EXPERIMENTAL

The detergent-free recombinant IN protein was produced in *Escherichia coli* and purified as previously described.[1] All oligonucleotides were synthesized by the phosphoramidite method on an automatic ABI 3400 DNA synthesizer (Applied Biosystems, USA) under conditions recommended by manufacturer and purified by electrophoresis in a 20% polyacrylamide/7 M urea gel. Modified oligonucleotides were additionally purified by RP-HPLC with acetonitrile gradient in 0.1 M ammonium acetate (pH 7). HPLC purification was carried out on AKTA Purifier equipped with Jupiter C18 or C5 column (Phenomenex, size 4.6*250 mm, 5 μm) with UV-Vis detector. Analytical HPLC was carried out on Agilent 1200 equipped with Ultasphere octyl (Beckman Coulter, size 4.6*250 mm, 5 μm), 45°C 1 ml/min with diode array detector. 5'-FAM, FAM, TET, HEX phosphoramidites were purchased from Metkinen Chemistry Oy; dT, dA(Bz), dG(iBu), dC(Ac), ddR, 1,3-propanediol phosphoramidites and dT p-methyl phosphonamidite - from ChemGenes, JOE phosphoramidite – from Primetech LLC. Oligonucleotides with eosin and oleic acid at 3'-end were synthesized as described previously.[2] Phosphorothioate oligonucleotides were synthesized accordingly to Krotz et al..[3] Oligonucleotides with methylphosphonate linkages were synthesized as described by Arnold and coworkers.[4] MALDI MS spectra were registered on AutoFlex (Bruker Вфдещтшсы) using 2,4,6-trihydroxyacetophenone/ ammonium cirtrate[5] or 3-hydroxypicolinic acid/ ammonium cirtrate[6] as a matrix.

3'-Processing activity assay. A DNA duplex consisting of oligonucleotides U5B (5'-GTGTGGAAAATCTCTAGCAGT-3') and U5A (5'-ACTGCTAGAGATTTTCACAC-3'), and mimicking the end of the HIV-1 U5 LTR was used as an IN substrate. The U5B oligonucleotide (10 pmol) was labeled with T4 polynucleotide kinase (Fermentas, Lithuania) and 50 μCi [γ-^{32}P]ATP (3000 Ci/mmol). After 1 h of incubation at 37°C, the T4 polynucleotide kinase was inactivated by the addition of EDTA followed by heating at 65°C for 5 minutes. The U5B oligonucleotide was then annealed with an equimolar amount of complementary oligonucleotide, U5A. The resulting U5B/U5A duplex was then purified from unincorporated [γ-^{32}P]ATP by centrifugation through MicroSpin G-25 Columns (GE Healthcare, UK). The ^{32}P-labeled substrate, U5B/U5A (3 nM), was

incubated in 20 μl of 20 mM Hepes, pH 7.2, 7.5 mM $MgCl_2$, 1 mM DTT with 50 nM IN, in the presence of increasing concentrations of an oligonucleotide inhibitor (0.01-10 μM) at 37°C for 2 h. The reaction was stopped by adding 80 μl of a stop solution (7 mM EDTA, 0.3 M sodium acetate, 10 mM Tris-HCl, pH 8). IN was extracted by phenol/chloroform, and DNA fragments were precipitated with ethanol. The reaction products were suspended in loading dye (80% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol) and separated by electrophoresis in a 20% polyacrylamide/7 M urea gel. Gel images were recorded on a STORM 840™ PhosphorImager (Molecular Dynamics, USA) and quantified with Image QuaNT™ 4.1 software (USA).

