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Supporting Information

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A Combinatorial Approach to Catalytic Peptide Dendrimers

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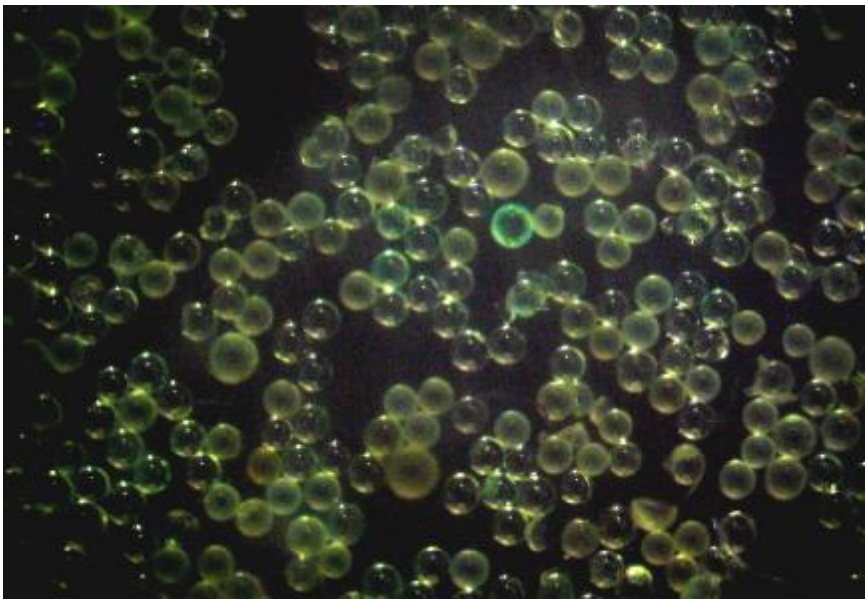
- **Analytical HPLC profiles and mass spectra (ESI MS) data for the resynthesized peptide dendrimers**
- **Experimental details for kinetic measurements and Michaelis-Menten kinetic plots for catalysis by dendrimers 8, C11 and C12**

A



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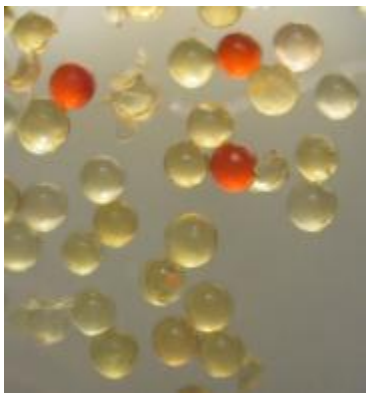


Figure S1. Screening of catalytic peptide dendrimer library. **A.** Microscope picture under illumination with UV 356 nm. Beads were soaked with 80 µM 8-butyryloxypyrene-1,3,6-trisulfonate in aq. 20 mM bis-tris pH. 6.0 and spread out in a petri-dish. The green bead near the center contains a catalytic sequence. **B.** Screening for binding to vitamin B_{12}. Conditions : 30 min. equilibration in aq. PBS (10 mM phosphate, 160 mM NaCl, pH 7.4) containing 400 µM cyanocobalamin (vitamin B_{12}), followed by washing with PBS and water.

HPLC-integration data used for sequence determination of single peptide dendrimers on resin beads.

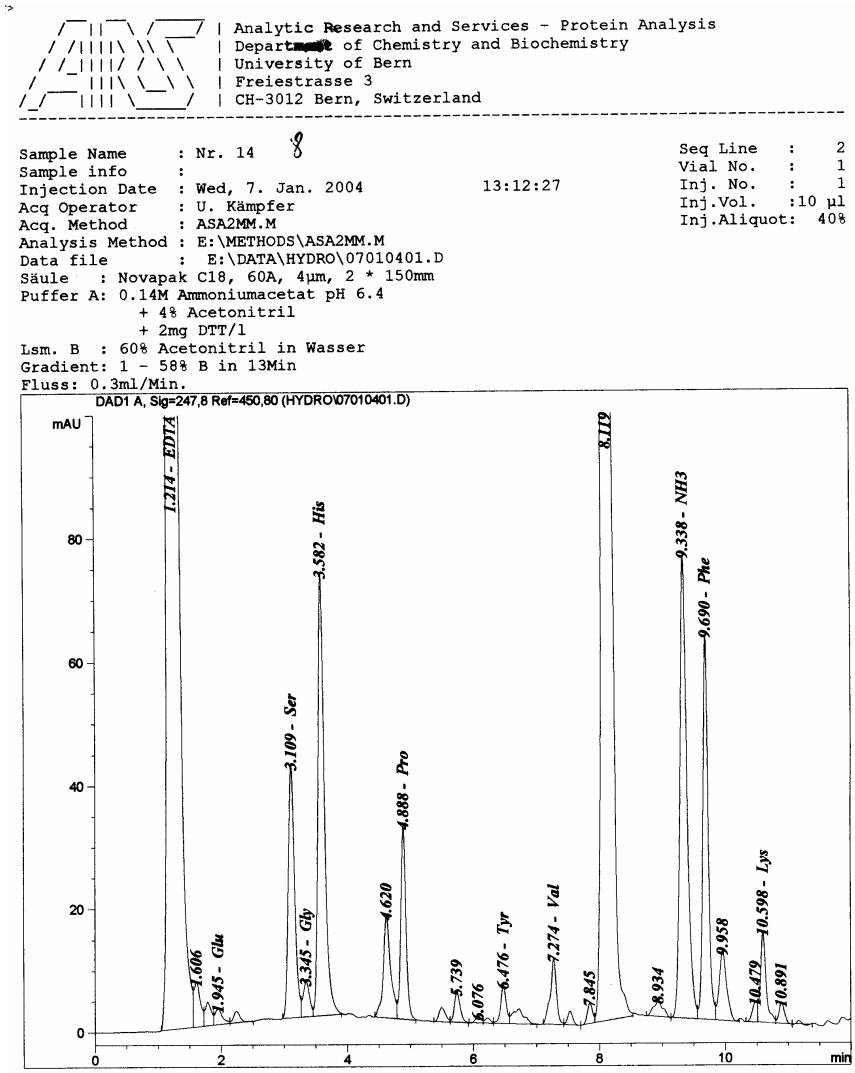
aminoacid	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala
t_R / min	1.88	2.07	3.25	3.52	3.8	4.22	4.54	4.75
ref.	9.9	12.0	10.7	10.0	10.9	8.9	4.1	10.2
1			50.3 (A7)	24.2 (A6)		36.2(A5)		10.8 (A1)
2	5.8 (A1)		32.6 (A5)		134.4 (A8+A6)			7.4 (A3)
3				37.1 (A6)	133.6 (A8)		17.8 (A3)	
4				31.2 (A6)	76.7 (A8)		23.9 (A3)	13.8 (A1)
5		80.3 (A8)	73.2 (A5)	36.1 (A6)		101.1 (A7)		
6	5.7 (A1)			38.4 (A8)		67.7 (A7+A5)		
7	7.3 (A1)		37.0 (A7)		40.6 (A8)			32.3 (A3)
8			106.1 (A7)	77.6 (A6)	145.2 (A8)	55.4 (A5)		26.4 (A3)
9			140.3 (A7)		239.6 (A8+A6)			
10	23.5 (A3)			223.2 (A8+A6)		123.8 (A7)		
13			50.8 (A5)		69.9 (A6)			36.6 (A3)
14	10.2 (A1)	116.3 (A8)		83.6 (A6)		95.9 (A5)	15.8 (A3)	
15	27.3 (A1)	192.6 (A6)	162.5 (A5)					74.8 (A3)
16				393.1 (A8)				
17	11.1 (A1)	77.2 (A6)		173.5 (A8)		186.3 (A7+A5)	8.2 (A3)	
18	17.2 (A1)			83.4 (A6)	182.1 (A8)	54.5 (A5)		
19		55.4 (A6)	48.8 (A5)			114.5 (A7)		

