

## **Electronic Supplementary Information:**

### **Aldolase Peptide Dendrimers from Combinatorial Libraries**

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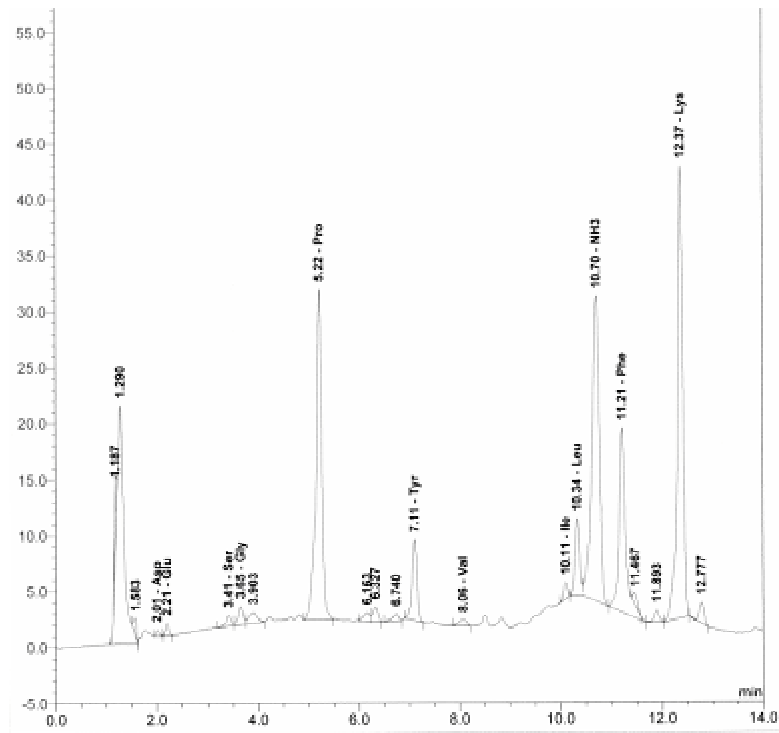
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jean-louis.reymond@ioc.unibe.ch

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

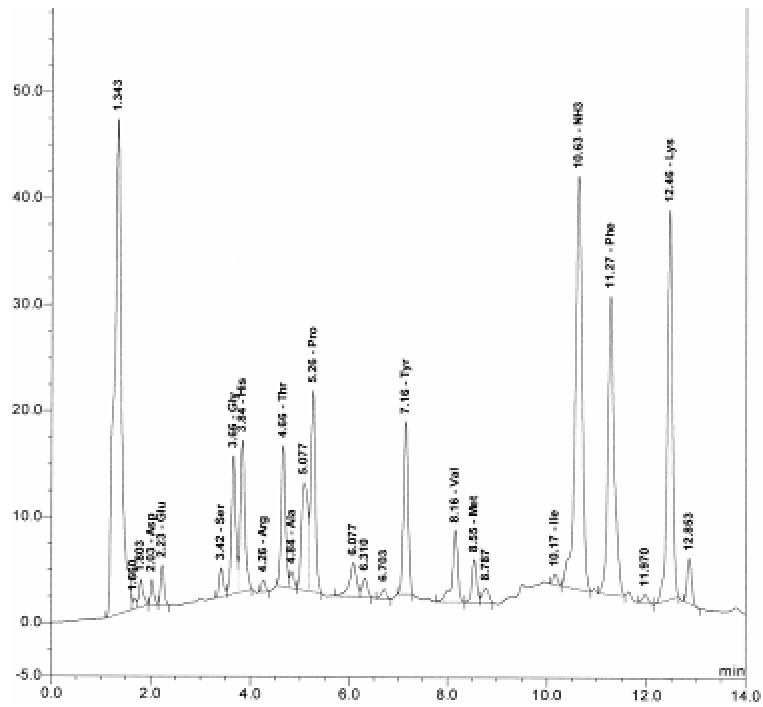
HPLC-integration data for amino acid analysis of single beads of dendrimers after total hydrolysis with 6M HCl solution at 110°C for 22 h and derivitization with phenyl isothiocyanate (PITC). The sequence of dendrimers is deduced from the HPLC-peak integration of each amino acid PITC-derivative relative to the reference integration of this derivative. The Dap (2,3-diaminopropanoic acid) branching unit co-elutes with phenylalanine (relative integration =91.0). The value is subtracted before calculating integration for this amino acid.

**HPLC-trace for analysis of the bead of catalytic dendrimer L2D1 identified as catalytic.**



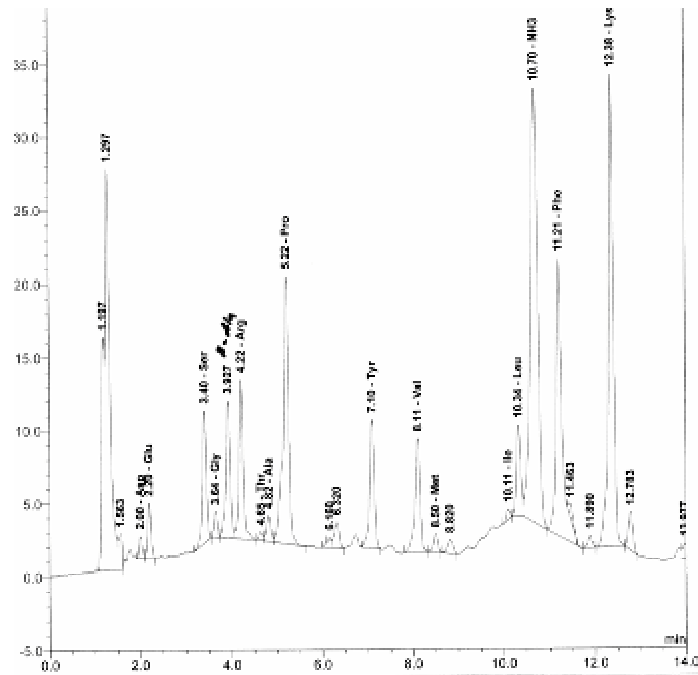
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
2.01	2.00	0.087	0.04	0.55	3.0	Asp
2.21	2.20	0.072	0.06	1.07	6.3	Glu
3.41	3.40	0.098	0.12	0.96	6.7	Ser
3.65	3.64	0.087	0.19	1.64	10.9	Gly
5.22	5.22	0.102	3.89	29.49	181.3	Pro
7.11	7.09	0.102	0.83	7.25	51.1	Tyr
8.06	8.10	0.187	0.10	0.52	3.8	Val
10.11	10.10	0.089	0.13	1.47	10.1	Ile
10.34	10.33	0.099	0.73	6.92	50.7	Leu
10.70	10.69	0.152	4.61	27.26	540.7	NH3
11.21	11.23	0.115	2.52	16.45	124.7	Phe
12.37	12.37	0.105	5.05	40.34	165.5	Lys
<b>Total:</b>			18.292	133.923	1154.80	

**HPLC-trace for analysis of the bead of catalytic dendrimer L2K4 identified as catalytic.**



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
2.03	2.03	0.065	0.18	2.35	6.5	Asp
2.23	2.23	0.074	0.30	3.73	6.7	Glu
3.42	3.41	0.084	0.25	2.70	8.9	Ser
3.66	3.66	0.090	1.30	13.00	50.7	Gly
3.84	3.83	0.092	1.51	14.28	98.8	His
4.26	4.26	0.081	0.09	1.10	3.9	Arg
4.66	4.66	0.090	1.32	13.31	93.7	Thr
4.84	4.84	n.a.	0.14	1.57	7.1	Ala
5.26	5.26	0.096	1.97	18.92	134.8	Pro
7.16	7.15	0.096	1.74	16.25	55.0	Tyr
8.16	8.16	0.099	0.90	6.81	31.8	Val
8.55	8.55	0.099	0.45	4.03	10.1	Met
10.17	10.17	0.096	0.10	0.96	7.0	Ile
10.63	10.63	0.138	6.23	38.84	728.7	NH3
11.27	11.31	0.119	4.08	28.06	203.8	Phe
12.46	12.46	0.104	4.32	36.59	150.2	Lys
<b>Total:</b>			24.880	202.502	1599.40	

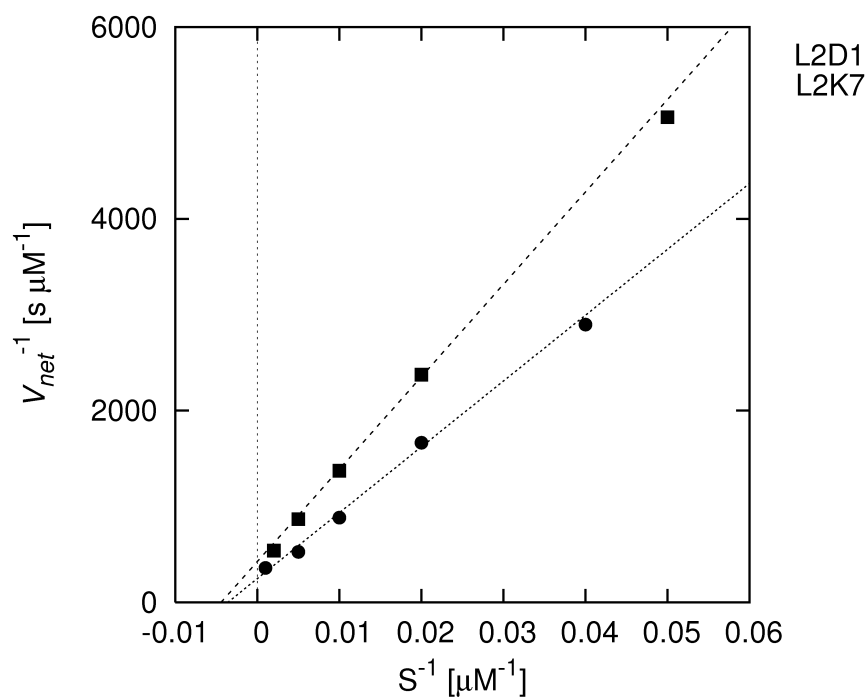
HPLC-trace for analysis of the bead of catalytic dendrimer L2K7 identified as catalytic.



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
2.00	2.00	0.071	0.11	1.49	8.0	Asp
2.20	2.20	0.076	0.32	3.76	22.1	Glu
3.40	3.40	0.089	0.91	9.05	62.6	Ser
3.64	3.64	0.084	0.16	1.78	11.8	Gly
4.22	4.22	0.091	1.17	10.93	75.3	Arg
4.65	4.63	0.111	0.06	0.54	4.3	Thr
4.82	4.81	0.101	0.20	1.76	12.5	Ala
5.22	5.22	0.104	2.57	18.30	112.5	Pro
7.10	7.09	0.105	1.06	8.80	62.1	Tyr
8.11	8.10	0.108	1.02	7.79	56.4	Val
8.50	8.49	0.103	0.14	1.22	9.6	Met
10.11	10.10	0.085	0.06	0.74	5.1	Ile
10.34	10.33	0.102	0.70	6.26	45.9	Leu
10.70	10.69	0.154	5.12	29.72	589.3	NH3
11.21	11.23	0.120	3.03	18.98	143.9	Phe
12.38	12.37	0.107	4.11	32.25	132.4	Lys
<b>Total:</b>			20.723	153.370	1353.65	

**Kinetic measurements with 6.** The kinetic measurements were carried out by using a Cytofluor II plate reader from Perseptive Biosystems ( $\lambda_{\text{ex}}= 460 \text{ nm}$ ,  $\lambda_{\text{em}}= 530 \text{ nm}$ ) at  $25 \text{ }^\circ\text{C}$ .  $200 \text{ }\mu\text{L}$  assays were followed in individual wells of round-bottom polypropylene 96-well-plates (Costar). Kinetic experiments were followed for 6 hrs. The dendrimers were stored at  $-20^\circ\text{C}$  in  $1 \text{ mM}$  stock solution in acetonitrile/water (1:1, v/v). Dendrimer stock solutions were freshly diluted to  $80.0 \text{ }\mu\text{M}$  solution in  $20 \text{ mM}$  aq. bicine buffer pH 8.5. The  $20 \text{ mM}$  bicine buffer, pH 8.5 was prepared using MilliQ deionized water. Initial reaction rates were calculated from the steepest part observed during the first 5000 sec of each curve. In a typical experiment,  $140 \text{ }\mu\text{L}$  of aq. bicine pH 8.5 ( $20 \text{ mM}$ ) were first added in a well, then  $50 \text{ }\mu\text{L}$  of a dendrimer solution ( $80.0 \text{ }\mu\text{M}$  in aq. bicine pH 8.5, concentration in the well:  $20 \text{ }\mu\text{M}$ ), and last  $10 \text{ }\mu\text{L}$  of substrate solution ( $1.0 \text{ mM}$  in aq. bicine buffer pH=8.5/acetonitrile (10:1, v/v), concentration in the well:  $50 \text{ }\mu\text{M}$ ). 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  $V_{\text{app}}$ .  $V_{\text{uncat}}$  is the initial rate observed under the same conditions without dendrimer. Michaelis-Menten parameters were obtained from the linear double reciprocal plot of  $1/V_{\text{net}}$  ( $V_{\text{net}} = V_{\text{app}} - V_{\text{uncat}}$ ) vs.  $1/[\text{S}]$  measured similarly with  $20 \text{ }\mu\text{M}$  dendrimer ( $V_{\text{app}}$ ) or no dendrimer ( $V_{\text{uncat}}$ ) and  $20, 50, 100, 200, 500 \text{ }\mu\text{M}$  substrate. The catalytic rate constant  $k_{\text{cat}}$  for the hydrolysis is given by  $k_{\text{cat}} = V_{\text{max}}/[\text{D}]$ , where  $[\text{D}]$  indicates the concentration of dendrimers. The second order rate constants  $k_2$  were calculated from linear regression of the experimentally measured pseudo first order rate constants  $k'$  as a function of proline or lysine concentration.

Double-reciprocal Lineweaver-Burk plot for dendrimer catalyzed reactions with ketone 6. Conditions: 20  $\mu\text{M}$  dendrimer, 20, 50, 100, 200 and 500  $\mu\text{M}$  ketone 6 in 20 mM aq. bicine buffer pH=8.5.

