Comparing dendritic with linear esterase peptides by screening SPOT arrays for catalysis

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Supporting Information

SPOT Library synthesis and screening

SPOT-Library synthesis: The peptides were synthesized on standard amino-modified acid stable cellulose membrane with PEG-Spacer from AIMS Scientific Products GmbH (Germany) in 96-well format using a fully automated MultiPep robot from Intavis Bioanalytical Instruments (Germany) equipped with the AutoSpot module. In the first step, The loading is 400 nmol/cm^2 from the supplier and was reduced by coupling a 1:4 mixture of Fmoc-Gly-OH and Ac-Gly-OH giving a final loading of approx. 100 nmol/cm^2. Couplings were performed in triple using HOAt/DIPCDI/collidine preactivation (0.4 µl 1 M DIC in NMP, 0.2 µl 1 M collidine in NMP, 1.2 µl 0.3 M Fmoc amino acid in 0.3 M HOAt in NMP per SPOT for 30 min). Fmoc deprotection was performed by treating each membrane with 20% piperdine in NMP (2 x 6 ml) for 10 min. Membrane washing was with NMP (6 x 6 ml) and EtOH (6 x 6ml) *via* the robot manifold followed by drying. The membrane was derivatized with the first C-terminal amino acid followed by capping with NMP/Ac_2O/DIPEA (94:5:1) (SPOT definition) followed by washing. After Fmoc removal, the peptides were synthesized using the repetitive cycle of deprotection, coupling and washing. After synthesis the cellulose membrane was washed with DCM (5 x 20 ml), dried, and the sidechain protection groups were removed by a 2 hour treatment with TFA/TIPS/water (92.5/5.0/2.5 v/v, 50 ml) followed by washings with DCM (5 x 20 ml), NMP (5 x 20 ml), and EtOH (5 x 20 ml). The dried membrane was placed on a transilluminator irradiating at 365 nm to identify the SPOTs. The SPOTs were punched out using a multi punch device available from Intavis Bioanalytical Instruments (Germany) and placed in a 96-well miroplate.

Table S1. Composition of the SPOT esterase library.[a)]

Type	no. of sequences	no. of residues	no. of histidines
Linear peptides	10	11	3-11
G1 dendrimers	2	13	5
G2 dendrimers	6	20-29	9-12
G3 dendrimers with His:			
- at the core (like **RG3**)	7	37	1-6
- in intermediate branches	3	37	8-10
- in the outer branches	18	37	8-12
- throughout (like **A3C**)	50	26-54	12-26

a) The library was assembled by 11 consecutive coupling of amino acids or diamino acid branching points on a 96-well format cellulose support using Fmoc synthesis. All sequences were acetylated at the N-terminus. See Supporting information Table S2 for a complete listing of sequences.

Table S2. List of all SPOT library members ordered by decrease activity in the fluorescence assay with substrate **1** (relative reading at 6 h reaction). Branching of the chain occurs at Dap (= bis-Fmoc-2,3-diaminopropanoic acid) and Lyb (= bis-Fmoc-lysine). Bla = beta-alanine,The N-termini are acetylated.

order	X11	X10	X9	X8	X7	X6	X5	X4	X3	X2	X1	no of AA	NHis	Activity	Act per his
P1	His	His	His	His	His	His	His	His	His	His	His	11	11	44.64	22.73
P2	His	Thr	His	Thr	His	Dap	Thr	Dap	His	Dap	Thr	54	26	34.34	7.40
P3	His	Thr	His	Dap	Thr	Dap	His	Thr	Dap	His	Thr	41	19	32.81	9.67
P4	His	Bla	His	Bla	His	Dap	Bla	Dap	His	Dap	Bla	54	26	30.82	6.64
P5	His	Dap	Thr	His	Thr	Dap	His	Thr	Dap	His	Thr	33	15	29.59	11.05
P6	His	Bla	His	Dap	Bla	Dap	His	Bla	Dap	His	Bla	41	19	28.99	8.55
P7	his	Bla	Dap	his	Bla	Dap	his	Bla	Dap	his	Bla	37	15	28.13	10.50
P8	Hyp	His	Dap	Hyp	His	Dap	Hyp	His	Dap	Hyp	His	37	15	27.58	10.30
P9	His	Thr	Ala	His	Thr	Dap	His	Thr	Dap	His	Thr	29	11	27.52	14.01
P10	His	Bla	Dap	his	Bla	Dap	his	Bla	Dap	his	Bla	37	15	27.20	10.15
P11	Ser	His	Dap	Ser	His	Dap	Ser	His	Dap	Ser	His	37	15	27.20	10.15
P12	Lys	His	Dap	Lys	His	Dap	Lys	His	Dap	Lys	His	37	15	27.18	10.15
P13	His	Thr	Dap	His	Thr	Ala	His	Thr	Dap	His	Thr	23	9	26.57	16.53
P14	Thr	His	Dap	Thr	His	Dap	Thr	His	Dap	Thr	His	37	15	26.29	9.82
P15	His	Lys	Dap	His	Lys	Dap	His	Lys	Dap	Lys	Lys	37	14	25.40	10.16
P16	Aib	His	Dap	Aib	His	Dap	Aib	His	Dap	Aib	His	37	15	25.14	9.38
P17	Aib	His	Dap	Aib	His	Dap	Aib	His	Dap	Aib	His	37	15	24.91	9.30
P18	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	11	6	24.54	22.91
P19	Thr	His	Dap	Thr	His	Dap	Thr	His	Dap	Thr	His	37	15	24.15	9.02
P20	His	Thr	Dap	His	Thr	Dap	His	Thr	Ala	His	Thr	20	12	24.13	11.26
P21	His	Dap	Bla	His	Bla	Dap	His	Bla	Dap	His	Bla	33	15	23.75	8.87
P22	His	Thr	Lyb	His	Thr	Lyb	His	Thr	Lyb	His	Thr	37	15	23.60	8.81
P23	Bla	His	Dap	Bla	His	Dap	Bla	His	Dap	Bla	His	37	15	23.37	8.72
P24	Gly	His	Dap	Gly	His	Dap	Gly	His	Dap	Gly	His	37	15	22.50	8.40
P25	His	Lys	His	Lys	His	Lys	His	Lys	His	Lys	His	11	6	22.46	20.97
P26	His	Arg	His	Arg	His	Arg	His	Arg	His	Arg	His	11	6	21.96	20.50
P27	Pro	His	Dap	Pro	His	Dap	Pro	His	Dap	Pro	His	37	15	21.52	8.03
P28	Tyr	His	Dap	Tyr	His	Dap	Tyr	His	Dap	Tyr	His	37	15	21.40	7.99
P29	His	Dap	Bla	Dap	His	Dap	Bla	His	Bla	His	Bla	26	12	20.73	9.68
P30	His	Bla	Dap	His	Bla	Dap	His	Bla	Ala	His	Bla	20	12	20.53	9.58
P31	Leu	His	Dap	Leu	His	Dap	Leu	His	Dap	Leu	His	37	15	20.30	7.58
P32	pro	His	Dap	pro	His	Dap	pro	His	Dap	pro	His	37	15	20.27	7.57
P33	His	Bla	Dap	His	Bla	Ala	His	Bla	Dap	His	Bla	23	9	19.70	12.26
P34	His	Aib	Dap	His	Aib	Dap	His	Aib	Dap	His	Aib	37	15	19.63	7.33
P35	His	Bla	Lyb	His	Bla	Lyb	His	Bla	Lyb	His	Bla	37	15	19.29	7.20
P36	Arg	His	Dap	Arg	His	Dap	Arg	His	Dap	Arg	His	37	15	19.25	7.19
P37	His	Gly	Dap	His	Gly	Dap	His	Gly	Dap	His	Gly	37	15	19.00	7.09
P38	His	Val	Dap	His	Bla	Dap	His	Bla	Dap	His	Bla	37	15	18.76	7.01
P39	His	Tyr	Dap	His	Tyr	Dap	His	Tyr	Dap	His	Tyr	37	15	18.76	7.01
P40	Ala	His	Dap	Ala	His	Dap	Ala	His	Dap	Ala	His	37	15	18.76	7.01
P41	leu	His	Dap	leu	His	Dap	leu	His	Dap	leu	His	37	15	17.03	6.36
P42	His	Bla	Ala	His	Bla	Dap	His	Bla	Dap	His	Bla	29	11	16.57	8.44
P43	Tyr	Gly	Dap	Tyr	Ser	Dap	His	His	Dap	His	His	37	6	15.76	14.71

order	X11	X10	X9	X8	X7	X6	X5	X4	X3	X2	X1	no of AA	NHis	Activity	Act per his
P44	Tyr	Gly	Dap	Tyr	Ser	Dap	His	His	Dap	Lys	Lys	37	4	15.49	21.68
P45	His	Leu	Dap	His	Leu	Dap	His	Leu	Dap	His	Leu	37	15	15.34	5.73
P46	His	Thr	Dap	His	Thr	Ala	His	Thr	Ala	His	Thr	13	5	14.97	16.77
P47	His	leu	Dap	His	leu	Dap	His	leu	Dap	His	leu	37	15	14.88	5.56
P48	His	Thr	Dap	Arg	Thr	Dap	His	Thr	Dap	Arg	Thr	37	10	14.79	8.28
P49	His	Hyp	Dap	His	Hyp	Dap	His	Hyp	Dap	His	Hyp	37	15	14.65	5.47
P50	His	His	Dap	Pro	Pro	Dap	Pro	Pro	Dap	Gly	Gly	37	16	14.12	4.94
P51	His	Arg	Dap	His	Arg	Dap	His	Arg	Dap	His	Arg	37	15	14.09	5.26
P52	His	Bla	Dap	His	Bla	Dap	His	Bla	Dap	His	Bla	37	15	13.83	5.16
P53	His	Asn	Dap	His	Asn	Dap	His	Asn	Dap	His	Asn	37	15	13.77	5.14
P54	His	pro	Dap	His	pro	Dap	His	pro	Dap	His	pro	37	15	13.70	5.11
P55	His	His	Dap	Arg	Arg	Dap	Thr	Thr	Dap	Thr	Thr	37	16	13.45	4.71
P56	Val	His	Dap	Val	His	Dap	Val	His	Dap	Val	His	37	15	13.24	4.94
P57	His	Bla	Dap	His	Bla	Ala	His	Bla	Ala	His	Bla	13	5	13.16	14.74
P58	his	Bla	Dap	His	Bla	Dap	His	Bla	Dap	His	Bla	37	15	12.80	4.78
P59	His	Leu	Lyb	His	Leu	Lyb	His	Leu	Lyb	His	Leu	37	15	12.47	4.66
P60	His	Thr	Ala	His	Thr	Ala	His	Thr	Ala	His	Thr	11	4	12.27	17.18
P61	His	Dap	Thr	Dap	His	Dap	Thr	His	Thr	His	Thr	26	12	11.78	5.50
P62	His	Ser	Dap	His	Pro	Dap	Lys	Val	Dap	Phe	Val	37	12	9.69	4.52
P63	His	Pro	Dap	His	Pro	Dap	His	Pro	Dap	His	Pro	37	15	9.66	3.61
P64	Tyr	Gly	Dap	Tyr	Ser	Dap	His	His	Dap	Arg	Arg	37	4	9.60	13.44
P65	Tyr	Gly	Dap	Tyr	Ser	Dap	Lys	Lys	Dap	Thr	His	37	1	9.21	51.59
P66	His	Tyr	Arg	His	Tyr	Arg	His	Tyr	Arg	His	Tyr	11	4	8.96	12.55
P67	Tyr	Gly	Dap	Tyr	Ser	Dap	His	Arg	Dap	His	Arg	37	3	8.00	14.93
P68	Thr	Thr	Dap	His	His	Dap	Arg	Asp	Dap	Arg	Asp	37	8	7.85	5.49
P69	Leu	His	Arg	Leu	His	Arg	Leu	His	Arg	Leu	His	11	4	7.46	10.45
P70	His	Arg	Leu	His	Arg	Leu	His	Arg	Leu	His	Arg	11	4	7.44	10.42
P71	His	Ser	Dap	His	Leu	Dap	Phe	Ala	Dap	Phe	Asp	37	12	7.31	3.41
P72	His	Thr	Dap	His	Thr	Dap	His	Thr	Dap	His	Thr	37	15	7.30	2.73
P73	His	His	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	16	6.51	2.28
P74	His	Ser	Dap	Gly	Arg	Dap	Lys	Val	Dap	Ile	Ala	37	8	6.23	4.36
P75	His	Bla	Ala	His	Bla	Ala	His	Bla	Ala	His	Bla	11	4	5.83	8.16
P76	Tyr	Gly	Dap	Tyr	Ser	Dap	Arg	Arg	Dap	Thr	His	37	1	5.31	29.74
P77	His	Ser	Dap	Gly	Arg	Dap	Ile	Ala	Dap	Ile	Val	37	8	5.01	3.50
P78	His	Leu	Dap	His	Ser	Dap	Tyr	Ala	Dap	Ile	Asp	37	12	5.00	2.33
P79	Gly	Tyr	Dap	His	Arg	Dap	Thr	Leu	Dap	Ser	Gly	37	4	4.71	6.59
P80	His	Arg	Dap	Gly	Ser	Dap	Ile	Val	Dap	Ile	Val	37	8	4.56	3.19
P81	His	Pro	Dap	Gly	Pro	Dap	Lys	Thr	Dap	Ile	Ala	37	8	3.76	2.63
P82	His	Bla	Dap	Arg	Bla	Dap	His	Bla	Dap	Arg	Bla	37	10	2.89	1.62
P83	Asp	His	Dap	Asp	His	Dap	Asp	His	Dap	Asp	His	37	15	2.62	0.98
P84	His	Ser	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	2.60	1.82
P85	His	Gly	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	2.56	1.80
P86	His	Tyr	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	2.44	1.71
P87	His	Ala	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	1.98	1.39
P88	His	Thr	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	1.82	1.27
P89	His	Thr	Dap	His	Thr	Dap	Asp	Asp	Dap	Asp	Asp	37	12	1.80	0.84
P90	His	Asp	Dap	His	Asp	Dap	His	Asp	Dap	His	Asp	37	15	1.75	0.65
P91	His	Leu	Dap	Gly	Leu	Dap	Tyr	Thr	Dap	Ile	Val	37	8	1.65	1.16
P92	Thr	His	Dap	His	Thr	Dap	Arg	Lys	Dap	Lys	Gly	37	12	1.62	0.75
P93	His	Pro	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	1.48	1.03
P94	His	Leu	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	1.45	1.02
P95	Asp	Ser	His	Leu	Asp	Ser	His	Leu	Asp	Ser	His	11	3	1.17	2.18
P96	His	Phe	Dap	Gly	Gly	Dap	Gly	Gly	Dap	Gly	Gly	37	8	1.00	0.70

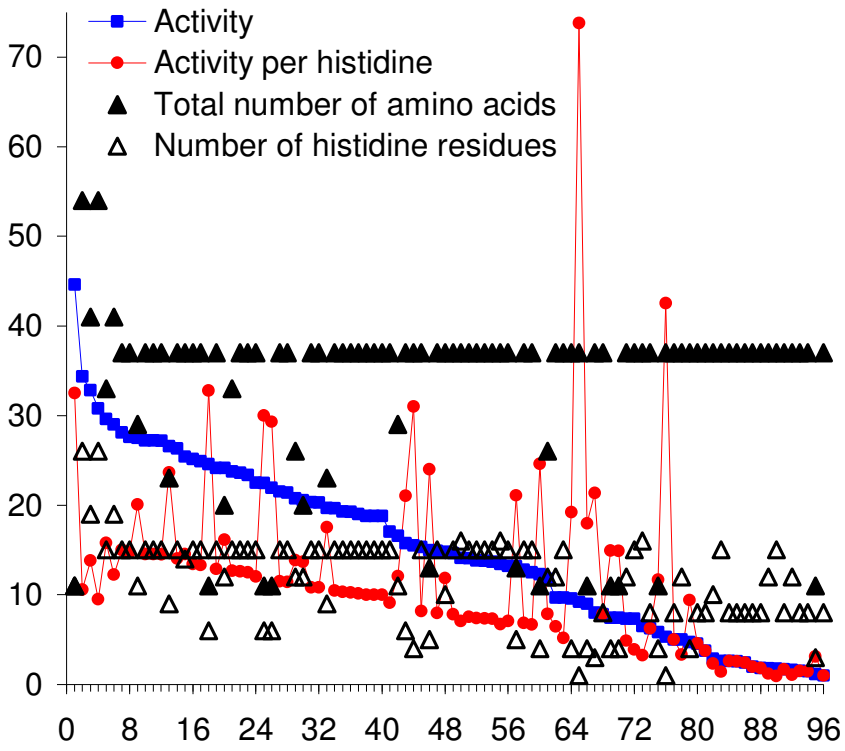


Figure S1. Screening of the 96-member SPOT library for hydrolysis of 1-acetoxy-3,6,8-pyrene trisulfonate **1**. The catalysts are ordered by decreasing activity and labeled as PosX (SPOT-library member) or **PX** (resynthesized, purified product, see Table 1). Conditions: paper disks with each SPOT-library member were cut out of the synthesis paper, transferred to a 96-well microtiter plate, washed with water and methanol, and suspended in 100 µL each of a solution of 80 µM substrate **1** in 5 mM aqueous citrate buffer pH 5.5. The formation of product **2** was recorded by fluorescence at 3h and 6 h from the fluorescence reading (λ_{exc} = 450±25 nm, λ_{em} = 530±12 nm). The activity is given at the 6h timepoint as the relative fluorescence intensity compared to the lowest reading in the library (Pos96). The activity per histidine is expressed relative to the smallest calculated activity per histidine, which is for the peptide dendrimer sequence with 8 histidine residues Pos96: $(AcHisPhe)_8(\textit{Dap}GlyGly)_4(\textit{Dap}GlyGly)_2\textit{Dap}GlyGly$.

Synthesis

Materials and Reagents. Peptide syntheses were performed manually in a syringe reactor. All reagents, amino acids and their derivatives were either purchased from Aldrich, Fluka (Switzerland) or Advanced Chemtach (USA); resins from Rapp Polymere GmbH, Germany. Amino acids were used as the following derivative: Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-His(trt)-OH, Fmoc-Ser(*t*-Bu)-OH**,** Fmoc-Thr(*t*-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(*t*-Bu)-OH. For the dendrimer, as branching unit was used Fmoc-Dap(Fmoc)-OH (where Dap is Diaminopropionic Acid). Analytical HPLC was carried out with HPLC-grade acetonitrile and miliQ deionized water on Waters 600 system with Waters 996 photodiode array detector (Column: Waters Atlantis dC18 5 μm, 100 x 4.6 mm) for analytical HPLC or Waters PrepLC Preparative Chromatography System with Waters 2489 absorbance Detector (Column: Atlantis ® PrepT3, OBD^{TM} C18, 5 μm, 30 x 100 mm) for preparative HPLC. For dendrimer purification and identification solvent A is 0.1% TFA in H_2O and solvent D is 0.1% TFA inH_2O/CH_3CN 40/60.MS spectra were provided by the Service of Mass Spectrometry of the Department of Chemistry and Biochemistry, University of Berne. Kinetic measurements were carried out using a CytoFluor® Series 4000 multi-well plate reader from PerSeptive Biosystems.

General procedures for peptide synthesis.

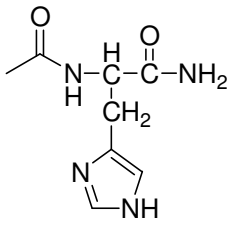
Procedure A (manual synthesis in syringe reactors): Prior to every reaction the resin was swelled in CH2Cl2. The resins Tentagel HL RAM from Rapp Poylmere (0.39 mmol/gm), were acylated with each amino acid (3.0 eq) using PyBOP (3.0 eq) and DIEA (5.0 eq) in NMP. The Fmoc protecting groups were removed with a solution of 20% piperidine in DMF (2×20 min). At the end of the synthesis, the resin was acylated with acetic anhydride/CH2Cl2 (1:1) for 30 mins. The cleavage was carried out with TFA/TIS/H_2O (94:5:1) for 4 h. The peptide was precipitated with methyl tert-butyl ether (in case of unsuccessful precipitation, the solution was evaporated) and then dissolved in a water/acetonitrile mixture then subjected to purification. All peptides were purified by preparative HPLC and obtained as TFA salts after lyophilization.

Procedure B (using automated peptide synthesizer PSW 1100 from Chemspeed Technology, Switzerland for major part of the synthesis): Prior to every reaction the resin was swelled in CH2Cl2. The resins Tentagel S RAM (0.24 mmol/gm) from Rapp Poylmere, were acylated with each amino acid (3.0 eq) using procedure PyBOP (3.0 eq) and DIEA (5.0 eq) in NMP. The Fmoc protecting groups were removed with a solution of 20% piperidine in DMF (2×20 min). At the end of the synthesis,The resin was taken out of the synthesizer and it was acylated with acetic anhydride/CH2Cl2 (1:1) for 30 mins. The cleavage was carried out with TFA/TIS/H2O (94:5:1) for

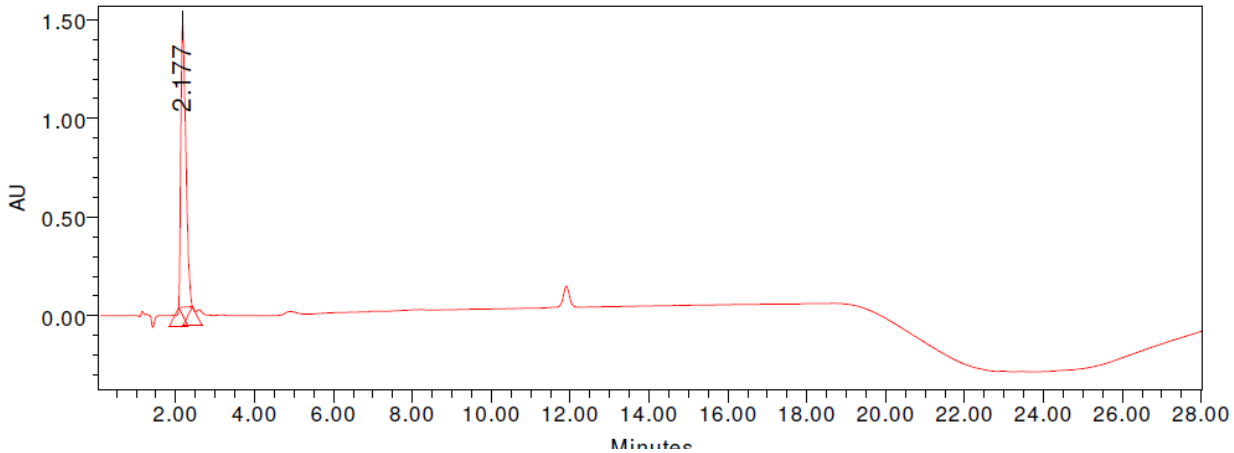
4 h. The peptide was precipitated with methyl tert-butyl ether (in case of unsuccessful precipitation, the solution was evaporated) and then dissolved in a water/acetonitrile mixture then subjected to purification. All peptides were purified by preparative HPLC and obtained as TFA salts after lyophilization.

His1: AceHis-NH_2

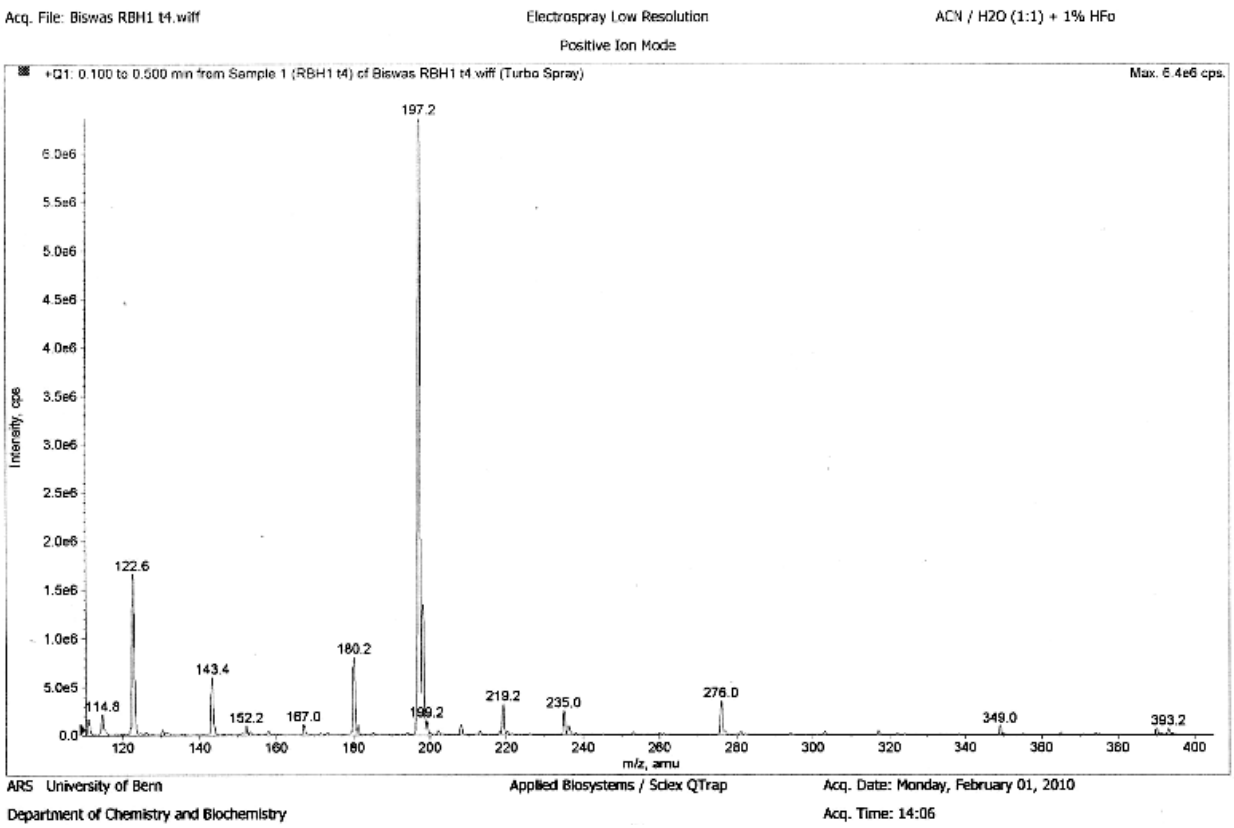
Starting with 100 mg Tentagel HL RAM from Rapp Poylmere (0.39 mmol/gm), the peptide 1-His was obtained using procedure A as colorless solid after cleavage from the resin and preparative RP-HPLC purification (2.3 mg, 23.9 %).