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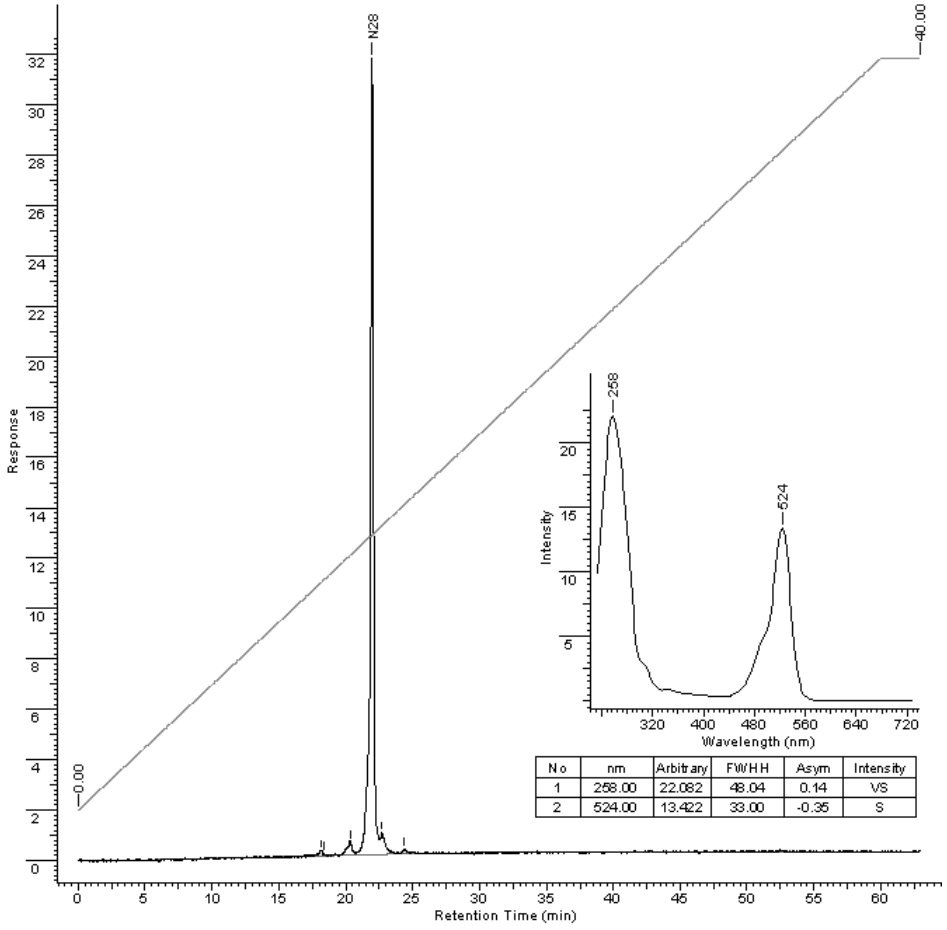
Table 1. MALDI MS analysis of the oligonucleotides used in the study.