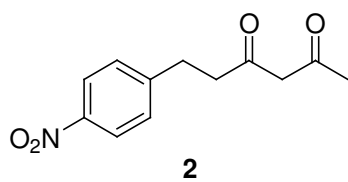


**Kinetic parameters for reaction with coumarin probe 6 with peptide dendrimers from library L2, regular series R1 and R2 and aldolase antibody 38C2.[a]**

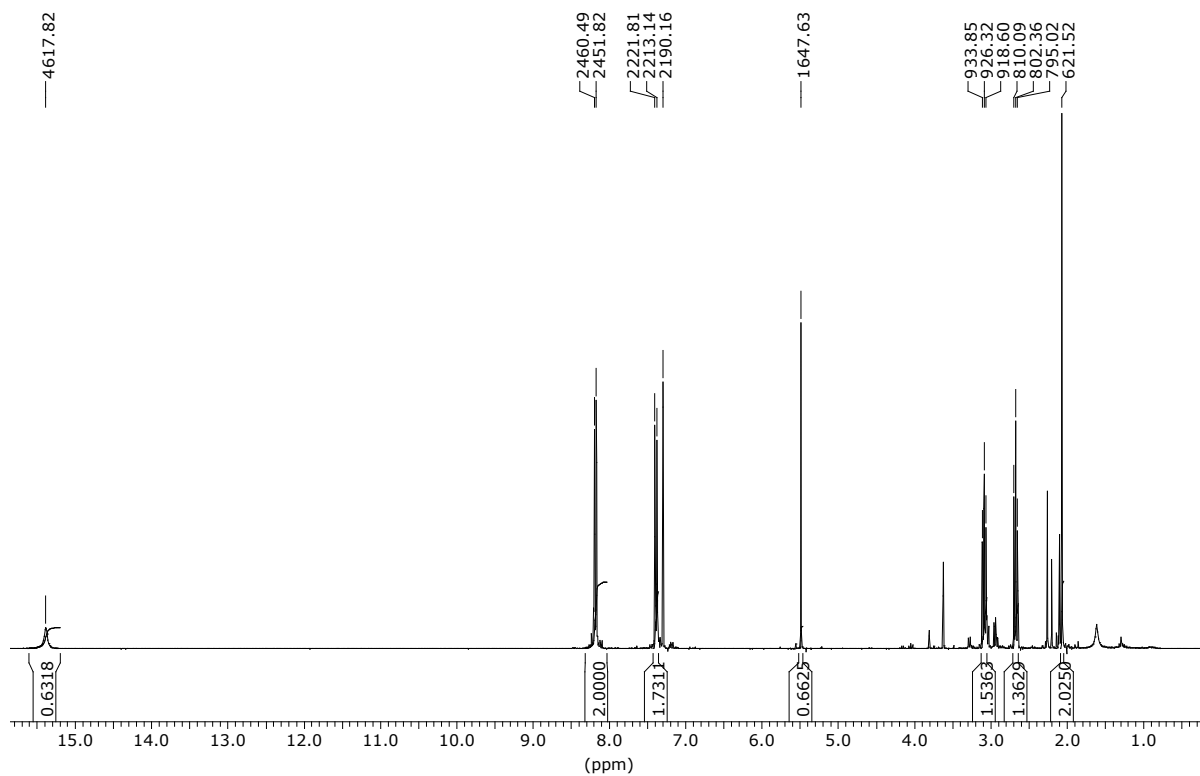
Catalyst		$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$K_{\text{M}}$ ( $\mu\text{M}$ )	$k_{\text{cat}}/k_{\text{uncat}}$	$k_{\text{cat}}/K_{\text{M}}$ ( $\text{s}^{-1} \mu\text{M}^{-1}$ )	$(k_{\text{cat}}/K_{\text{M}})/k_2$
PK.PK.YL.IG	<b>L2D1</b>	$1.2 \times 10^{-4}$	230	56	$5.2 \times 10^{-7}$	5'900
EK.SK.YA.FV	<b>L2D5</b>	$2.0 \times 10^{-4}$	460	98	$4.3 \times 10^{-7}$	4'300 <sup>[b]</sup>
SK.SK.YG.FG	<b>L2D6</b>	$1.1 \times 10^{-4}$	140	51	$7.5 \times 10^{-7}$	7'400 <sup>[b]</sup>
PK.ER.βAG.FV	<b>L2D7</b>	$2.0 \times 10^{-4}$	340	92	$3.6 \times 10^{-7}$	6'500
PK.TH.TG.FV	<b>L2K4</b>	$2.4 \times 10^{-4}$	380	120	$6.3 \times 10^{-7}$	7'200
PK.SR.βAV.YL	<b>L2K7</b>	$2.0 \times 10^{-4}$	280	96	$7.3 \times 10^{-7}$	8'300
EK.ED.IG.YA	<b>L2K8</b>	$3.0 \times 10^{-4}$	820	150	$3.7 \times 10^{-7}$	3'700 <sup>[b]</sup>
PT.PT	<b>R1G1</b>	$9.3 \times 10^{-5}$	210	45	$4.4 \times 10^{-7}$	5'000
PT.PT.PT	<b>R1G2</b>	$9.4 \times 10^{-5}$	160	45	$6.0 \times 10^{-7}$	6'800
PT.PT.PT.PT	<b>R1G3</b>	$1.3 \times 10^{-4}$	180	61	$7.1 \times 10^{-7}$	8'100
PK.PK	<b>R2G1</b>	$6.3 \times 10^{-5}$	530	30	$1.2 \times 10^{-7}$	1'400
PK.PK.PK	<b>R2G2</b>	$1.7 \times 10^{-4}$	470	82	$3.6 \times 10^{-7}$	4'100
PK.PK.PK.PK	<b>R2G3</b>	$1.9 \times 10^{-4}$	300	92	$6.4 \times 10^{-7}$	7'300
Antibody <b>38C2</b>		$2.6 \times 10^{-3}$	80	1800	$3.2 \times 10^{-5}$	320'000 <sup>[b]</sup>

[a] Conditions: 25–500  $\mu\text{M}$  ketone **6**, 20  $\mu\text{M}$  dendrimer, 20 mM aqueous bicine buffer pH 8.5, 25°C. The kinetic constants given are derived from the linear double-reciprocal plots of  $1/V_{\text{net}}$  versus  $1/[\text{S}]$ .  $V_{\text{net}} = V_{\text{app}} - V_{\text{uncat}}$  with  $V_{\text{app}}$  being the apparent hydrolysis rate in the presence of dendrimer and  $V_{\text{uncat}}$  the hydrolysis rate in buffer alone ( $k_{\text{uncat}} = 2.1 \times 10^{-6} \text{ s}^{-1}$ ).  $k_2 = 8.8 \times 10^{-11} \mu\text{M}^{-1} \text{ s}^{-1}$  is the catalytic rate constant for  $\beta$ -elimination catalyzed by L-proline under the same conditions. [b]  $k_2 = 1.01 \times 10^{-10} \mu\text{M}^{-1} \text{ s}^{-1}$  is the catalytic rate constant for  $\beta$ -elimination catalyzed by L-lysine under the same conditions.

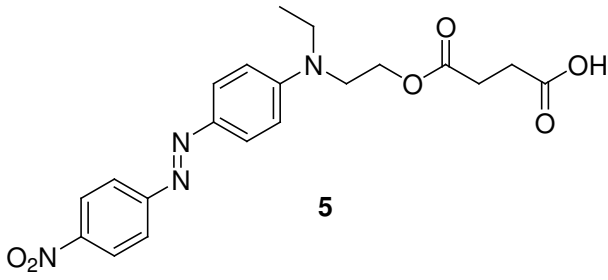


**6-(4-Nitro-phenyl)-hexane-2,4-dione (5)**

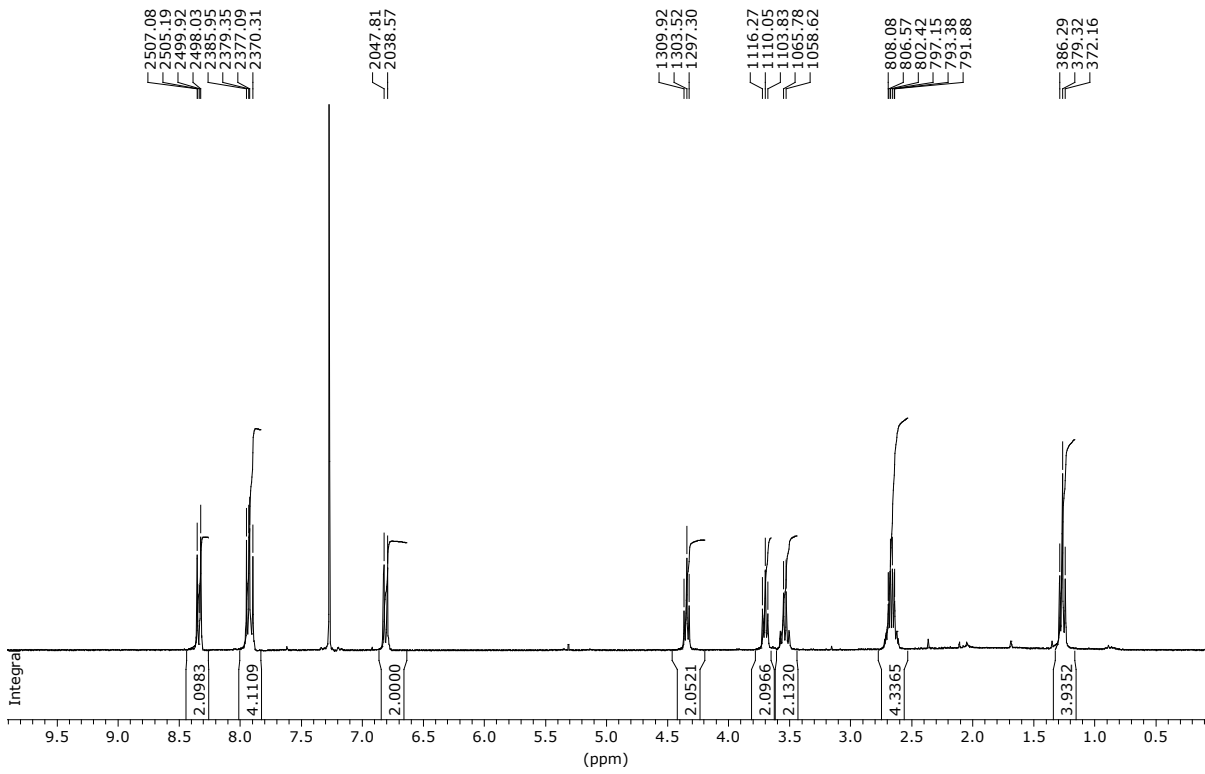
$C_{12}H_{13}NO_4$   
Mol. Wt.: 235.24



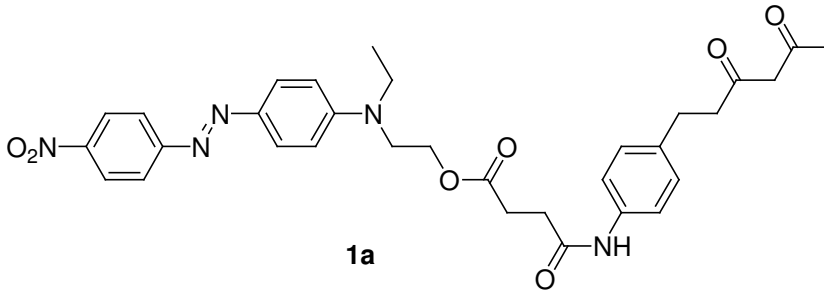
Succinic acid mono-(2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethyl) ester (8)



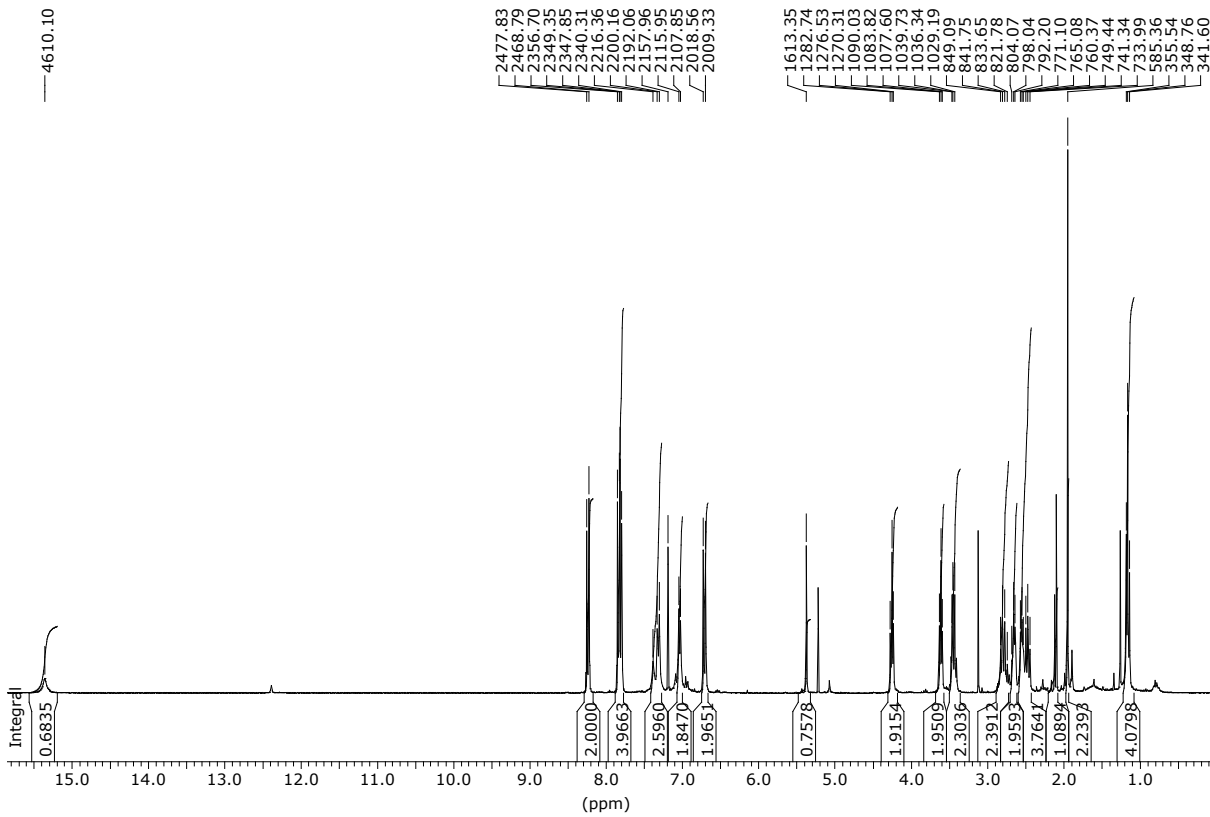
$C_{20}H_{22}N_4O_6$   
Mol. Wt.: 414.41

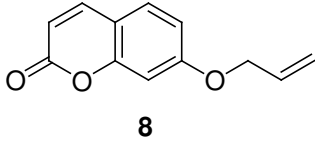


***N*-[4-(3,5-Dioxo-hexyl)-phenyl]-succinamic acid 2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethyl ester (**1a**)**

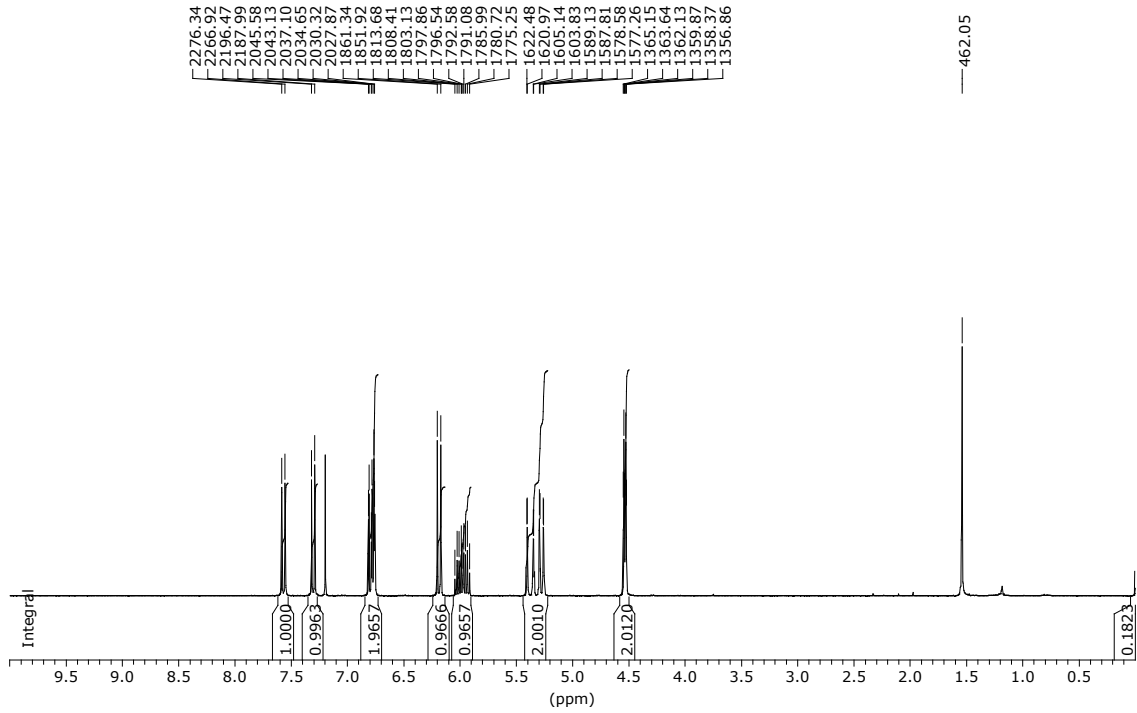


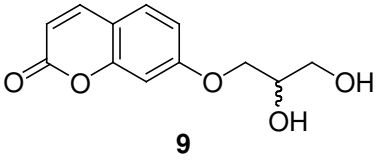
**1a**  
 $C_{32}H_{35}N_5O_7$   
 Mol. Wt.: 601.65