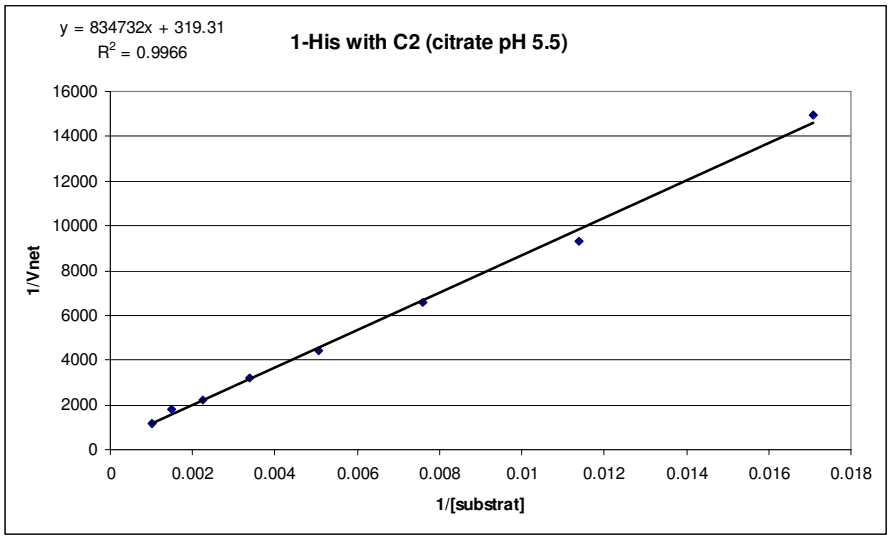
Analytical RP-HPLC: *t*R = 2.2 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_8H_{12}N_4O_2$ $[M+H]^+$ 197.2, found: 197.2

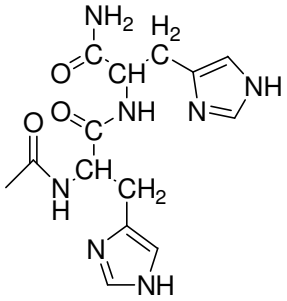


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

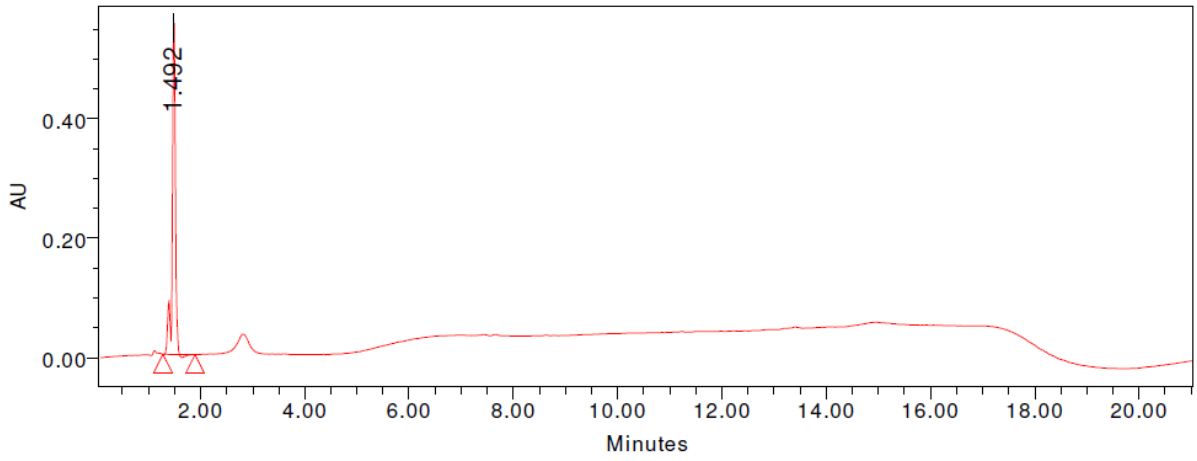


His2: AcHisHis-NH_2

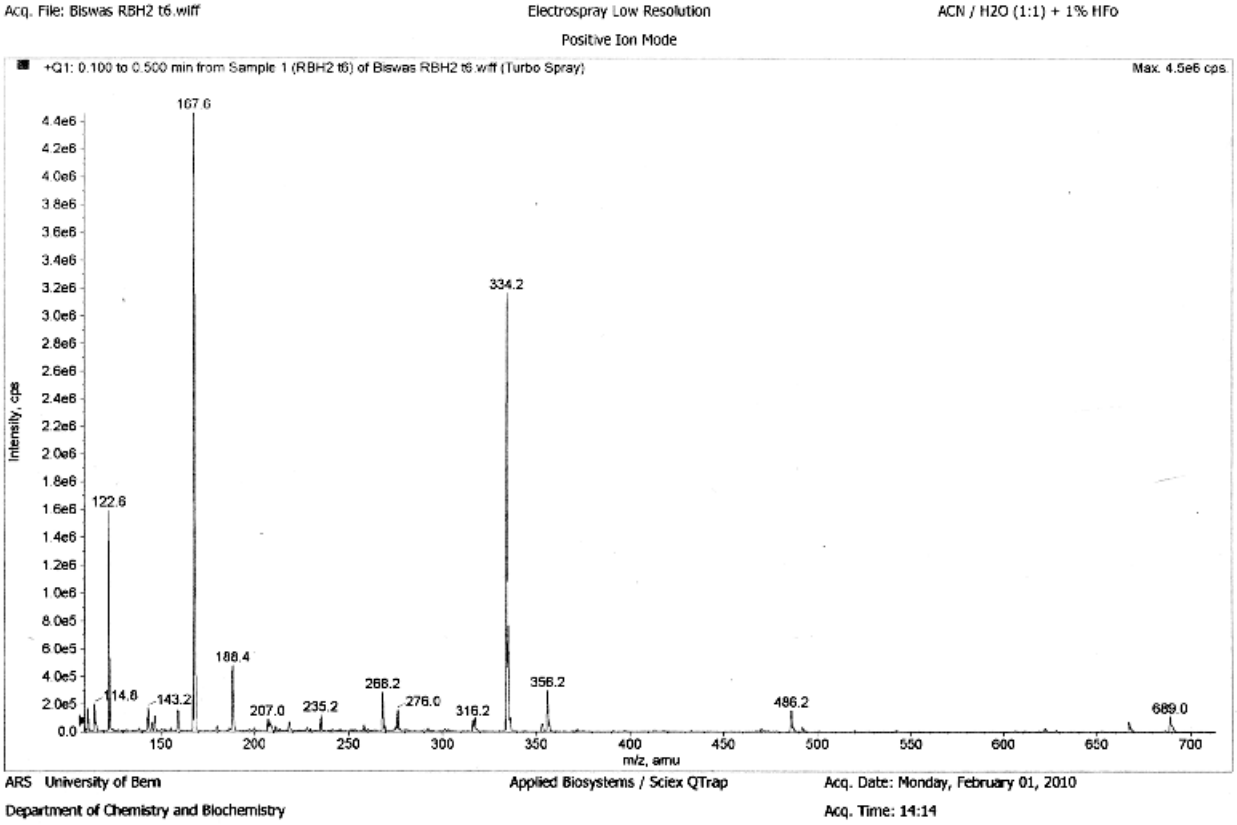
Starting with 100 mg Tentagel HL RAM from Rapp Poylmere (0.39 mmol/gm), the peptide 2-His was obtained using procedure A as colorless solid after cleavage from the resin and preparative RP-HPLC purification (2.9 mg, 13.2 %).



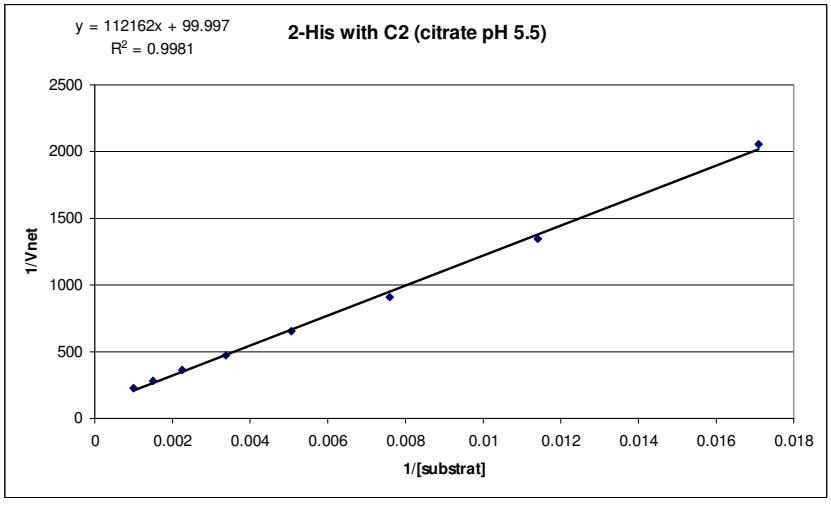
Analytical RP-HPLC: *t*R = 1.49 min (A/D = 100/0 to A/D = 0/100 in 10 min)



MS (ES+) calcd for $C_{14}H_{19}N_7O_3$ $[M+H]^+$ 334.3, found: 334.2; $[M+2H]^{2+}/2$: 167.6, found: 167.6.

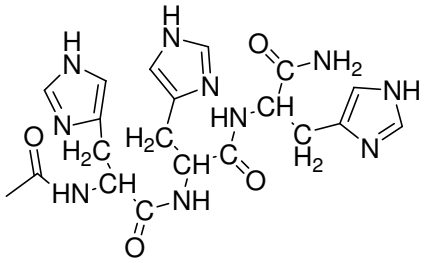


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

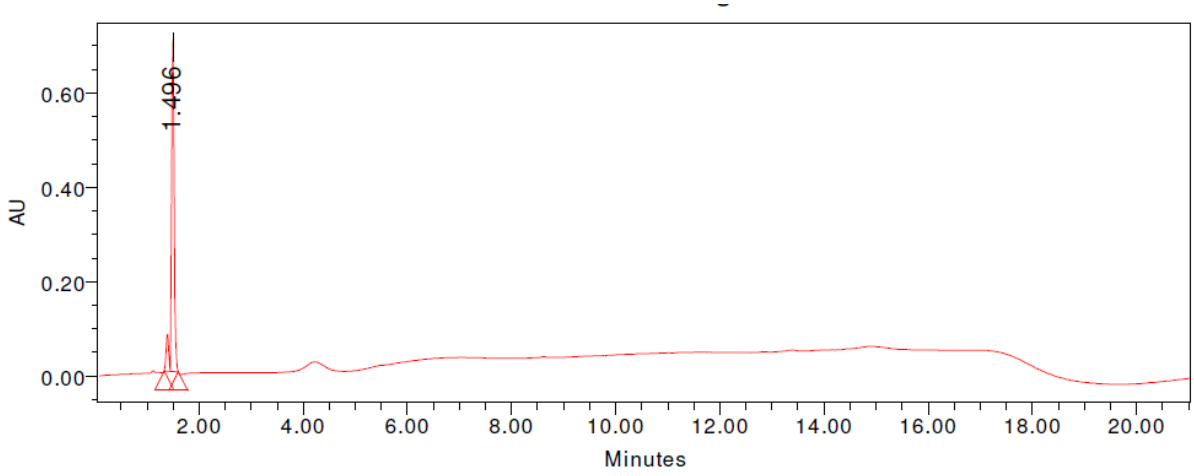


His3: AcHisHisHis-NH_2

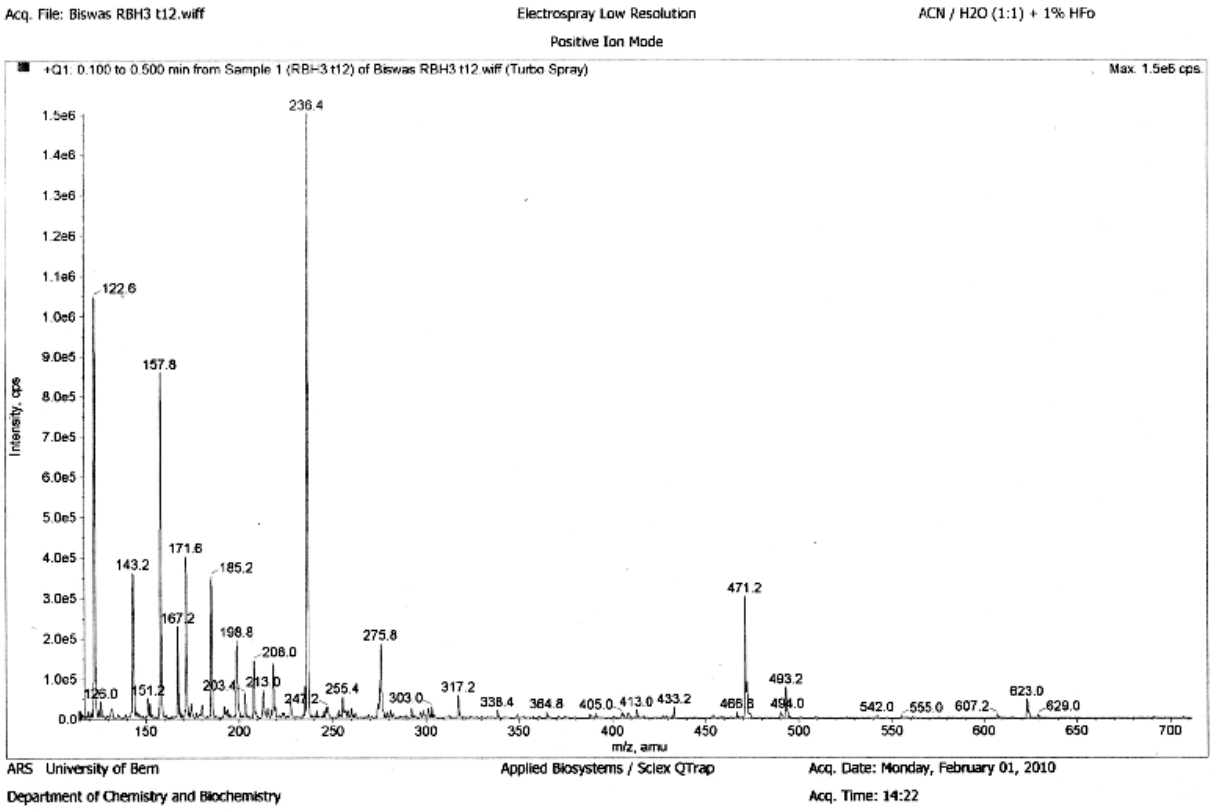
Starting with 100 mg Tentagel HL RAM from Rapp Poylmere (0.39 mmol/gm), the peptide 3-His was obtained using procedure A as colorless solid after cleavage from the resin and preparative RP-HPLC purification (2.3 mg, 7.2 %).



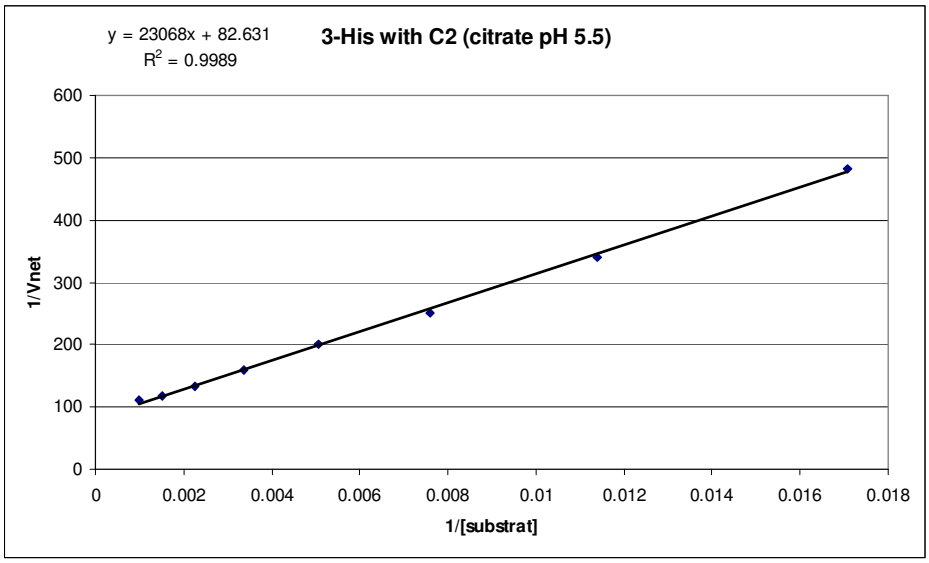
Analytical RP-HPLC: *t*R = 1.5 min (A/D = 100/0 to A/D = 0/100 in 10 min)



MS (ES+) calcd for $C_{20}H_{26}N_{10}O_4$ $[M+H]^+$ 471.49, found: 471.2; $[M+2H]^{2+}/2$: 236.2, found:236.4; $[M+3H]^{2+}/3$ 157.3, found: 157.8.

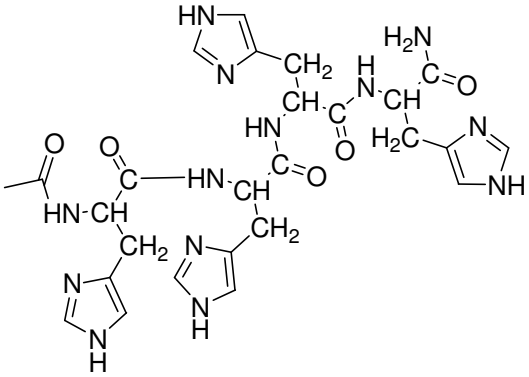


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

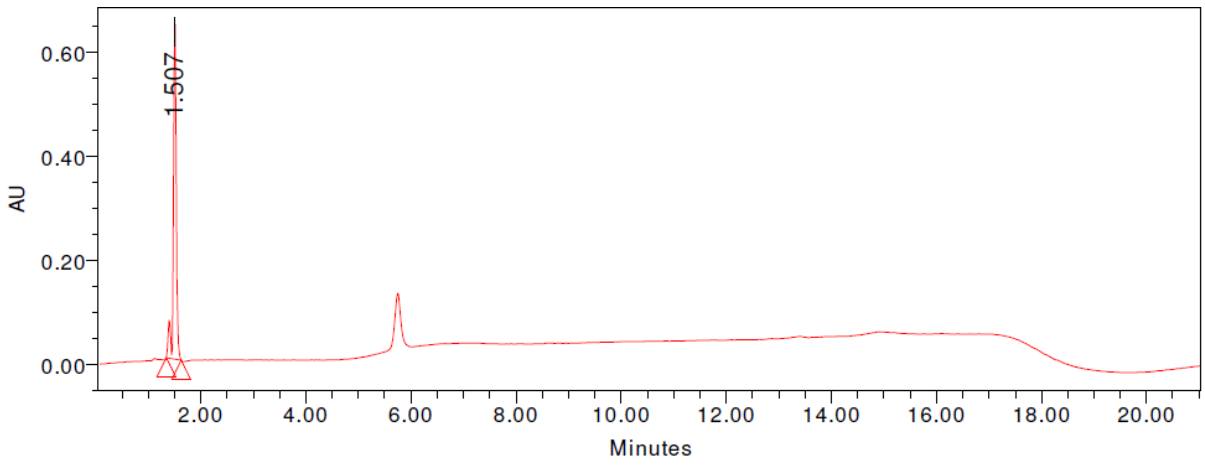


His4: AcHisHisHisHis-NH_2

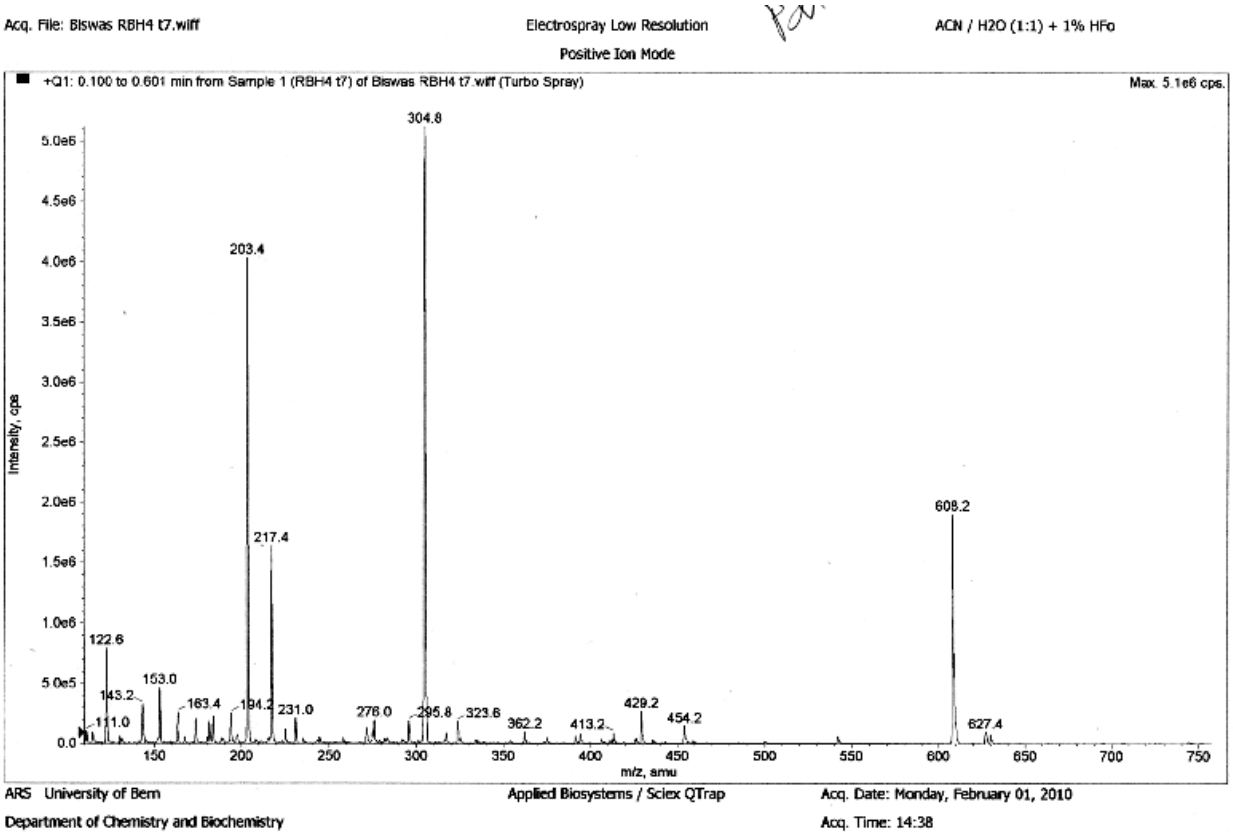
Starting with 100 mg Tentagel HL RAM from Rapp Poylmere (0.39 mmol/gm), the peptide 4-His was obtained using procedure A as colorless solid after cleavage from the resin and preparative RPHPLC purification (2.5 mg, 6 %).



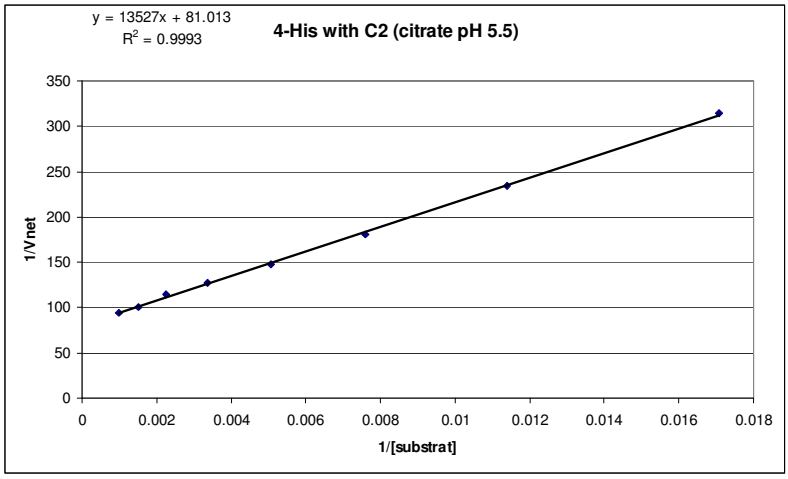
Analytical RP-HPLC: *t*R = 1.5 min (A/D = 100/0 to A/D = 0/100 in 10 min)



MS (ES+) calcd for $C_{26}H_{33}N_{13}O_5$ $[M+H]^+$ 608.6, found: 608.2; $[M+2H]^{2+}/2$: 304.8, found:304.8; $[M+3H]^{3+}/3$: 203.5, found: 203.4; $[M+4H]^{4+}/4$: 152.9, found: 153.0; $[M+5H]^{4+}/5$:122.5, found:122.6.