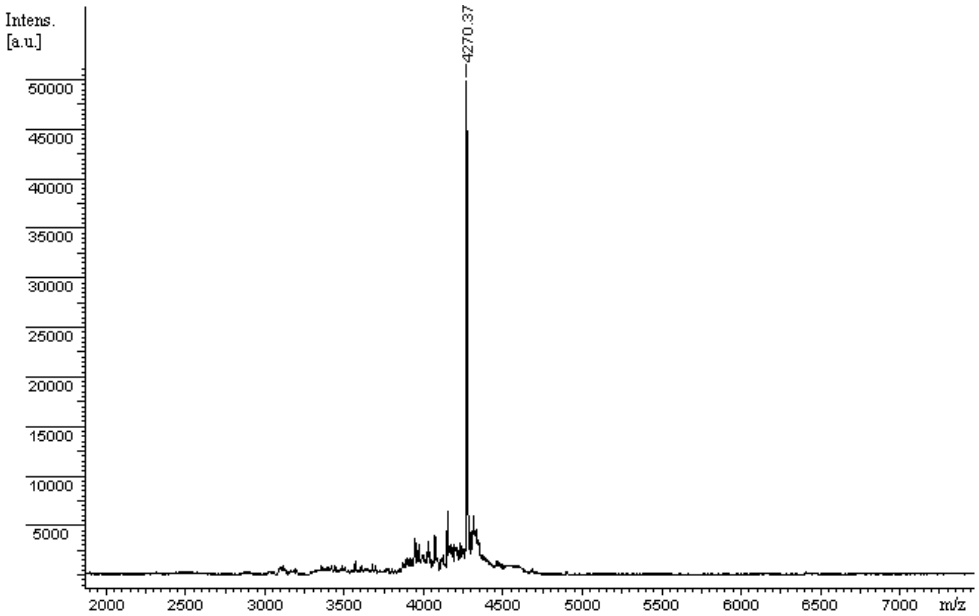
N	Conjugate	Calculated mass, $M+H^+$	Found mass, $M+H^+$
1	**11D-E**	4269.3	4270.4
2	**11D-E2**	4091.2	4091.4
3	**5'-FAM1-11D**	4035.8	4036.6
4	**5'- FAM2-11D**	3924.7	3923.9
5	**11D-3'-FITC**	3953.7	3954.2
6	**HEX-11D**	4129.4	4130.2
7	**HEX_{Acr}-11D**	4128.4	4126.3
8	**TET-11D**	4060.4	4060.2
9	**JOE-11D**	4049.6	4049.4
10	**11D-Ole**	3860.8	3860.9
11	**FAM-11D-Ole**	4511.4	4512.4
12	**11D- FAM-Ole**	4511.4	4512.4
13	**11DX-E-1**	4263.4	4264.2
14	**11DX-E-2**	4263.4	4263.9
15	**11DX-E-3**	4263.4	4264.2
16	**11DS-E-1**	4430.0	4432.9
17	**11DS-E-2**	4301.5	4302.7
18	**11DS-E-3**	4301.5	4304.0
19	**11DS-E-4**	4301.5	4299.5
20	**HEX-11-ddR**	2788.3	2788.6
21	**HEX-11D-ddR-1**	3334.7	3337.9
22	**HEX-11D-ddR-2**	3334.7	3335.2
23	**HEX-11-PD**	2368.0	2368.9
24	**HEX-11D-PD-1**	3082.5	3082.1
25	**HEX-11D-PD-2**	3082.5	3083.1
26	**11M-E**	4501.4	4501.6
27	**HEX-11M**	4129.3	4361.9
28	**HEX-11MS**	4538.2	4537.1
29	**HEX-11MS-Ole**	5029.8	5028.5

11D-E



No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	22.082	48.04	0.14	VS
2	524.00	13.422	33.00	-0.35	S

Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Asymmetry	Resolution	Plate Number
N28	21.968	500927.625	91.924	0.318	1.000	1.531	76157.781