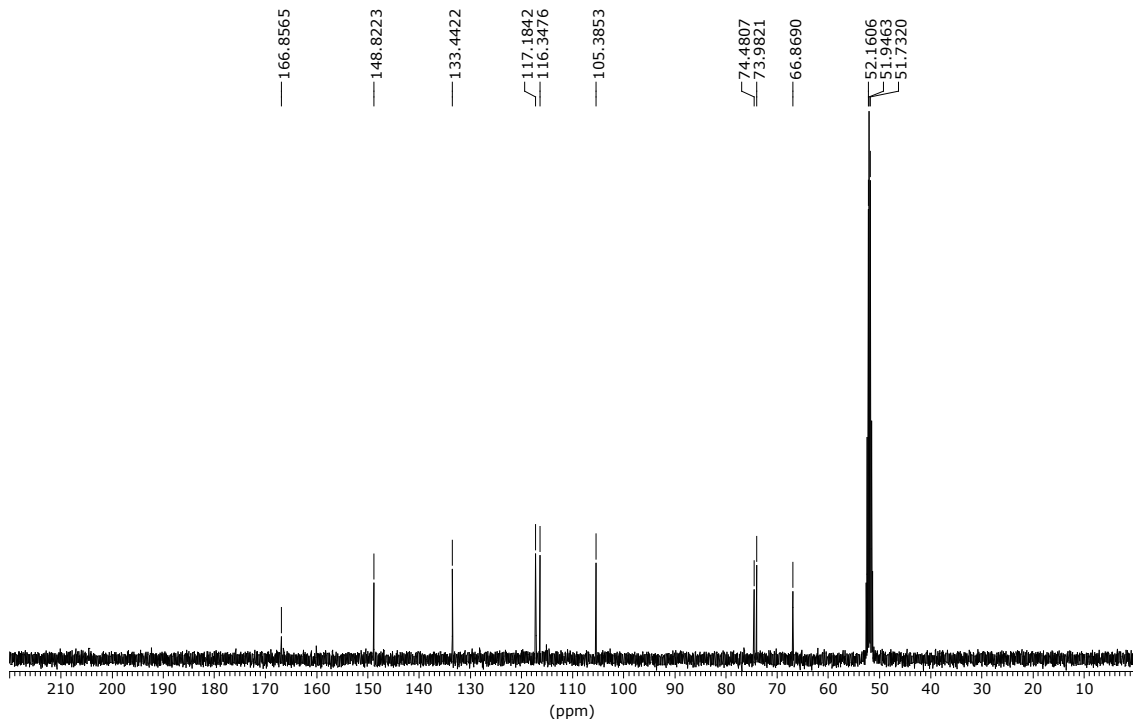
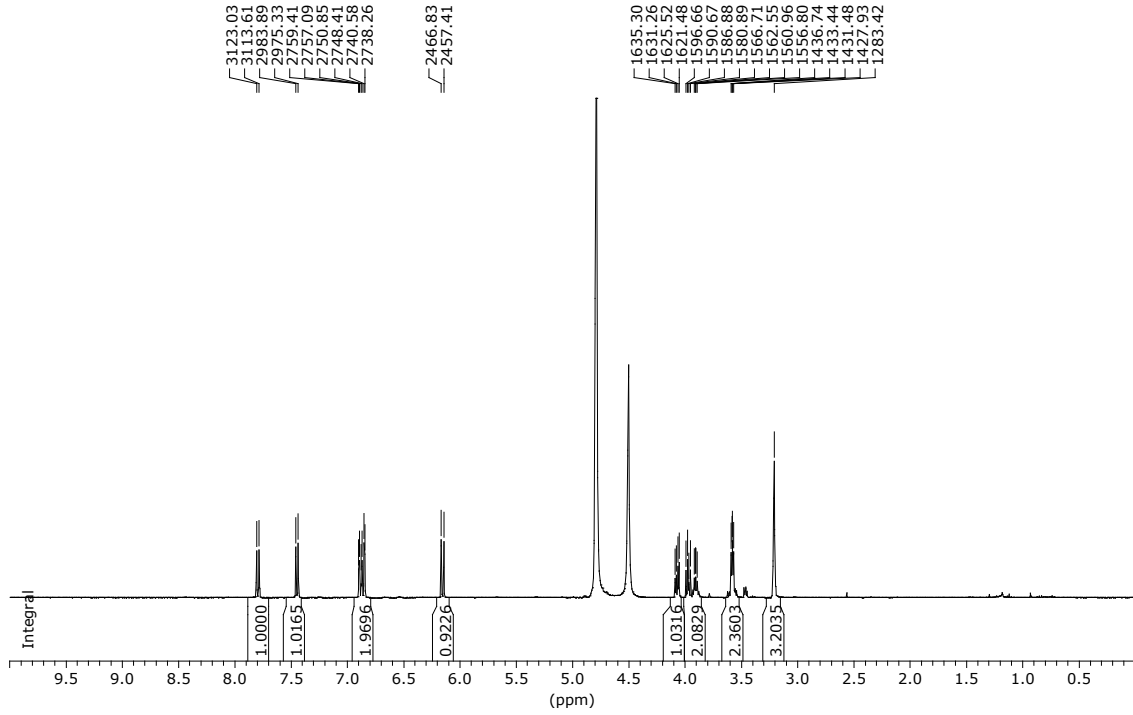


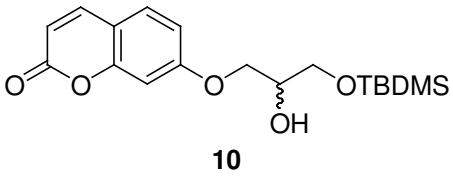
$C_{12}H_{10}O_3$   
 Mol. Wt.: 202.206  
 C, 71.28; H, 4.98; O, 23.74



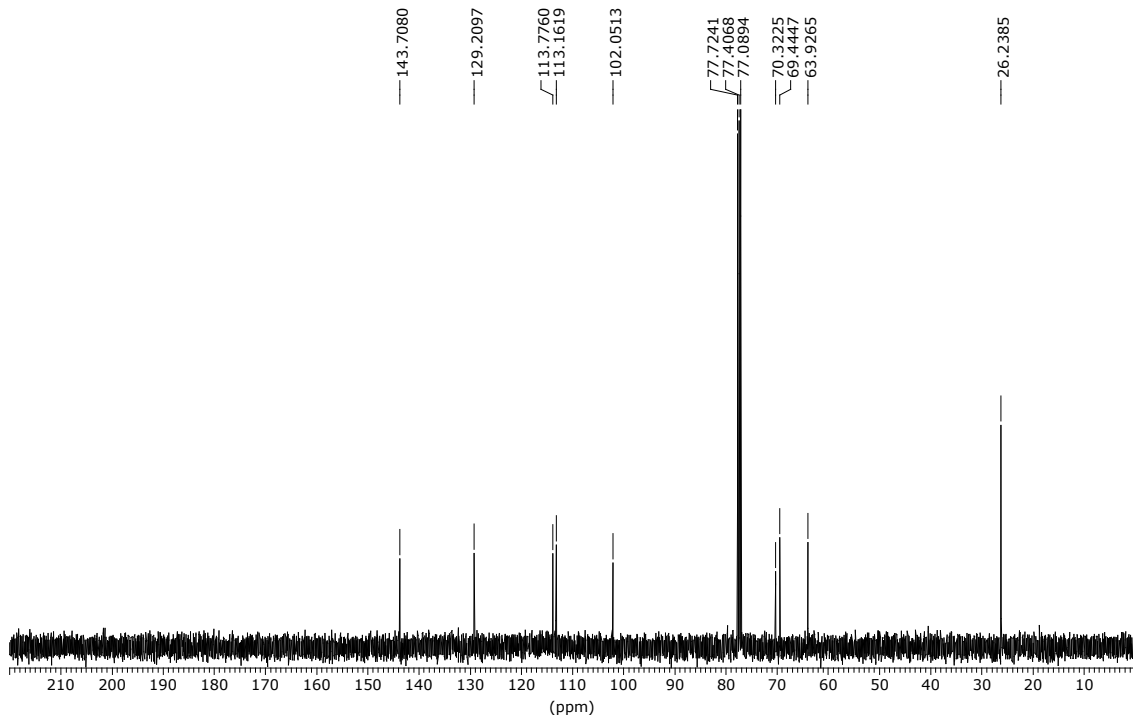
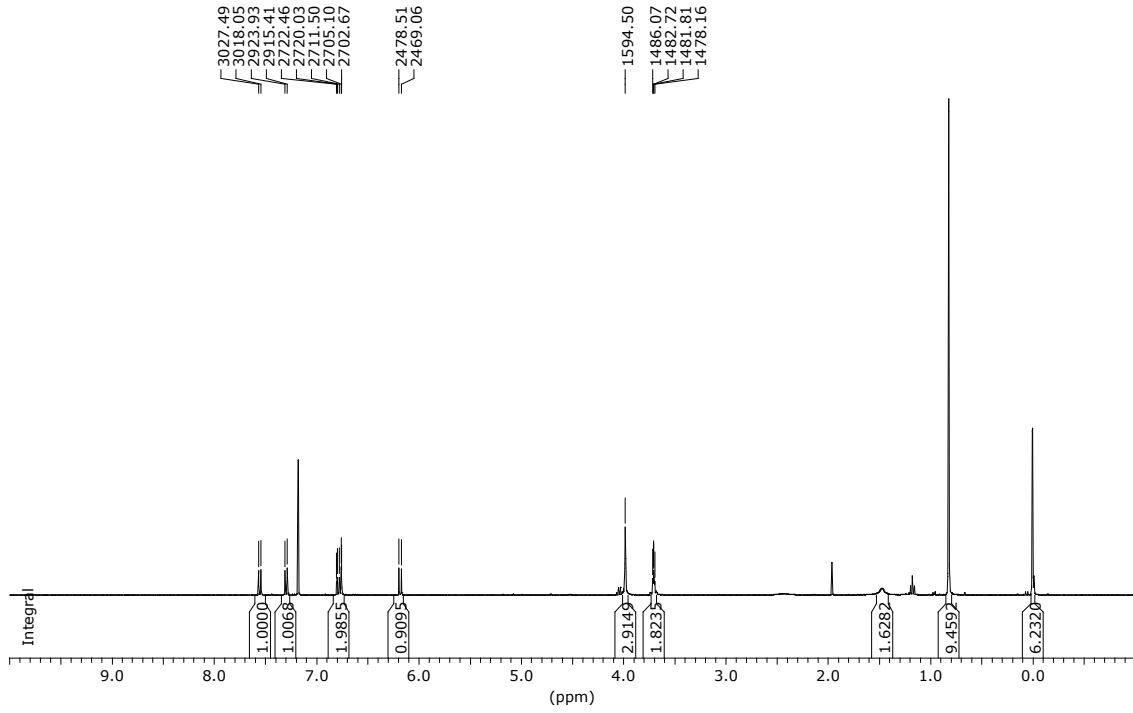


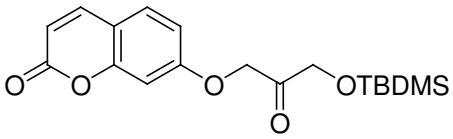
$C_{12}H_{12}O_5$   
 Mol. Wt.: 236.22068  
 C, 61.01; H, 5.12; O, 33.87





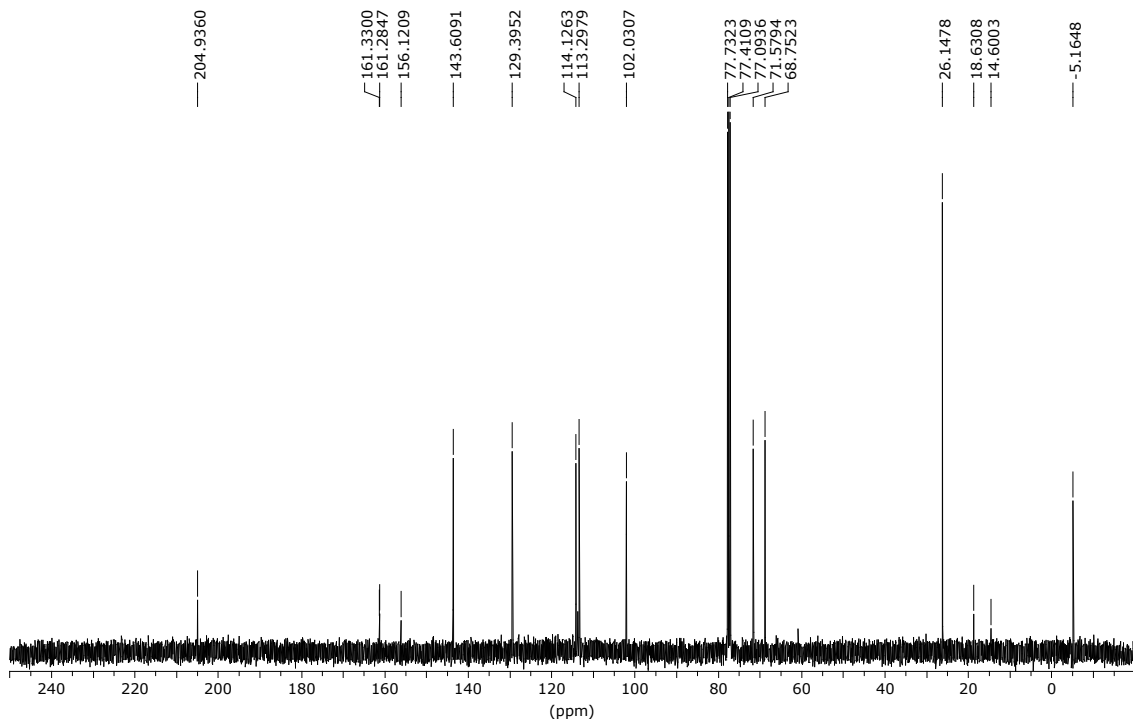
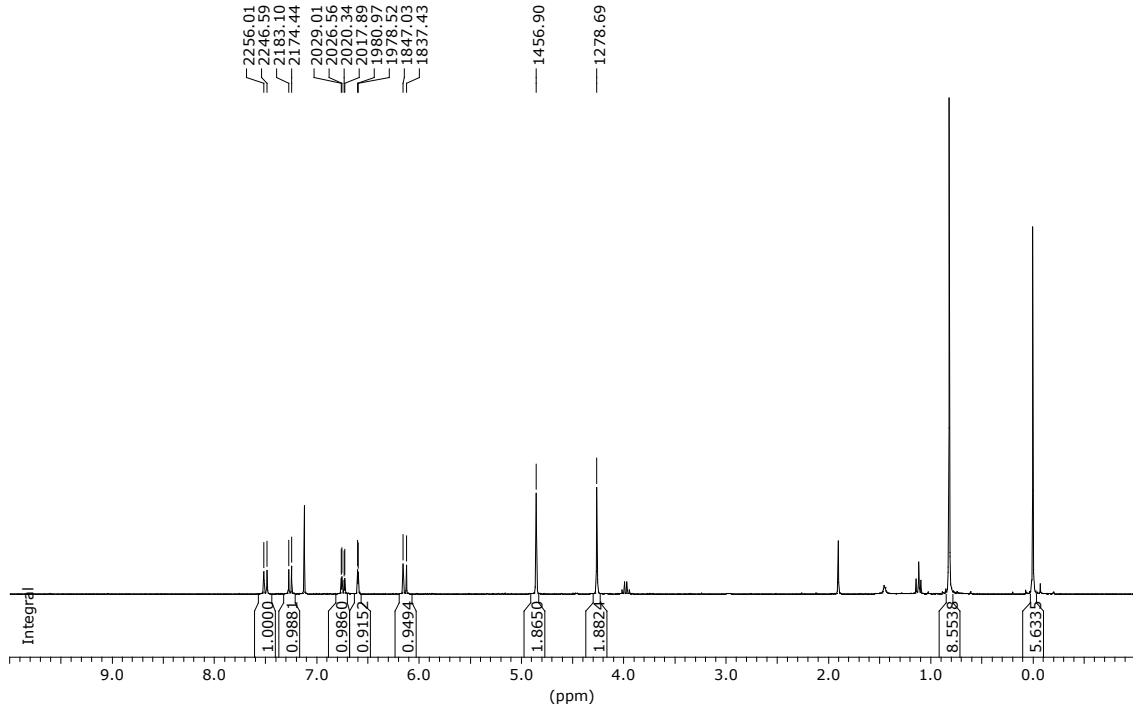
$C_{18}H_{26}O_5Si$   
 Mol. Wt.: 350.48154  
 C, 61.68; H, 7.48; O, 22.82; Si, 8.01