aminoacid	Pro	Tyr	Val	Ile	Leu	Phe	Lys	Trp
t_R / min	5.08	6.72	7.54	9.18	9.36	10.06	10.98	n.d.
ref.	13.8	9.8	7.5	5.3	11.6	11.3	7.9	0.0
1			15.8 (A3)	16.9 (A2)			37.7 (A4)	(A8)
2		13.9 (A4)		4.5 (A2)	79.8 (A7)			
3		21.7 (A4)	15.2 (A1)	11.2 (A2)	163.0 (A7+A5)			
4	250.2 (A7+A5)			9.5 (A2)			27.1 (A4)	
5			37.8 (A3+A1)	40.7 (A4+A2)				
6			8.6 (A3)	12.7 (A4)				(A6)
7					32.1 (A5)	36.7 (A4+A2)		
8			9.6 (A1)	25.1 (A4+A2)				
9	75.4 (A5)		27.1 (A3+A1)			12.6 (A2)	21.8 (A4)	
10	69.4 (A5)		10.1 (A1)	23.3 (A4+A2)				
13				22.3 (A4)	110.1 (A7)		12.2 (A2)	(A8)
14	106.9 (A7)	21.2 (A4+A2)						
15	67.7 (A7)	41.3 (A4+A2)						(A8)
16	643.1 (A7+A5)	114.4 (A4)	80.7 (A3+A1)				23.1 (A2)	(A6)
17				19.5 (A4+A2)				
18			34.9 (A3)		154.0 (A7)		47.2 (A4+A2)	
19			17.2 (A3+A1)	16.5 (A4+A2)				(A8)

Table S1. HPLC-intergration data for amino acid analysis of single beads of dendrimers after total hydrolysis with 6M HCl solution at 110°C for 22 h and derivatization with phenyl isothiocyanate (PITC). The sequence of dendrimers is deduced from the HPLC-peak-integration of each amino acid PITC-derivative relative to the reference integration of this derivative. The assigned position is indicated in parentheses. Trp is assigned by default to the missing amino acid position after all other amino acids have been assigned. The Dap (2,3-diaminopropanoic acid) branching unit co-elutes with phenylalanine (relative integration =

91.0), and the aminohexanoyl spacer coelutes with tyrosine (rel. int. = 9.8). Both values are substracted before calculating integration for these two amino acids.

HPLC-trace for analysis of the bead of catalytic dendrimer **8** identified as catalytic.



HPLC-integration report for HPLC-analysis of dendrimer **8** shown above.

Analytic Research and Services - Protein Analysis
Department of Chemistry and Biochemistry
University of Bern
Freiestrasse 3
CH-3012 Bern, Switzerland

Customized Report: ASA

Sample Name :Nr. 14
Calib. Data modified:Wed, 7. Jan. 2004 15:01:51

RT [min]	Exp. RT	Type	Peak Width	Peak Area	Peak Height	Amount pmol injected	Name
1.21	1.17	BV	0.113	6796.71	942.79	178.96	EDTA
1.61	0.00	VV	0.098	51.51	7.37	0.00	
1.78	1.78	VV	0.100	26.66	3.86	10.34	Asp
1.94	1.94	VV	0.121	21.91	2.68	8.26	Glu
2.24	2.28	VP	0.112	13.55	1.90	6.96	CMC
3.11	3.11	BV	0.108	287.43	40.91	140.35	Ser
3.34	3.35	VV	0.111	40.46	5.74	19.42	Gly
3.58	3.58	VB	0.105	504.04	71.50	239.62	His
0.00	4.00		0.000	0.00	0.00	0.00	Arg
0.00	4.31		0.000	0.00	0.00	0.00	Thr
0.00	4.47		0.000	0.00	0.00	0.00	Ala
4.62	0.00	BV	0.118	136.26	16.61	0.00	
4.89	4.86	VB	0.085	178.86	31.47	75.44	Pro
5.50	0.00	PV	0.108	17.05	2.33	0.00	
5.74	0.00	VB	0.097	31.54	4.95	0.00	
6.08	0.00	PV	0.094	6.82	1.10	0.00	
6.23	0.00	VV	0.089	5.04	0.84	0.00	
6.48	6.46	VV	0.092	37.46	6.26	15.11	Tyr
6.72	0.00	VB	0.162	30.09	2.46	0.00	
7.27	7.28	BV	0.094	72.57	10.80	27.06	Val
7.53	7.58	VP	0.092	12.84	2.26	6.31	Met
7.85	0.00	VV	0.087	19.40	3.33	0.00	
8.12	0.00	VB	0.109	3586.80	480.65	0.00	
0.00	8.20		0.000	0.00	0.00	0.00	C-
0.00	8.52		0.000	0.00	0.00	0.00	C-C
0.00	8.85		0.000	0.00	0.00	0.00	Ile
8.93	0.00	PP	0.131	27.21	2.75	0.00	
0.00	9.03		0.000	0.00	0.00	0.00	Leu
9.34	9.33	VV	0.116	582.86	75.03	138.56	NH3
9.69	9.69	VV	0.087	364.54	62.31	161.12	Phe
9.96	0.00	VB	0.121	92.21	11.33	0.00	
10.48	0.00	BV	0.082	18.13	3.37	0.00	
10.60	10.62	VV	0.092	92.28	14.85	21.74	Lys
10.89	0.00	VP	0.094	20.86	3.41	0.00	
11.17	0.00	VB	0.058	2.22	0.61	0.00	
Totals:					1813.47	1049.26	