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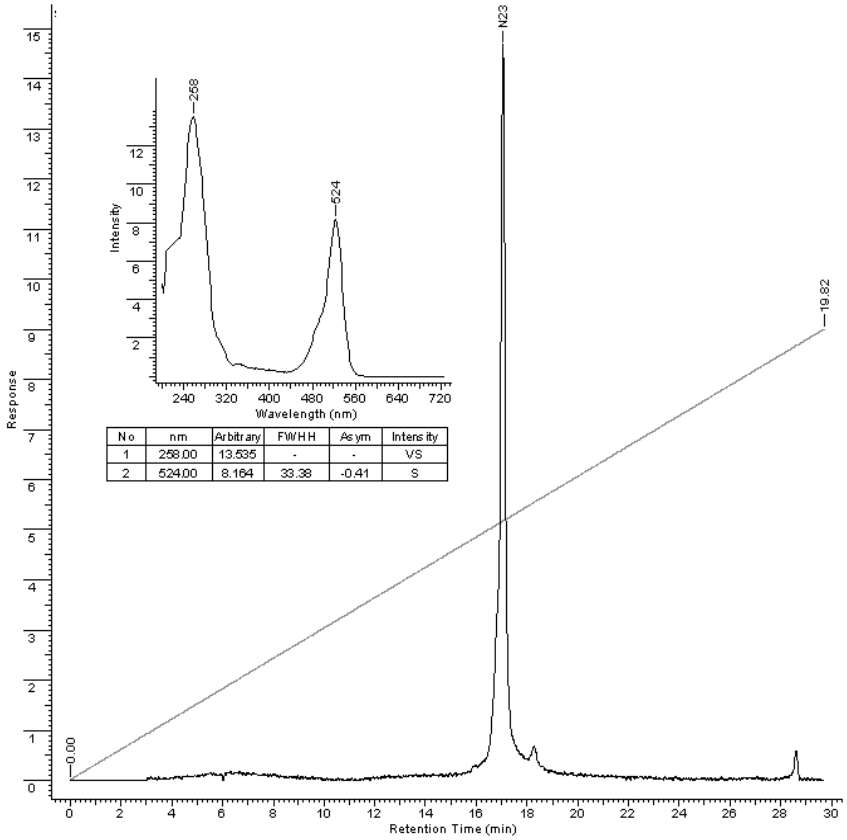
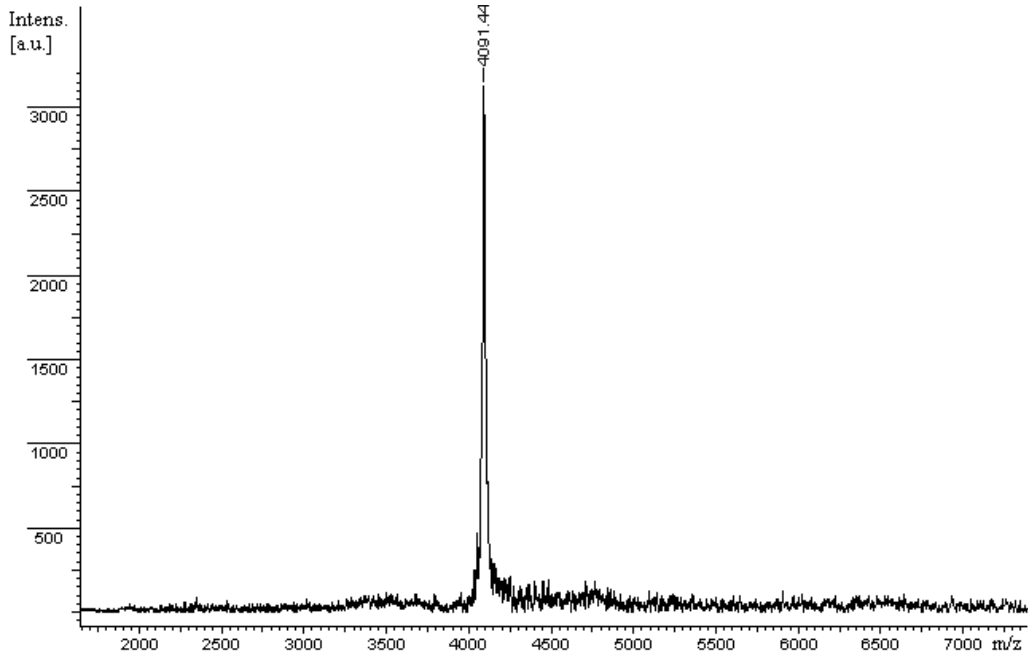
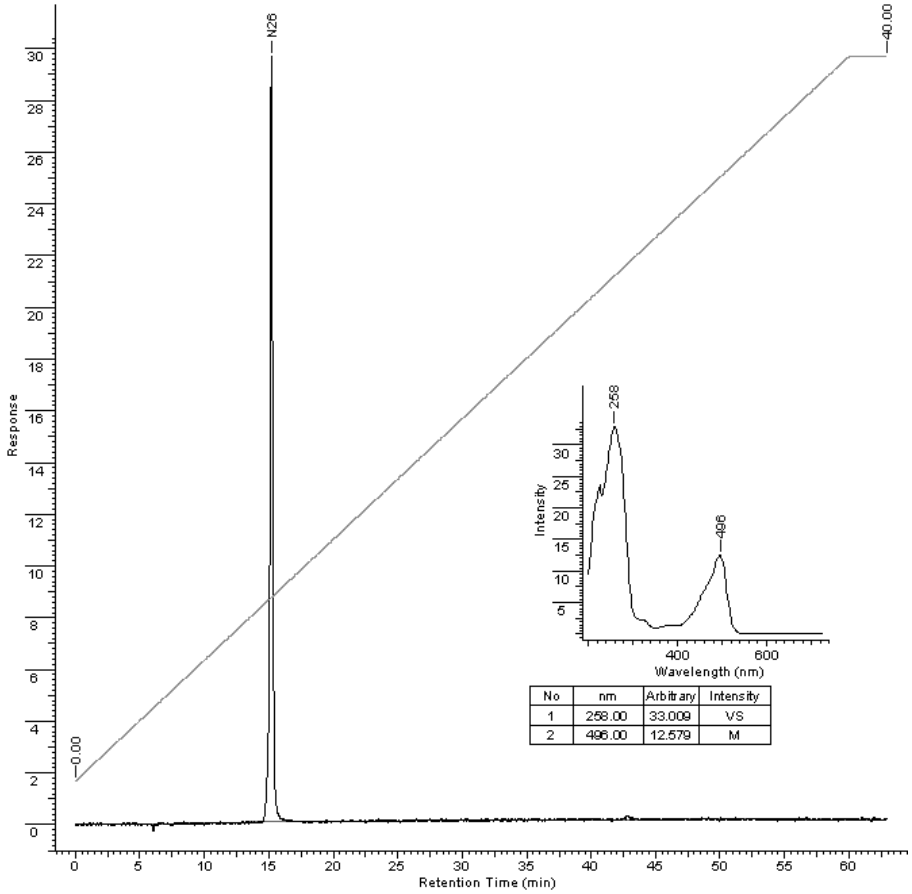