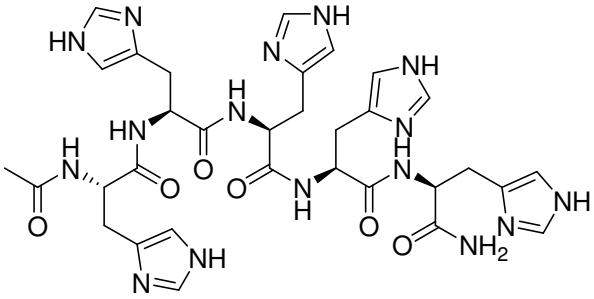


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

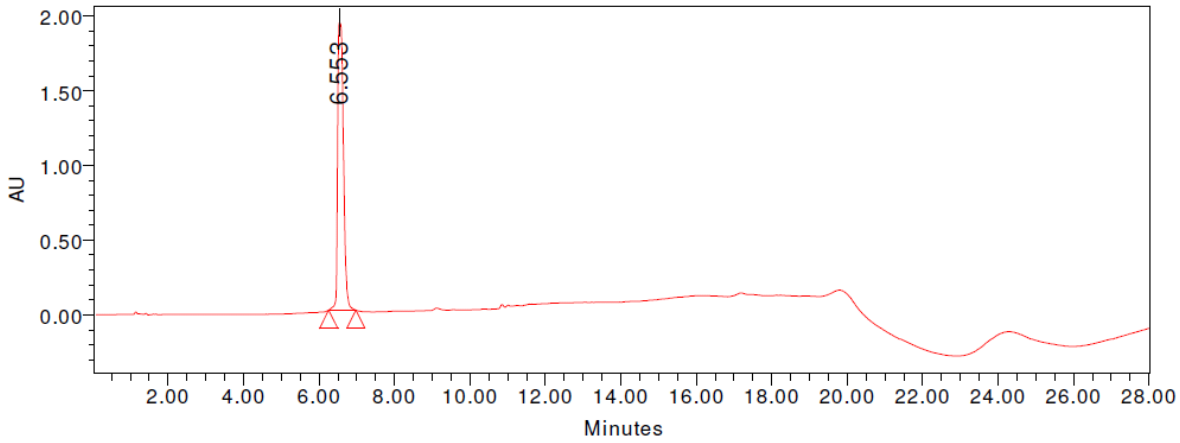


His5: AcHisHisHisHisHis-NH_2

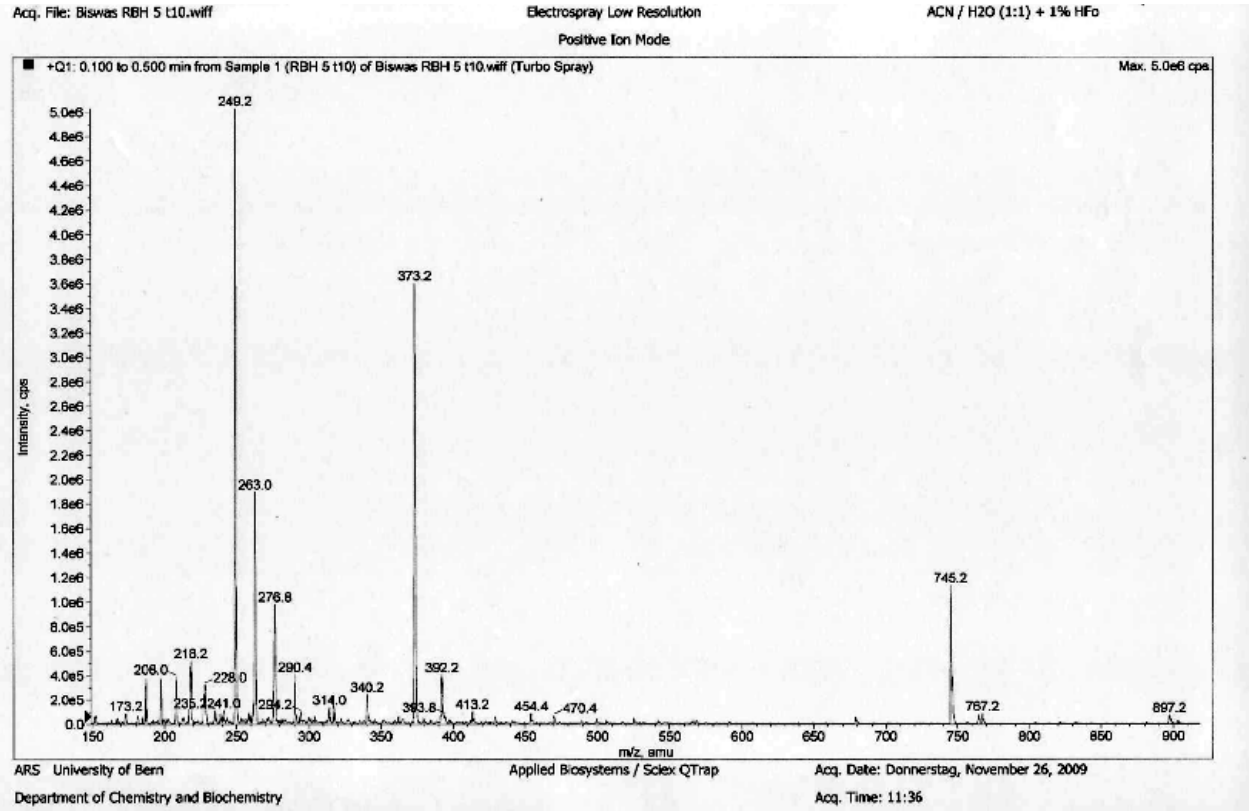
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 5-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (10 mg, 21 %).



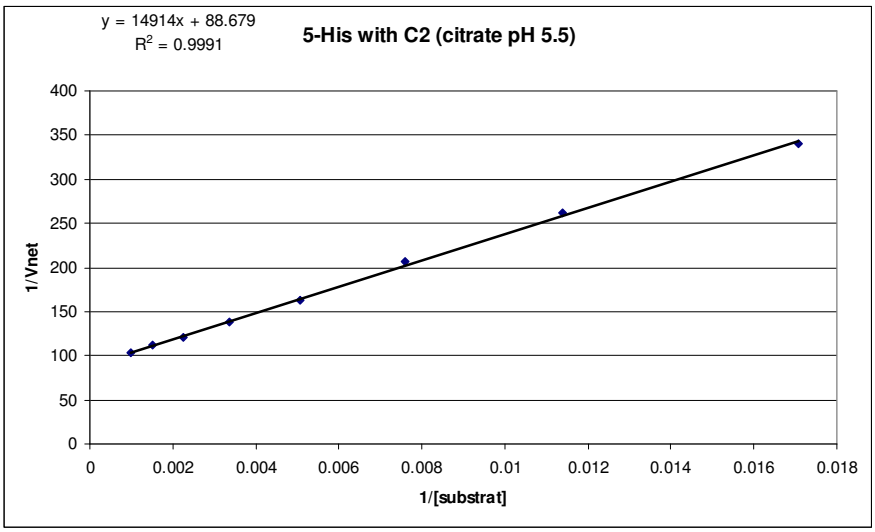
Analytical RP-HPLC: *t*R = 6.53 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{32}H_{40}N_{16}O_6$ $[M+H]^+$ 745.7, found: 745.2; $[M+2H]^{2+}/2$: 373.3, found:373.2; $[M+3H]^{3+}/3$: 249.1, found: 249.2.

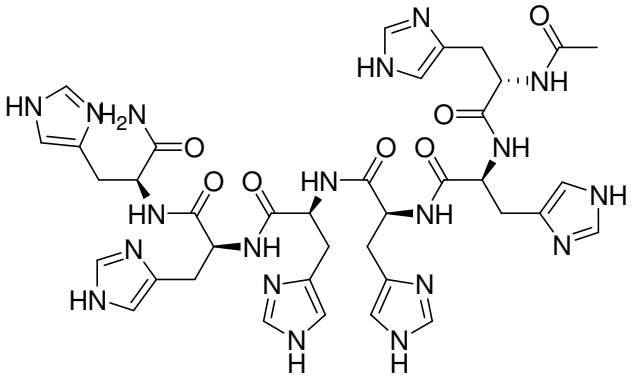


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

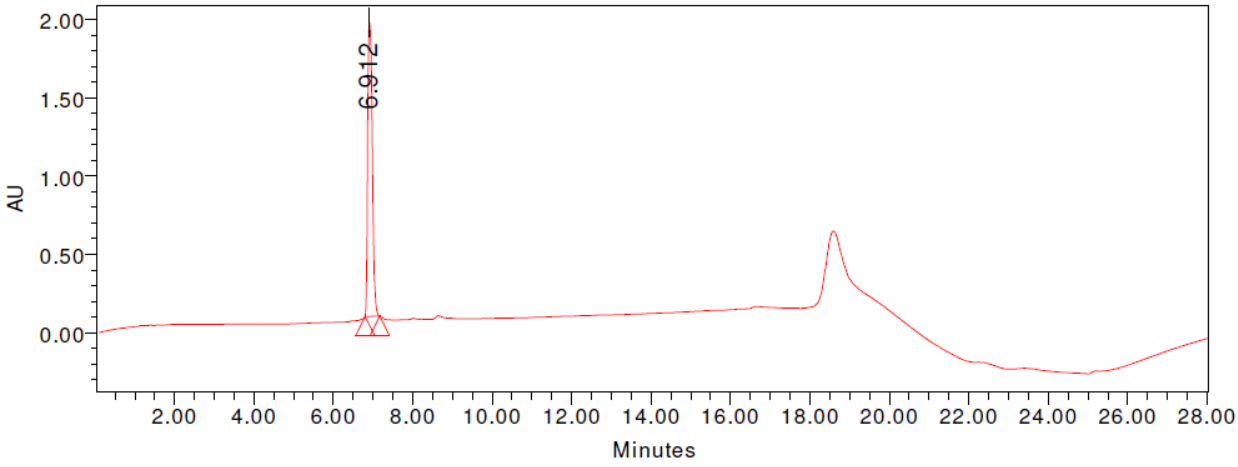


His6: AcHisHisHisHisHisHis-NH_2

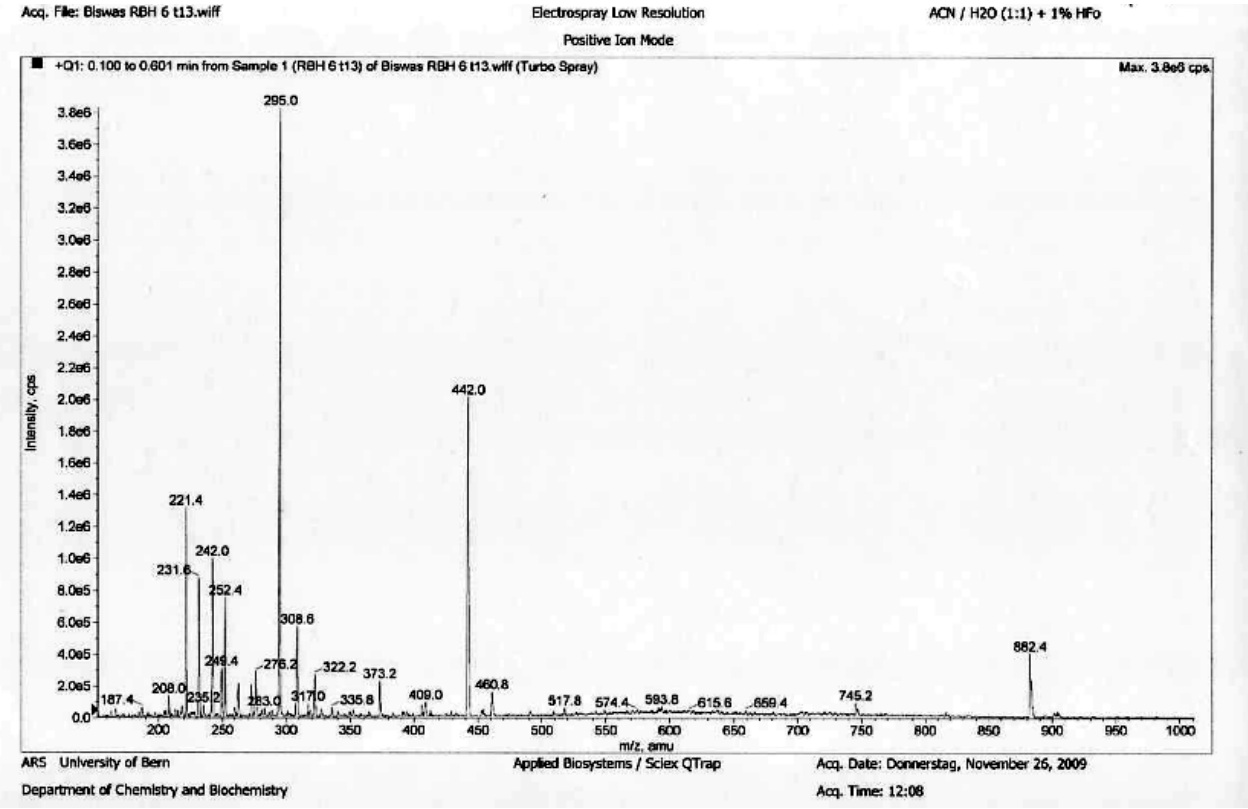
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 6-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (12.3 mg, 22 %).



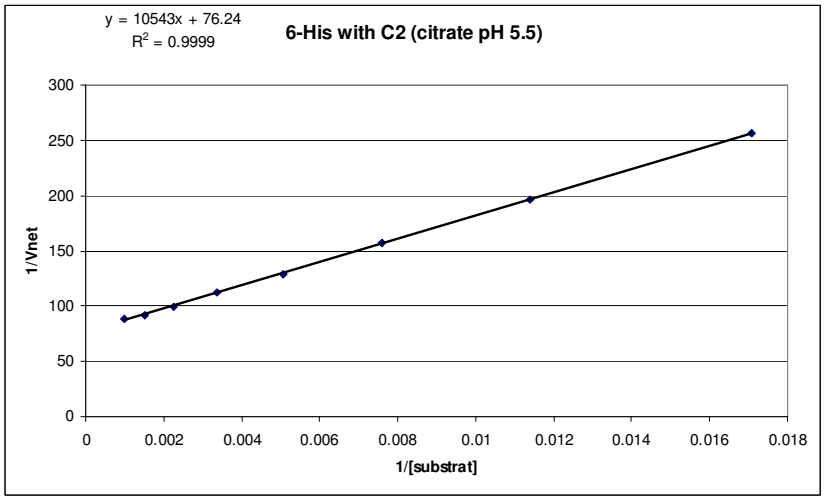
Analytical RP-HPLC: *t*R = 6.9 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{38}H_{47}N_{19}O_7$ $[M+H]^+$ 882.9, found: 882.4; $[M+2H]^{2+}/2$: 441.9, found:442; $[M+3H]^{3+}/3$: 294.9, found: 295; $[M+4H]^{4+}/4$: 221.4, found: 221.4.

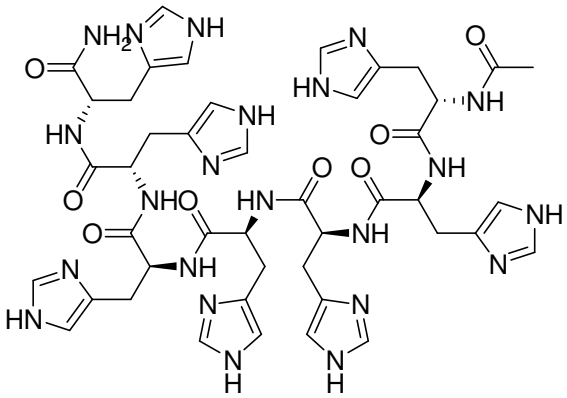


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

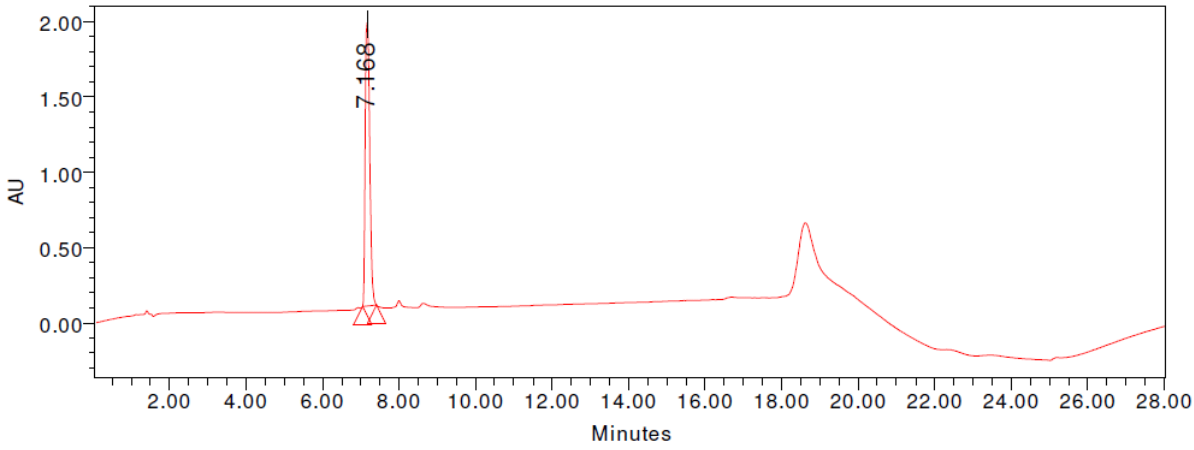


His7: AcHisHisHisHisHisHisHis-NH_2

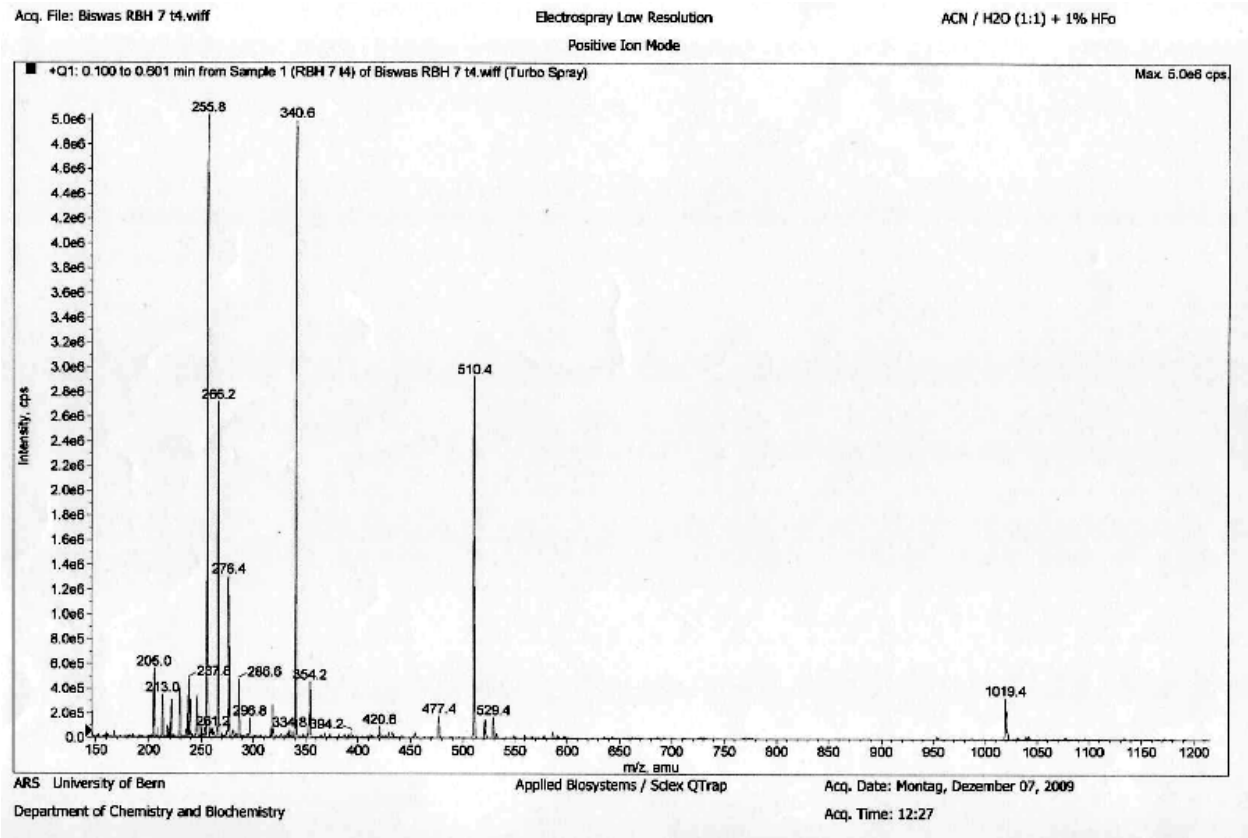
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 5-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (13 mg, 17 %).



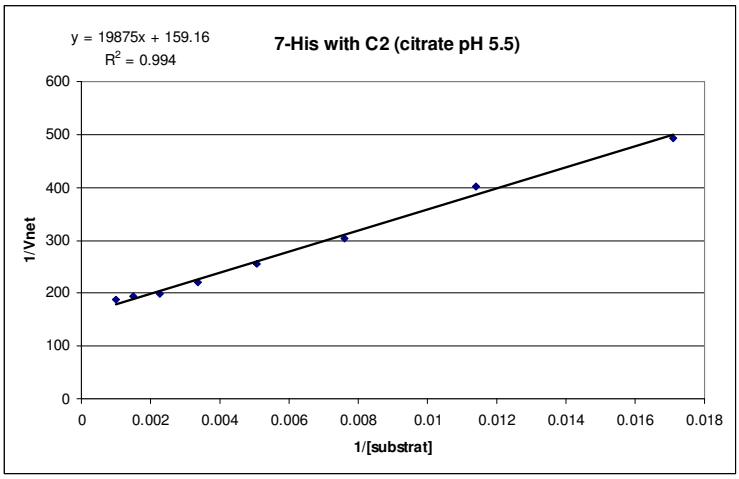
Analytical RP-HPLC: *t*R = 7.2 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{44}H_{54}N_{22}O_8$ $[M+H]^+$ 1020.0, found: 1019.4; $[M+2H]^{2+}/2$: 510.5, found:510.4; $[M+3H]^{3+}/3$: 340.6, found: 340.4; $[M+4H]^{4+}/4$: 255.8, found: 255.8.

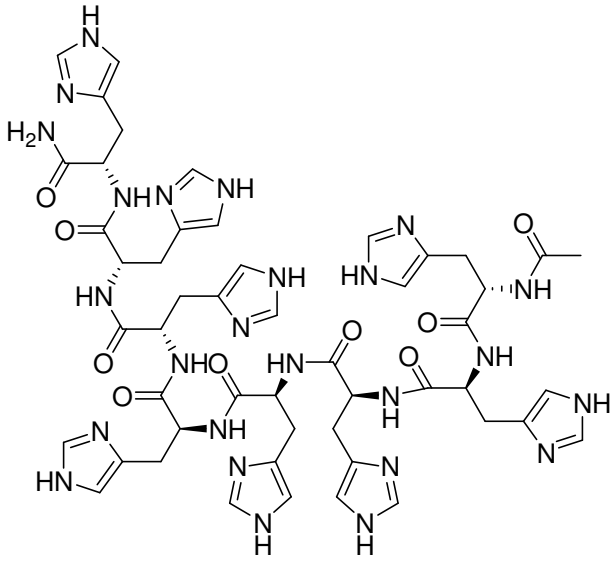


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

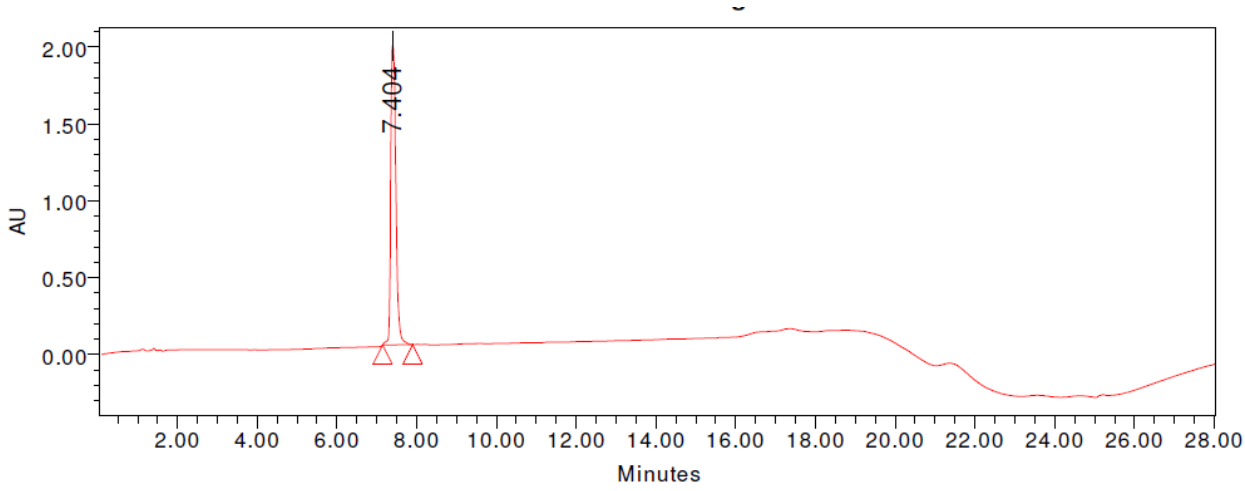


His8: AcHisHisHisHisHisHisHisHis-NH_2

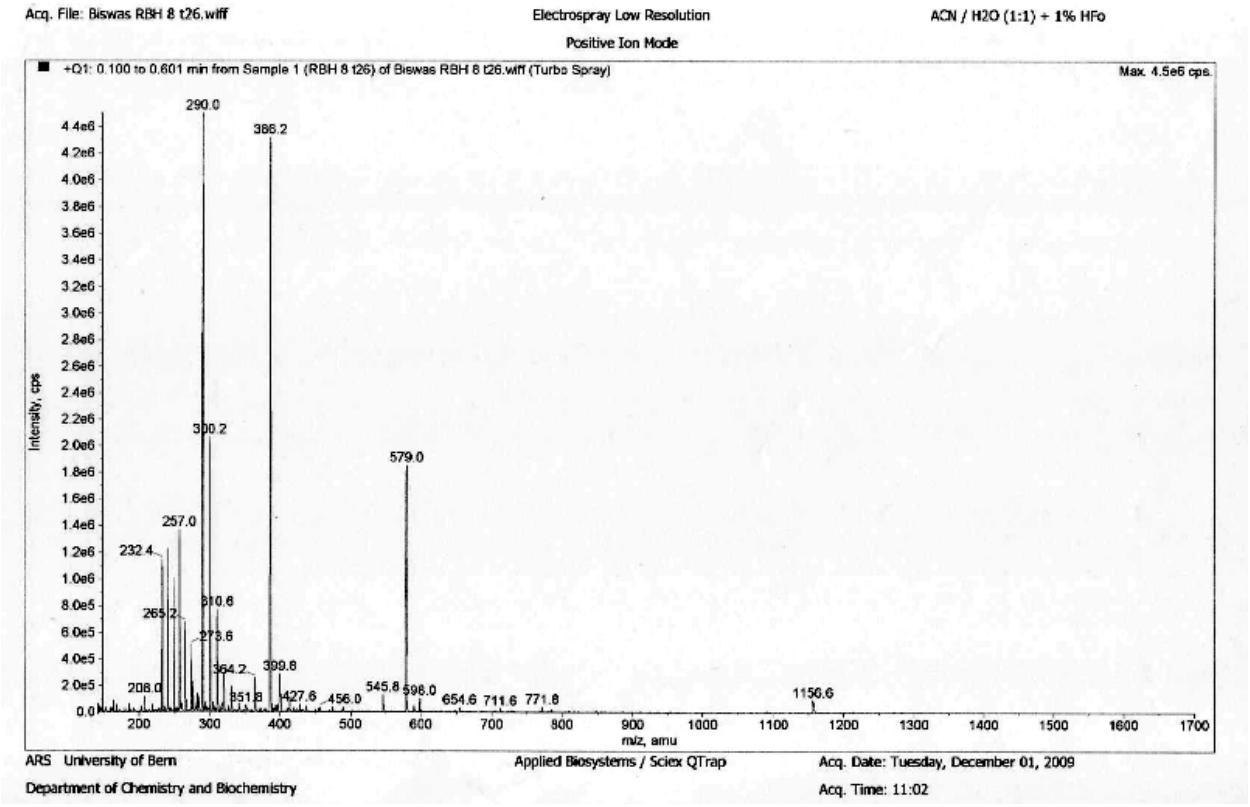
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 8-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (10.5 mg, 14 %).