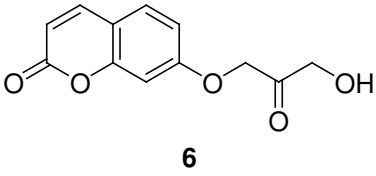




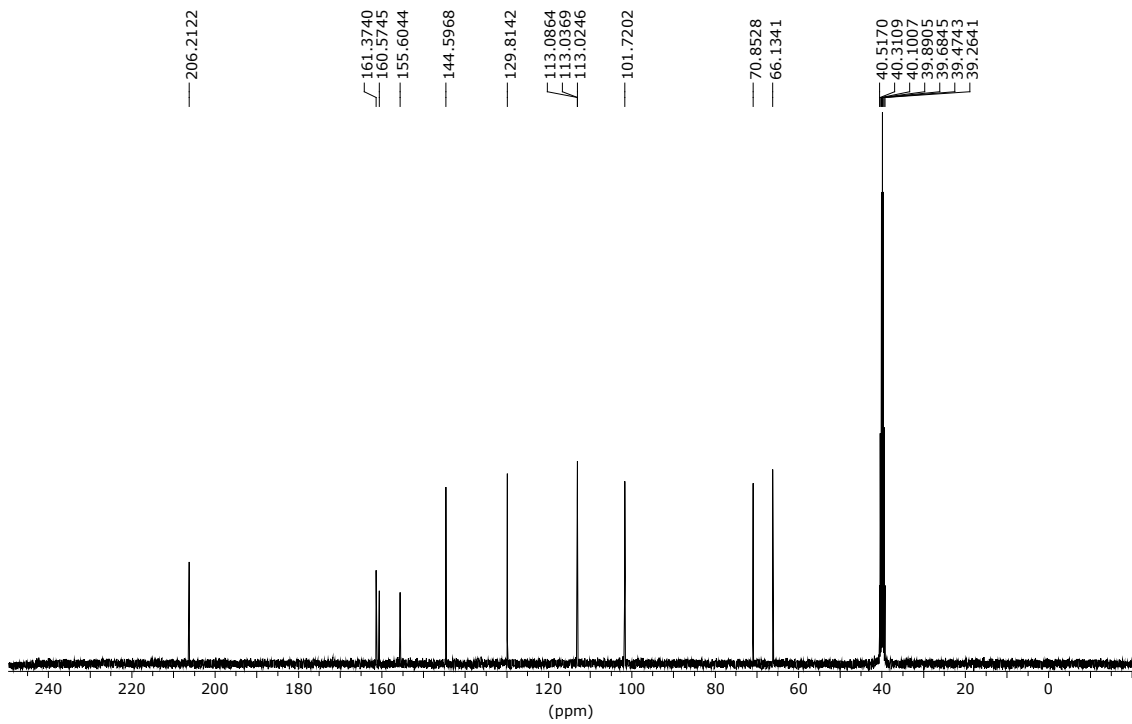
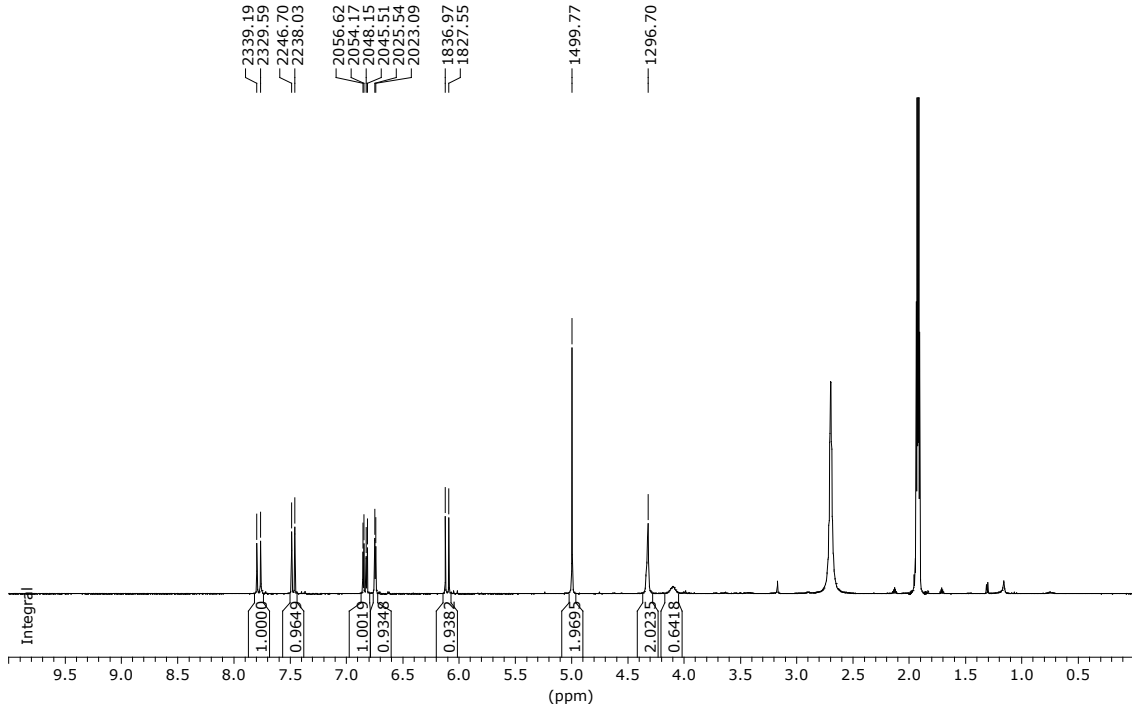
11

$C_{18}H_{24}O_5Si$   
 Mol. Wt.: 348.46566  
 C, 62.04; H, 6.94; O, 22.96; Si, 8.06



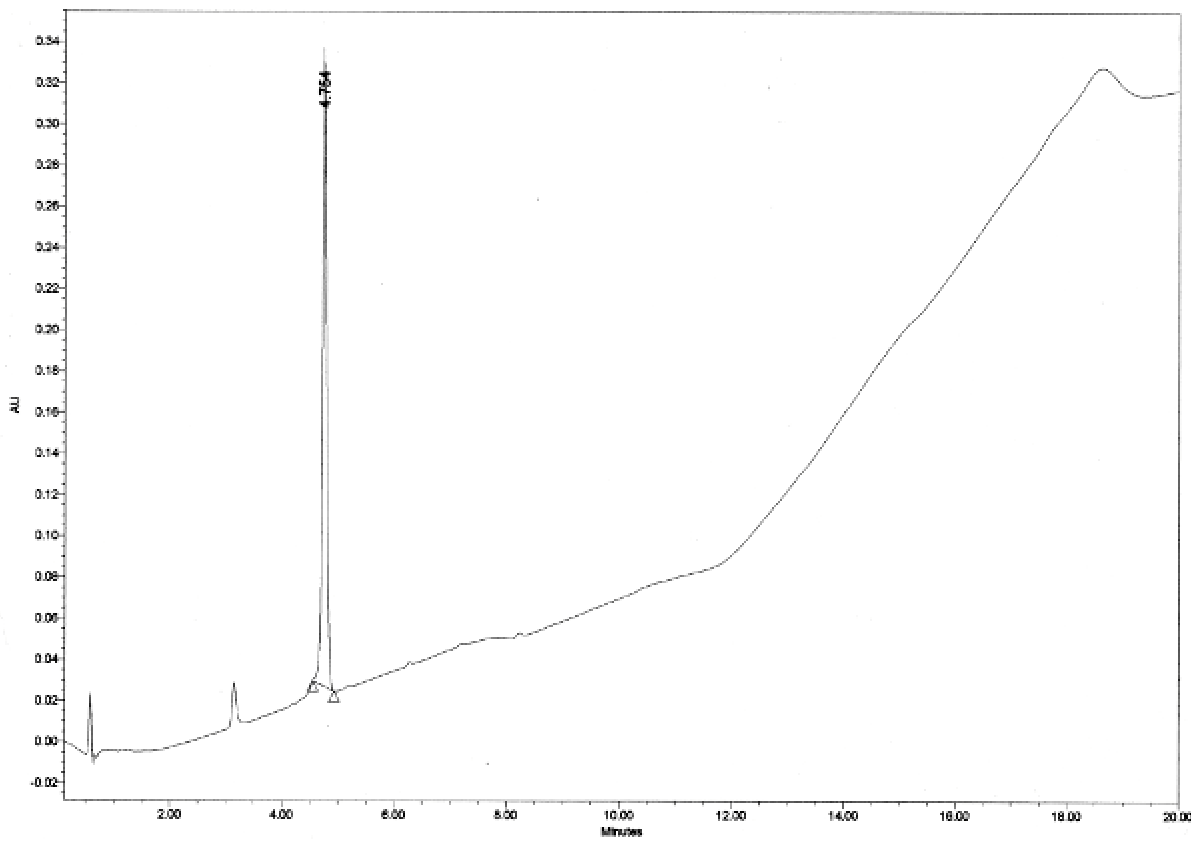
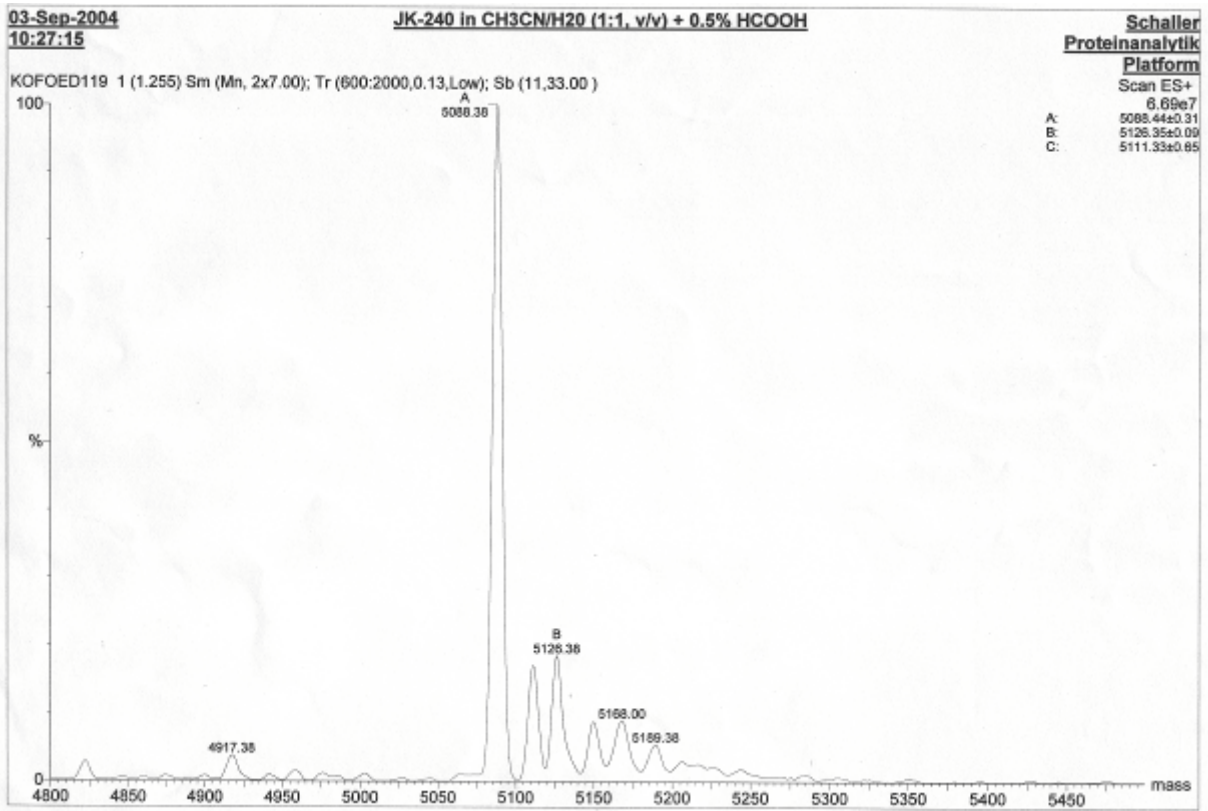


$C_{12}H_{10}O_5$   
 Mol. Wt.: 234.2048  
 C, 61.54; H, 4.30; O, 34.16

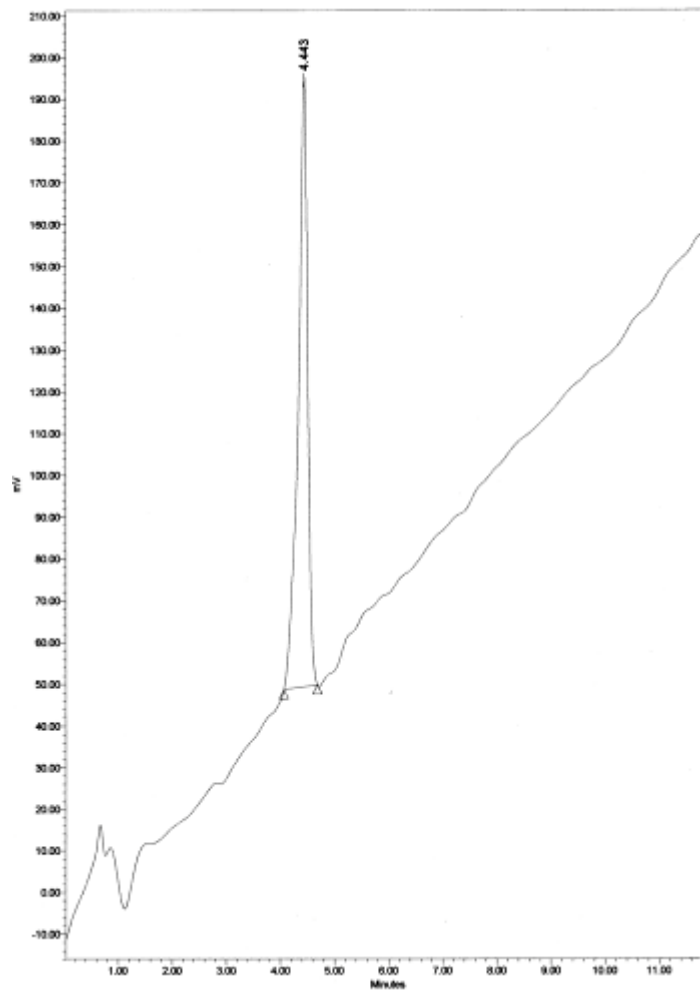
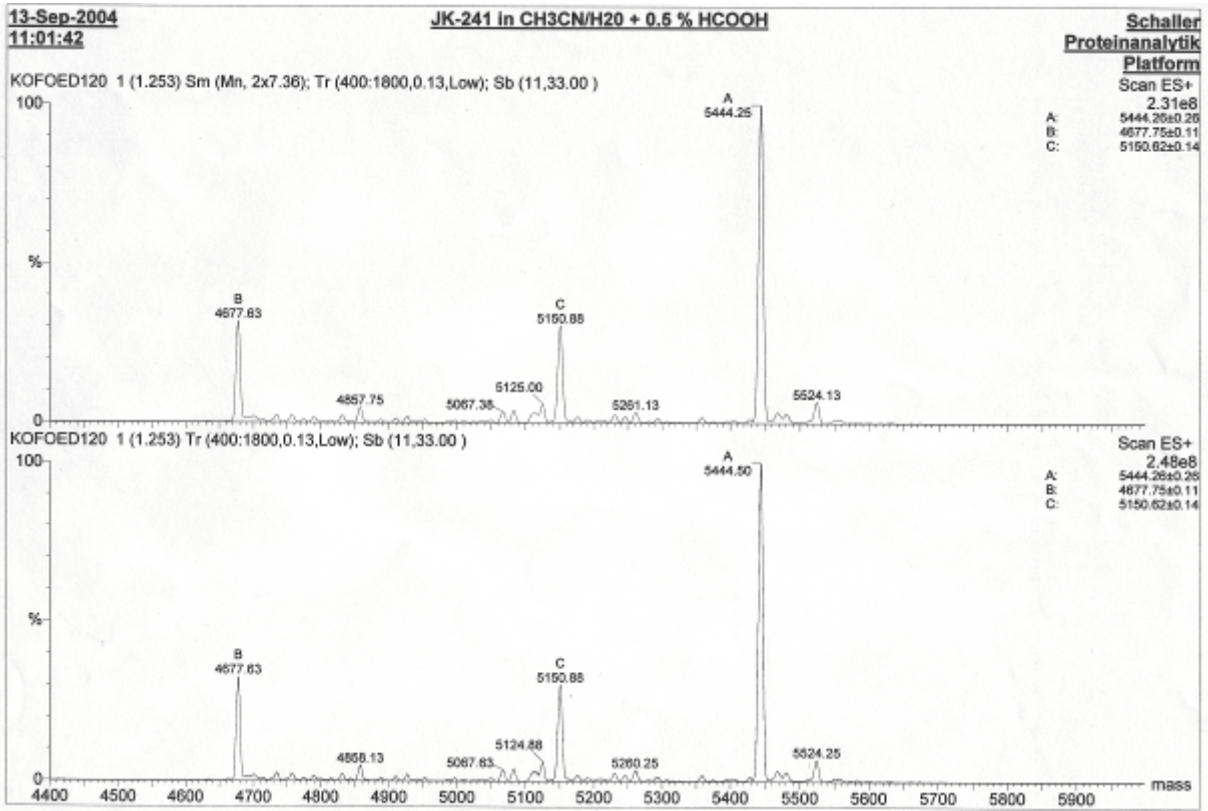