*** End of Report ***

All dendrimers were synthesized on TGR resin (tentagel-Rink-amide resin) by Fmoc-SPPS and purified by preparative RP-C18-HPLC as described before,. [**Error! Bookmark not defined.**b]

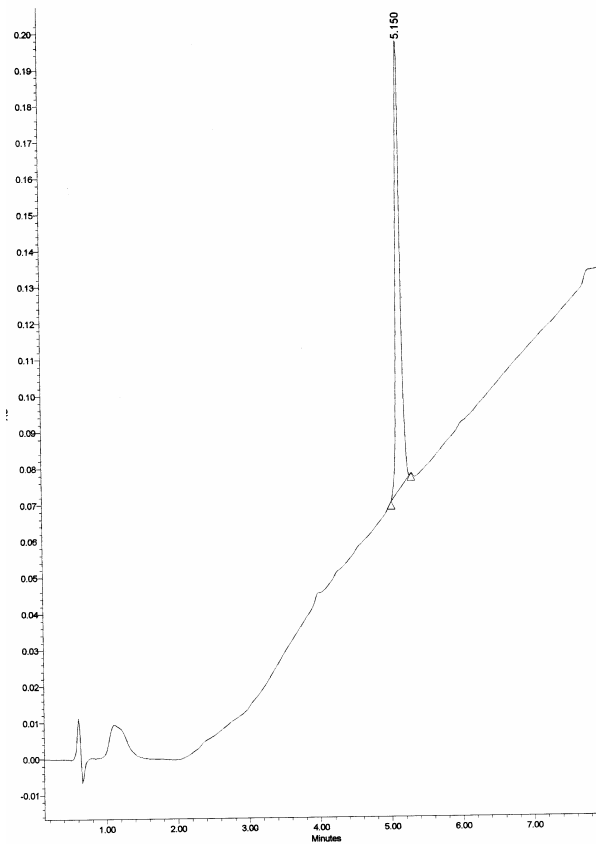
Analytical HPLC profiles and Mass spectra (ESI MS) data for the peptide dendrimers

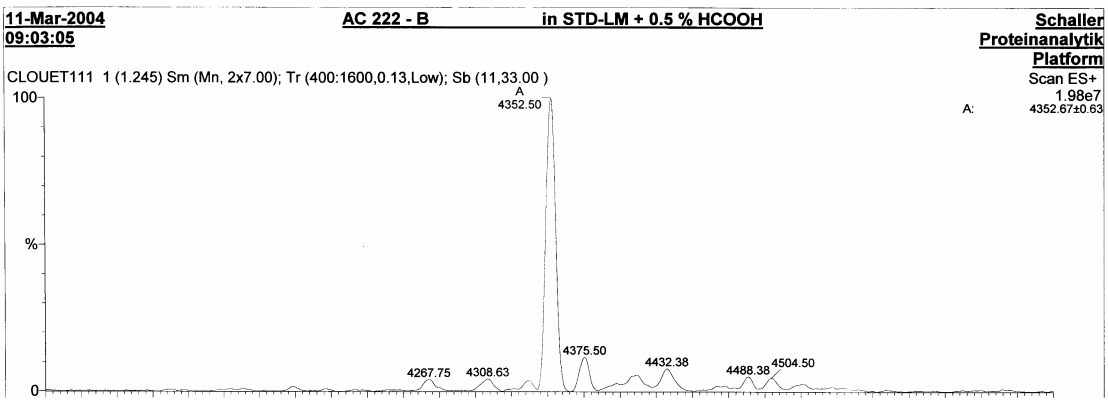
Analytical HPLC columns: Chromolith Performance RP-18e , 0.46 × 10 cm, flow 3 mL.min^{-1}; detection by UV at 234 nm, solvent systems: A = 0.1% TFA in H_2O; B = 40% H_2O / 60% CH_3CN, 0.1% TFA.

HPLC conditions for dendrimer 14,15,19,C20,C21: A/B = 80/20 to A/B = 50/50 in 40 min.

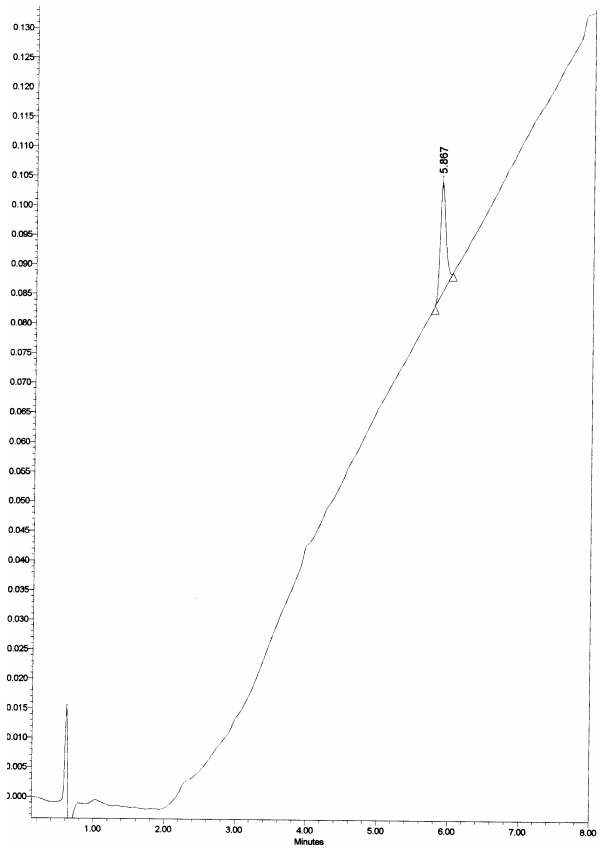
HPLC conditions for dendrimer 8,10,C11,C12: A/B = 85/15 to A/B = 60/40 in 35 min.