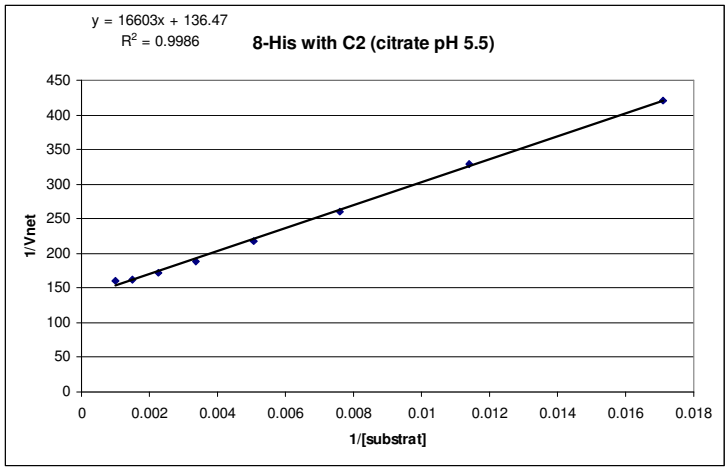
Analytical RP-HPLC: *t*R = 7.4 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{50}H_{61}N_{25}O_9$ $[M+H]^+$ 1157.2, found: 1156.6; $[M+2H]^{2+}/2$: 579.1, found:579.0; $[M+3H]^{3+}/3$: 386.4, found: 386.2; $[M+4H]^{4+}/4$: 290.0, found: 290.0.

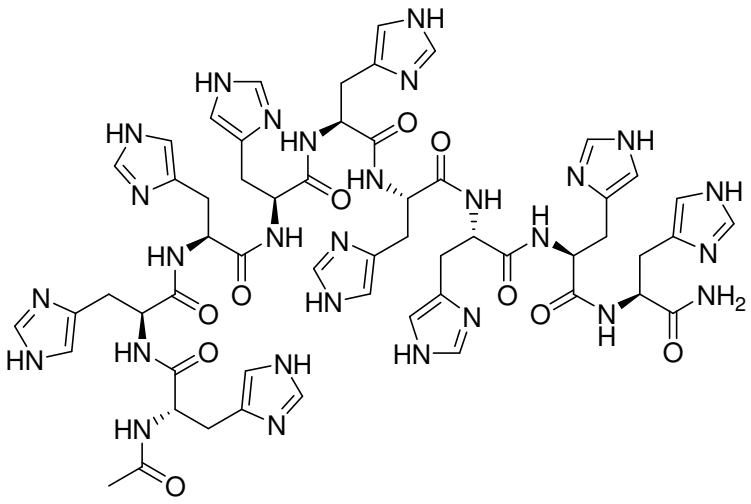


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

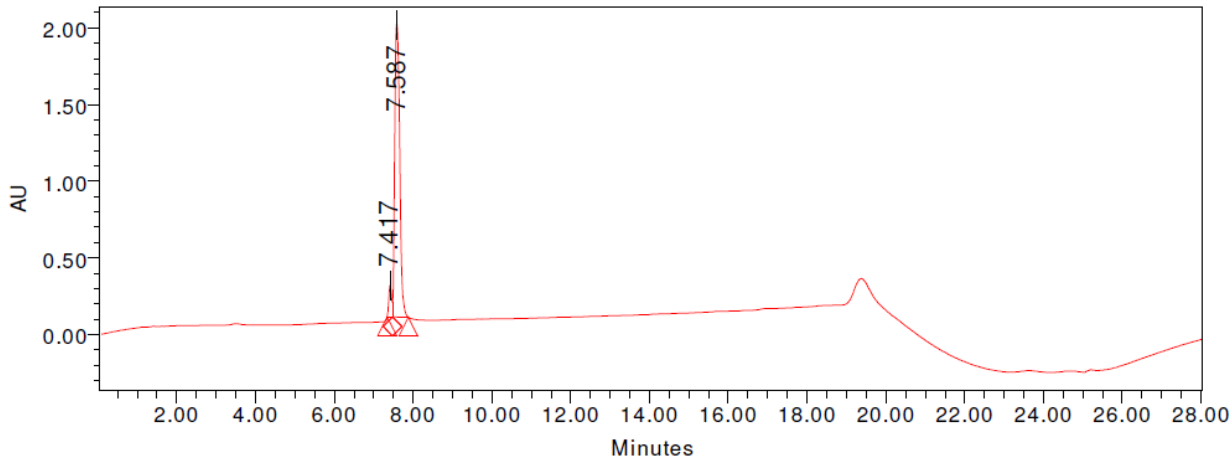


His9: AcHisHisHisHisHisHisHisHisHis-NH_2

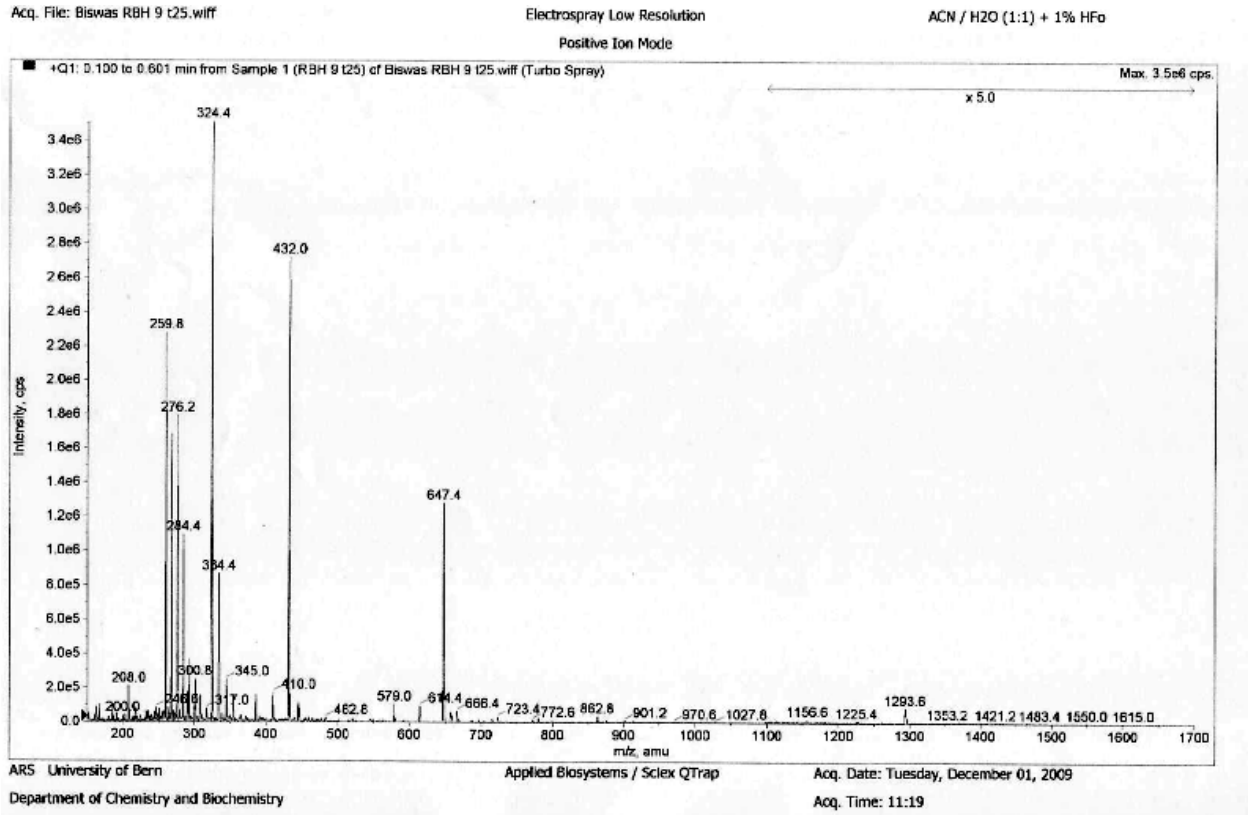
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 5-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (14.3 mg, 17 %).



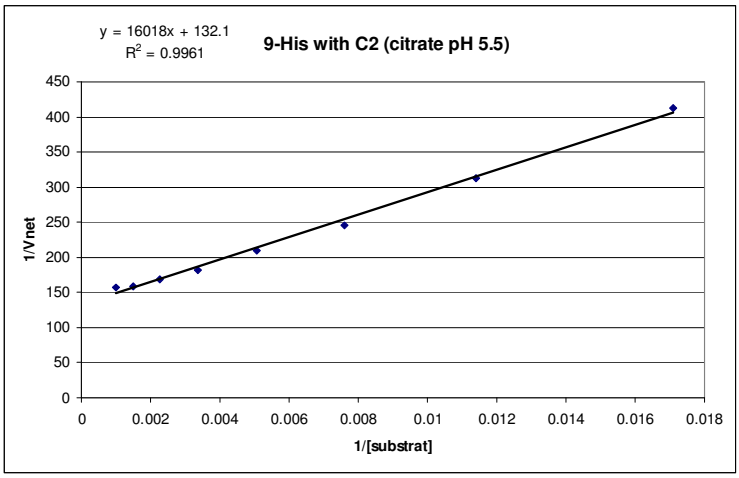
Analytical RP-HPLC: *t*R = 7.6 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{56}H_{68}N_{28}O_{10}$ $[M+H]^+$ 1294.2, found: 1293.6; $[M+2H]^{2+}$/2: 647.6, found:647.4; $[M+3H]^{3+}$/3: 432.0, found: 432.0; $[M+4H]^{4+}$/4: 324.3, found: 324.4; $[M+5H]^{5+}$/5: 259.6, found: 259.8.



Representative Michaelis-Menten plots for determination of *kcat* and *K*M



His10: AcHisHisHisHisHisHisHisHisHisHis-NH_2

Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 10-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (14.9 mg, 16 %)

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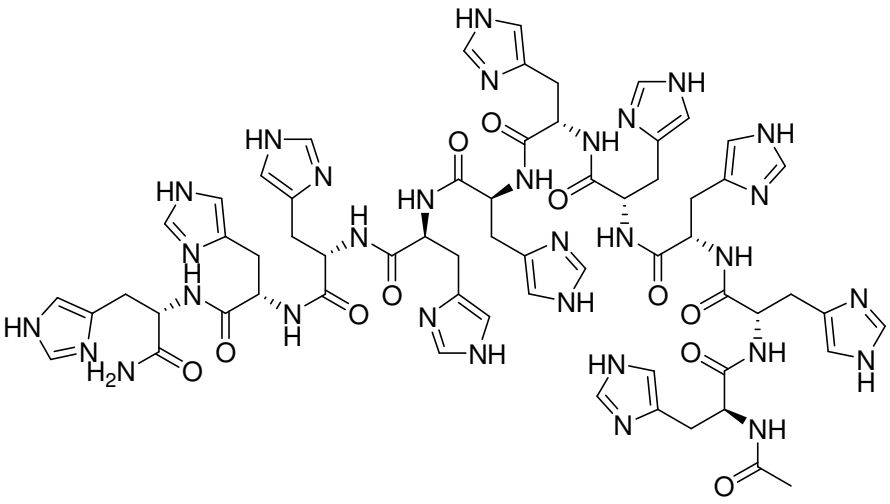
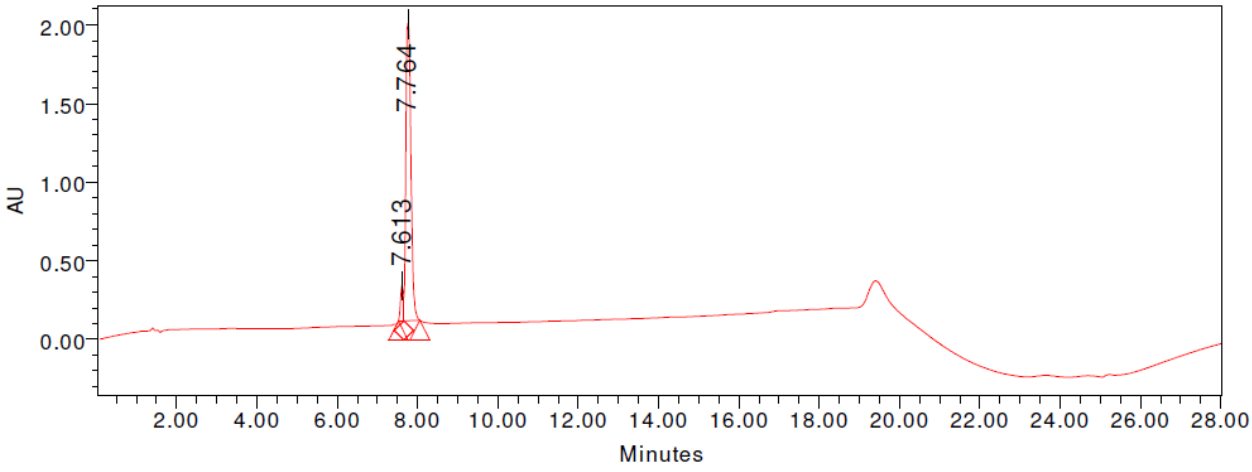
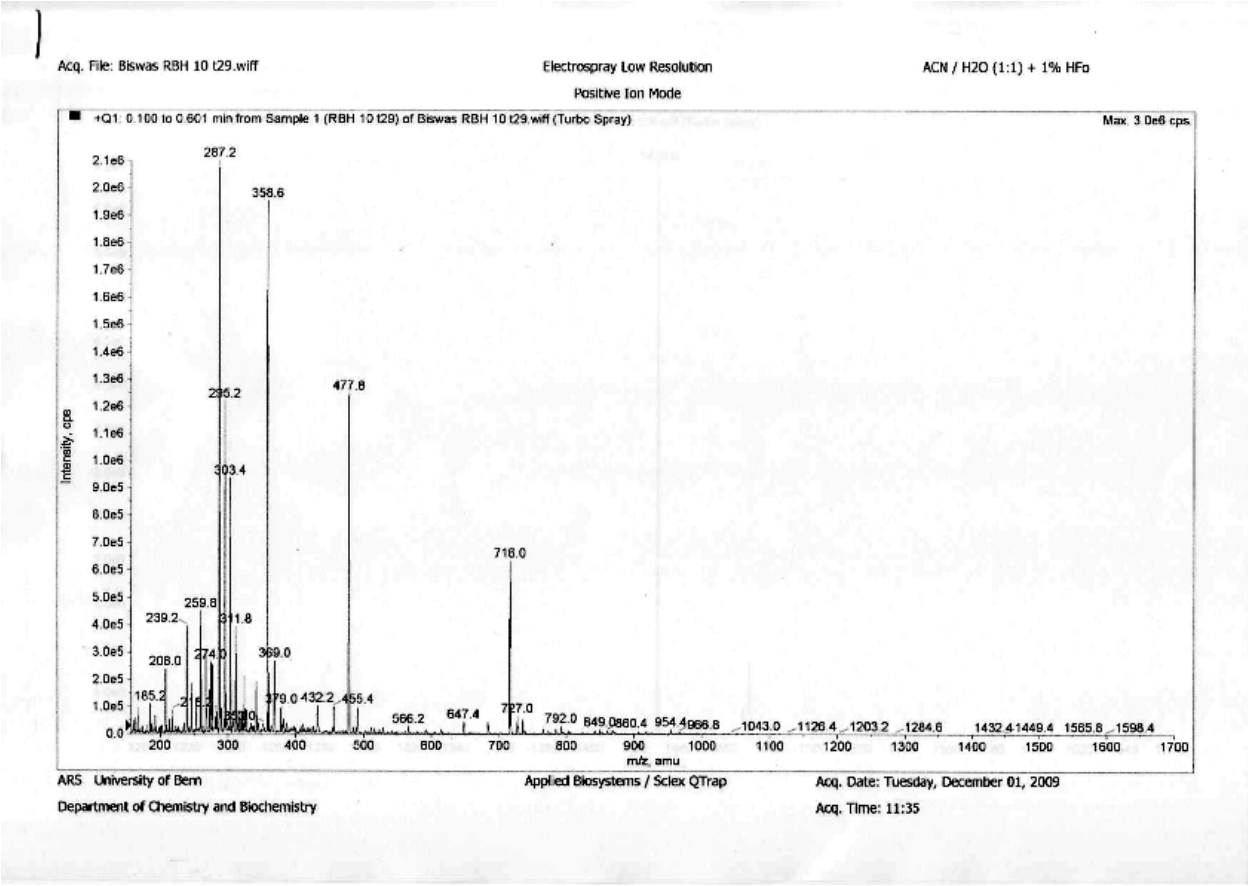


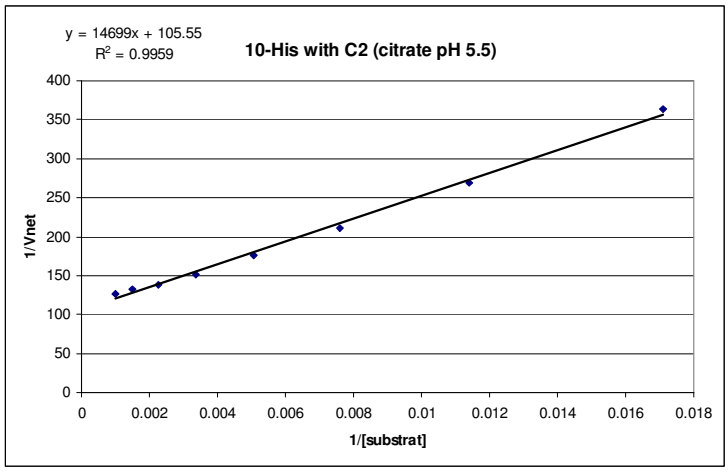
Analytical RP-HPLC: *t*R = 7.8 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{62}H_{75}N_{31}O_{11}$ M: 1430.46, $[M+2H]^{2+}/2$: 715.7, found:716; $[M+3H]^{3+}/3$: 477.8.0, found: 477.8; $[M+4H]^{4+}/4$: 324.3, found: 324.4; $[M+5H]^{5+}/5$: 259.6, found: 259.8.

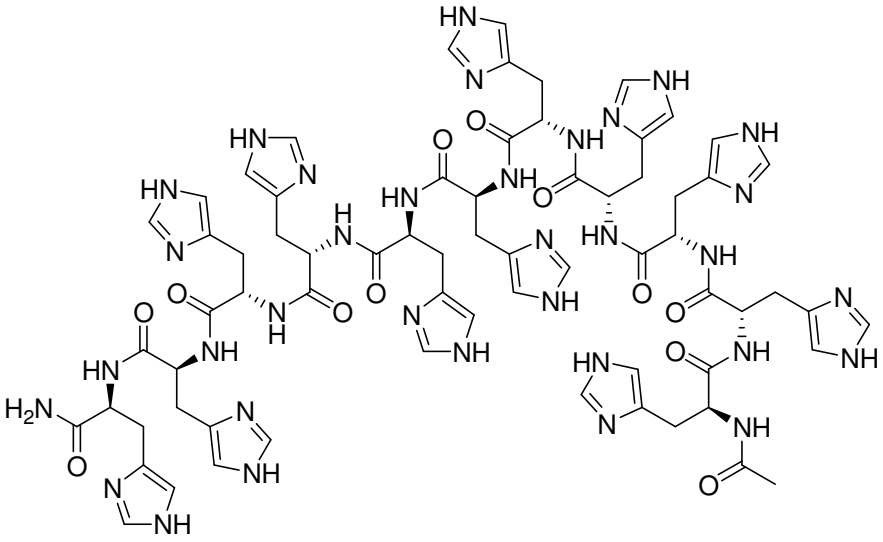


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

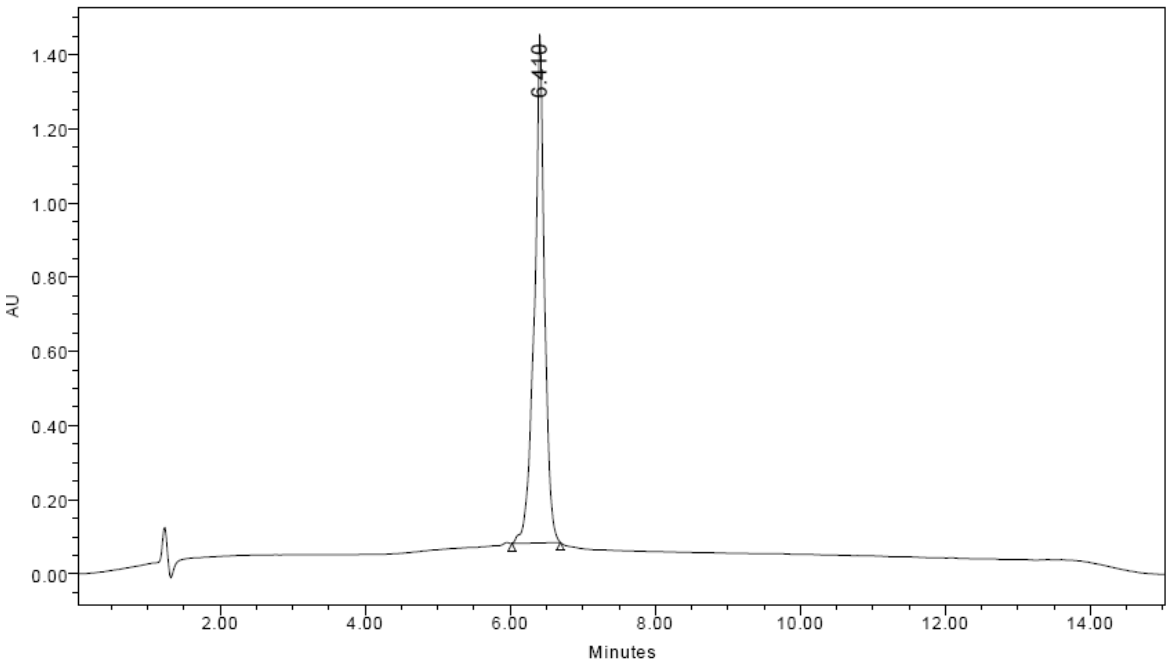


His11: AcHisHisHisHisHisHisHisHisHisHisHis-NH_2

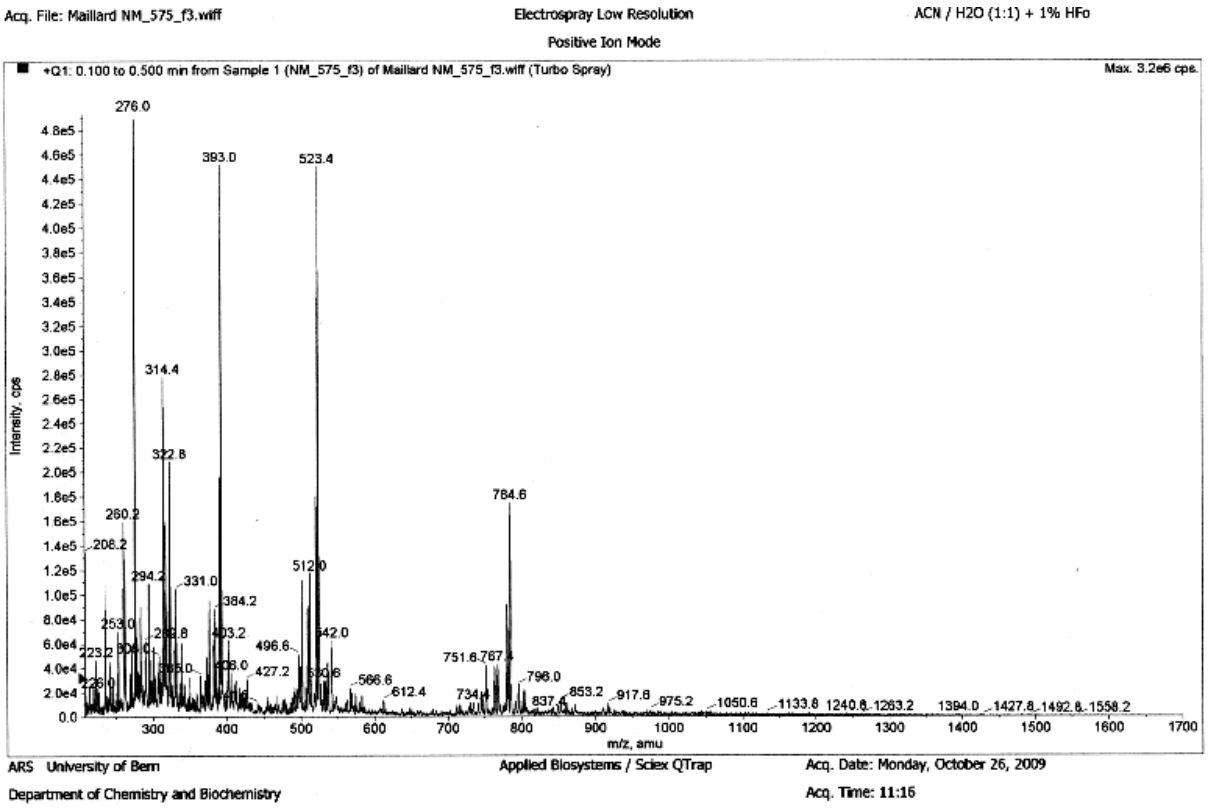
Starting with 300 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 11-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (18 mg, 9%).



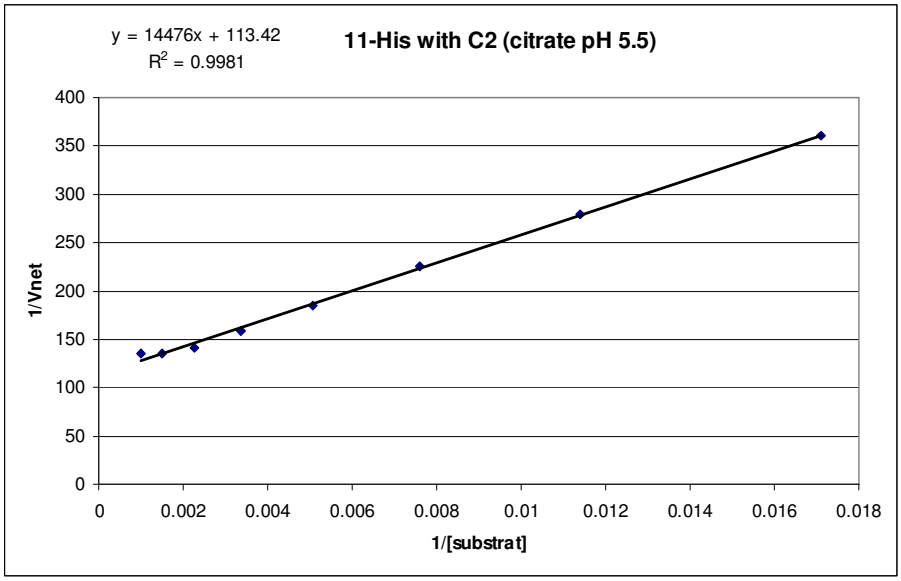
Analytical RP-HPLC: *t*R = 6.4 min (A/D = 100/0 to A/D = 0/100 in 10 min)



MS (ES+) calcd for $C_{68}H_{83}N_{34}O_{12}$ M: 1566.7, $[M+2H]^{2+}/2$: 784.3, found:784.8; $[M+3H]^{3+}/3$: 523.2, found: 523.4; $[M+4H]^{4+}/4$: 392.6, found: 393.0; $[M+5H]^{5+}/5$: 314.3, found: 314.4.

