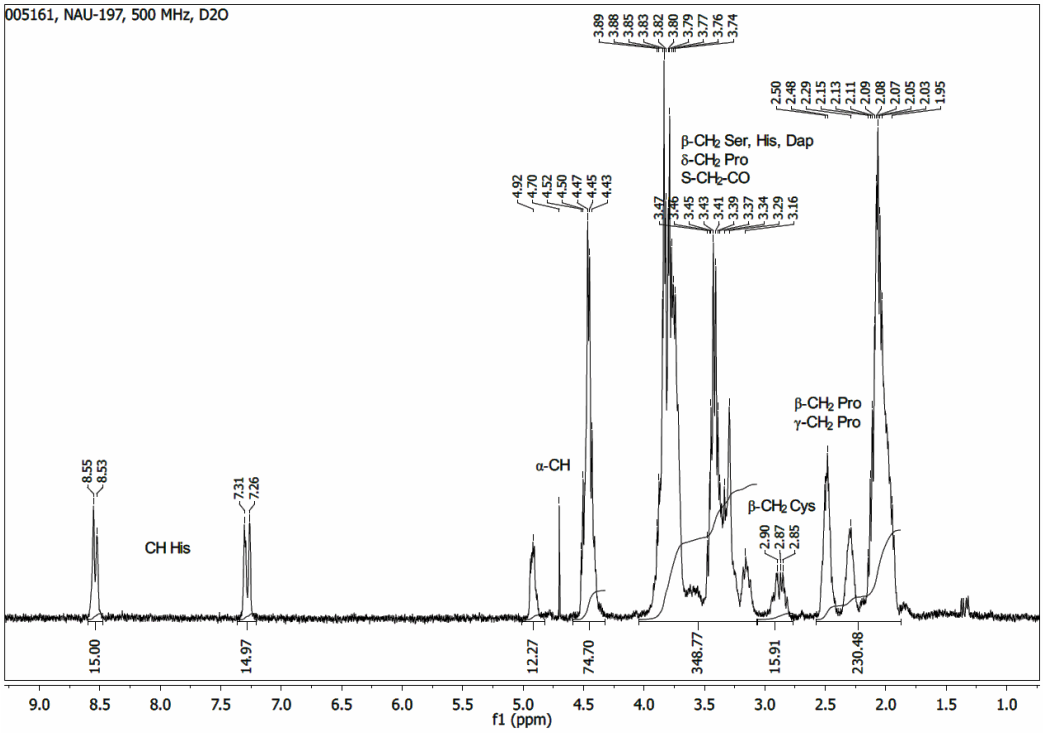


Amino Acid Analysis:

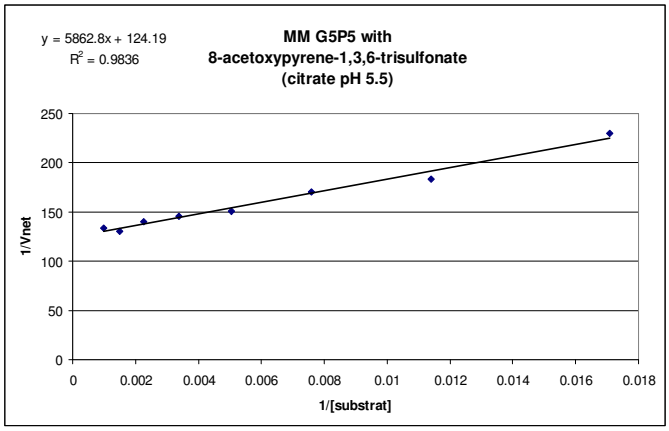


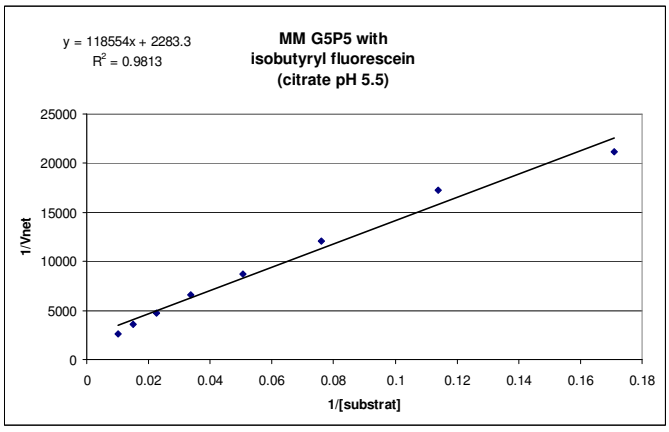
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,17	1,17	0,093	6,71	65,70		517,5	EDTA
2,03	2,07	0,067	0,54	8,18		43,5	Asp
2,73	2,73	0,073	14,00	170,18		957,3	CM-Cys
3,57	3,59	0,080	90,36	1019,94		6544,6	Ser
4,05	4,06	0,079	30,92	337,59		2254,7	His
5,58	5,64	0,084	133,14	1439,55		7878,0	Pro
11,80	11,77	0,092	92,12	910,10		3347,1	Dap
Total:						21542,77	

^{1}H-NMR:



Representative Michaelis-Menten plots for determination of k_{cat} and K_M:



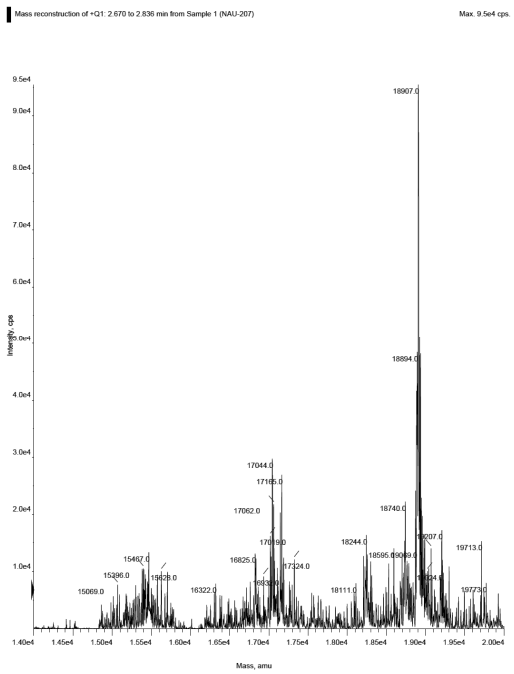


Peptide Dendrimer G5P6

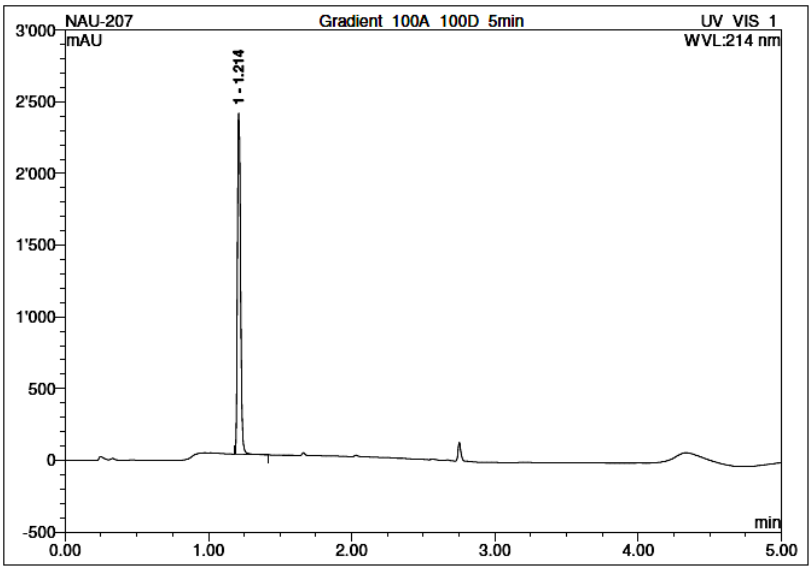
(Pro-Asp)$_{32}$(*Dap*-Pro-Asp)$_{16}$(*Dap*-Pro-Asp-Cys-x-Pro-Ser)$_8$(*Lys*-Pro-Ser)$_4$(*Lys*-Pro-Ser)$_2$*Lys*-Pro-Ser-NH$_2$

From starting materials **G3C3** and **G2M4** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G5P6** was obtained as foamy colourless solid after preparative RP-HPLC (11.8 mg). Amino Acid Analysis: protein content 80.0% (9.41 mg, 0.46 µmol, yield 66%); found/calc. (%) Pro 39.6/39.2, Asp 31.7/30.9, Dap 12.3/13.3, Ser 7.5/8.3, CM-Cys 4.6/4.4, Lys 4.3/3.9 (Dap coeluted with Phe, 3501.1 pmol). Analytical RP-UPLC: t_R = 1.214 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (LC-MS+): $C_{778}H_{1147}N_{221}O_{316}S_8$ (8-fold ligation product, main peak) found/calc. 18907.0/18908.2 $[M]^+$. $C_{703}H_{1038}IN_{199}O_{284}S_7$ (7-fold ligation product with one unreacted iodoacetyl group, peak height 28% of main peak) found/calc. 17165.0/17172.3 $[M]^+$. $C_{628}H_{929}I_2N_{177}O_{252}S_6$ (6-fold ligation product with two unreacted iodoacetyl groups, peak height 11% of main peak) found/calc. 15467.0/15436.3 $[M]^+$. $D = 6.61 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 3.07 ± 0.05 nm.

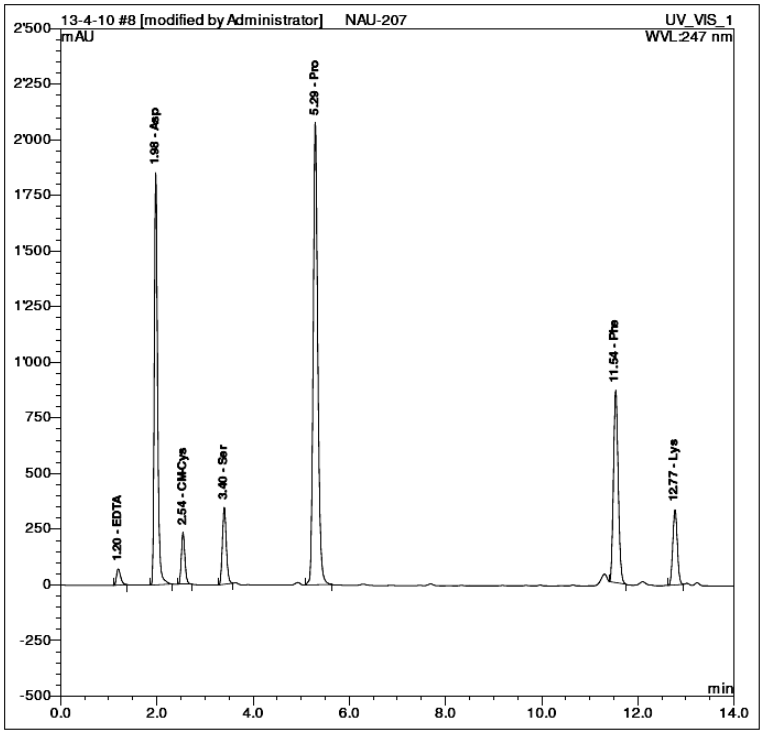
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