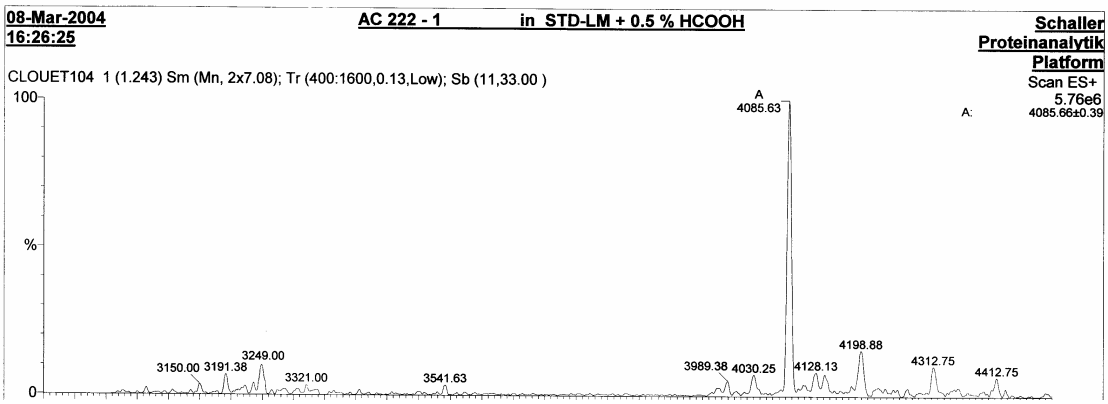
Dendrimer 8 . From Tentagel Rink amide resin (160 mg, 0.25 mmol/g), the dendrimer **8** was obtained as colorless foamy solid after preparative HPLC purification (6.4 mg, 12.8%); RP-HPLC: t_R = 22.4 min; MS (ES+): calcd. for $C_{178}H_{283}N_{75}O_{55}$: 4351.16, found: 4352.50



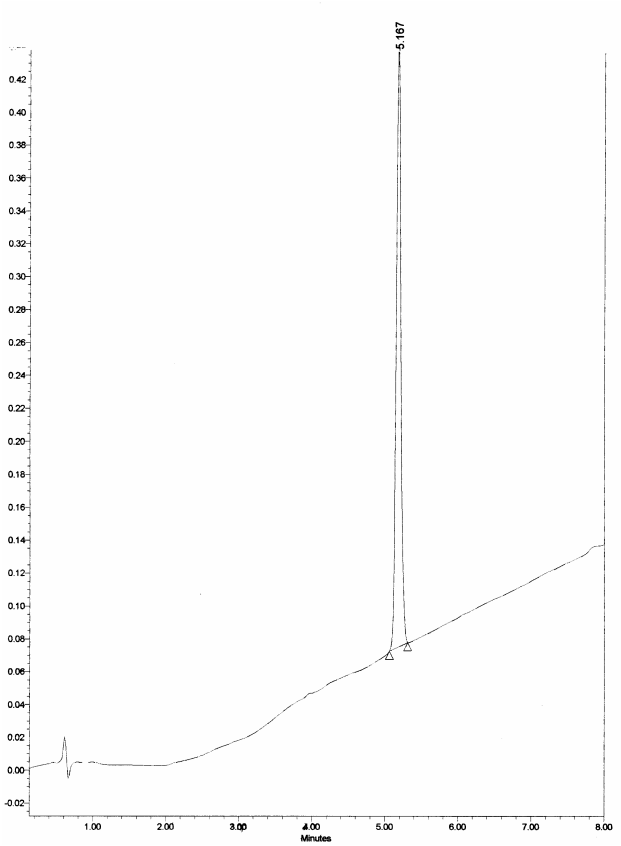


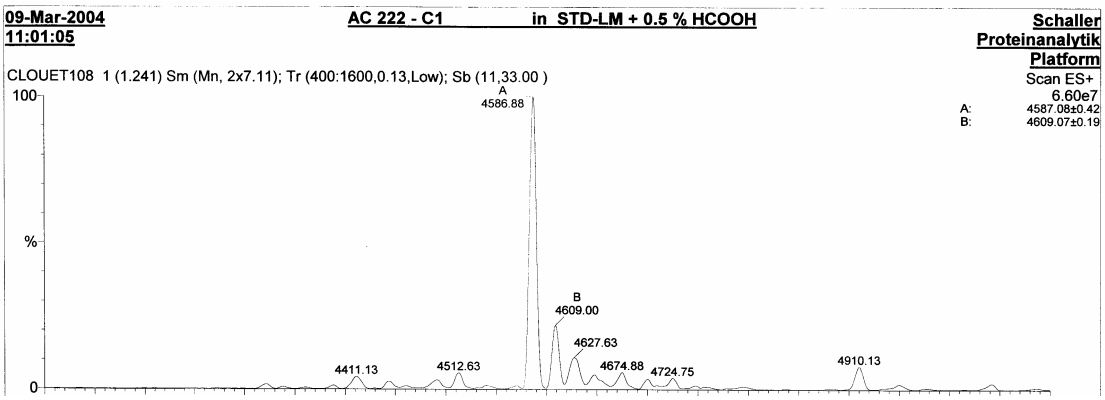
Dendrimer 10 . From Tentagel Rink amide resin (160 mg, 0.25 mmol/g), the dendrimer **10** was obtained as colorless foamy solid after preparative HPLC purification (3.8 mg, 7.6%); RP-HPLC: t_R = 23.8 min; MS (ES+): calcd. for $C_{170}H_{295}N_{71}O_{47}$: 4083.28, found: 4085.63