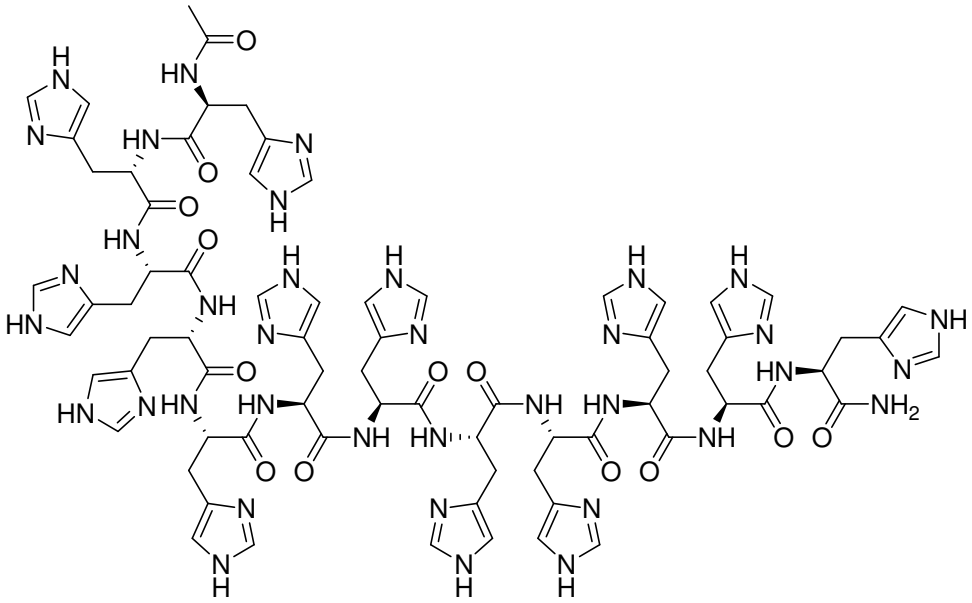


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

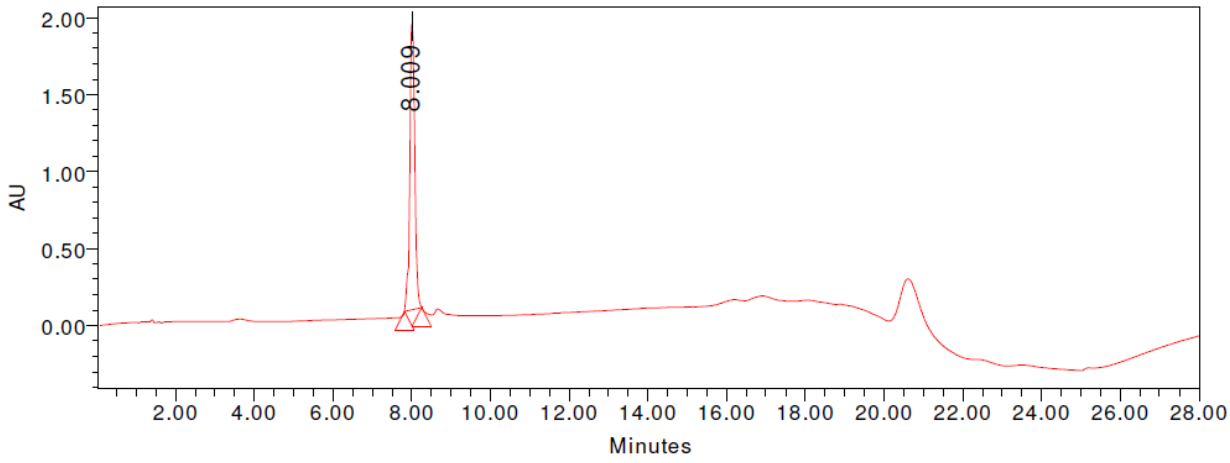


His12: AcHisHisHisHisHisHisHisHisHisHisHis-NH_2

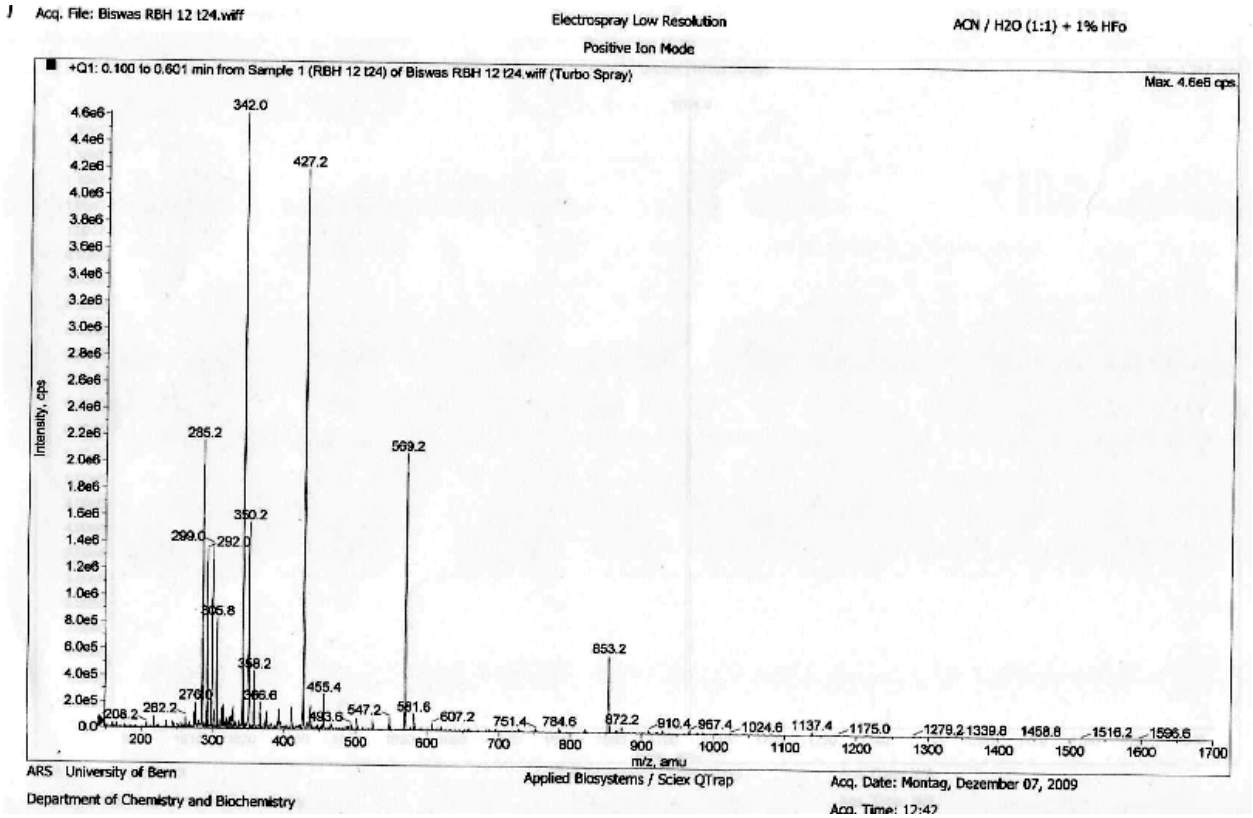
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 12-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (15.3 mg, 14 %).



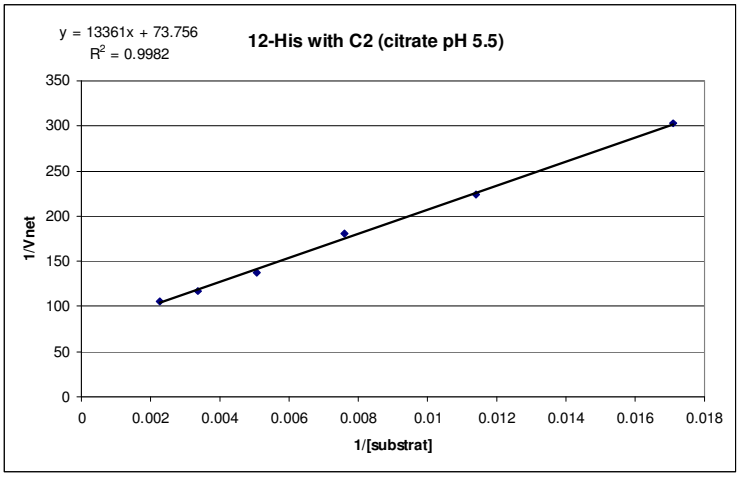
Analytical RP-HPLC: *t*R = 8.0 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{74}H_{89}N_{37}O_{13}$ M: 1704.7, $[M+2H]^{2+}$/2: 853.3, found:853.2; $[M+3H]^{3+}$/3: 569.2, found: 569.2; $[M+4H]^{4+}$/4: 427.2, found: 427.2; $[M+5H]^{5+}$/5: 341.9, found: 342.0; $[M+6H]^{6+}$/6: 285.1, found 285.2.

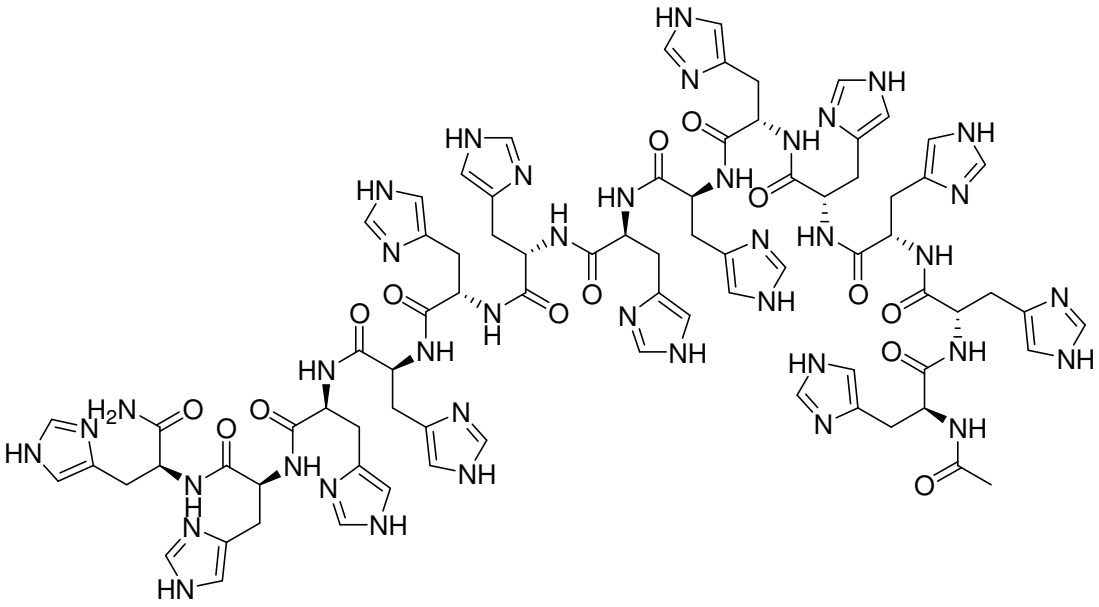


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

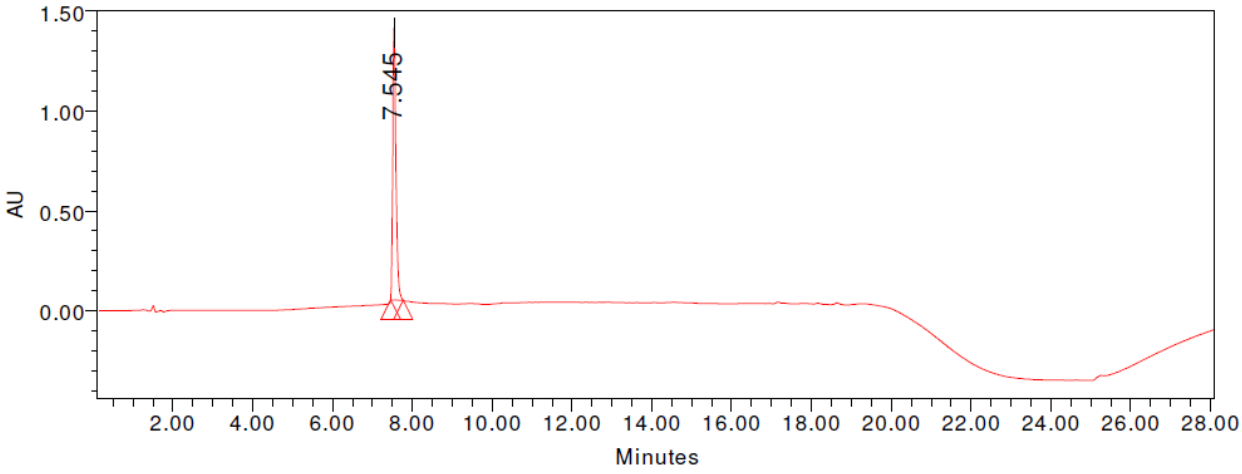


His13: AcHisHisHisHisHisHisHisHisHisHisHisHis-NH_2

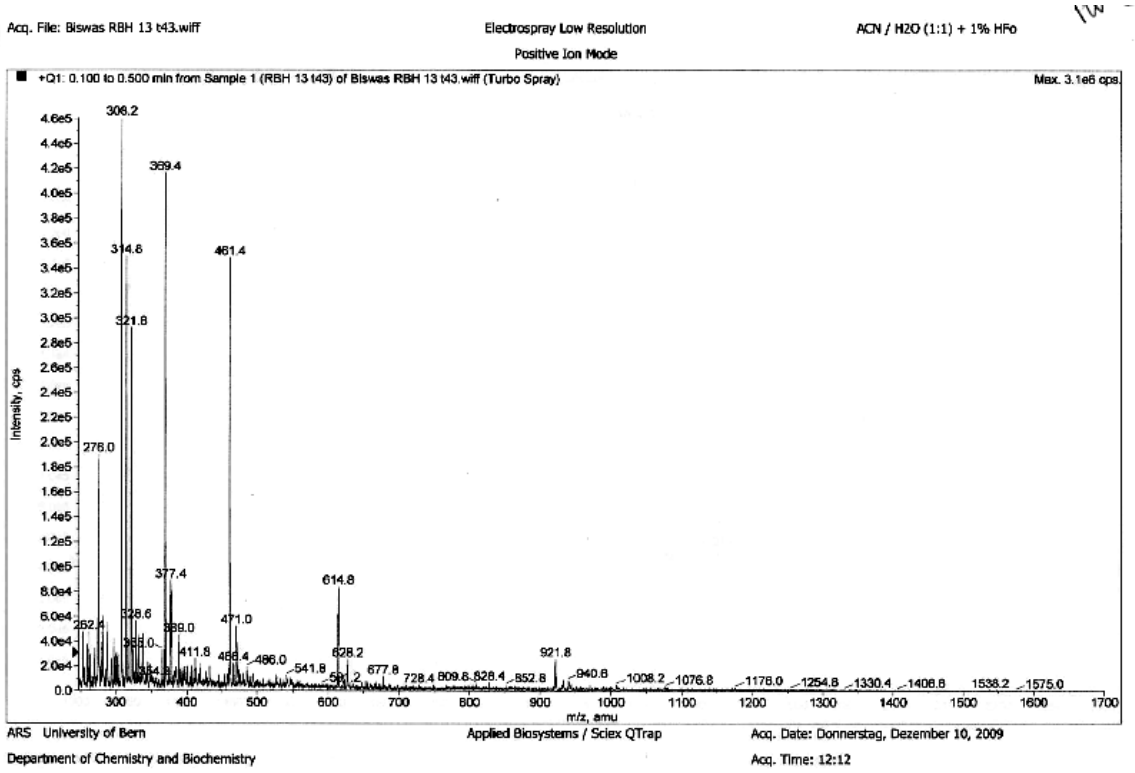
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 13-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (16mg, 13 %).



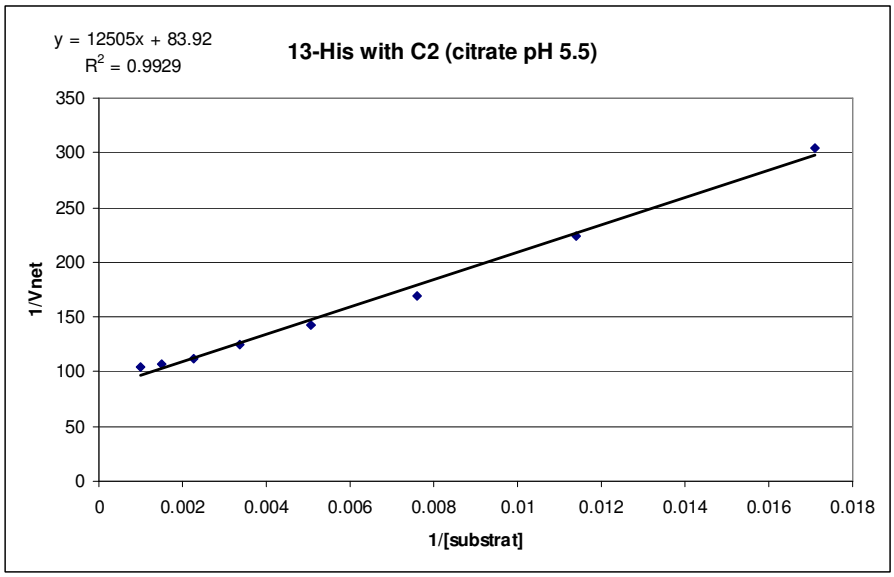
Analytical RP-HPLC: *t*R = 7.5 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{80}H_{96}N_{40}O_{14}$ M: 1841.9, $[M+2H]^{2+}$/2: 921.5, found:921.8; $[M+3H]^{3+}$/3: 614.9, found: 614.8; $[M+4H]^{4+}$/4: 461.5, found: 461.4; $[M+5H]^{5+}$/5: 369.4, found: 369.4; $[M+6H]^{6+}$/6: 308.0, found 308.2.

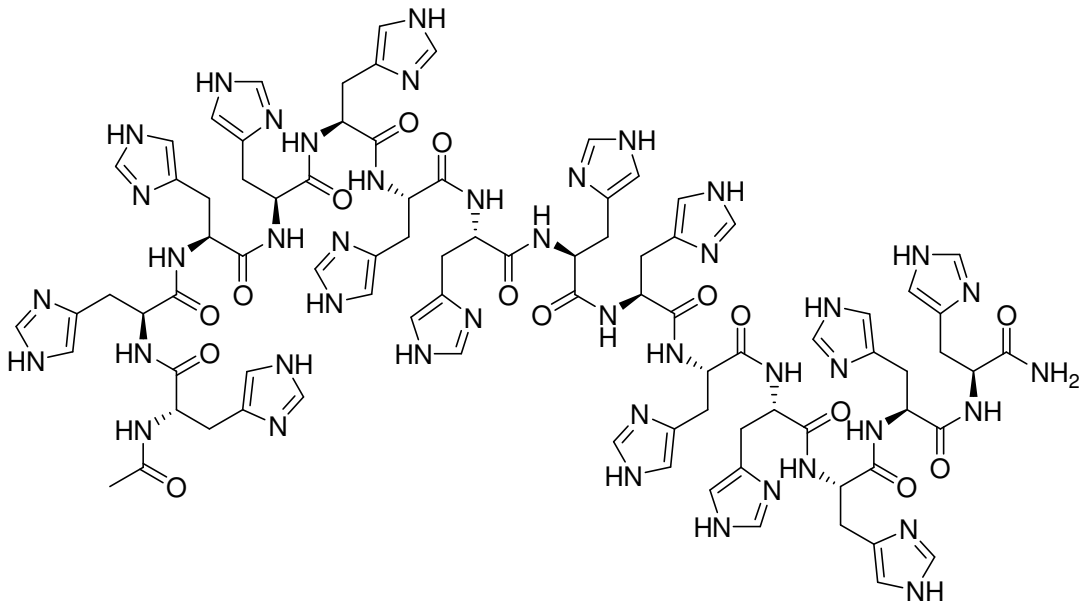


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

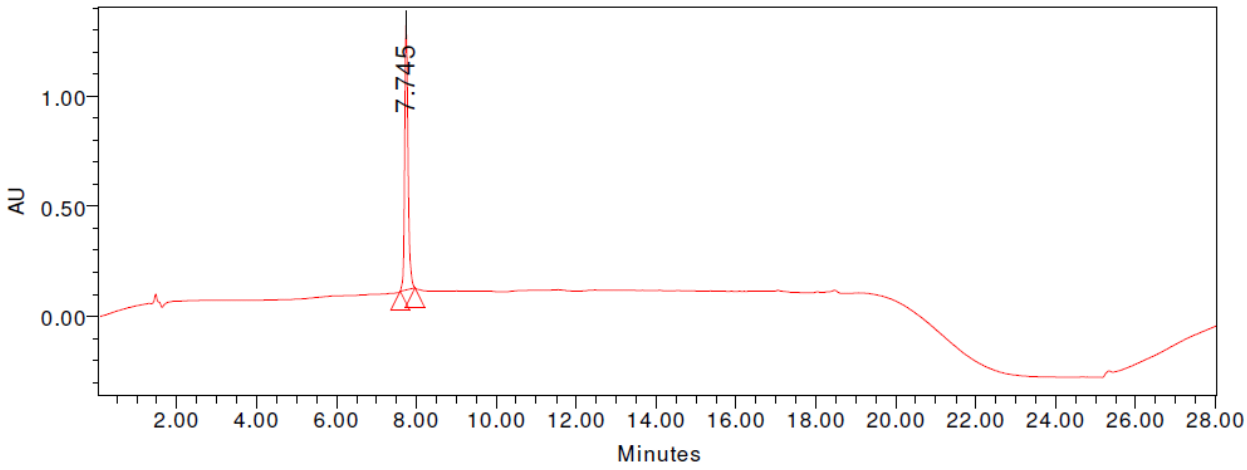


His14: AcHisHisHisHisHisHisHisHisHisHisHisHisHis-NH_2

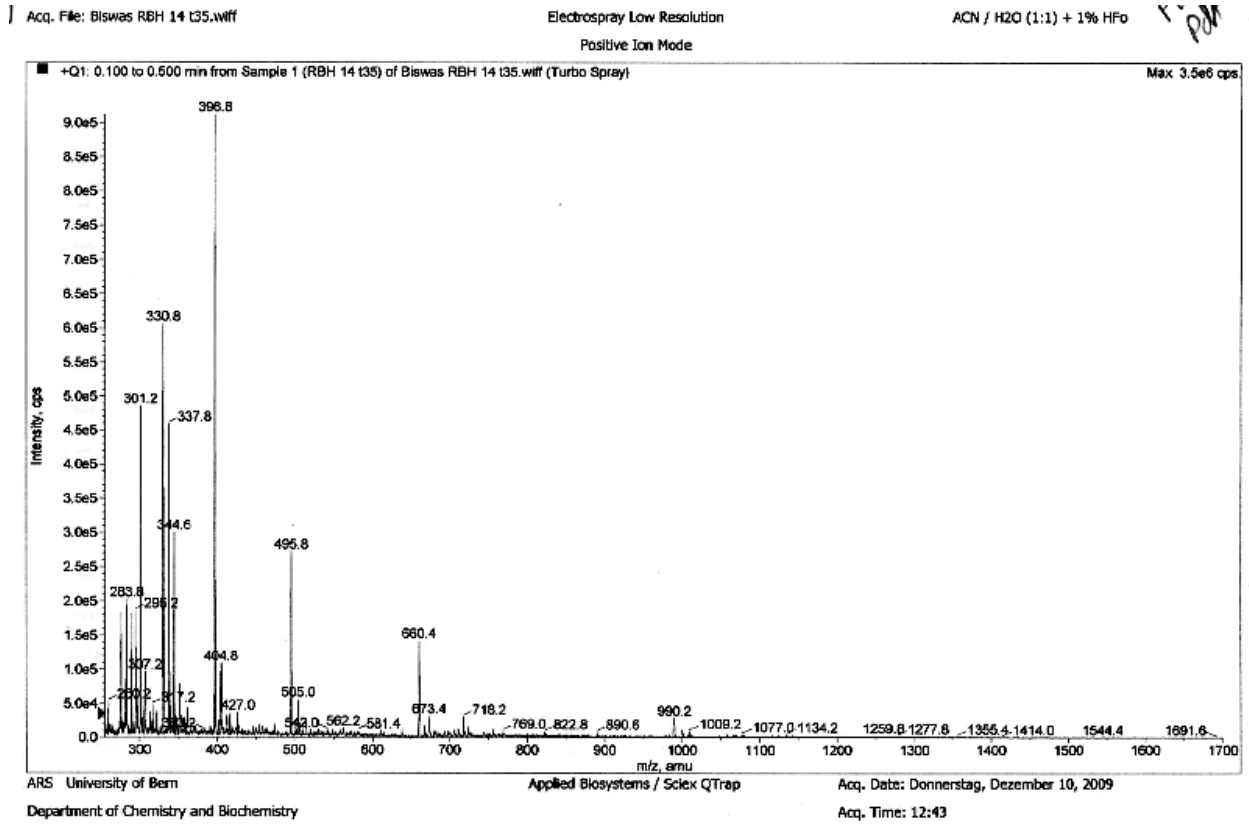
Starting with 150 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 14-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (7.4mg, 6 %).