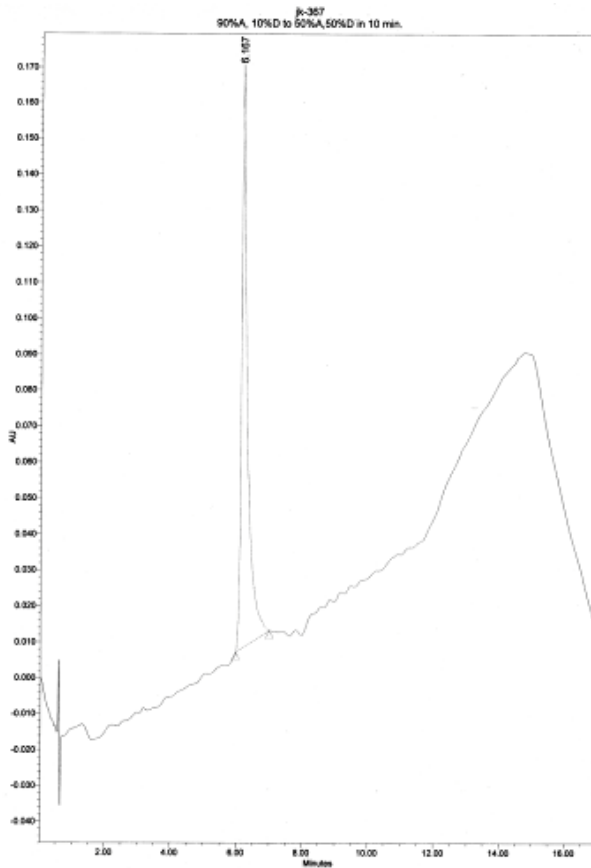
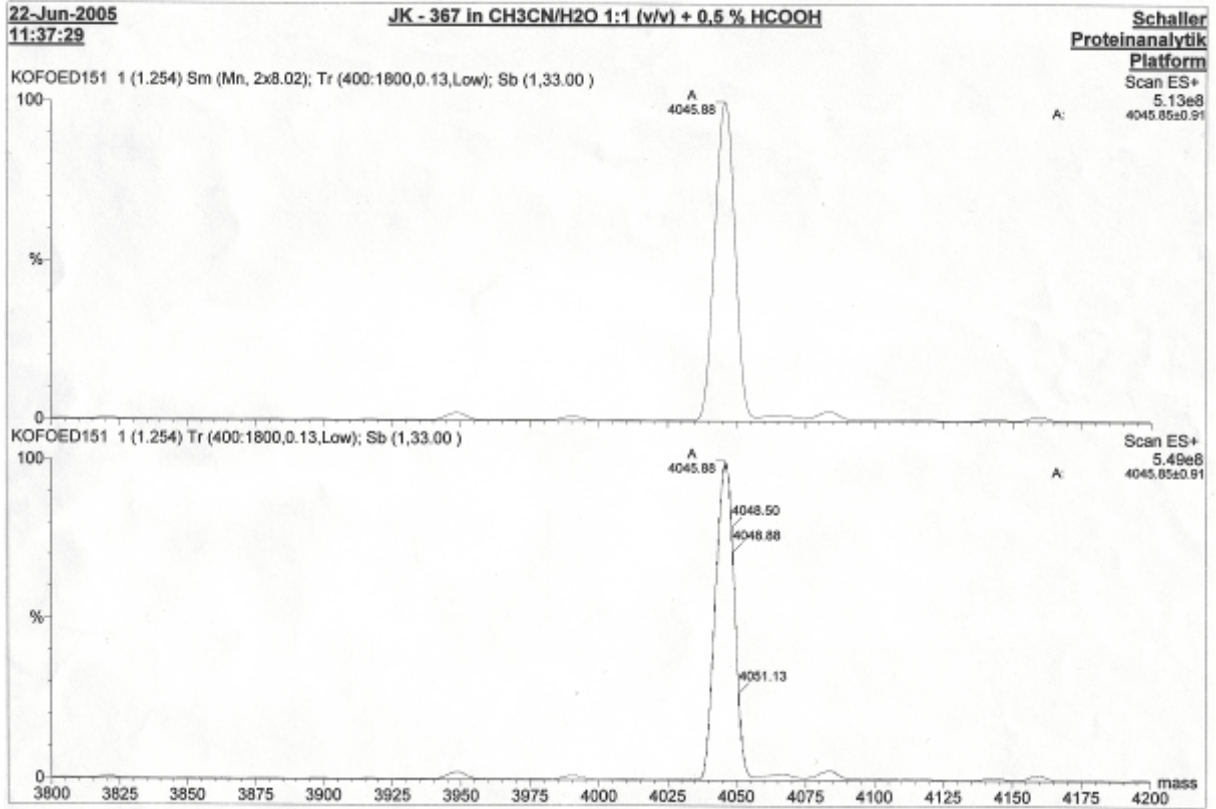
Dendrimer L1D5



Dendrimer L1D6

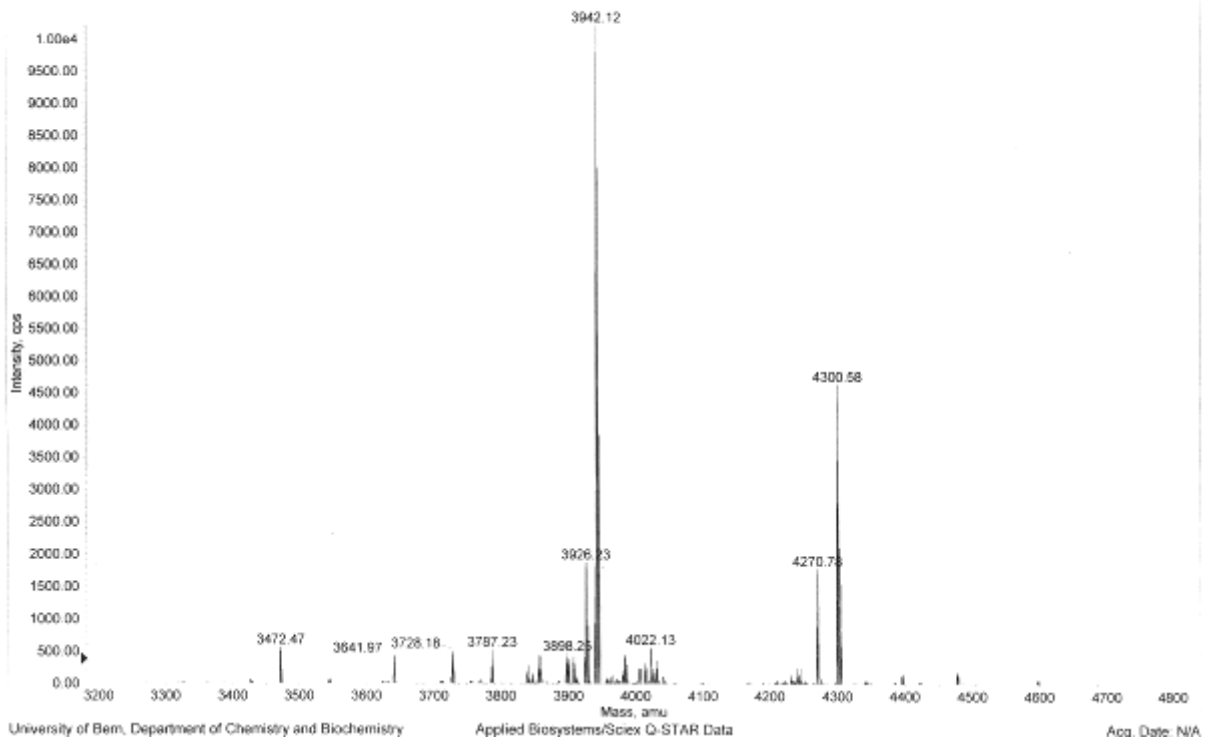


Dendrimer L2D1



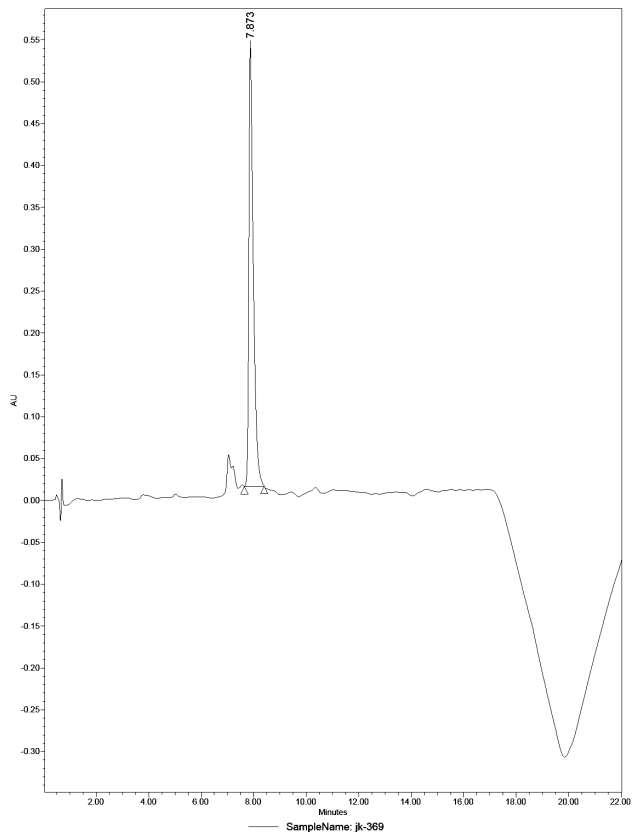
Dendrimer L2D5

Sample Name: N/A  
 Mass reconstruction of +TOF MS: 0.700 to 2.851 min from JK\_369.wiff  
 a=3.56172594560657950e-004, y0=4.69637173806758260e+000  
 ESI-MS positive mode  
 Max: 1.0 cps.



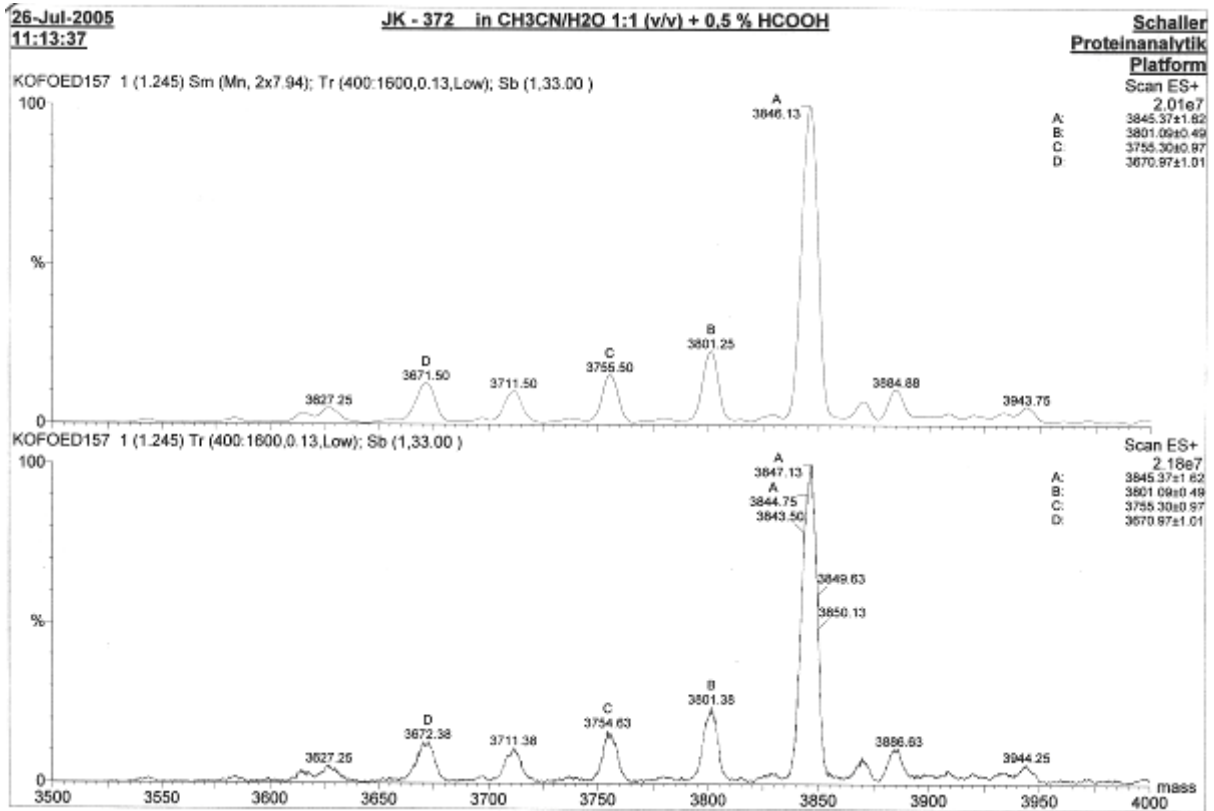
University of Bern, Department of Chemistry and Biochemistry Applied Biosystems/Sciex Q-STAR Data Acc. Date: N/A

Time	Flow	%A	%B	%C	%D	Curve
0.01	3.00	90.0	0.0	0.0	10.0	6
15.003.00	50.0	0.0	0.0	0.0	50.0	6