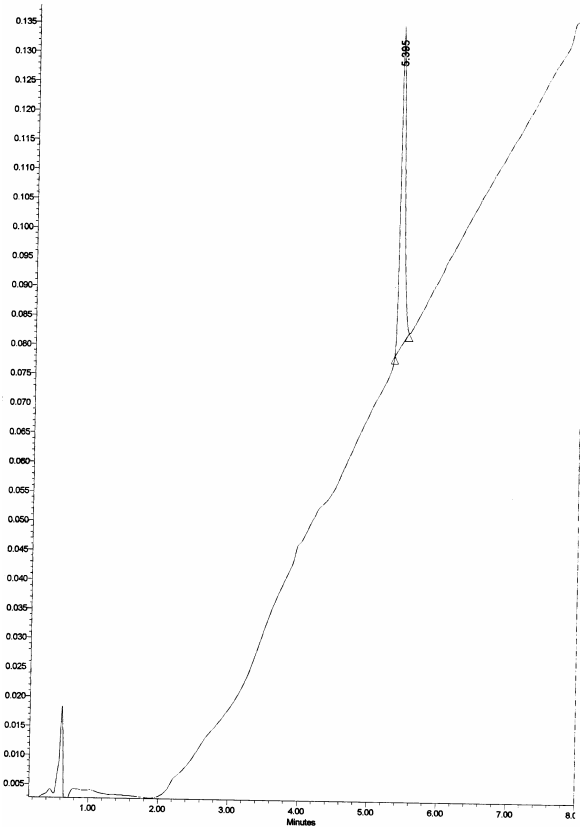


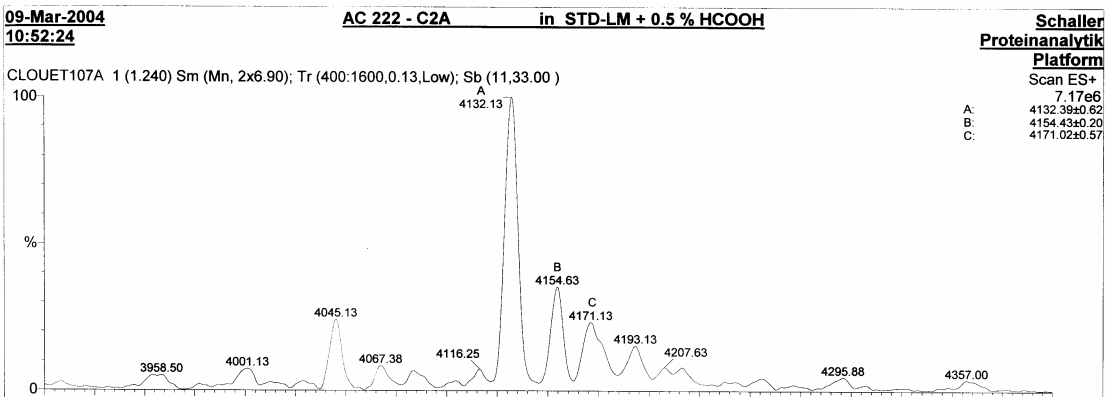
Dendrimer C11 . From Tentagel Rink amide resin (160 mg, 0.25 mmol/g), the dendrimer **C11** was obtained as colorless foamy solid after preparative HPLC purification (19.7 mg, 39.4%); RP-HPLC: t_R = 21.9 min; MS (ES+): calcd. for $C_{199}H_{305}N_{71}O_{56}$: 4585.32, found: 4586.88



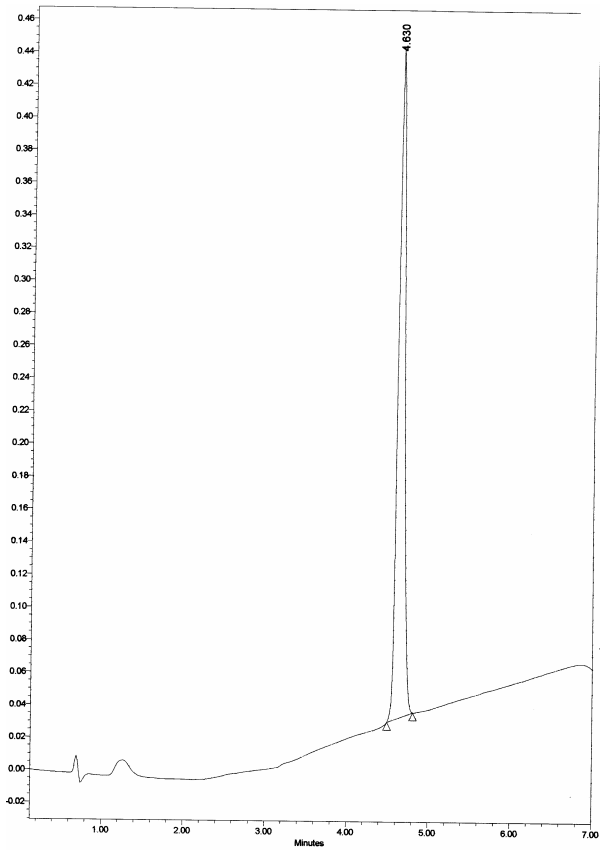


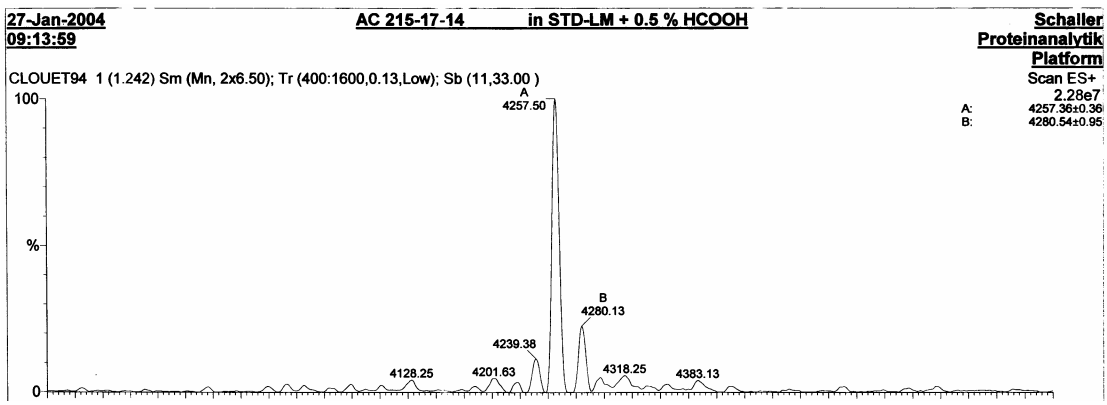
Dendrimer C12 . From Tentagel Rink amide resin (160 mg, 0.25 mmol/g), the dendrimer **C12** was obtained as colorless foamy solid after preparative HPLC purification (4.2 mg, 8.4%); RP-HPLC: t_R = 22.5 min; MS (ES+): calcd. for $C_{170}H_{273}N_{63}O_{59}$: 4130.95, found: 4132.13