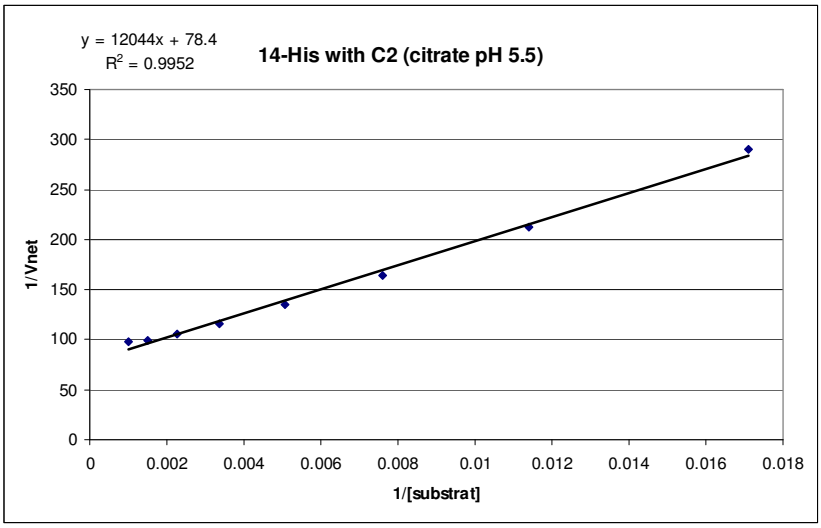
Analytical RP-HPLC: *t*R = 7.7 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{86}H_{103}N_{43}O_{15}$ M:1979.0, $[M+2H]^{2+}$/2: 990.5, found:990.2; $[M+3H]^{3+}$/3: 660.6, found: 660.4; $[M+4H]^{4+}$/4: 495.7, found: 495.8; $[M+5H]^{5+}$/5: 396.8, found: 396.8; $[M+6H]^{6+}$/6: 330.8, found 330.8.

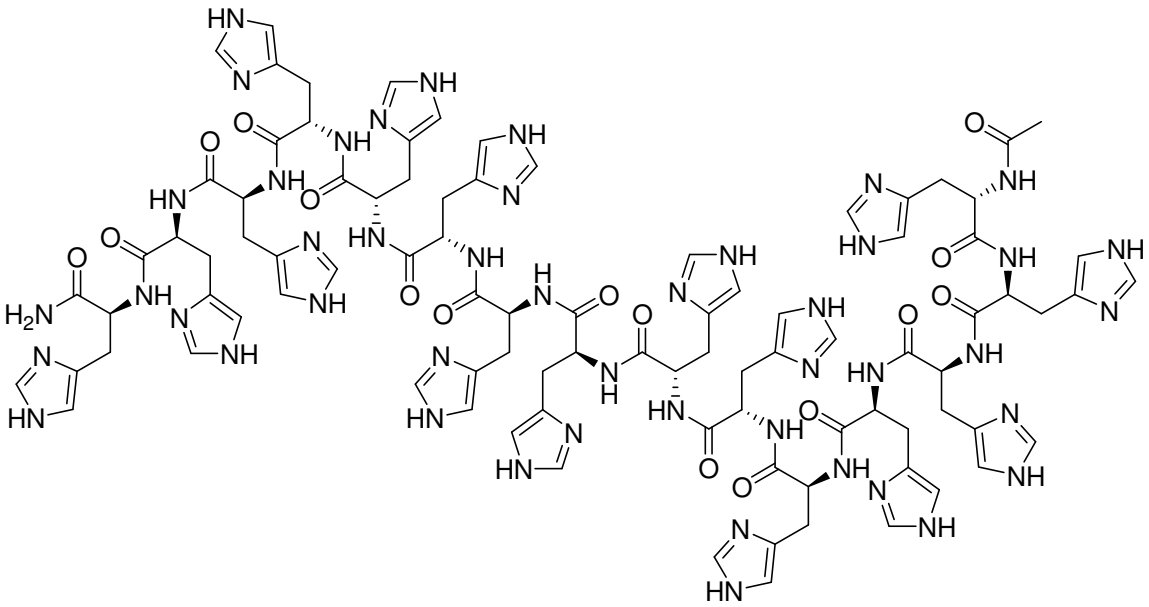


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

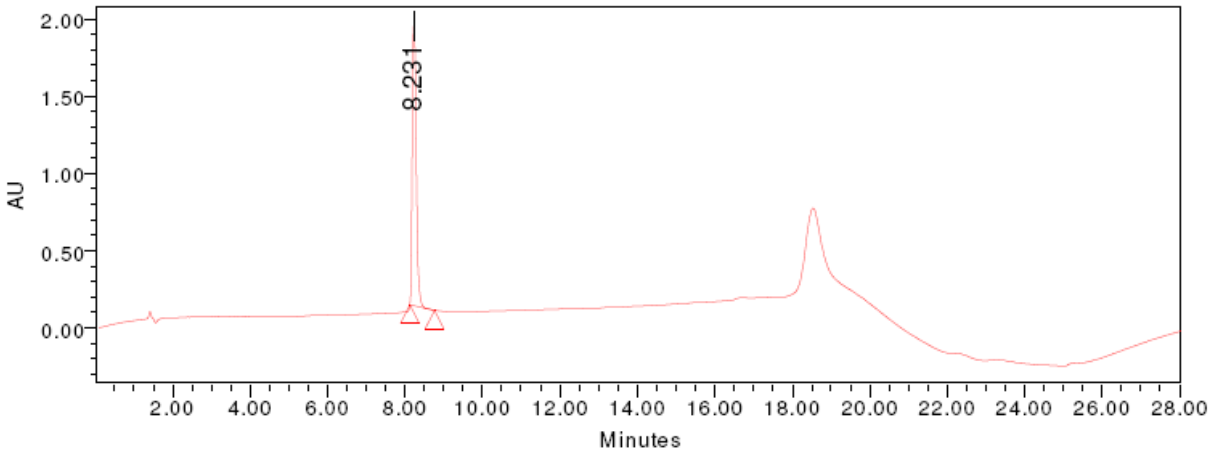


His15: AcHisHisHisHisHisHisHisHisHisHisHisHisHisHis-NH_2

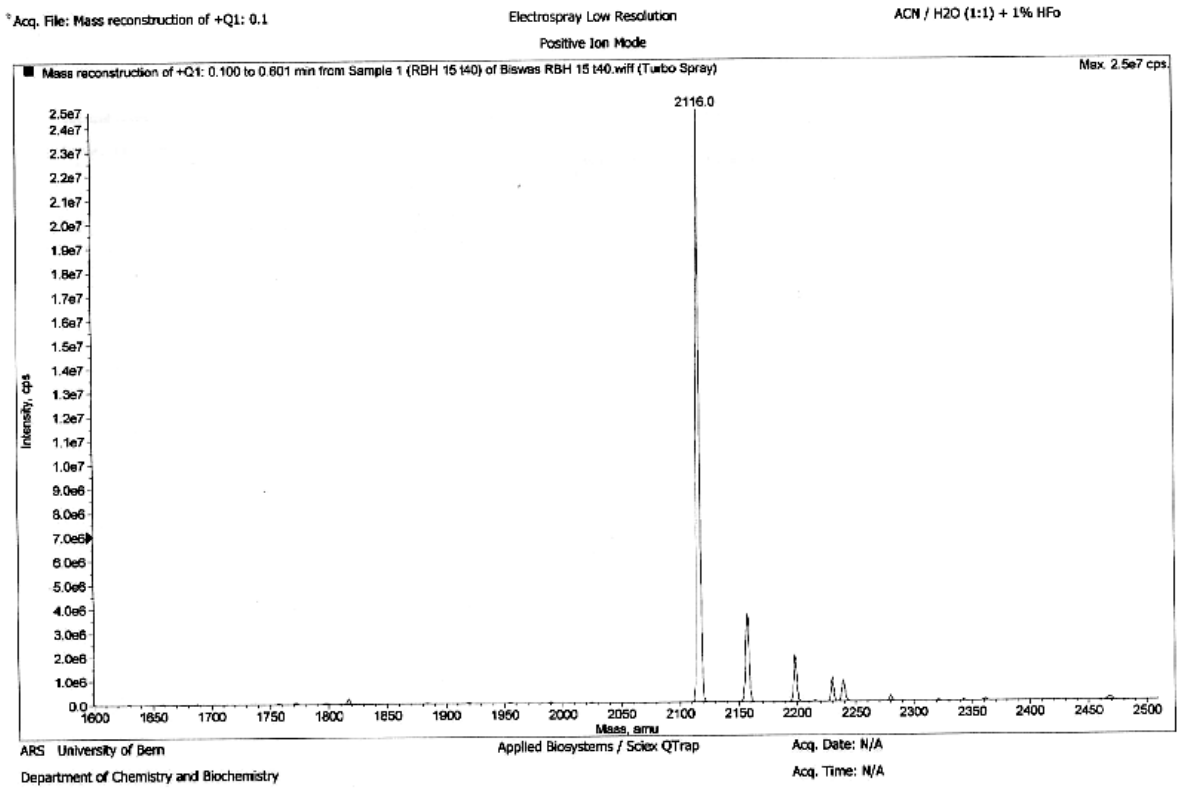
Starting with 300 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide 15-His was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (34mg, 12 %).



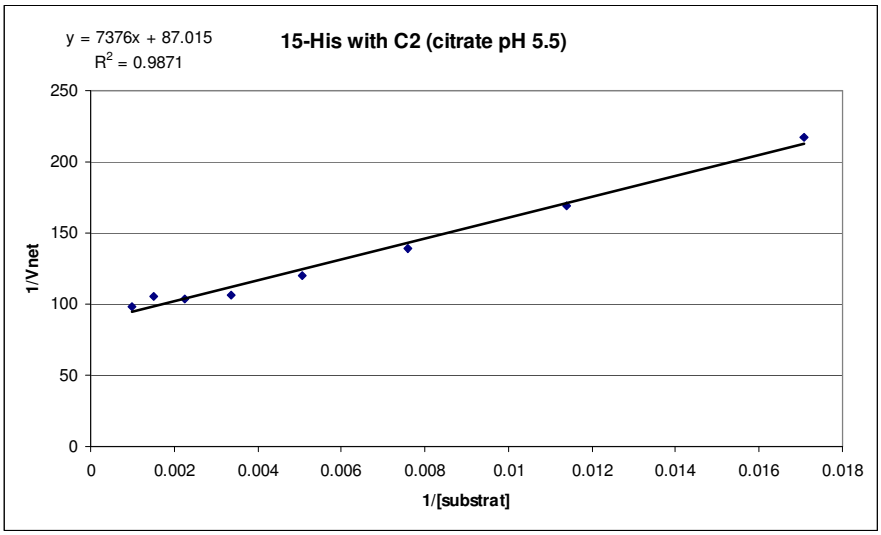
Analytical RP-HPLC: *t*R = 8.2 min (A/D = 100/0 to A/D = 0/100 in 15 min)



MS (ES+) calcd for $C_{92}H_{110}N_{46}O_{16}$ M:2116.16, found 2116.0

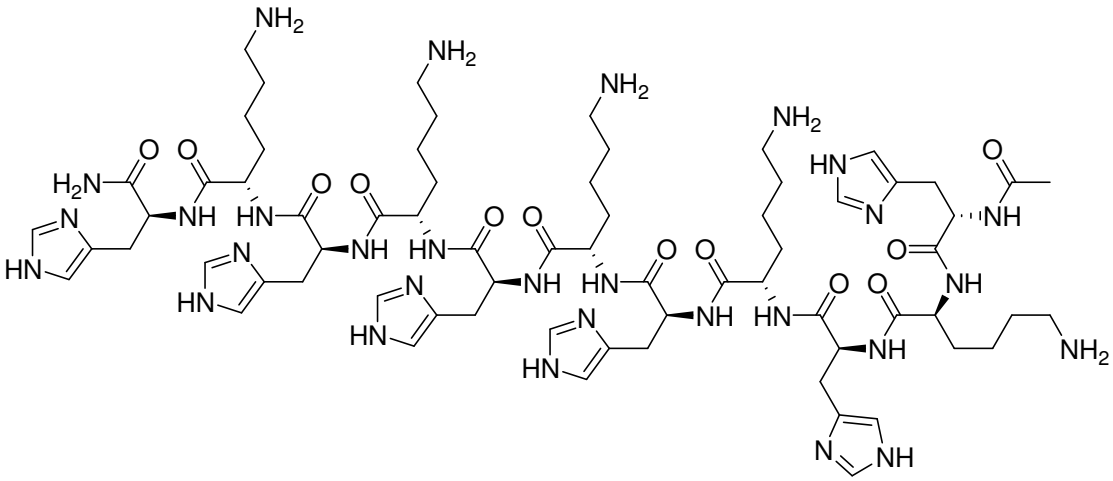


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

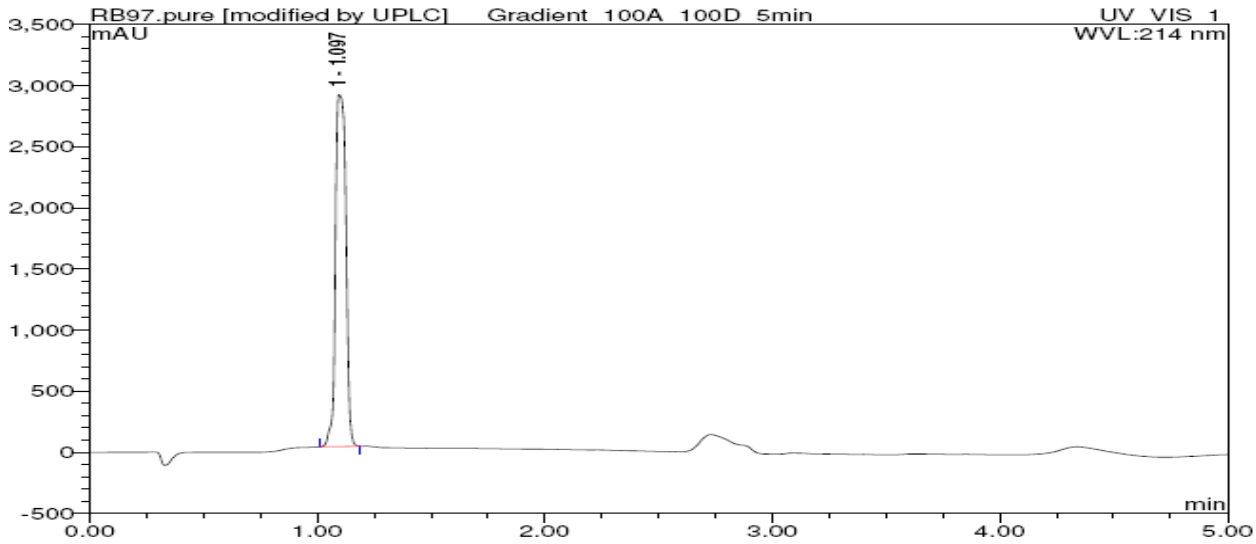


P25: AcHisLysHisLysHisLysHisLysHisLysHis-NH_2

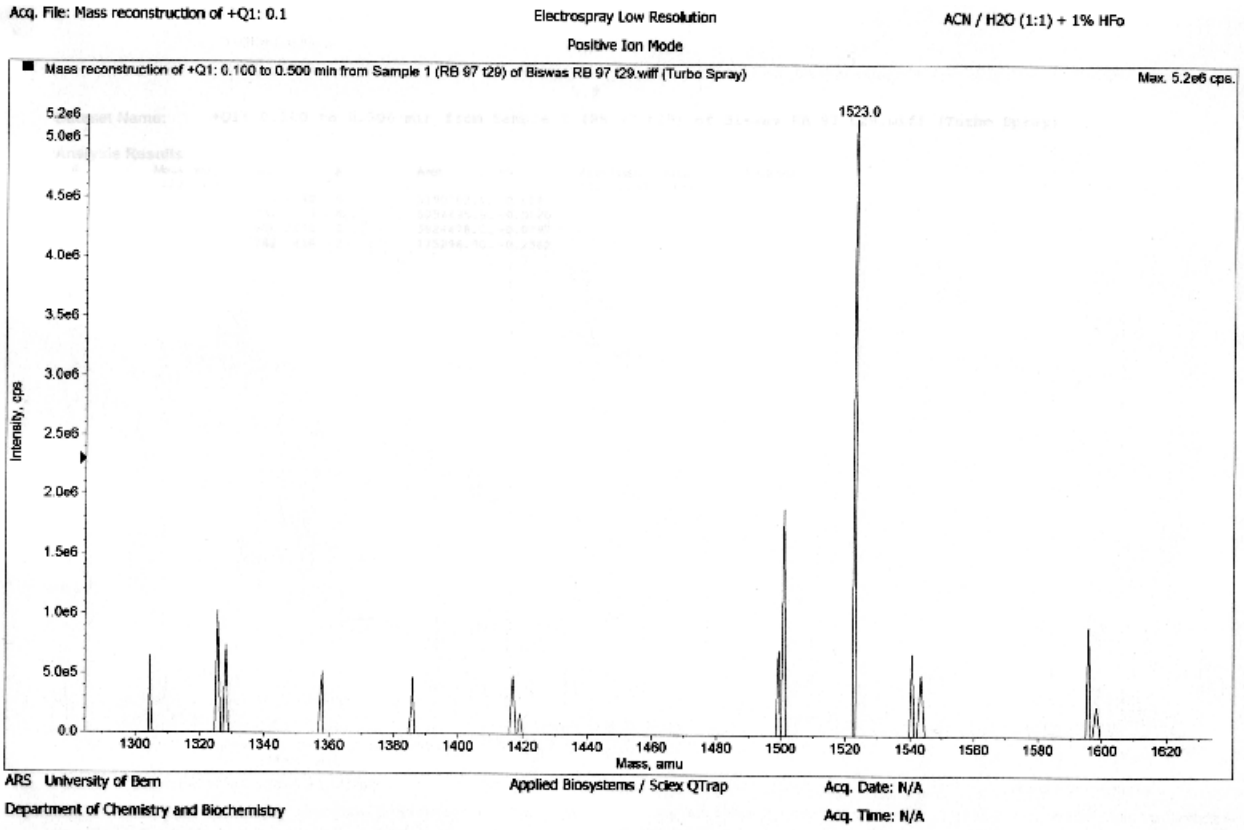
Starting with 300 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide $(His\text{-}Lys)_n$ was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (55.42 mg, 50 %).



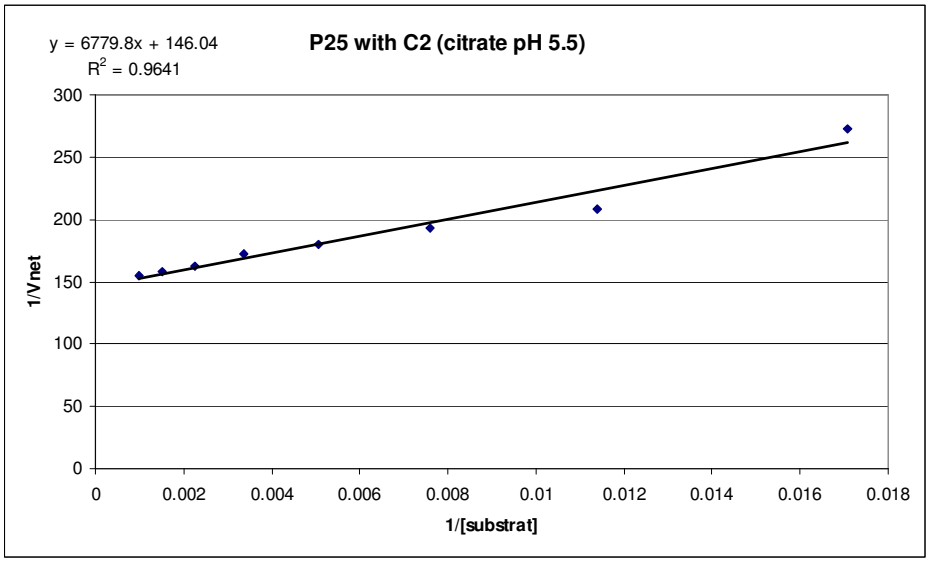
Analytical RP-UPLC: *t*R = 1.097 min (A/D = 100/0 to A/D = 0/100 in 2.2 min)



MS (ES+) calcd for $C_{68}H_{107}N_{29}O_{12}$ M:1522.76, found 1523.03

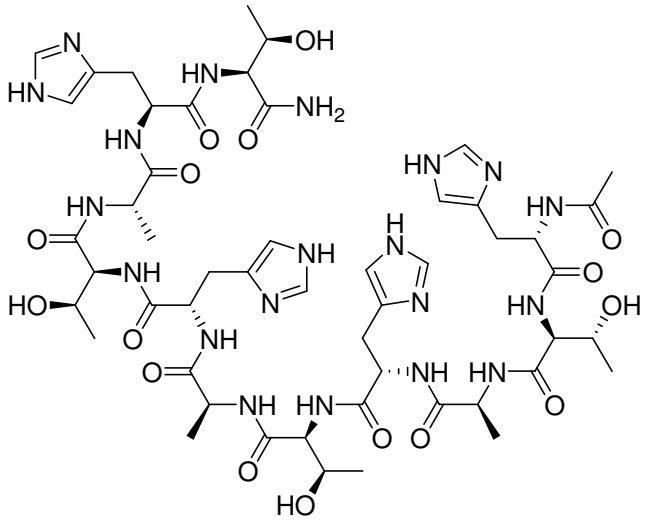


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

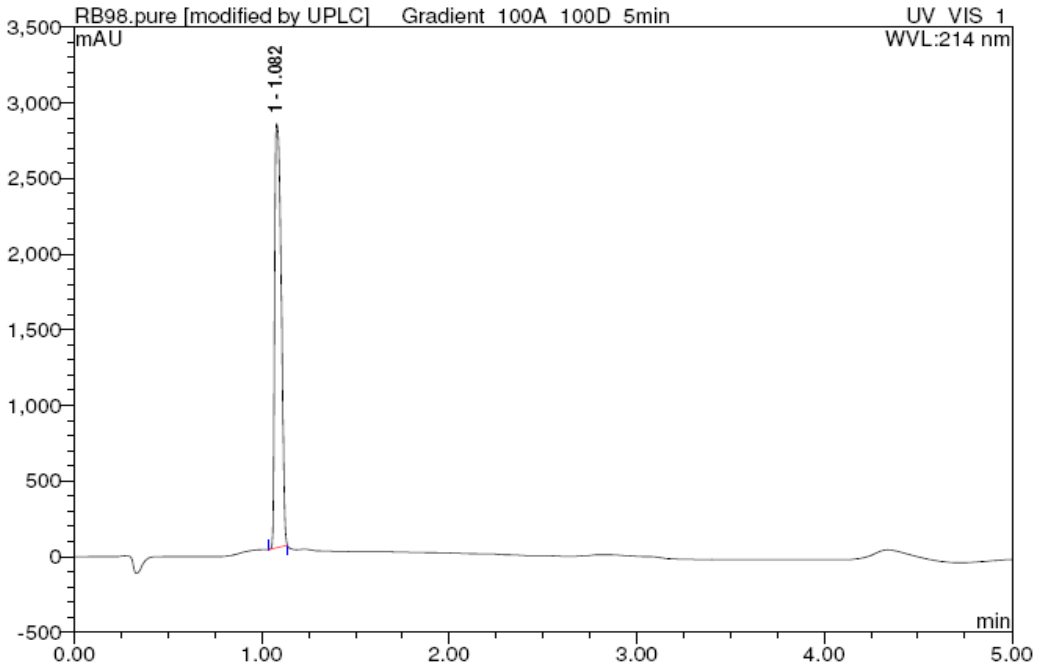


P60: AcHisThrAlaHisThrAlaHisThrAlaHisThr-NH_2:

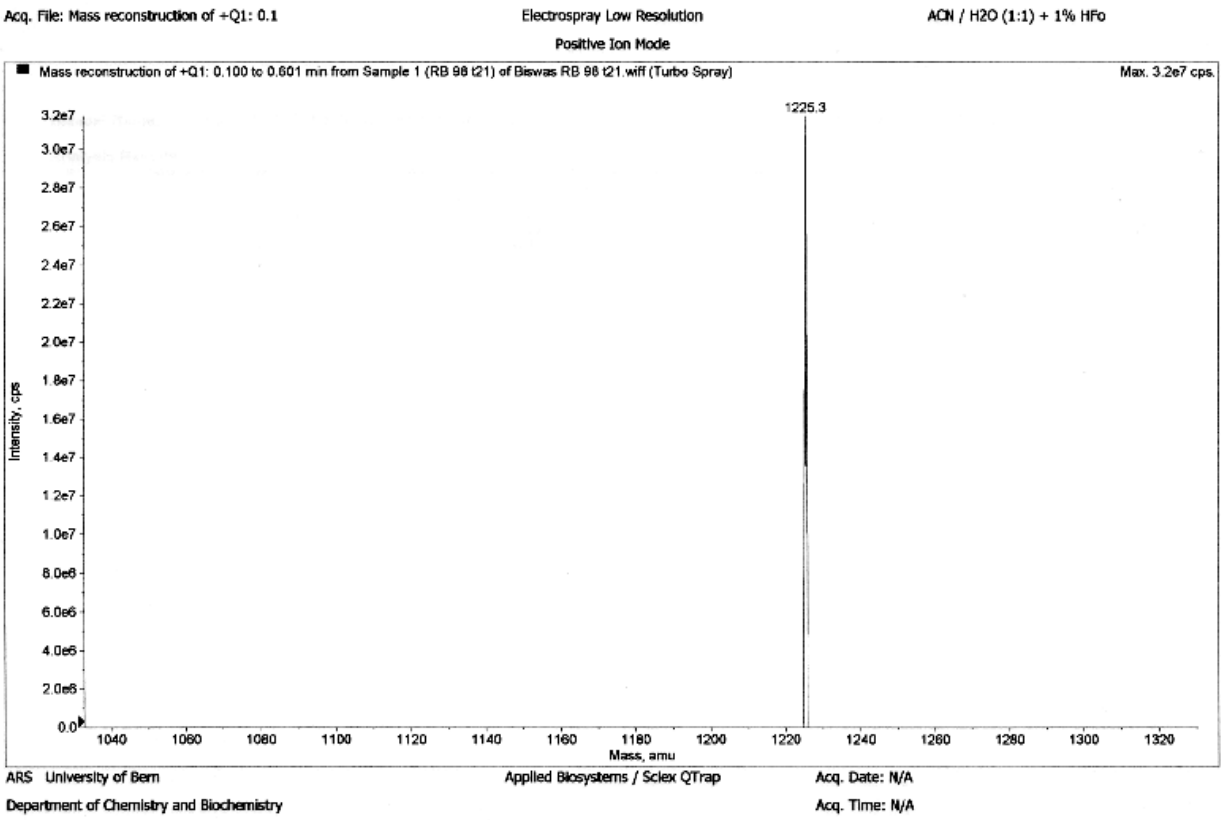
Starting with 300 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide $(HisThrAla)_n$ was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (29.4 mg, 33 %).