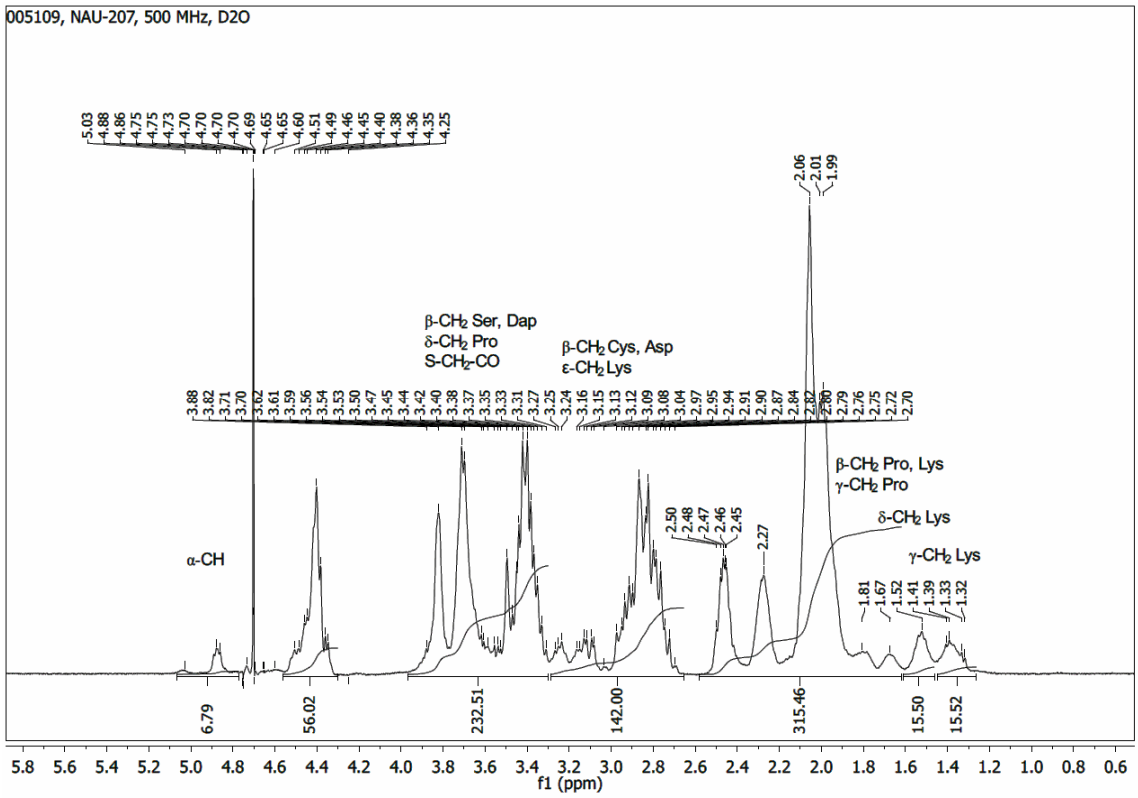


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,20	1,19	0,091	7,17	72,64		448,4	EDTA
1,98	1,97	0,069	144,79	1852,22		9006,1	Asp
2,54	2,54	0,078	20,00	234,09		1297,4	CM-Cys
3,40	3,39	0,087	32,88	345,30		2139,7	Ser
5,29	5,32	0,099	230,18	2078,98		11247,0	Pro
11,54	11,55	0,106	98,99	864,92		5802,3	Phe
12,77	12,75	0,102	36,83	338,42		1222,0	Lys
Total:						31162,91	

^{1}H-NMR:

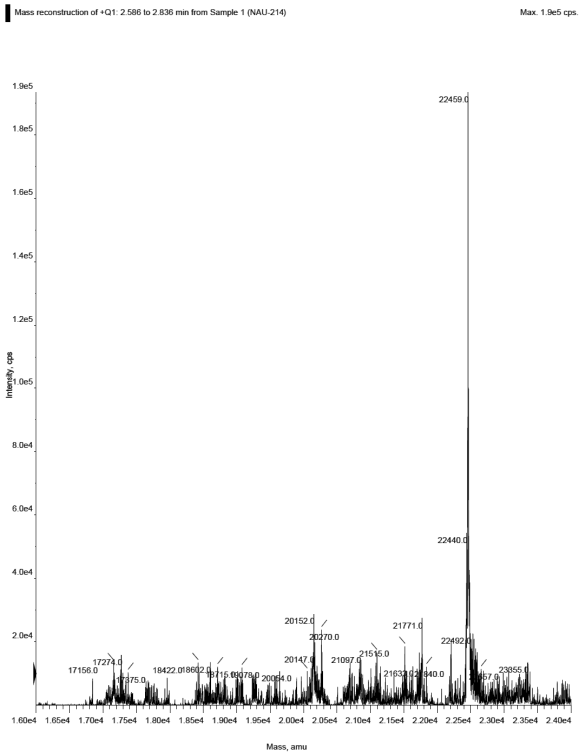

005109, NAU-207, 500 MHz, D2O
β-CH2 Ser, Dap
δ-CH2 Pro
S-CH2-CO
β-CH2 Cys, Asp
ε-CH2 Lys
β-CH2 Pro, Lys
γ-CH2 Pro
δ-CH2 Lys
γ-CH2 Lys
α-CH
6.79
56.02
232.51
142.00
315.46
15.50
15.52
f1 (ppm)