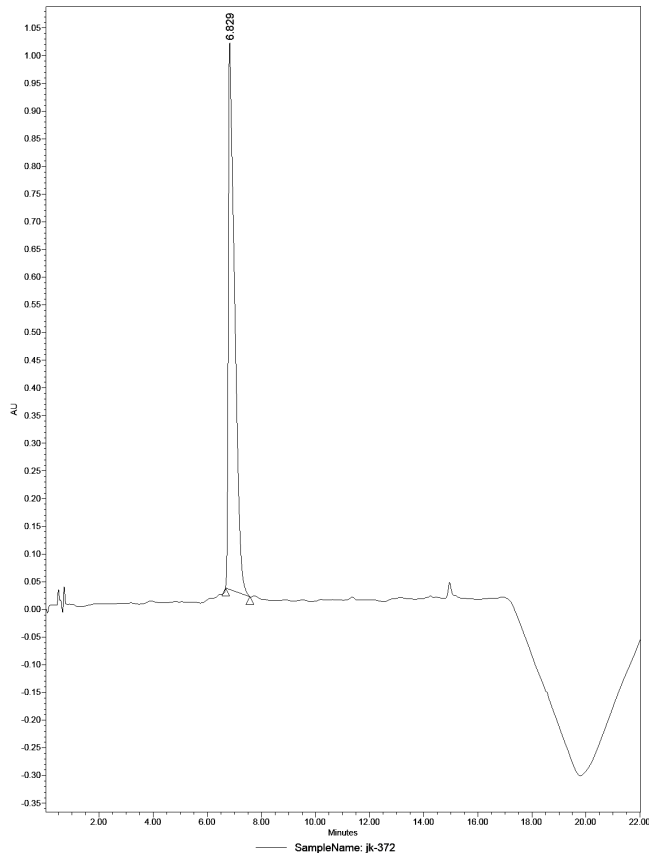


SampleName: jk-369

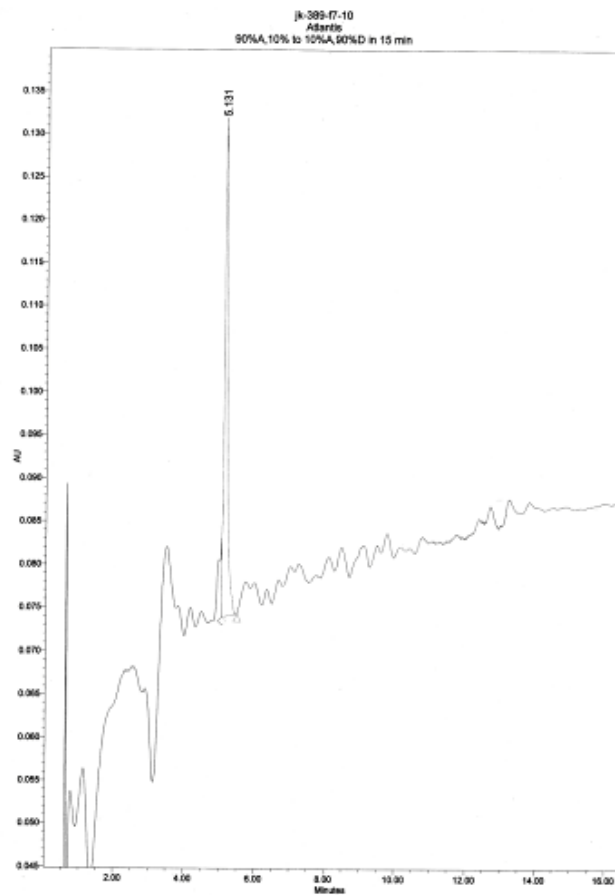
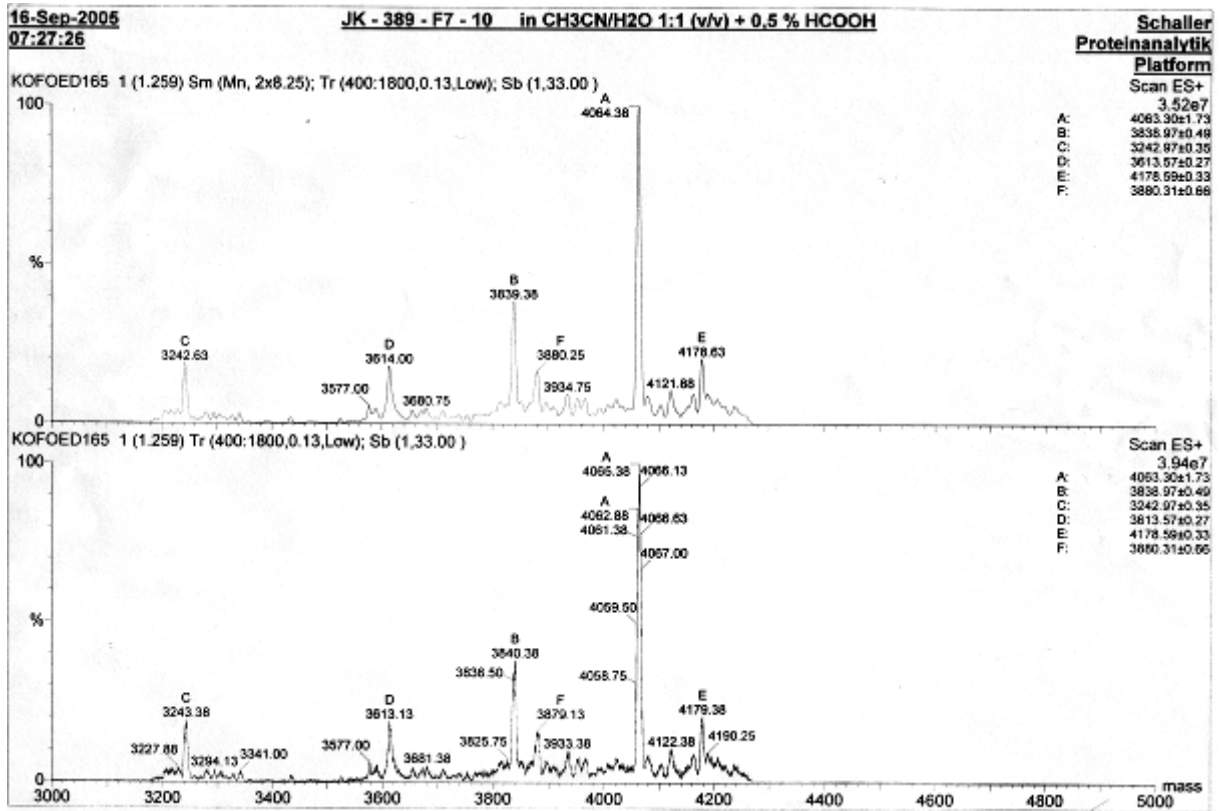
Dendrimer L2D6



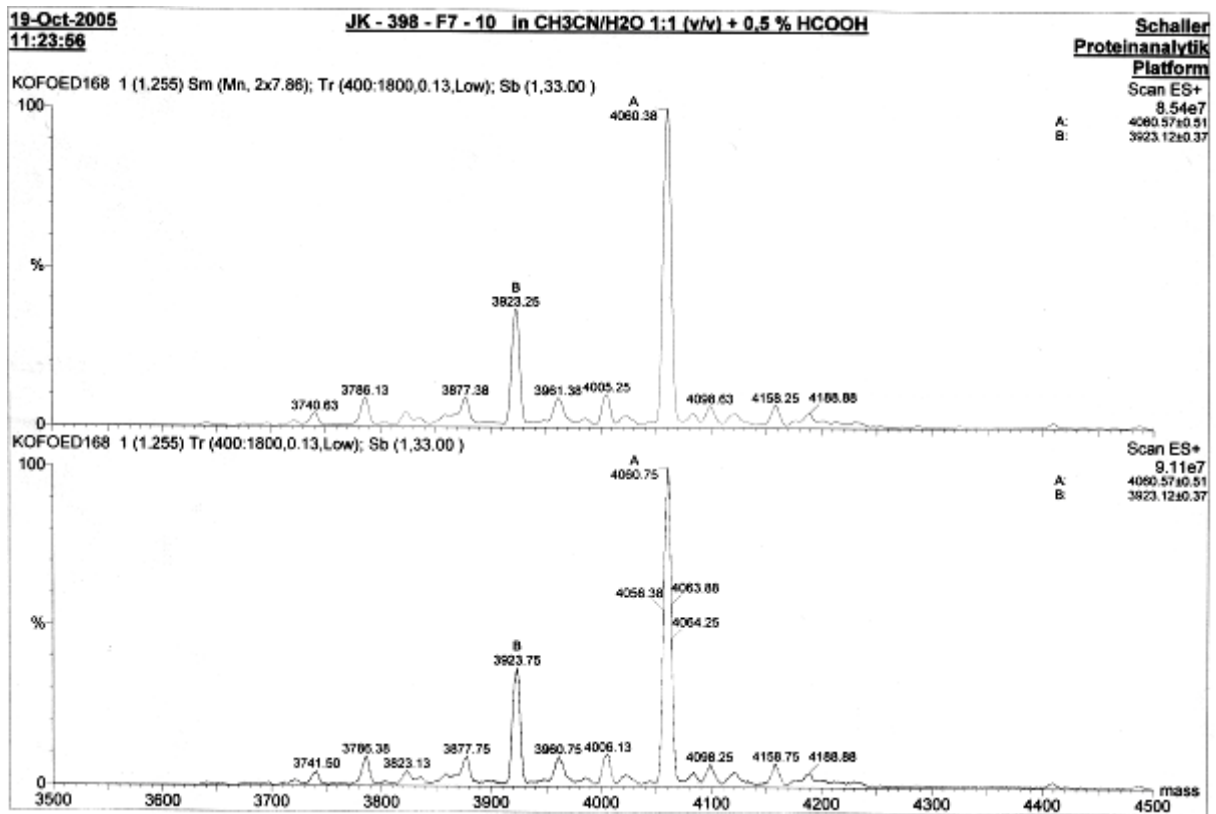
Time	Flow	%A	%B	%C	%D	Curve
0.01	3.00	90.0	0.0	0.0	10.0	6
15.003.00		50.0	0.0	0.0	50.0	6



Dendrimer L2D7

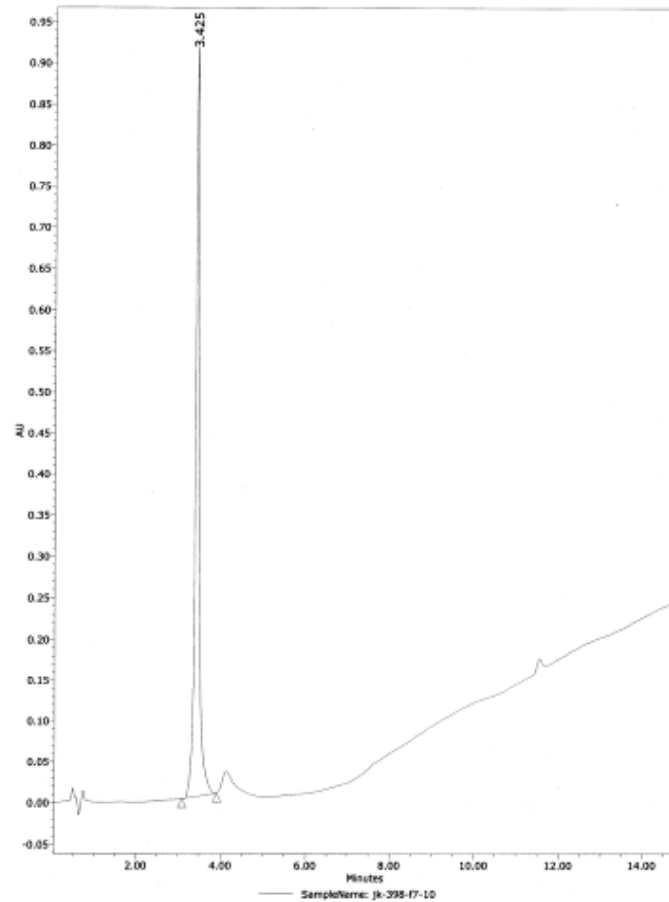


Dendrimer L2K4

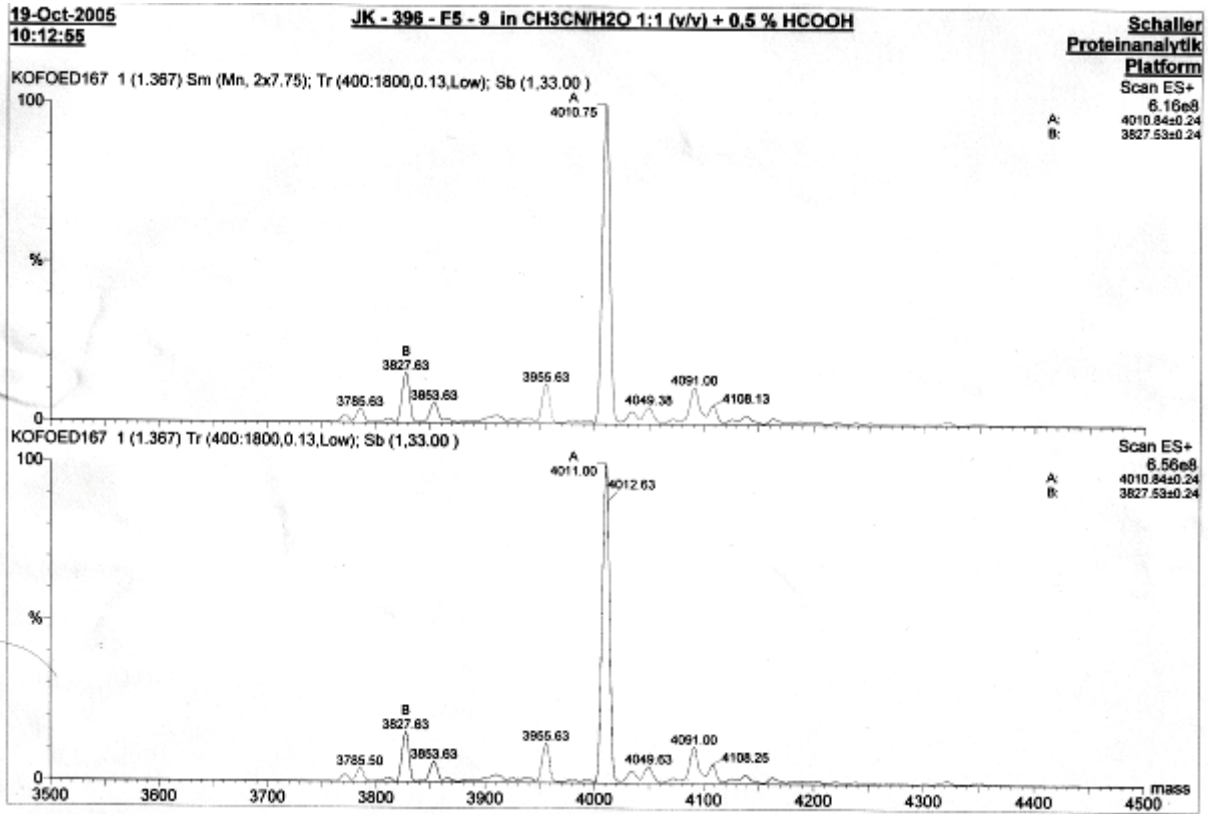


MSMS: dC18 Sam, 4.6mmx100mm

Time	Flow	%A	%B	%C	%D	Curve
1	0.00	3.00	90.0	0.0	0.0	10.0 6
2	15.00	3.00	10.0	0.0	0.0	90.0 6