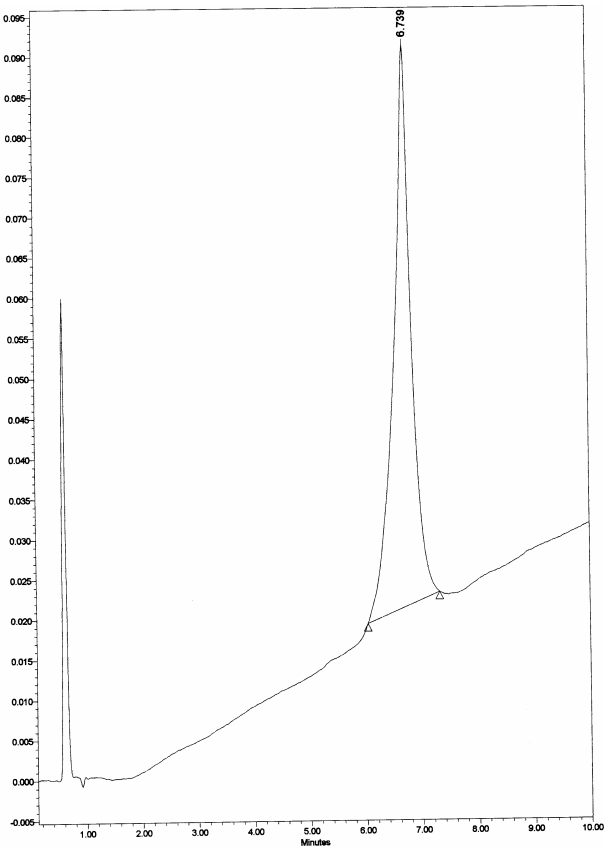


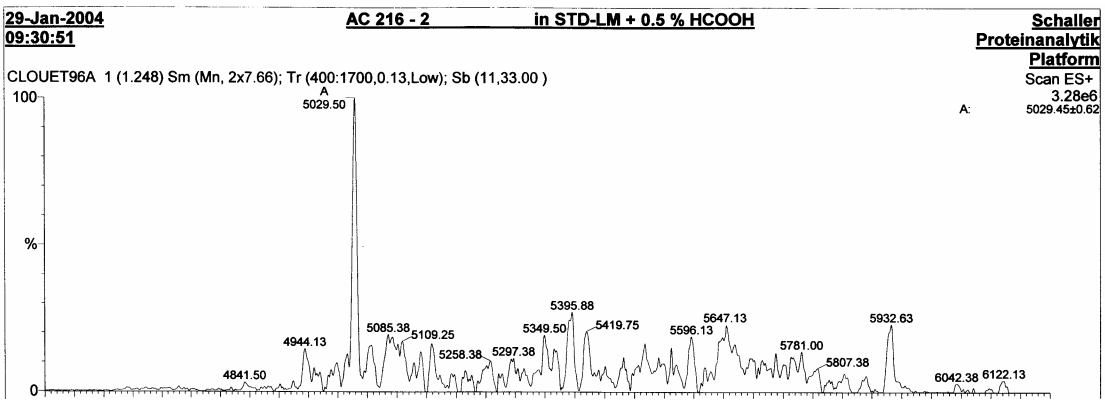
Dendrimer 14 . From Tentagel Rink amide resin (320 mg, 0.25 mmol/g), the dendrimer **14** was obtained as colorless foamy solid after preparative HPLC purification (37.6 mg, 11%); RP-HPLC: t_R = 25.7 min; MS (ES+): calcd. for $C_{190}H_{277}N_{59}O_{62}$: 4257.03, found: 4257.50.