Peptide Dendrimer G5P7

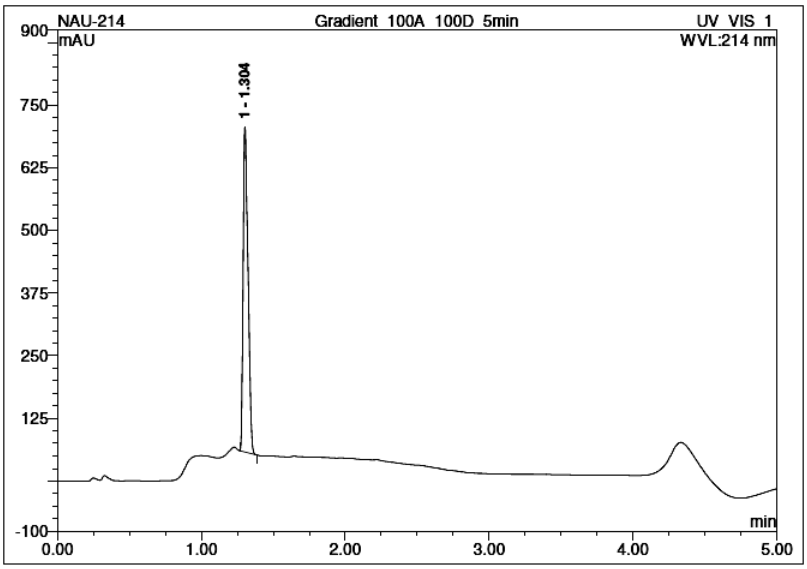
(DPro-Pro-Glu)$_{32}$(*Dap*-Pro-Asp)$_{16}$(*Dap*-Pro-Asp-Cys-x-Pro-Ser)$_8$(*Lys*-Pro-Ser)$_4$(*Lys*-Pro-Ser)$_2$*Lys*-Pro-Ser-NH$_2$

From starting materials **G3C3** and **G2M7** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G5P7** was obtained as foamy colourless solid after preparative RP-HPLC (15.6 mg). Amino Acid Analysis: protein content 60.7% (9.46 mg, 0.40 µmol, yield 56%); found/calc. (%) Pro 51.7/48.4, Glu 15.3/15.0, Asp 11.6/11.3, Dap 10.6/11.3, Ser 6.6/7.0, CM-Cys 4.2/3.8, Lys 3.7/3.3 (Dap coeluted with Phe, 2208.5 pmol). Analytical RP-UPLC: t_R = 1.304 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS (LC-MS+): $C_{970}H_{1435}N_{253}O_{348}S_8$ (8-fold ligation product, main peak) found/calc. 22459.0/22464.8 $[M]^+$. $C_{871}H_{1290}IN_{227}O_{312}S_7$ (7-fold ligation product with one unreacted iodoacetyl group, peak height 12% of main peak) found/calc. 20270.0/20271.9 $[M]^+$, no 6-fold ligation product observed (calc. 18117.8 $[M]^+$). $D = 6.31 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 3.21 ± 0.08 nm.

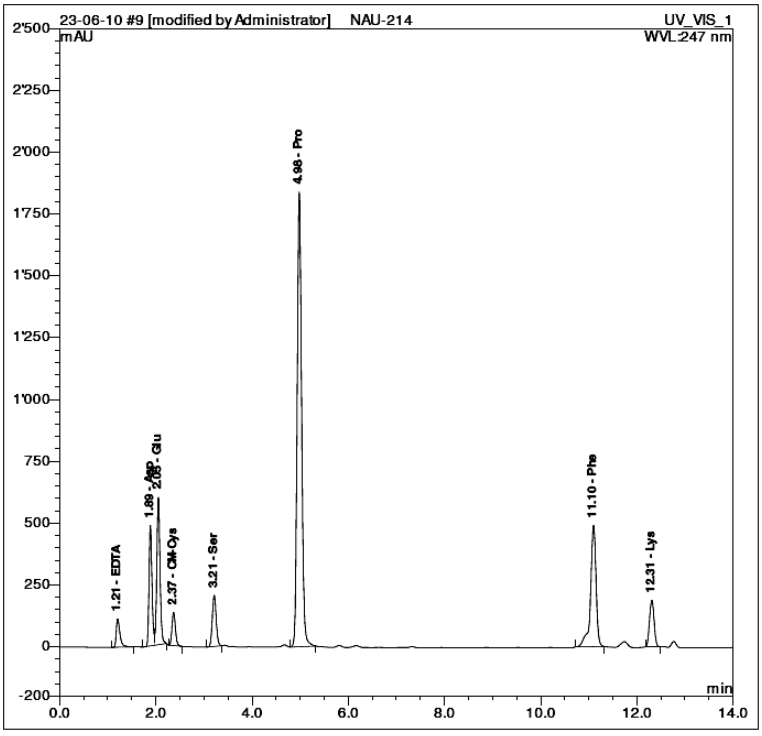
Mass spectrum, MS (ESI+):



Analytical RP-UPLC chromatogram:

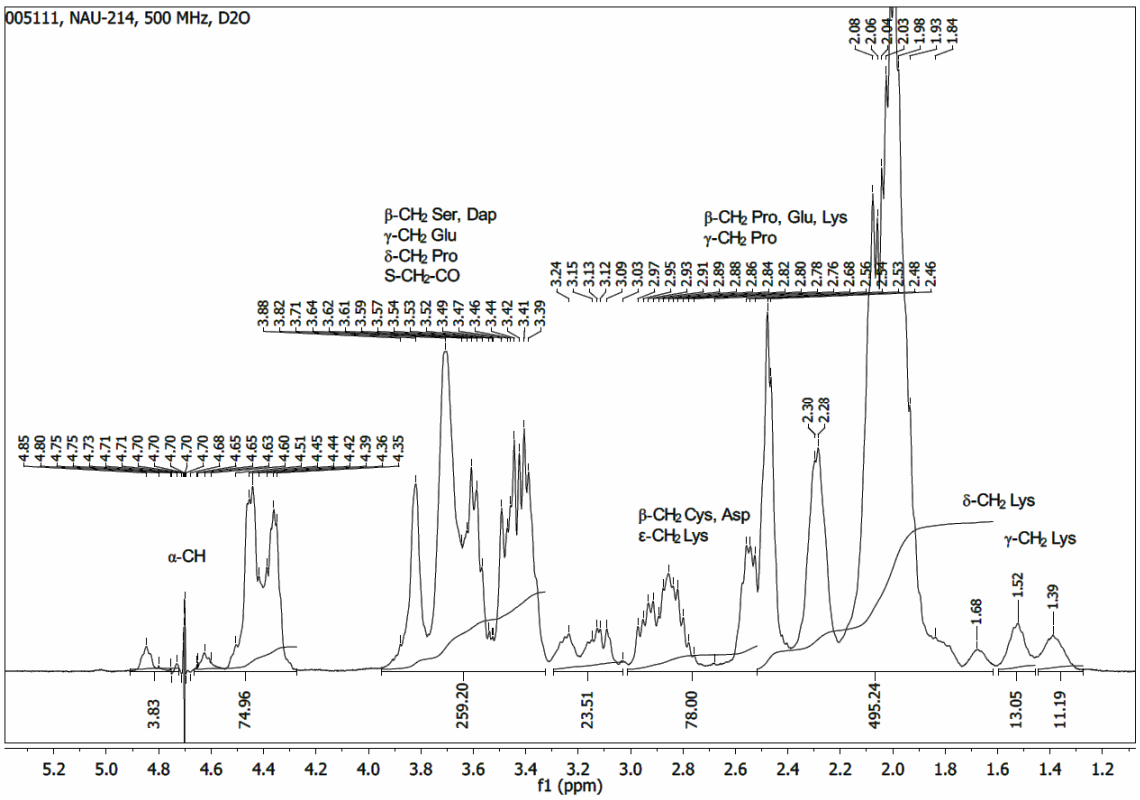


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,21	1,20	0,077	10,15	113,93		403,9	EDTA
1,89	1,88	0,065	34,55	487,42		2421,5	Asp
2,05	2,05	0,072	46,85	595,66		3187,4	Glu
2,37	2,37	0,076	11,02	134,10		875,7	CM-Cys
3,21	3,21	0,088	19,44	206,61		1371,5	Ser
4,98	5,01	0,098	196,05	1839,30		10773,1	Pro
11,10	11,11	0,112	65,24	490,87		3745,3	Phe
12,31	12,31	0,106	21,06	188,65		779,3	Lys
Total:						23557,69	

^{1}H-NMR:

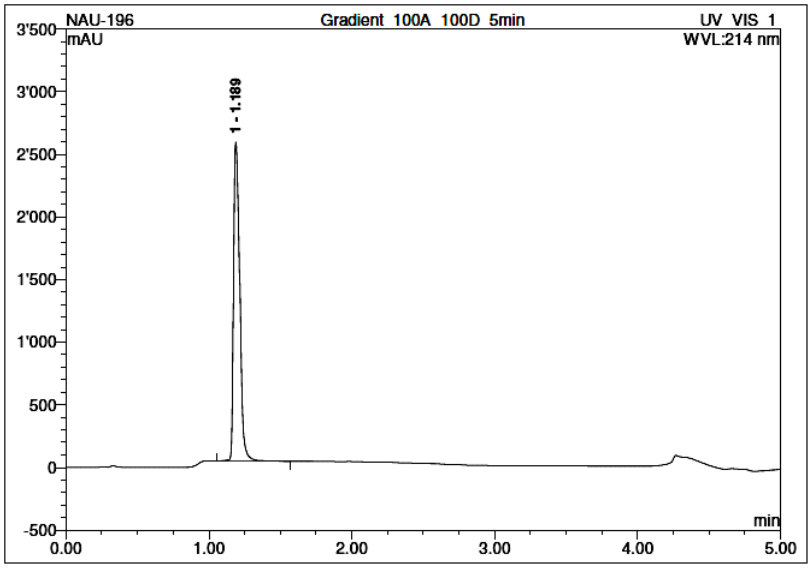

005111, NAU-214, 500 MHz, D2O
β-CH2 Ser, Dap
γ-CH2 Glu
δ-CH2 Pro
S-CH2-CO
β-CH2 Pro, Glu, Lys
γ-CH2 Pro
α-CH
β-CH2 Cys, Asp
ε-CH2 Lys
δ-CH2 Lys
γ-CH2 Lys
3.83
74.96
259.20
23.51
78.00
495.24
13.05
11.19
f1 (ppm)

Peptide Dendrimer G6P1

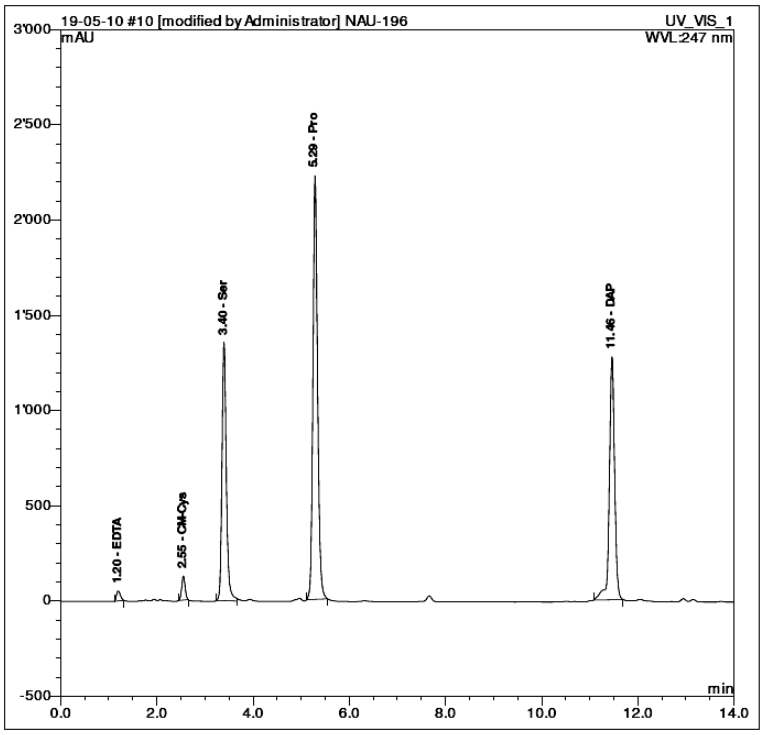
(Pro-Ser)$_{64}$(*Dap*-Pro-Ser)$_{32}$(*Dap*-Pro-Ser)$_{16}$(*Dap*-Pro-Ser-Cys-x-Pro-Ser)$_8$(*Dap*-Pro-Ser)$_4$(*Dap*-Pro-Ser)$_2$*Dap*-Pro-Ser-NH$_2$