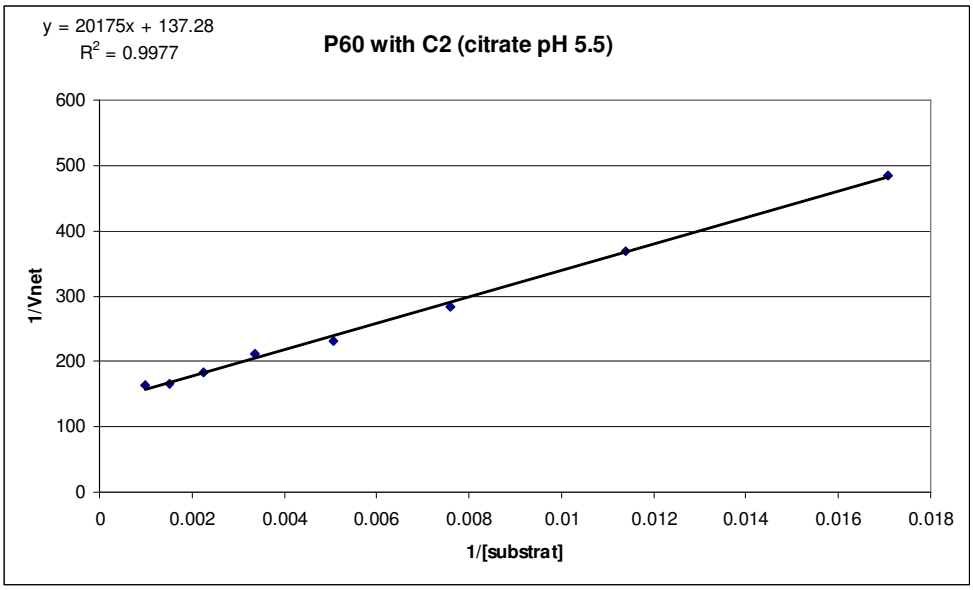
Analytical RP-UPLC: *t*R = 1.082 min (A/D = 100/0 to A/D = 0/100 in 2.2 min)



MS (ES+) calcd for $C_{51}H_{76}N_{20}O_{16}$ M: 1225.27, found 1225.32

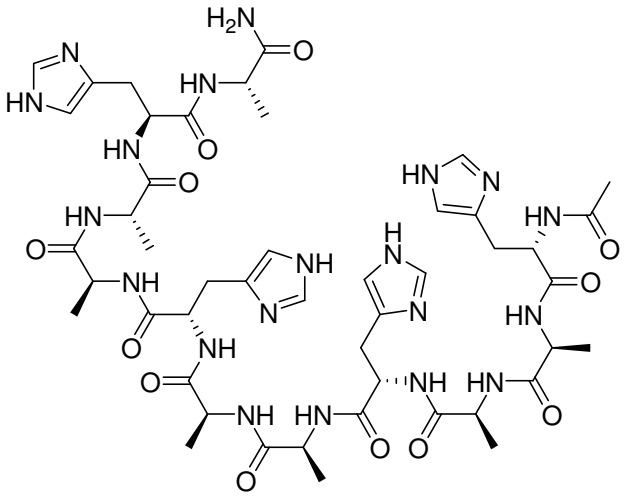


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

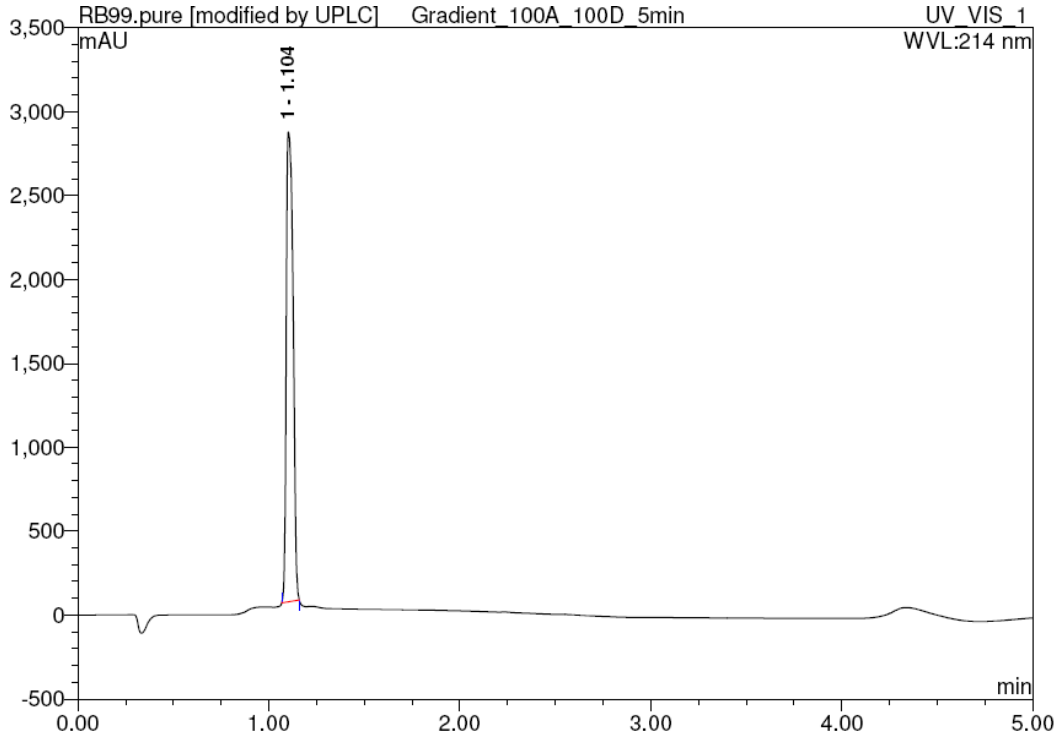


P60A: AcHisAlaAlaHisAlaAlaHisAlaAlaHisAla-NH_2

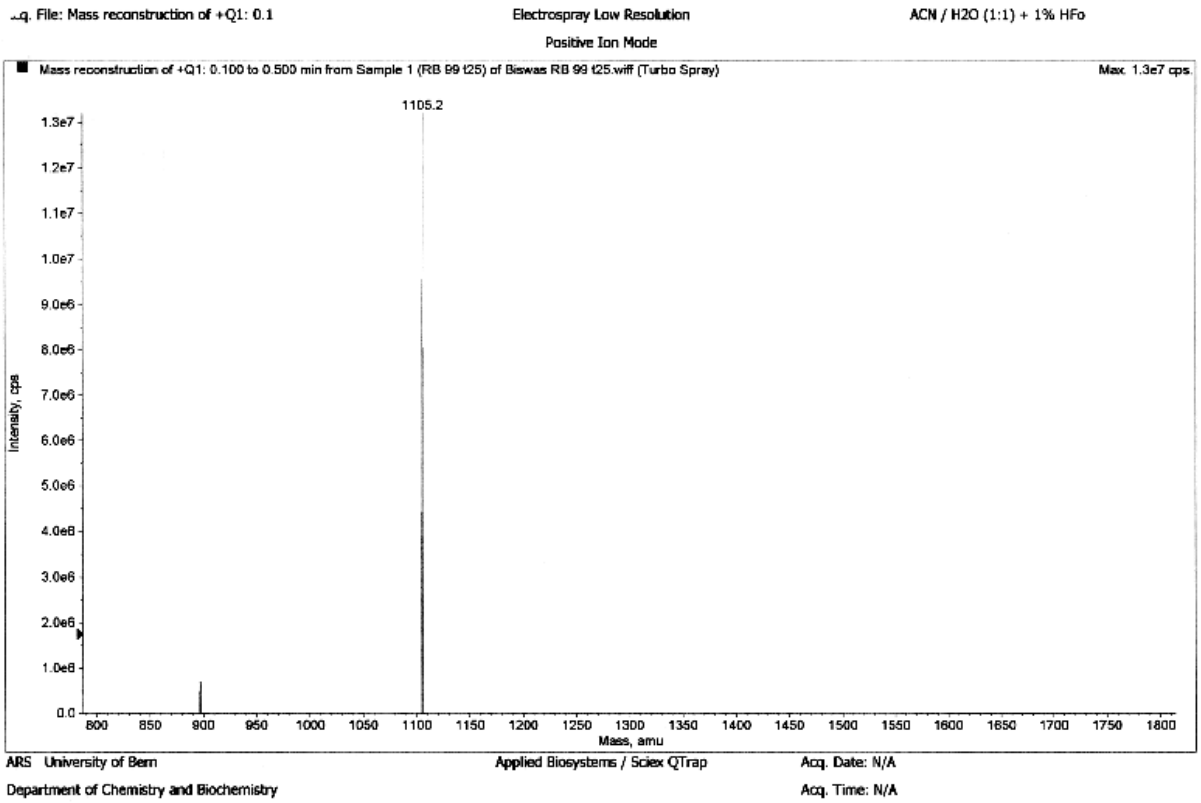
Starting with 300 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide $(HisAla)_n$ was obtained using procedure B as colorless solid after cleavage from the resin and preparative RP-HPLC purification (42 mg, 52 %).



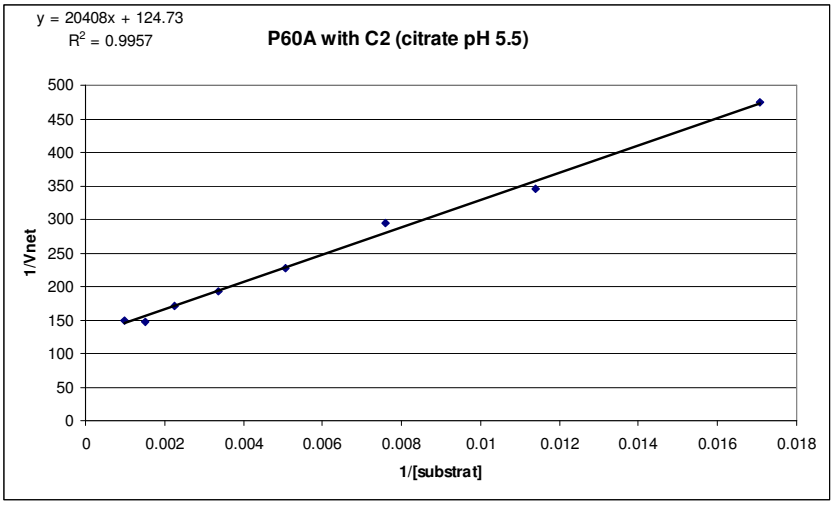
Analytical RP-UPLC: *t*R = 1.104 min (A/D = 100/0 to A/D = 0/100 in 2.2 min)



MS (ES+) calcd for $C_{47}H_{68}N_{20}O_{12}$ M: 1105.17, found 1105.20

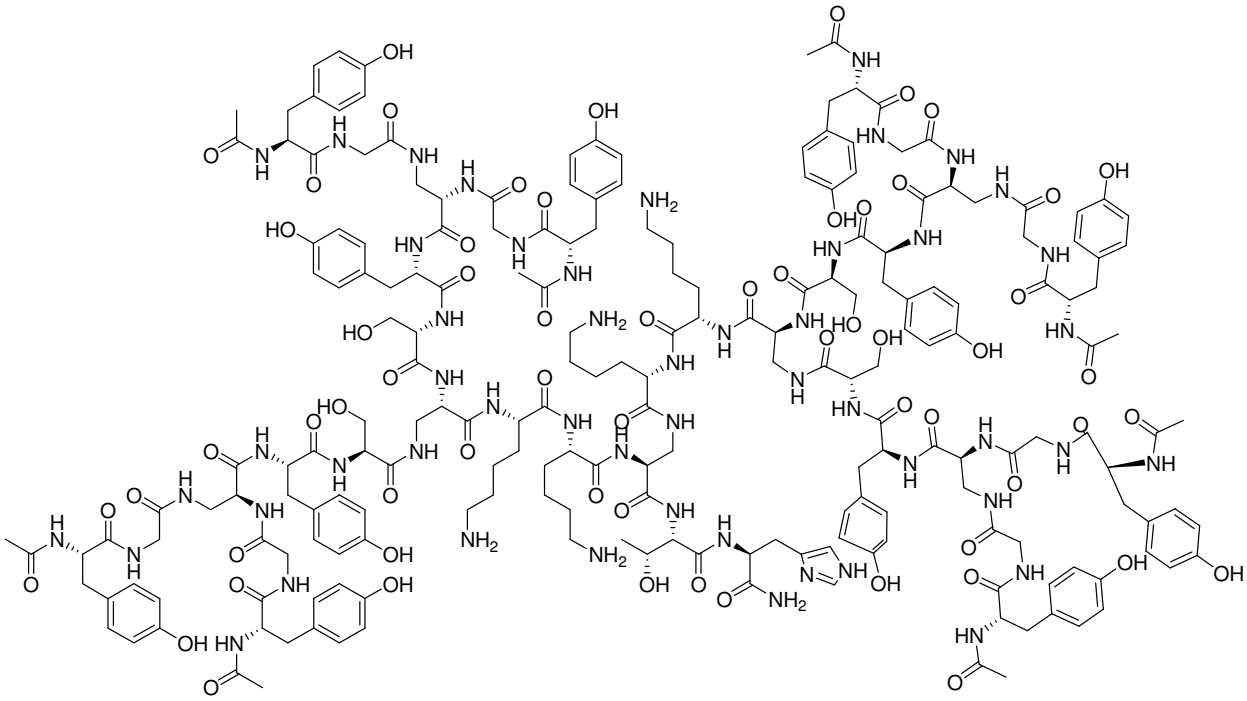


Representative Michaelis-Menten plots for determination of *kcat* and *K*M

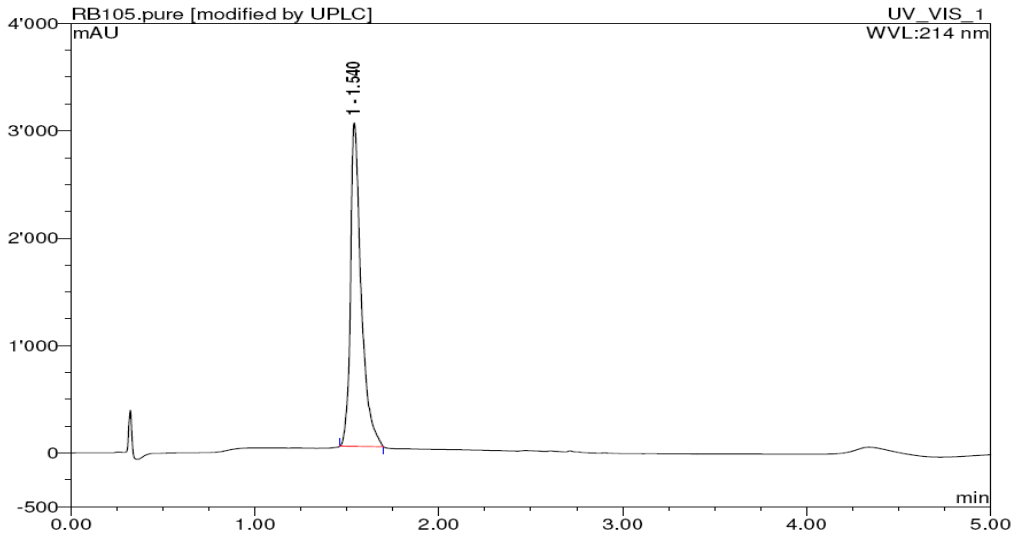


P65: **$(AcTyrGly)_8(DapTyrSer)_4(DapLysLys)_2(DapThrHis)\text{-}NH_2$**

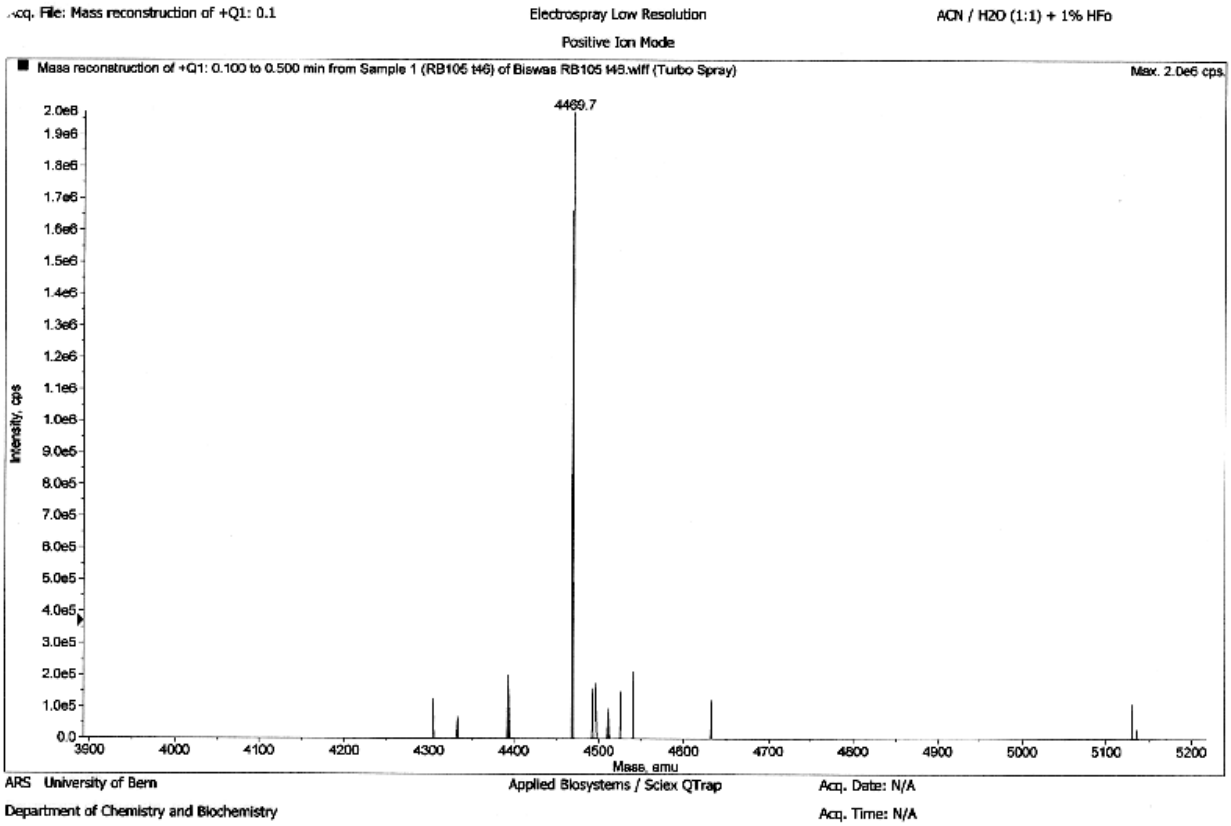
Starting with 250 mg Tentagel S RAM from Rapp Poylmere (0.24 mmol/gm), the peptide $(AceTyrGly)_8(DapTyrSer)_4(DapLysLys)_2(DapThrHis)\text{-}NH_2$ was obtained using procedure A as colorless solid after cleavage from the resin and preparative RP-HPLC purification (13.7 mg, 5 %)



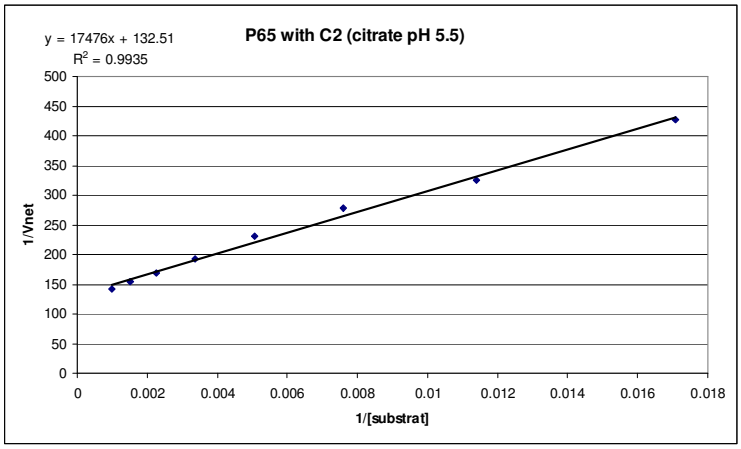
Analytical RP-UPLC: *t*R = 1.540 min (A/D = 100/0 to A/D = 0/100 in 2.2 min)



MS (ES+) calcd for $C_{207}H_{275}N_{51}O_{62}$ M: 4469.7, found 4469.7



Representative Michaelis-Menten plots for determination of *kcat* and *K*M



Kinetic Studies

Kinetic Measurements. Kinetic measurements were carried out using a CytoFluor Series 4000 multiwell plate reader from PerSeptive Biosystems. Peptides were used as 20 μM (1-His to 4-His), 15 μM (5-His, 6-His), or 5 μM (7-His to 14-His) freshly prepared solutions in milliQ water. Solutions were prepared by dissolving the dry TFA salts of peptides. Substrate solutions for the Michaelis-Menten kinetics were prepared by serial dilution by a factor 2/3 (7x) of a freshly prepared 3.0 mM solution of substrate in milliQ water (final concentration on the plate 60-1000 μM. Eight solutions of 8-hydroxypyrene-1,3,6-trisulfonic acid sodium (HPTS) salt ranging from 0 μM to 100 μM in buffer were used for the calibration curve. Bis-Tris 30 mM (pH7) or citrate 15 mM (pH5.5) was used as buffer, and the pH was adjusted to the desired value with HCl 1.0 M and NaOH 1.0 M using a Metrohm 692 pH/ion meter. In a typicalexperiment, using a multichannel pipet, 40 μL of dendrimer was mixed with 40 μL of buffer and 40 μL of substrate in a Costar flat-bottom polystyrene 96-well-plate (150 μL). The formation of **HPTS** was followed by fluorescence emission using absorbance filter 450/50 and emission filter 530/25. The gain was adjusted using the signal of the calibration curve prior to every experiment (typically a signal 45 000-55 000 for the 100 μM **HPTS** well). The calibration curve (40 μL of **HPTS**, 40 μL of buffer, and 40 μL of H_2O) and the blank (40 μL of substrate, 40 μL of buffer, and 40 μL of H_2O) were recorded for every experiment in the same time. The temperature inside the instrument was adjusted to 34.0 °C. Kinetic experiments were followed for typically 180 min. The data points were measured every 90 s. Fluorescence data were converted to product concentration by means of the calibration curve. Initial reaction rates were calculated from the steepest linear part observed in the curve that gives fluorescence versus time, typically between 4000 and 2000 s.

Measurement of Apparent Rate Enhancements and Kinetic Parameters *k*cat and *K*M. *V*cat is the apparent rate in the presence peptide catalyst; *V*un is the rate in buffer alone. The observed rate enhancement is defined as *V*net/*V*un with *V*net = *V*cat - *V*un. Michaelis- Menten parameters *k*cat (rate constant) and *K*M (Michaelis constant) were determined from the linear double reciprocal plot 1/*V*net versus 1/[S] (where [S] is the substrate concentration). The rate constant *k*uncat without catalyst was calculated from the slope of the linear curve that gives *V*un (as product concentration per time) v*ersus* substrate concentration [S].

Table S3. Synthesis and catalytic parameters of selected peptides.

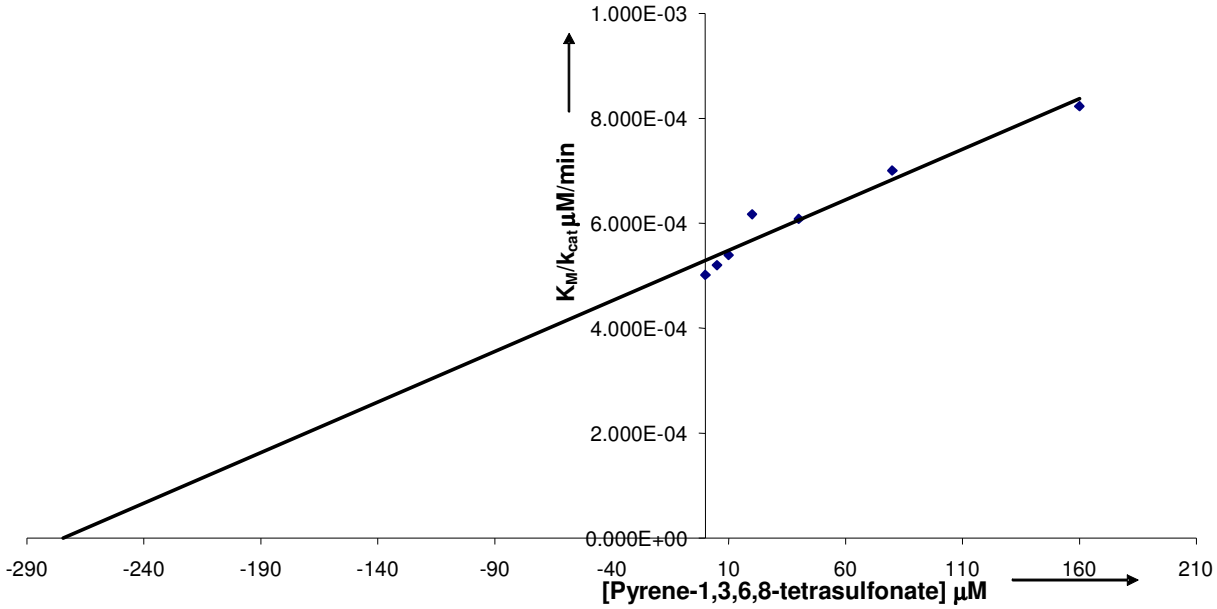
cpd	Sequence[a)]	mg[a)]	%	MS calc	MS obs	k_{cat}/k_{uncat}	K_M , μM	$(k_{cat}/K_M)/k_2$[c)]
His1	$AcHNH_2$	2.3	24	197.2	197.2	286 ±36	3044±588	3±0.18
His2	$AcHHNH_2$	2.9	13	334.3	334.2	840±86	1116±69	27±0.36
His3	$AcHHHNH_2$	2.3	7.2	471.5	471.2	1045±78	283±26	132±4
His4	$AcHHHHNH_2$	2.5	6	608.6	608.2	1108±42	181±18	219±6
His5	$AcHHHHHNH_2$	10	21	745.7	745.2	1333±43	178±8	268±7
His6	$AcHHHHHHNH_2$	12.2	22	882.9	882.4	1533±71	144±6	379±2
His7	$AcHHHHHHHNH_2$	13	17	1020.0	1019.4	2519±481	103±18	794±128
His8	$AcHHHHHHHHNH_2$	10.5	14	11.57.2	1156.6	3002±533	102±13	954±140
His9	$AcHHHHHHHHHNH_2$	14.3	17	1294.3	1293.6	3031±528	90±9	1092±131
His10	$AcHHHHHHHHHHNH_2$	14.9	16	1430.5	1430.6	3808±616	99±5	1232±108
His11	$AcHHHHHHHHHHHNH_2$	18	9	1566.7	1568	3536±482	95±5	1207±132
His12	$AcHHHHHHHHHHHHNH_2$	14.3	14	1704.7	1704.9	5186±917	109±11	1540±175
His13	$AcHHHHHHHHHHHHHNH_2$	16	13	1841.9	1842.0	4863±925	101±17	1561±255
His14	$AcHHHHHHHHHHHHHHNH_2$	7.4	6	1979.0	1978.9	5247±1010	100±13	1689±224
Hi15	$AcHHHHHHHHHHHHHHHNH_2$	34	12	2116.2	2116.0	6035±186	86±6	2201±191
P25	$AcHKHKHKHKHKHNH_2$	55.4	50	1522.8	1523.9	2948±58	47±4	2371±196
P60	$AcHTAHTAHTAHTNH_2$	29.4	33	1525.3	1525.3	768±13	143±3	203±6
P60A	$AcHAAHAAHAAHANH_2$	42	52	1105.2	1105.2	834±16	156±13	203±14
P65	$(AcYG)_8(BYS)_4(BKK)_2BTHNH_2$	13.7	5	4469.7	4469.7	620±10	122±4	192±3

[a)] *B* = branching diaminopropionic acid *Dap*. [b)] All products were prepared by Fmoc-SPPS on TGR resin and purified by preparative RP-HPLC. [c)] Assay conditions: aqueous 5 mM citrate buffer, pH 5.5, 34°C, Catalyst concentration 20 μM (for His1-His4),15 μM (for His5-His6, P60, P60A), 5 μM (for His7- His14), 3.75 μM (for His15,P25,P65) , 58.5-1000 μM substrate **1** (8 different concentrations). 120 μL assays in microtiterplate wells were followed by fluorescence (λ_{exc} = 450±25 nm, λ_{em} = 530±12 nm). All kinetics were run in triplicate. Parameters were obtained from Lineweaver-Burk plots. k_2 is the 2nd order rate constant for the hydrolysis of **1** under the same conditions, k_2 = 1.00 $M^{-1}min^{-1}$, and the spontaneous background reaction amounts to k_{uncat} = 3.58E-05 min^{-1}.