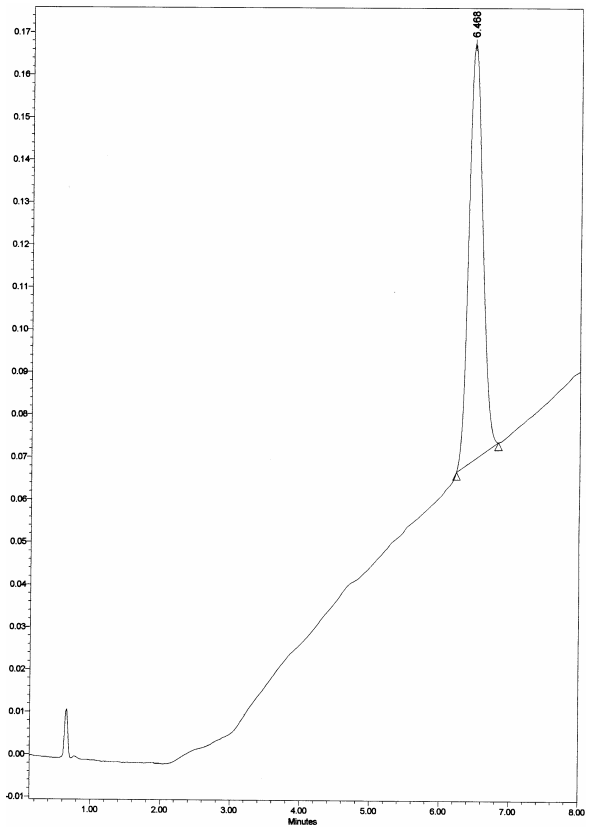


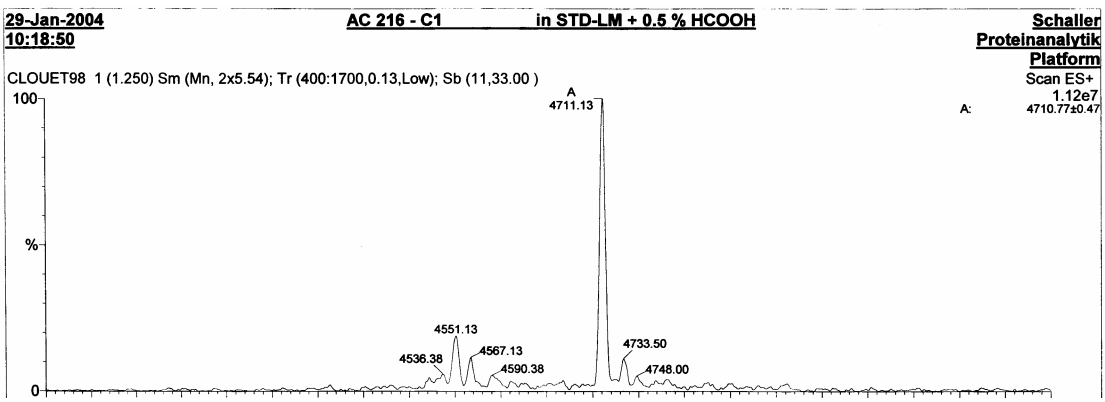
Dendrimer 19 . From Tentagel Rink amide resin (240 mg, 0.25 mmol/g), the dendrimer **19** was obtained as colorless foamy solid after preparative HPLC purification (14.2 mg, 4.7%); RP-HPLC: t_R = 32.4 min; MS (ES+): calcd. for $C_{230}H_{343}N_{79}O_{51}$: 5027.66, found: 5029.50.



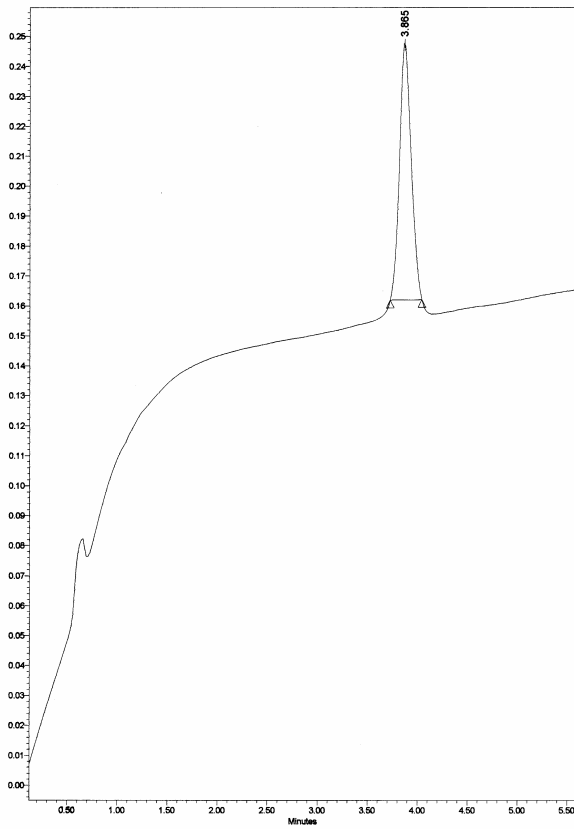


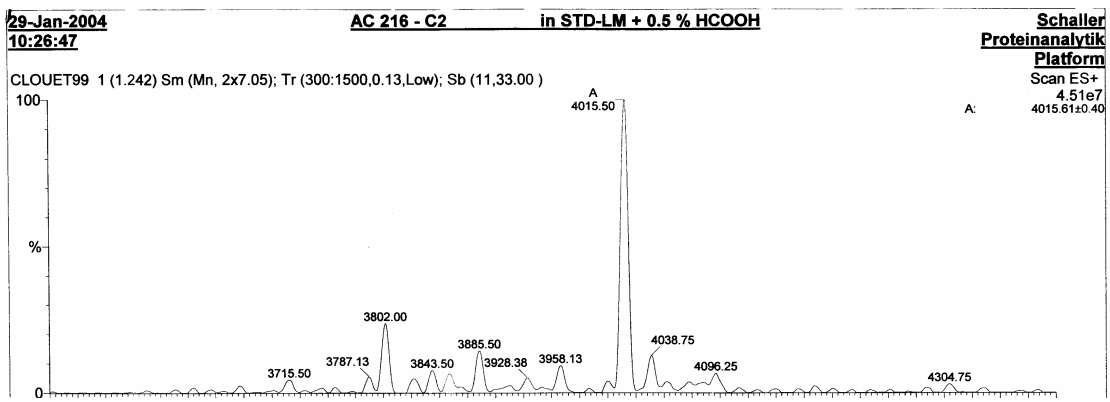
Dendrimer C20 . From Tentagel Rink amide resin (240 mg, 0.25 mmol/g), the dendrimer **C20** was obtained as colorless foamy solid after preparative HPLC purification (4.0 mg, 1.4%); RP-HPLC: t_R = 34.8 min; MS (ES+): calcd. for $C_{230}H_{305}N_{67}O_{44}$: 4709.36, found: 4711.13.





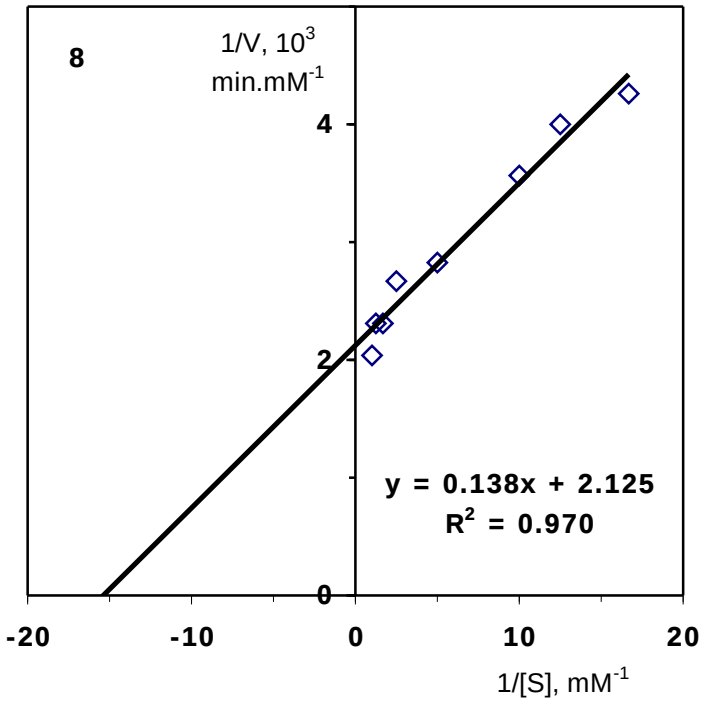
Dendrimer C21 . From Tentagel Rink amide resin (240 mg, 0.25 mmol/g), the dendrimer **C21** was obtained as colorless foamy solid after preparative HPLC purification (30.9 mg, 12.9%); RP-HPLC: t_R = 24.8 min; MS (ES+): calcd. for $C_{156}H_{284}N_{72}O_{53}$: 4014.17, found: 4015.50.