From starting materials **G3C2** and **G3M1** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G6P1** was obtained as foamy colourless solid after preparative RP-HPLC (17.7 mg). Amino Acid Analysis: protein content 65.8% (11.6 mg, 0.34 µmol, yield 45%); found/calc. (%) Pro 44.2/39.6, Ser 30.3/39.6, Dap 22.9/18.5, CM-Cys 2.6/2.3. Analytical RP-UPLC: t_R = 1.189 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS: $C_{1309}H_{2065}N_{413}O_{484}S_8$ (8-fold ligation product) found/calc. N.d./31588.4 $[M]^+$. D = $3.68 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 5.51 ± 0.07 nm.

Analytical RP-UPLC chromatogram:

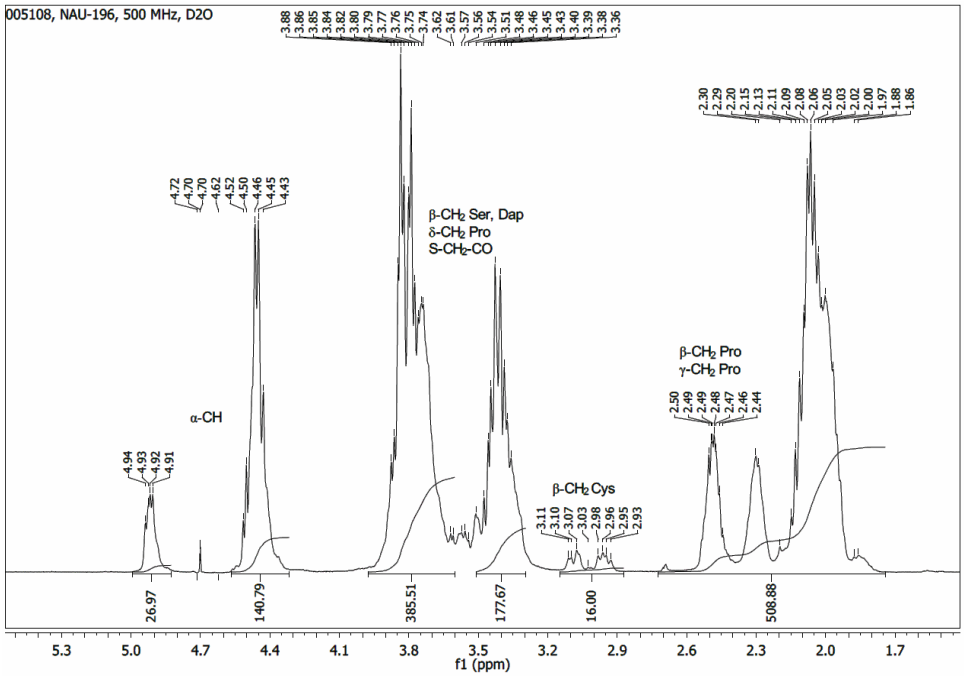


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,20	1,19	0,089	4,82	52,17		472,8	EDTA
2,55	2,55	0,077	10,19	125,26		675,6	CM-Cys
3,40	3,41	0,092	141,75	1357,27		7869,0	Ser
5,29	5,32	0,101	247,89	2223,93		11480,3	Pro
11,46	11,50	0,111	162,16	1275,08		5952,4	DAP
Total:						26450,22	

^{1}H-NMR:

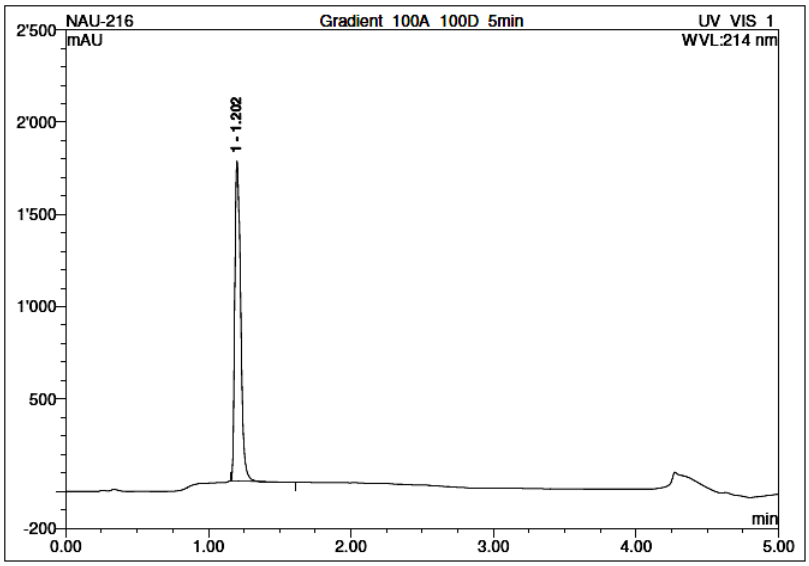


Peptide Dendrimer G6P2

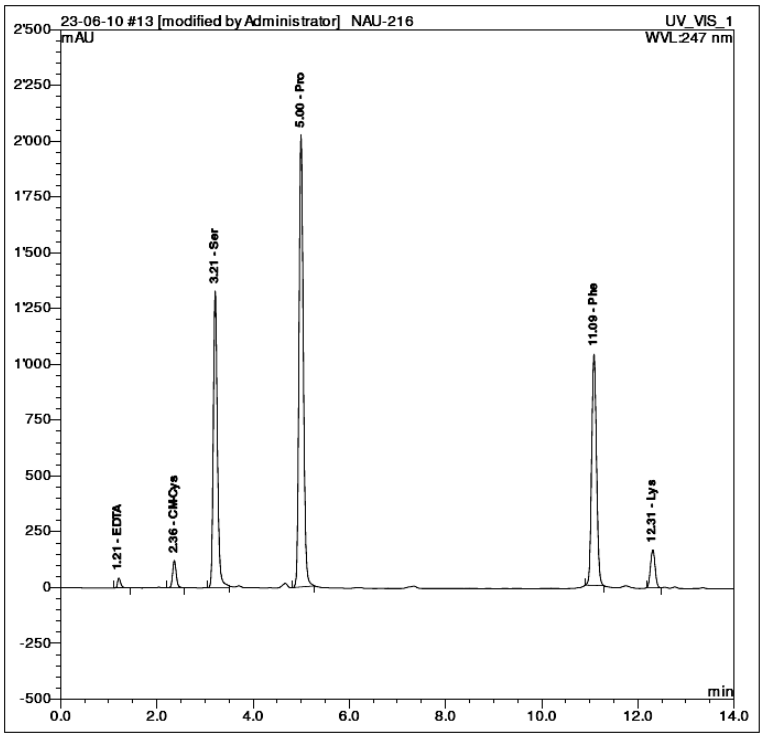
(Pro-Ser)$_{64}$(*Dap*-Pro-Ser)$_{32}$(*Dap*-Pro-Ser)$_{16}$(*Dap*-Pro-Ser-Cys-x-Pro-Ser)$_8$(*Lys*-Pro-Ser)$_4$(*Lys*-Pro-Ser)$_2$*Lys*-Pro-Ser-NH$_2$

From starting materials **G3C3** and **G3M1** using the general procedure described above (solvent: DMF/H_2O (1/1 v/v), 20 eq KI), **G6P2** was obtained as foamy colourless solid after preparative RP-HPLC (18.2 mg). Amino Acid Analysis: protein content 68.6% (12.5 mg, 0.36 µmol, yield 51%); found/calc. (%) Pro 44.2/39.6, Ser 32.9/39.6, Dap 17.4/16.4, CM-Cys 3.0/2.3, Lys 2.6/2.1 (Dap coeluted with Phe, 4658.9 pmol). Analytical RP-UPLC: t_R = 1.202 min (A/D 100/0 to 0/100 in 5 min, λ = 214 nm). MS: $C_{1330}H_{2107}N_{413}O_{484}S_8$ (8-fold ligation product) found/calc. N.d./31883.0 $[M]^+$. D = $4.15 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$; R_h = 4.88 ± 0.18 nm.

Analytical RP-UPLC chromatogram:

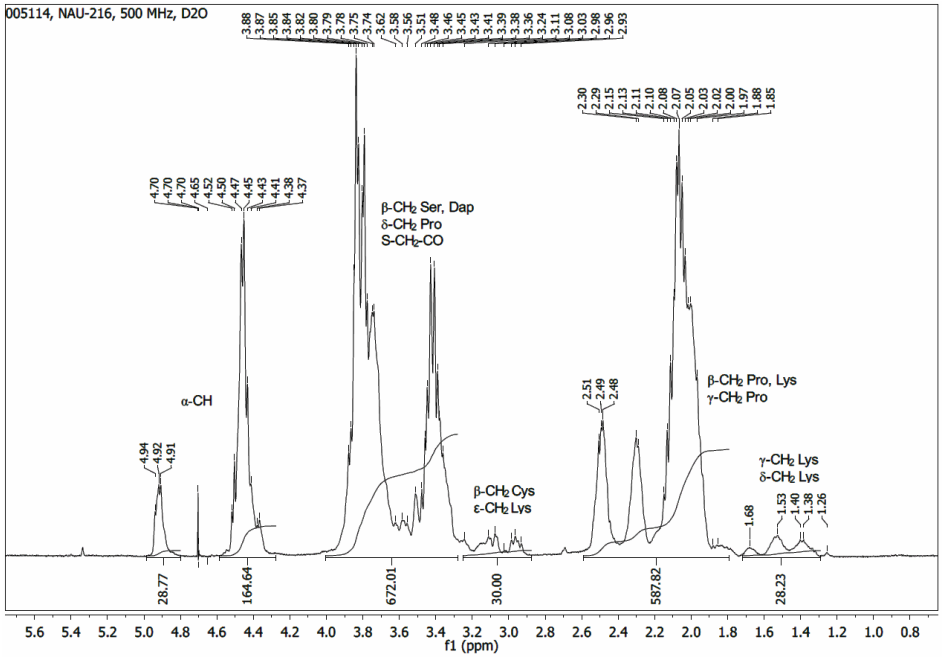


Amino Acid Analysis:



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1,21	1,20	0,067	3,49	44,85		159,0	EDTA
2,36	2,37	0,078	10,51	122,43		799,5	CM-Cys
3,21	3,21	0,088	130,82	1329,57		8825,6	Ser
5,00	5,01	0,096	210,28	2026,81		11871,4	Pro
11,09	11,11	0,110	121,18	1035,49		7900,6	Phe
12,31	12,31	0,106	18,72	168,58		696,4	Lys
Total:						30252,51	

^{1}H-NMR:



Amino Acid Analysis

Samples were hydrolyzed in the gas phase with 6M HCl containing 0.1% (v/v) phenol for 22 h at 115 °C under N_2 vacuum according to Chang and Knecht.[1] The liberated amino acids were coupled with phenylisothiocyanate (PITC), and the resulting phenylthiocarbamoyl (PTC) amino acids were analyzed by RP-HPLC on a Nova Pack C18 column (4 µm, 3.9 mm × 150 mm, Waters) with a Dionex Summit® HPLC system with an automatic injection system according to Bidlingmeyer et al.[2] The corresponding ammonium acetate buffer replaced the 0.14 M sodium acetate buffer, pH 6.3. Cysteine was detected as carboxymethyl cysteine (CM-Cys); AMBA = Amb = 4-aminomethylbezoic acid. Dap coeluted with Phe. For some compounds the amount of Dap (pmol) was calculated based on a separate standard and is indicated within the characterization data.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