Table S4: Influence of ionic strength (KCl), Pyrene-1,3,6,8-tetrasulfonic acid and citric acid on catalytic properties of His15 .

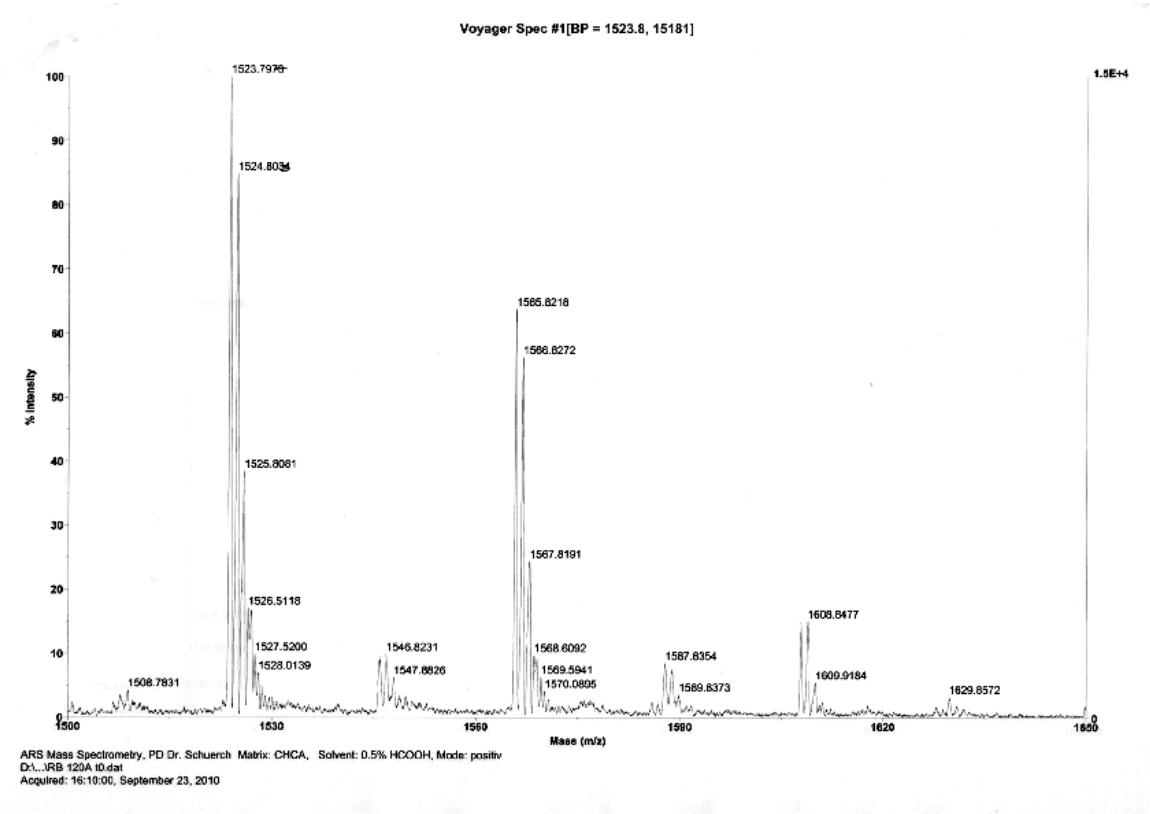
	k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($min^{-1}M^{-1}$)	k_{cat}/k_{uncat}	k_{uncat}(min^{-1})
KCl conc. (mM)					
0	0.19±5.82E-03	86±6	2207±191	6035±186	3.13E-05
5	0.19±1.07E-02	77±14	2578±383	5264±289	3.69E-05
10	0.21±2.69E-03	91±8	2277±240	5669±74	3.64E-05
50	0.19±6.58E-03	93±7	2025±218	5004±176	3.74E-05
100	0.18±9.14E-03	91±3	1945±165	4558±235	3.89E-05
200	0.16±9.60E-03	102±6	1579±142	3906±233	4.12E-05
300	0.16±8.23E-03	127±10	1282±206	3927±199	4.15E-05
500	0.13±7.49E-03	185±4	723±28	2913±163	4.58E-05
Pyrene-1,3.6,8-tetrasulfonate conc. (μM)					
0	0.16±1.34E-02	83±6	1992±164	4705±785	3.55E-05
5	0.16±1.24E-02	86±9	1921±205	4695±734	3.55E-05
10	0.17±1.09E-02	90±8	1853±178	4755±689	3.41E-05
20	0.17±1.29E-02	104±7	1620±146	4819±769	3.19E-05
40	0.17±1.35E-02	104±12	1644±200	4836±737	3.51E-05
80	0.16±1.39E-02	113±6	1427±94	4624±789	3.55E-05
160	0.16±1.26E-02	132±10	1215±95	4587±717	3.55E-05
Citrate conc.(mM)					
5	0.21±2.67E-03	107±2	1942±6	4314±114	4.81E-05
10	0.22±4.18E-03	119±10	1824±188	4447±251	4.87E-05
20	0.26±8.02E-03	210±3	1225±27	4834±265	5.34E-05
30	0.30±1.17E-02	373±63	837±119	5481±507	5.62E-05
40	0.37±1.27E-02	492±21	742±8	6452±364	5.67E-05

Figure S2. Inhibition of His15 catalysis by pyrene-1,3,6,8-tetrasulfonic acid for catalytic hydrolysis of **1** at pH 5.5

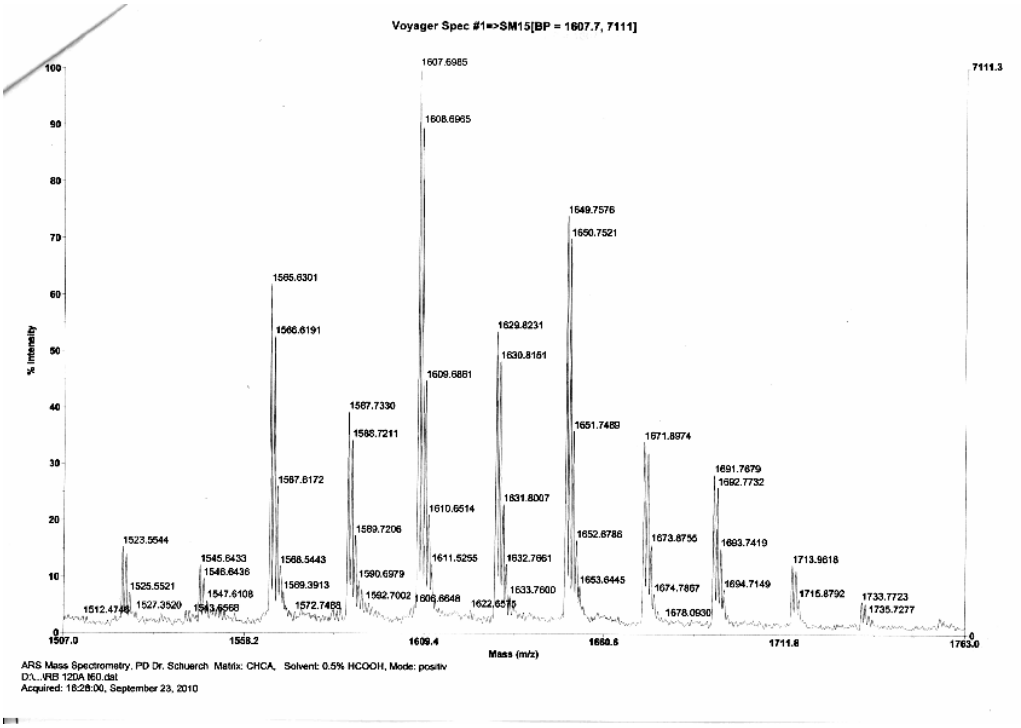


Analysis of lysine acylation. A 100 μM solution of peptide (**P25**, **P65** or **His11**) in aqueous 2 mM citrate buffer pH 5.5 containing 1 mM of substrate **1** (total volume 750 μL, final pH value 5.5) was incubated at 25°C and analyzed at 10 min, 30 min, 1 h, 2h, 6 h and 18 h. Ninhydrin tests. 0.3 microliter of reaction solution were deposited with a glass capilary on a TLC plate. The plate was dried and stained with a ninhydrin solution (1.0 g ninhydrin, 3 mL AcOH, 100 mL n-butanol). In each test, a control solution of the same peptide without substrate **1** was also analyzed. The ninhydrin test with **His11** was negative in all cases**.** Reactions of **P25** and **P65** with **1** gave colored spots until 6 h reaction indicative of free amino groups. At 18h (overnight) the test was negative. MALDI-TOF analysis. 5 μL aliquots were diluted in 0.1 mL 0.5% aq. HCOOH and applied to MALDI-TOF (positive mode). MS of **His11** did not show detectable acetylation even after 24 hours of reaction. For the linear peptide **P25** (five Lysines), a monoacetylated product (1564.8) and a trace of diacetylated (1606.8) product are visible after 10 min. reaction, but the non-acetylated peptide (1522.7) along with its Na and NaK adducts dominate the spectra. With increasing time the triacetylated (1648.8, tetraacetylated (1690.9), and pentaacetylated (1732.9) products are formed. For **P65** after 90 mins the non-acetylated dendrimer (4469.7) is the major peak, with small amounts of the monoacetylated product (4511.7). After 6 hours of reaction the diacetylated product predominates (MW4553.8).

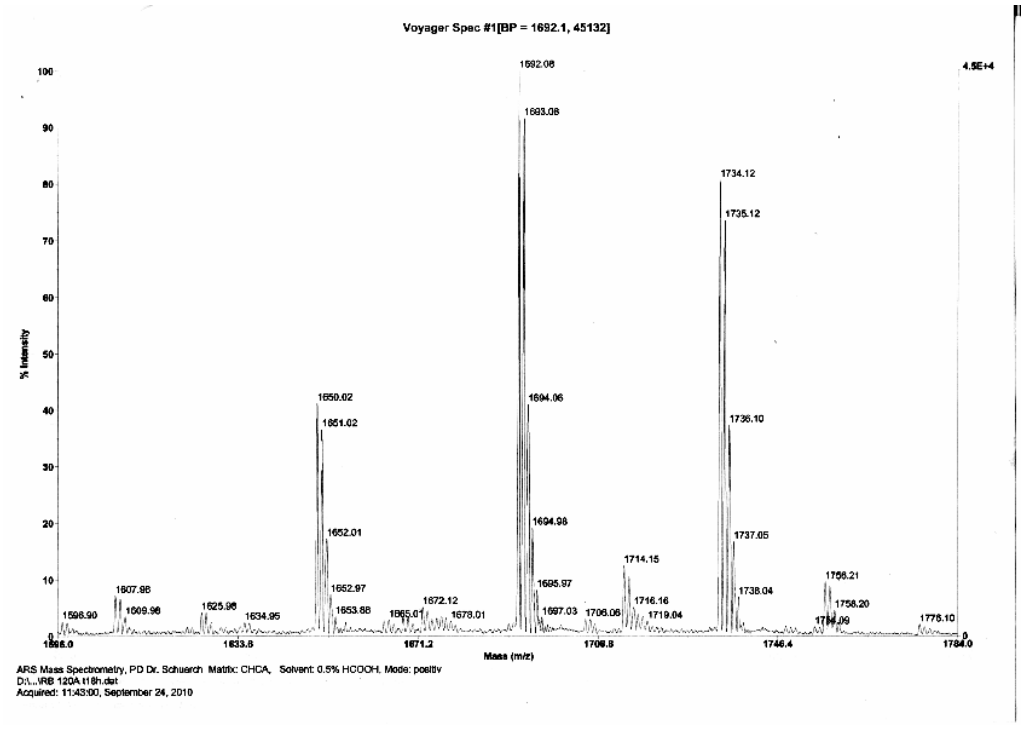
P25 after 10 mins of reaction: MALDI-TOF MS: **P25** calc. for $C_{68}H_{107}N_{29}O_{12}$ 1522.7, obs. 1523.8 (M^+), 1545.8 ($M+Na^+$); **monoacetyl P25** calc. for $C_{70}H_{109}N_{29}O_{13}$ 1564.8, Obs, 1565.8 (M^+); **diacetyl P25** calc. for $C_{72}H_{111}N_{29}O_{14}$ 1606.8 (M^+), obs. 1608.8.



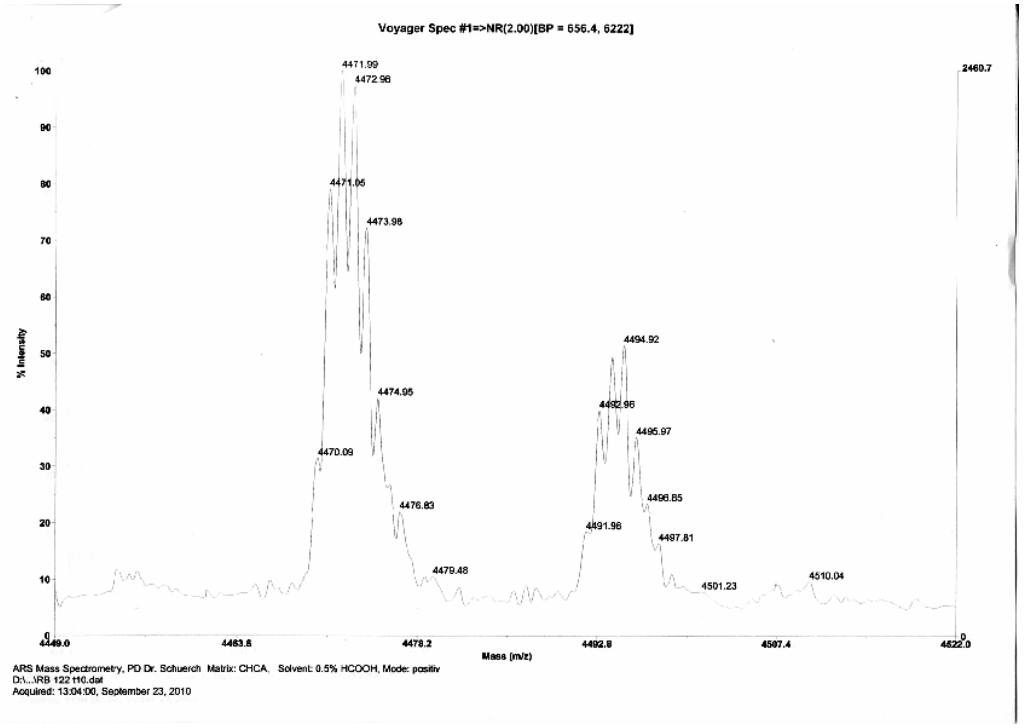
P25 after 70 mins of reaction: MALDI-TOF MS: **P25** calc. for $C_{68}H_{107}N_{29}O_{12}$ 1522.7, obs. 1523.5 (M^+), 1545.5 ($M+Na^+$), 1587.5 ($M+Na^+$+HCOOH); **monoacetyl P25** calc. for $C_{70}H_{109}N_{29}O_{13}$ 1564.8, Obs, 1565.8 (M^+); **diacetyl P25** calc. for $C_{72}H_{111}N_{29}O_{14}$ 1606.8 (M^+), obs. 1608.8; **triacetyl P25** calc. for $C_{74}H_{113}N_{29}O_{15}$ 1648.8 (M^+), Observed 1649.6; **tetraacetyl P25** calc. for $C_{76}H_{115}N_{29}O_{16}$ 1690.9 (M^+), obs.1691.8; **pentaaacetyl P25** calc. for $C_{78}H_{117}N_{29}O_{17}$ 1732.9 (M^+), obs. 1733.7.



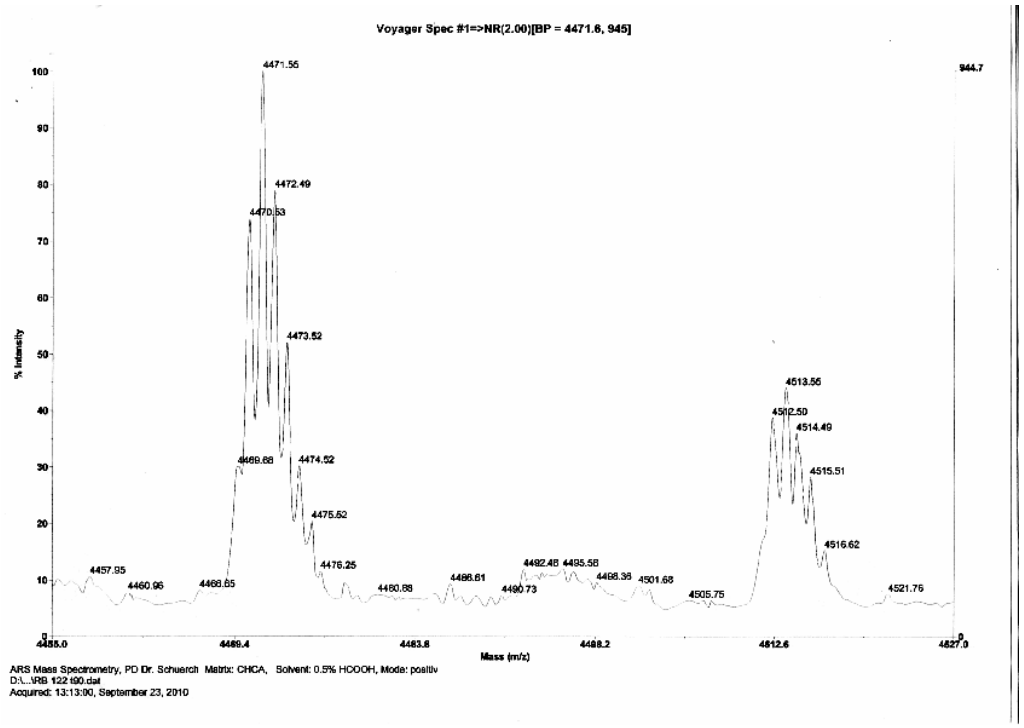
P25 after 18 hours of reaction: MALDI-TOF MS: **P25** calc. for $C_{68}H_{107}N_{29}O_{12}$ 1522.7, observed 1625.9 ($M+Na^+$+2HCOOH), 1714.1 (M^++Citric acid), 1756.2 (M^++Na_2Citrate); **monoacetyl P25** calc. for $C_{70}H_{109}N_{29}O_{13}$ 1564.8, Obs, 1565.6 (M^+); **diacetyl P25** calc. for $C_{72}H_{111}N_{29}O_{14}$ 1606.8 (M^+), obs. 1608.6; **triacetyl P25** calc. for $C_{74}H_{113}N_{29}O_{15}$ 1648.8 (M^+), Observed 1649.6; **tetraacetyl P25** calc. for $C_{76}H_{115}N_{29}O_{16}$ 1690.9 (M^+), obs.1691.8; **pentaaacetyl P25** calc. for $C_{78}H_{117}N_{29}O_{17}$ 1732.9 (M^+), obs. 1734.1.



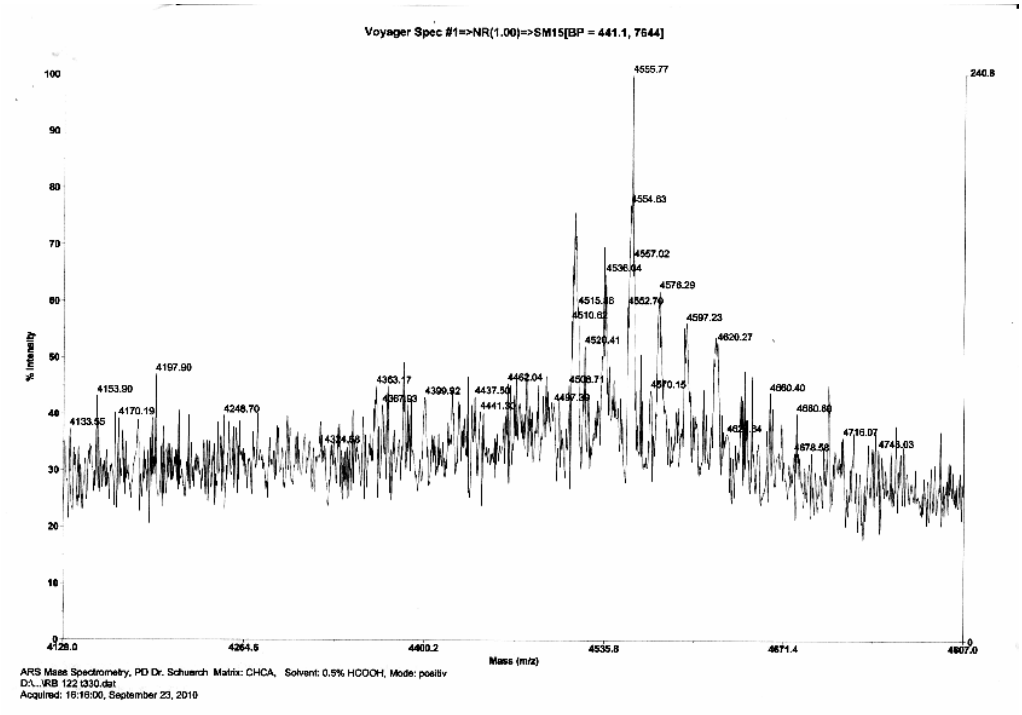
P65 after 10 mins of reaction:MALDI-TOF MS: **P65** calc. for $C_{207}H_{275}N_{51}O_{62}$ 4469.7. Observed 4470.0 (M^+), 4491.9 ($M+Na^+$).



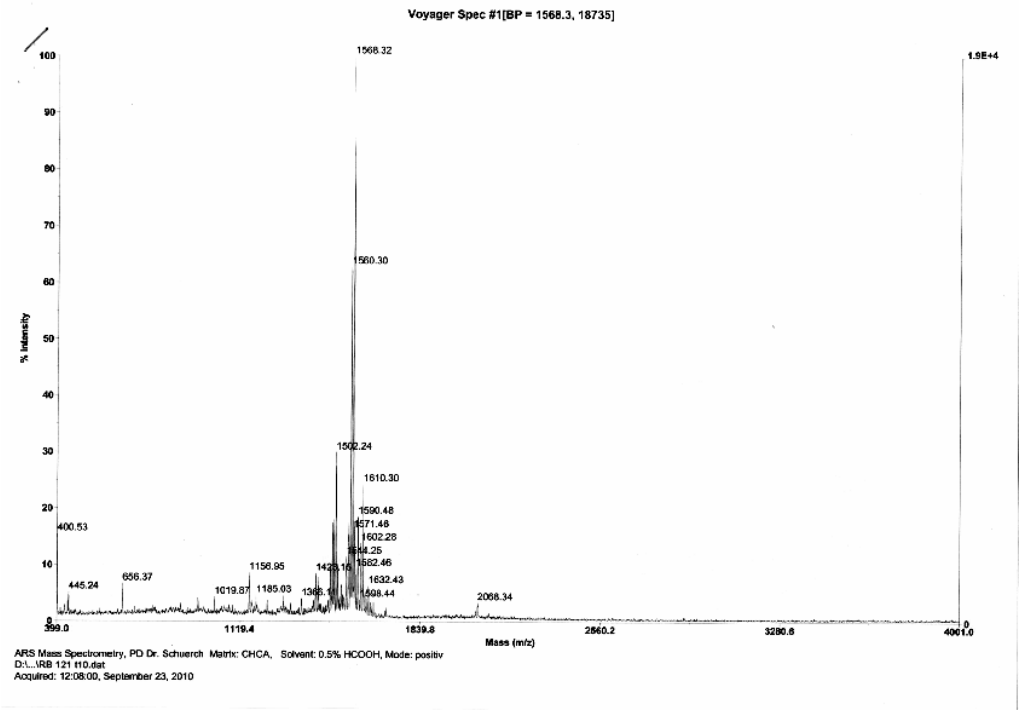
P65 after 100 mins of reaction:MALDI-TOF MS: **P65** calc. for $C_{207}H_{275}N_{51}O_{62}$ 4469.7, obs. 4470.0 (M^+). **Monoacetyl P65** calc. for $C_{209}H_{277}N_{51}O_{63}$ 4511.4, obs. 4512.5 (M^+).



P65 after 6 hours of reaction:MALDI-TOF MS: **Monoacetyl P65** calc. for $C_{209}H_{277}N_{51}O_{63}$ 4511.4, obs. 4512.5 (M^+); d**iacetyl P65** calc.for $C_{211}H_{279}N_{51}O_{64}$ 4553.8, obs. 4555.7(M^+).



His11 after 10 mins of reaction: MALDI-TOF MS: **His11 c**alc. for $C_{68}H_{82}N_{34}O_{12}$ 1567.6, obs. 1568.3(M^+)



His11 after 24 hours of reaction: MALDI-TOF MS: **His11 c**alc. for $C_{68}H_{82}N_{34}O_{12}$ 1567.6. Obs. 1569.6(M^+)