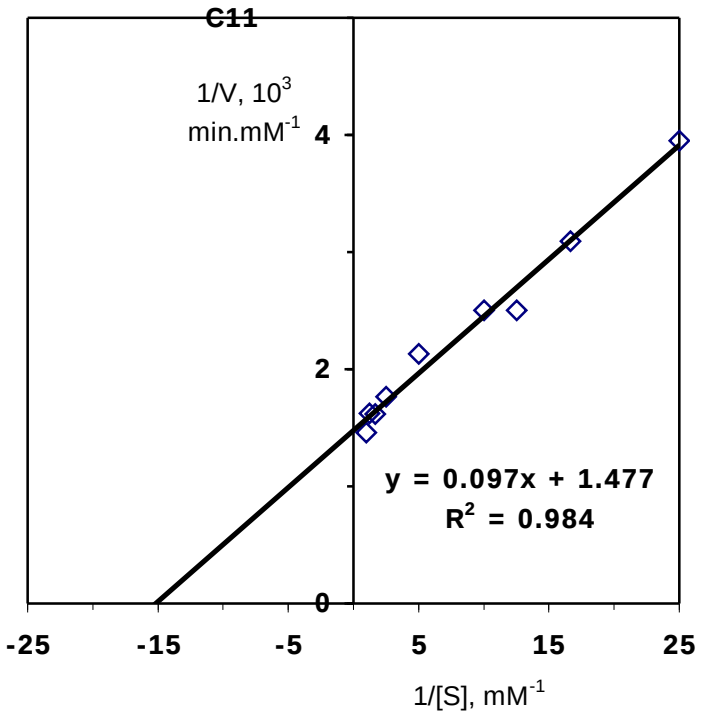


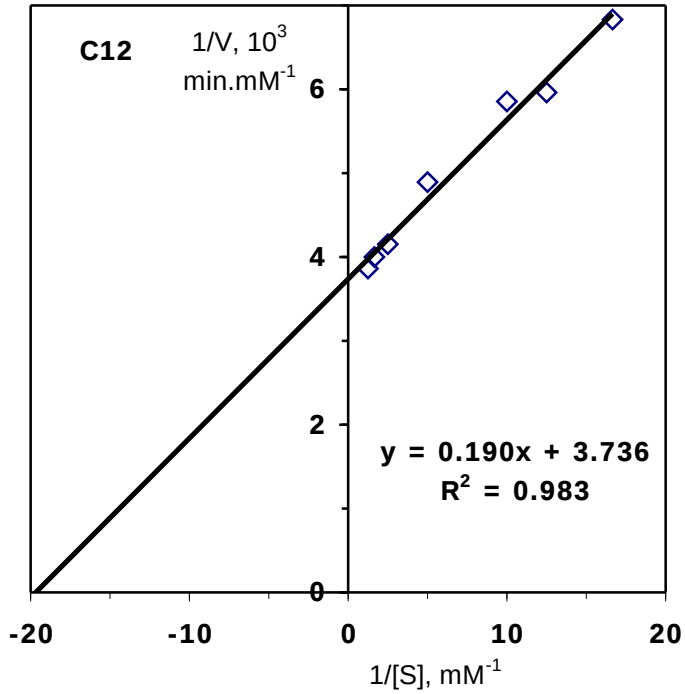


Kinetic measurements. The kinetic measurements were carried out by using a SPECTRAMax fluorescence plate reader (λ_{ex}= 460 nm, λ_{em}= 530 nm) at 25.2 °C. Assays were followed in individual wells of round-bottom polystyrene 96-well-plates (Costar). Kinetic experiments were followed for 2 h. The dendrimers were stored at -20°C in 1mM stock solution in acetonitrile/water: 1/1. Dendrimer stock solutions were freshly diluted to 0.05 mM solution in 20 mM aq. BisTris pH 6.0. The BisTris buffer, pH 6.0 was prepared using MilliQ deionized water. Initial reaction rates were calculated from the steepest part observed during the first 2000 sec of each curve. In a typical experiment, 20 µL of aq. BisTris pH 6.0 (20 mM) were first added in a well, then 2.5 µL of a dendrimer solution (0.05 mM in aq. BisTris pH 6.0, concentration in the well: 5 µM), and last 2.5 µL of substrate solution (e.g. 2 mM in acetonitrile/water: 1/1, concentration in the well: 200 µM). Fluorescence data were converted to product concentration by means of a calibration curve with pure product. The initial reaction rates observed under these conditions is the apparent rate V_{app}. V_{uncat} is the initial rate observed under the same conditions without dendrimer. The observed rate enhancement V_{net}/V_{uncat} is calculated as (V_{app}/V_{uncat})-1. Michaelis-Menten parameters were obtained from the linear double reciprocal plot of $1/V_{net}$ (Vnet = V_{app}-V_{uncat}) vs. 1/[S] measured similarly with 5 µM dendrimer (V_{app}) or no dendrimer (V_{uncat}) and 40, 60, 80, 100, 200, 400, 600, 800, 1000 µM substrate. The catalytic rate constant k_{cat} for the hydrolysis is given by k_{cat} = V_{max}/[D], where [D] indicates the concentration of dendrimers. The reaction rate with 4-methylimidazole (4-MeIm) was obtained under the same conditions with 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800 µM 4-MeIm and 200 µM substrate. The second order rate constants k_2 was calculated from linear regression of the experimentally measured pseudo first order rate constants k' as a function of 4-Me-Imidazole concentration.

Michaelis-Menten kinetic plots for catalysis by dendrimers 8, C11 and C12






C12
1/V, 10^3 min.mM^-1
6
4
2
0
y = 0.190x + 3.736
R^2 = 0.983
-20
-10
0
10
20
1/[S], mM^-1