To cast the gels (84 × 73 × 0.75 mm), Bio-Rad short plates/spacer plates were filled with resolving gel until 2 cm under the glass rim and covered with mQ-deionized H_2O. When polymerization was finished (~ 20 min) the water was removed and stacking gel was poured on top of the resolving gel. The pocket forming comb was inserted. Polymerization of the stacking gel was generally finished after 5 min. The gels were used in electrophoresis boxes purchased from Bio-Rad. After the addition of electrophoresis buffer, the gel pockets were washed with the latter and compounds (6-36 µg dissolved in a mixture of 12.5 µL mQ-deionized H_2O and 2.5 µL sample buffer) or molecular marker (2.5 µL of the prepared solution) were added using a 50 µL glass syringe. Power was supplied by a Consort E452

(1) Chang, J.-Y.; Knecht, R. *Anal. Biochem.* **1991**, *197*, 52-58.

(2) Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. *J. Chromatogr.* **1984**, *336*, 93-104.

(200 V) during 0.7 to 1.3 h. To develop the gels, they were removed from the glass plates and stained in a staining/fixation bath for 3 min in a microwave oven at 600 W followed by a background destaining bath (4 × 15 min). The gels were washed in a mixture of mQ-deionized H_2O/glycerol and slowly dried at rt between two permeable jam foils. Pictures of the gels were obtained by the use of a flat-bed scanner.

Preparation. All stock solutions and buffers were filtered prior to use.

Resolving gel 20% (for two gels). 1.5 M Tris·Base pH 8.7 (2.5 mL), aq. solution of SDS (10%, 0.1 mL), aq. solution of acrylamide (Rotiphorese Gel A®, 30%, 6.65 mL), aq. solution of bisacrylamide (Rotiphorese Gel B®, 2%, 0.325 mL), aq. solution of APS (10%, 33 µL), TMED (5 µL).

Stacking gel 4.3% (for two gels). mQ-deionized H_2O (2 mL), 0.25 M Tris·Base pH 6.8 with 0.2% of SDS and Coomassie® Brilliant Blue R-250 (2.5 mL), aq. solution of acrylamide (30%) and bisacrylamide (0.8%) (0.75 mL), aq. solution of APS (10%, 50 µL), TMED (10 µL).

Sample buffer. SDS (5 g), 1M Tris·Base pH 6.8 (15 mL), glycerol (22.5 mL), β-mercatoethanol (12.5 mL), bromphenol blue.

Molecular Marker. Calibration Kit 17-0446-01 purchased from GE Healthcare was used as molecular marker. The protein mixture containing phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) was dissolved in mQ-deionized H_2O (160 µL) and sample buffer (40 µL). The solution was heated at 100 °C for 5 min and cooled back down to rt prior to use on the gel.

Electrophoresis buffer. To a solution of glycine (72 g) and Tris·Base (15 g) in mQ-deionized H_2O (975 mL) a solution of SDS (10%, 25 mL) was added.

Staining/fixation bath. MeOH (0.5 L), acetic acid (99%, 0.125 L), mQ-deionized H_2O (0.625 L), Coomassie® Brilliant Blue R-250 (2.5 g).

Background destaining bath. MeOH (2.5 L), acetic acid (99%, 0.5 L), mQ-deionized H_2O (2.5 L).

Catalysis

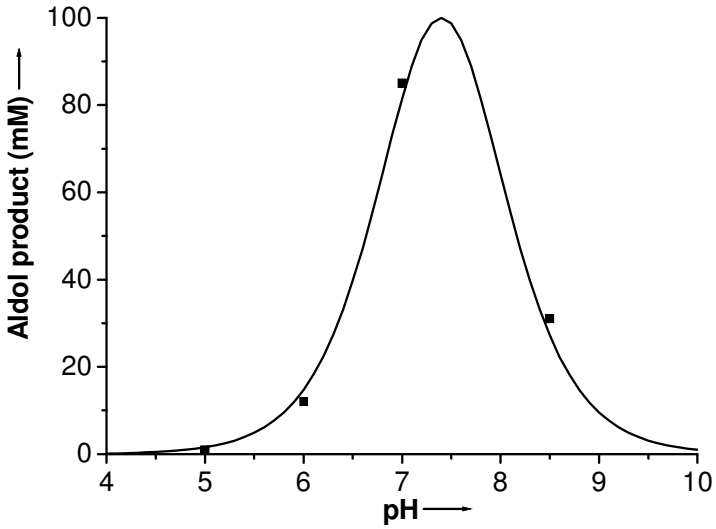


Figure S1. pH dependent activity profile of G6 dendrimer **G6P1** resulting from a bell-shaped fit assuming bifunctional catalysis (v = c [HA][A]), plotted for pKa(HA) = 7.4.

Table S1. Enantioselectivities (%) observed for aldol **6**.

ID	G4P	G5P1	G5P2	G5P3	G5P4	G5P5	G5P6	G5P7	G6P1	G6P2
ee[a]	24	16	18	14	27	15	12	<5	23	19
ee[b]	35	24	27	25	n.r.[c]	29	20	9	31	41

Determined after dendrimer-catalyzed aldol reaction in [a] 0.1 M HEPES buffer pH 7.0/acetone (1/1, v/v) or [b] DMSO/acetone (1/1, v/v), buffered with *x* eq. of NMM (where *x* = number of formic acid-amine salts for the dendrimer tested) on a chiral Daicel Chiralpak AS® column (amylose tris[(S)-α-methylbenzylcarbamate] coated on a silica support, 10 µm, 4.6 × 250 mm, flow rate 2.0 mL·min^{-1}); [c] no reaction.