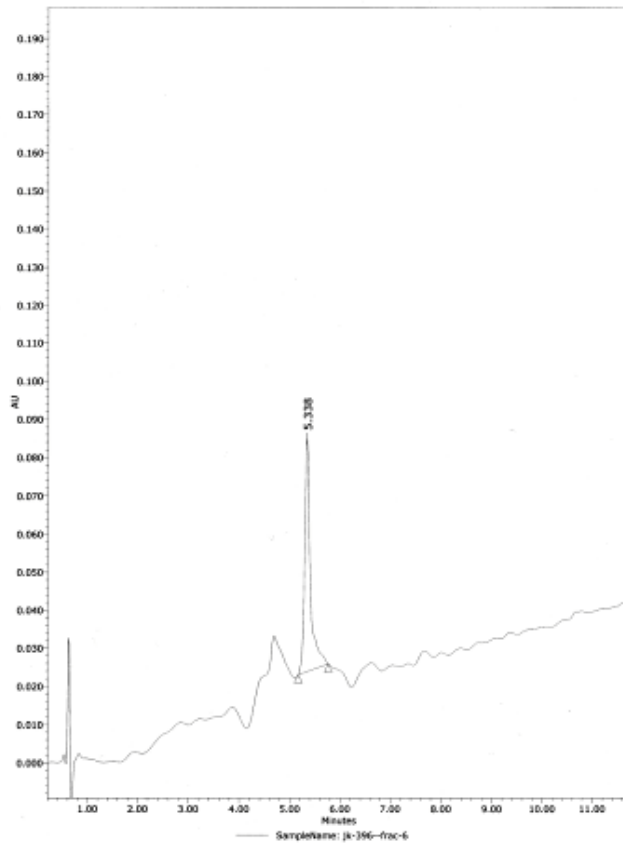


Dendrimer L2K7



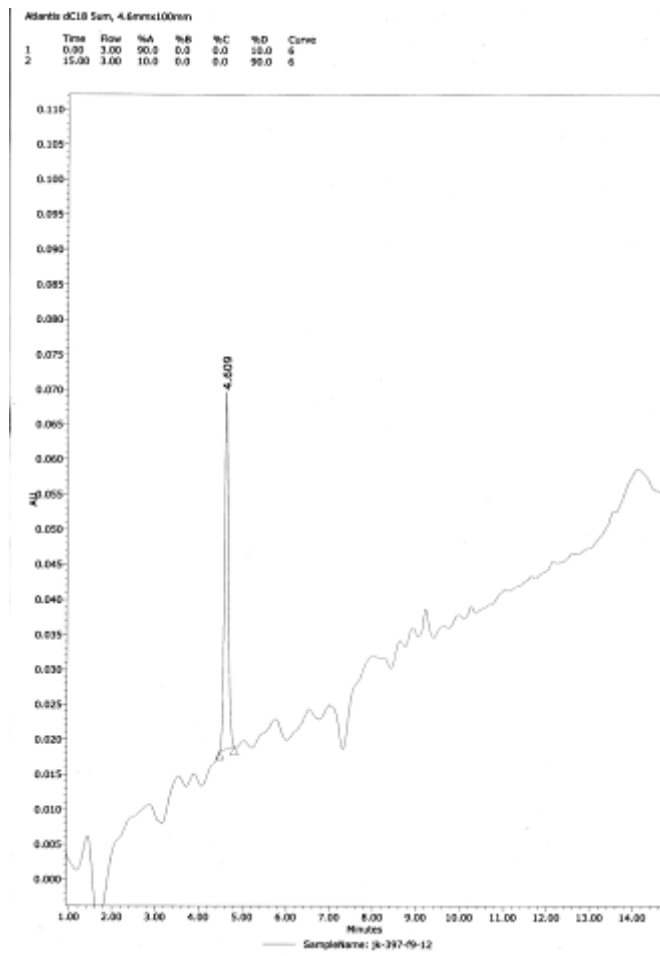
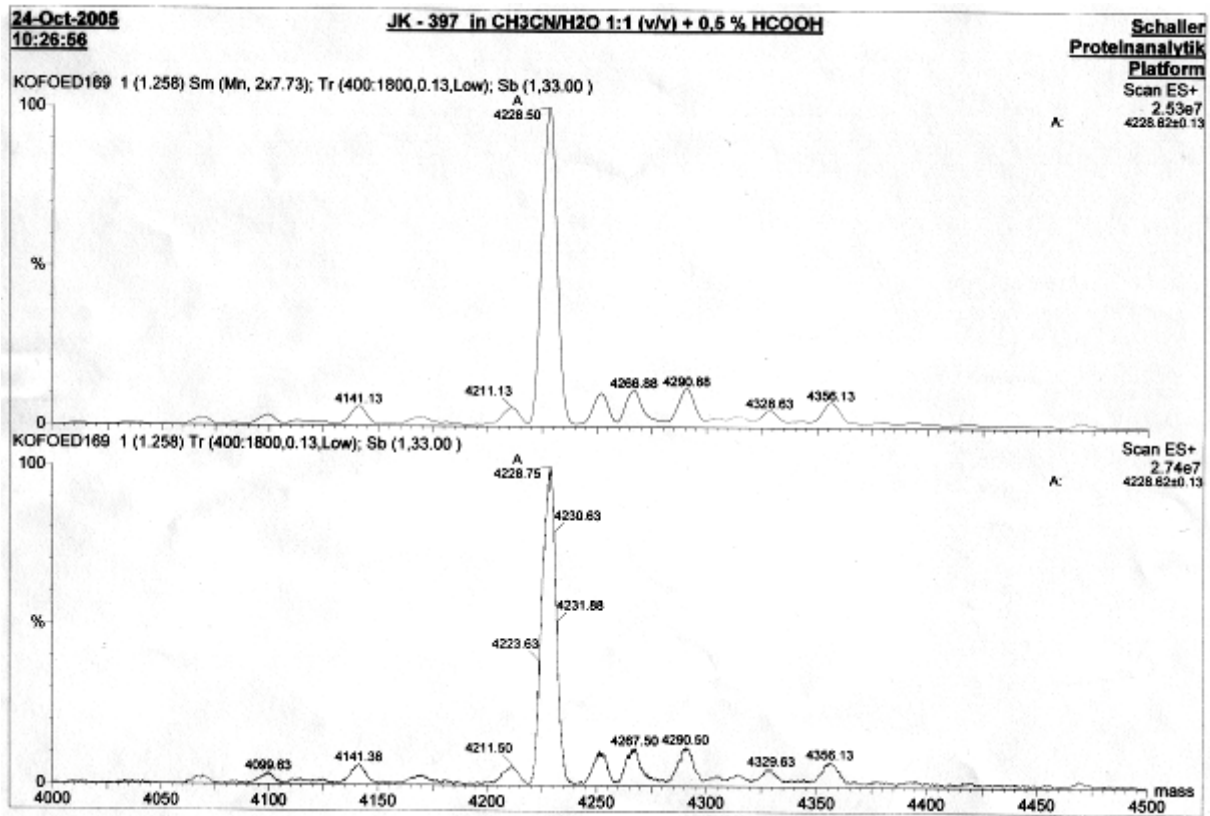
Atlantis dC18 Sun, 4.6mmx100mm

	Time	Flow	%A	%B	%C	%D	Curve
1	0.00	3.00	90.0	0.0	0.0	10.0	6
2	15.00	3.00	10.0	0.0	0.0	90.0	6

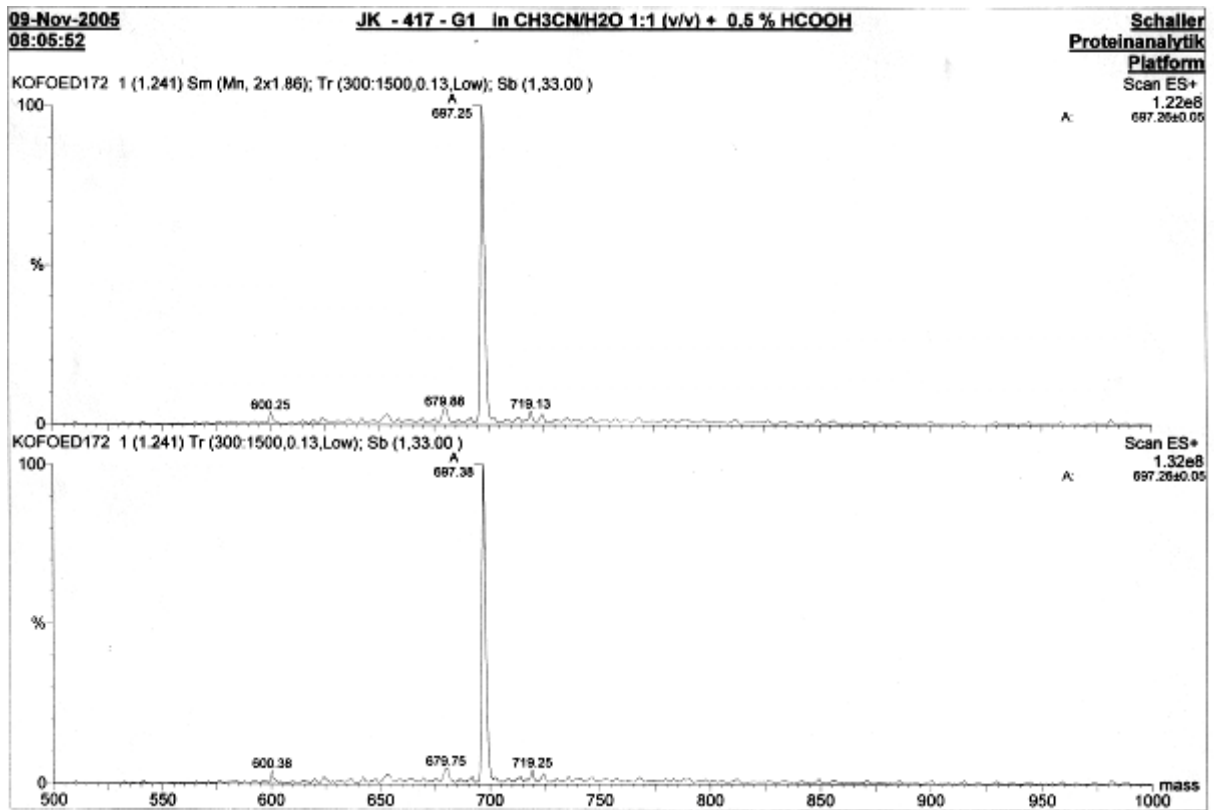




Dendrimer L2K8

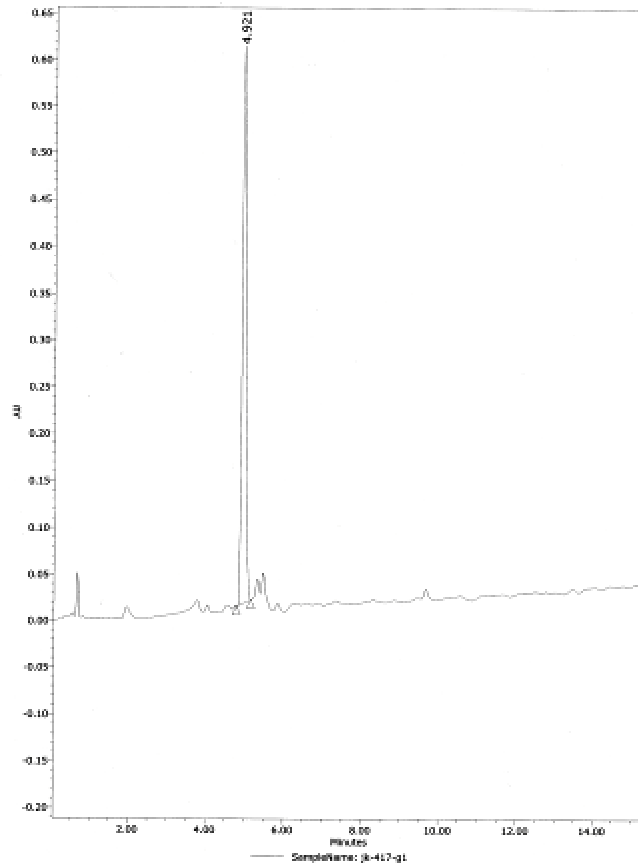


Dendrimer R1G1

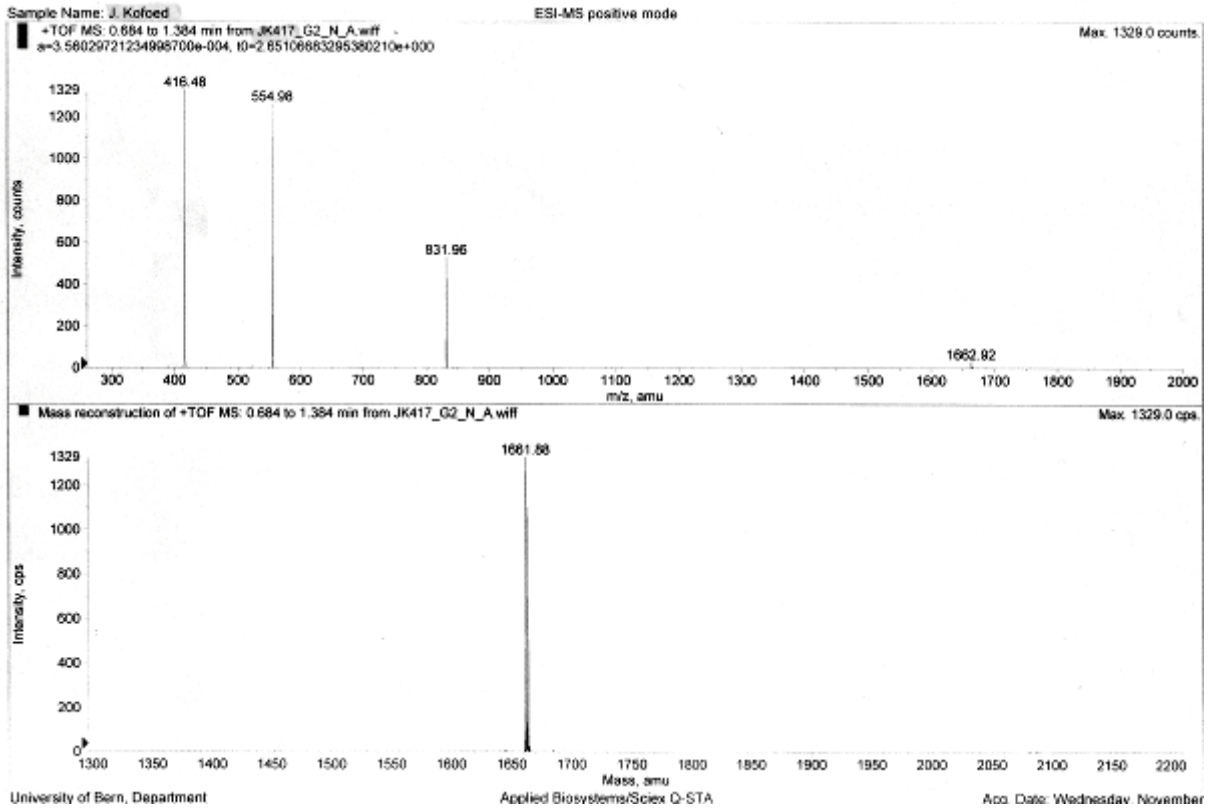


Atlantis dC18 Surr, 4.6mmx100mm

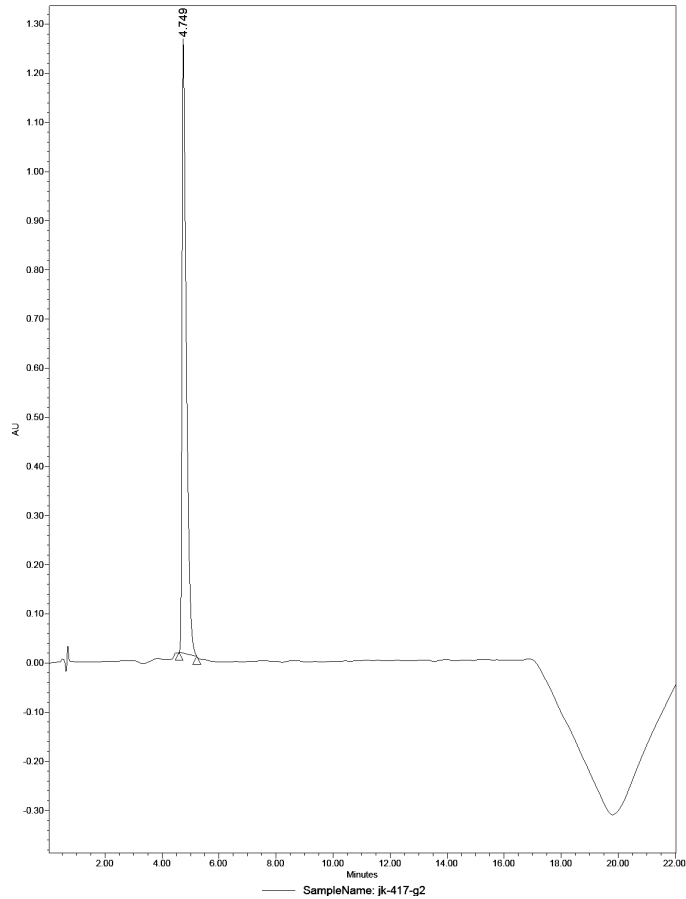
	Time	Flow	Flow	%B	%C	%D	Curve
1	0.00	1.00	10.0	0.0	0.0	0.0	1
2	15.00	1.00	10.0	0.0	0.0	0.0	2



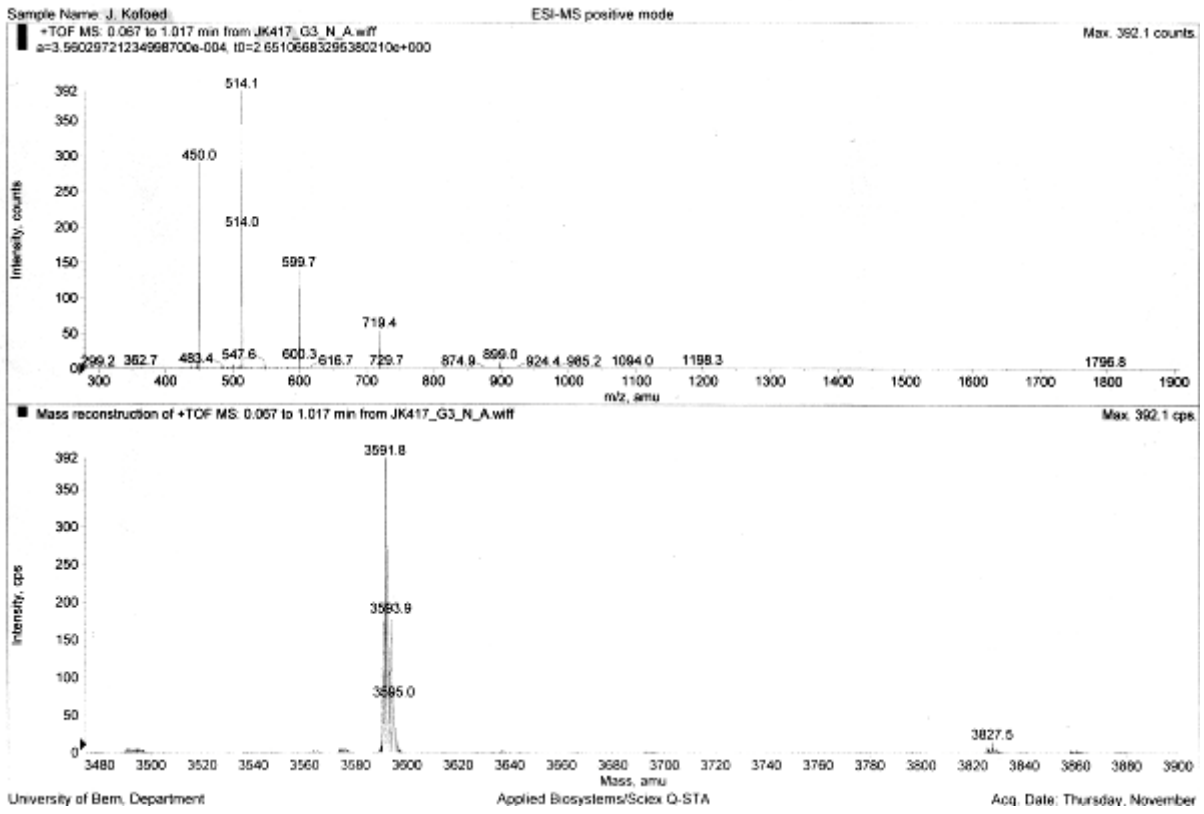
Dendrimer R1G2



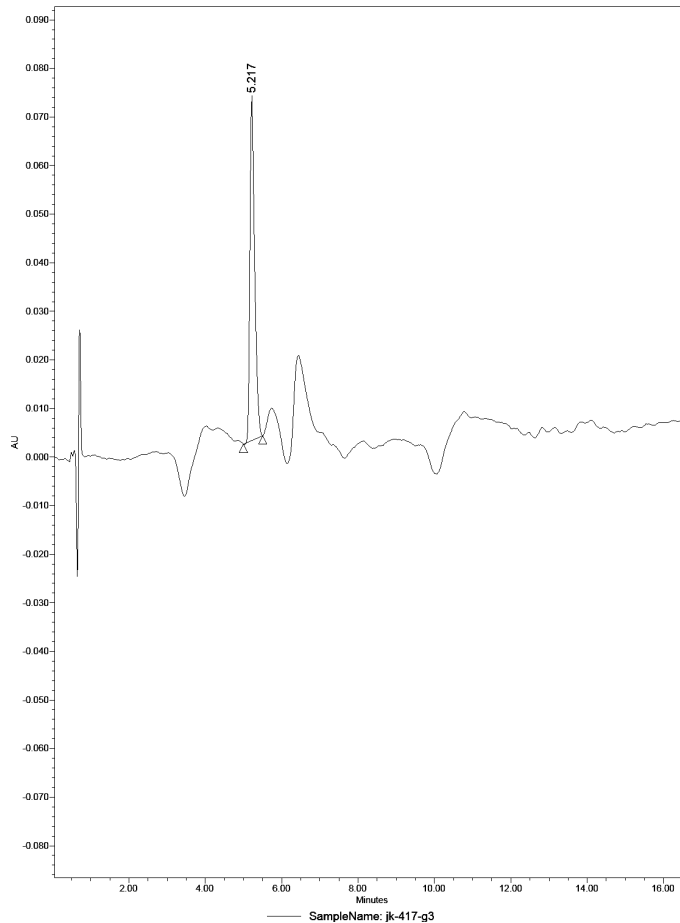
Time	Flow	%A	%B	%C	%D	Curve
0.01	3.00	90.0	0.0	0.0	10.0	6
15.003.00	50.0	0.0	0.0	50.0	50.0	6



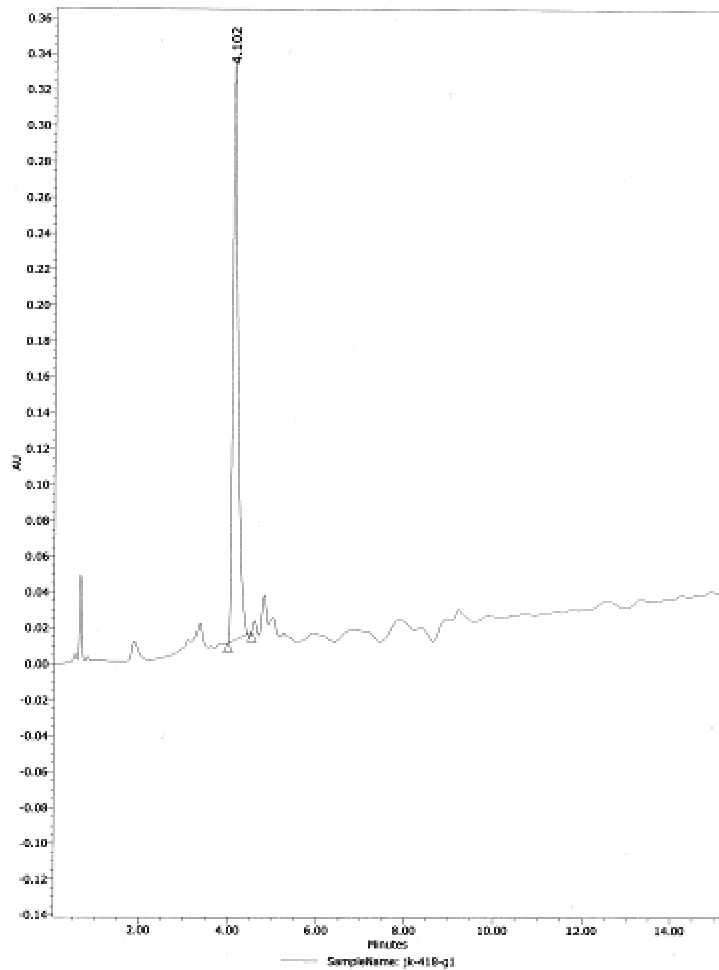
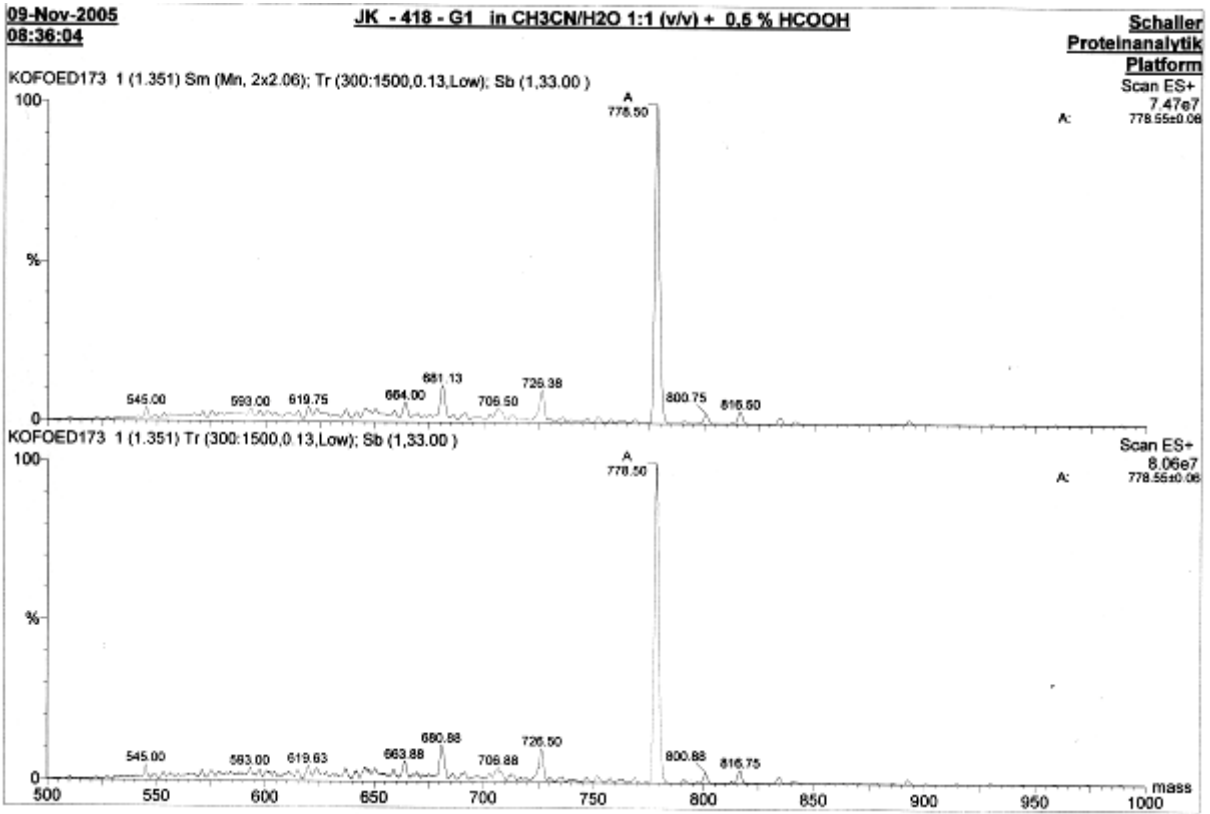
# Dendrimer R1G3



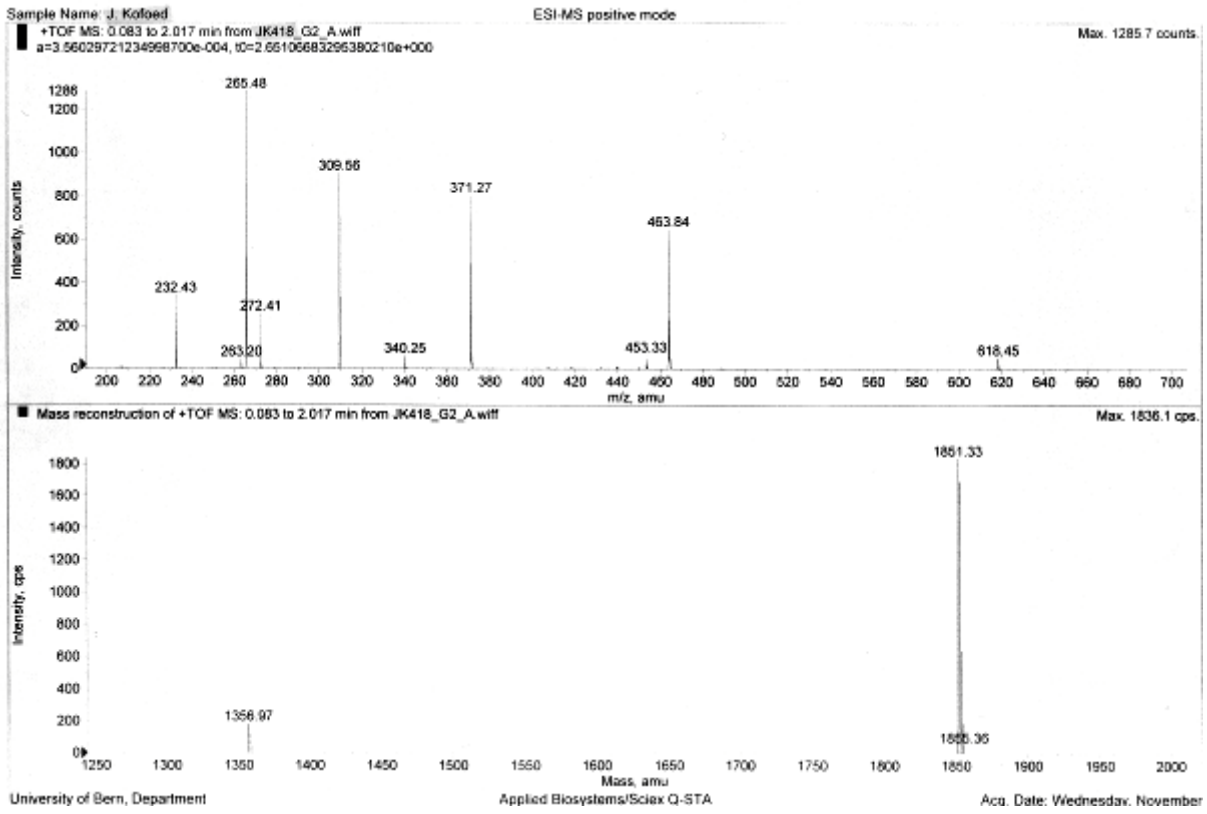
Time	Flow	%A	%B	%C	%D	Curve
0.01	3.00	90.0	0.0	0.0	10.0	6
15.003	3.00	50.0	0.0	0.0	50.0	6



Dendrimer R2G1

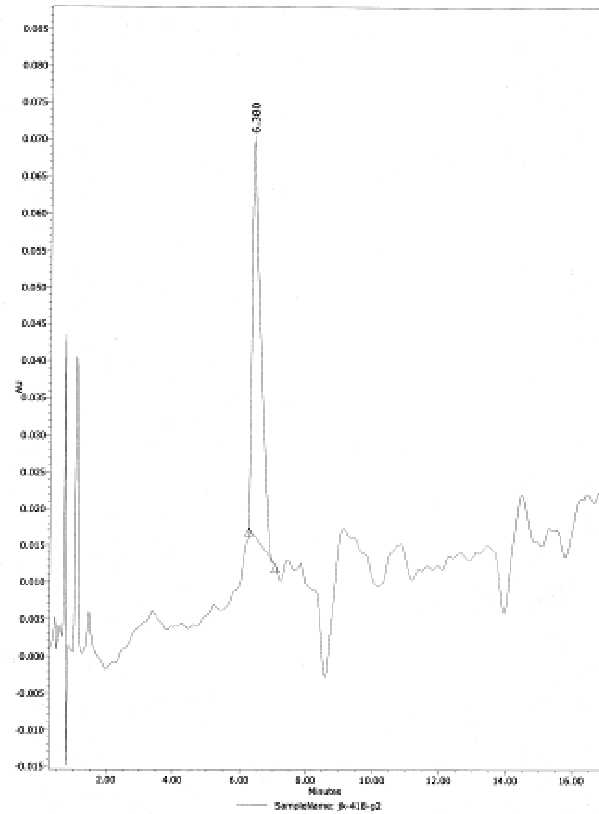


# Dendrimer R2G2

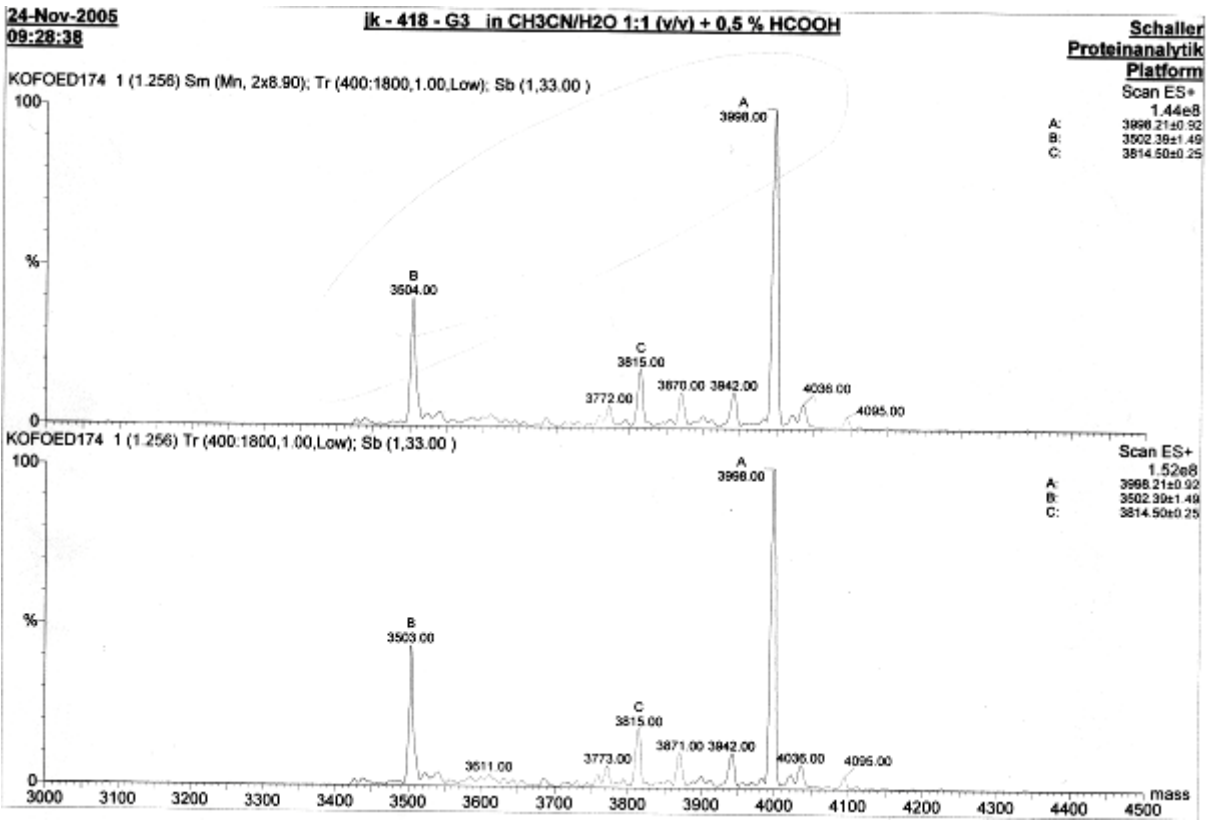


Atlanta vC18 Sum, 4.61min(1000Hz)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	2.00	95.0	0.0	0.0	3.0	5
2	15.00	3.00	70.0	0.0	0.0	30.0	6



Dendrimer R2G3



Time	Flow	%A	%B	%C	%D	Curve
0.01	3.00	90.0	0.0	0.0	10.0	6
15.003.00	50.0	0.0	0.0	50.0		6