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	13.535	-	-	VS
2	524.00	8.164	33.38	-0.41	S

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N23	17.053	239591.859	100.000	0.305	1.095	50088.266

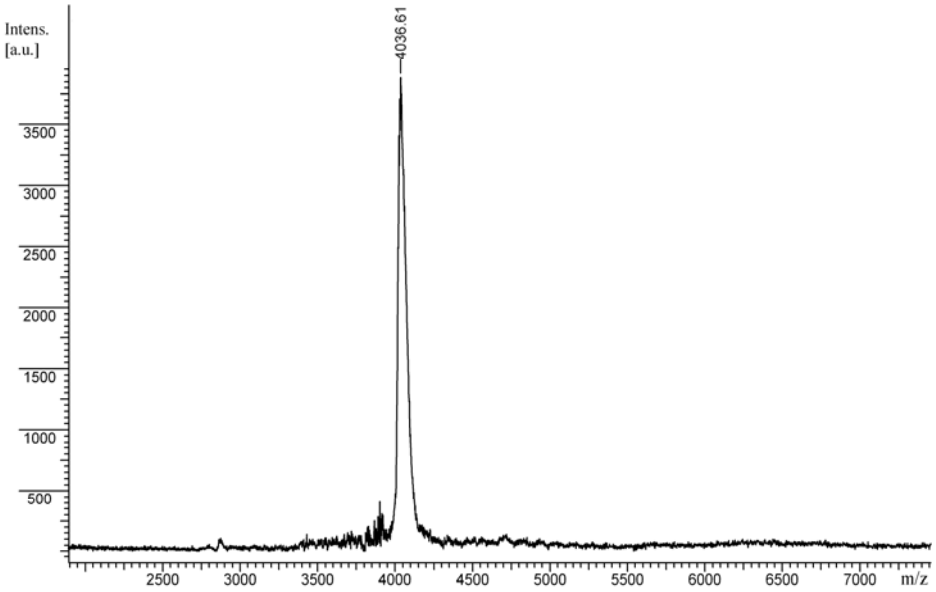


5’-FAM1-11D



No	nm	Arbitrary	Intensity
1	258.00	33.009	VS
2	496.00	12.579	M

Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Plate Number
N26	15.198	400013.188	100.000	0.279	1.000	47611.262



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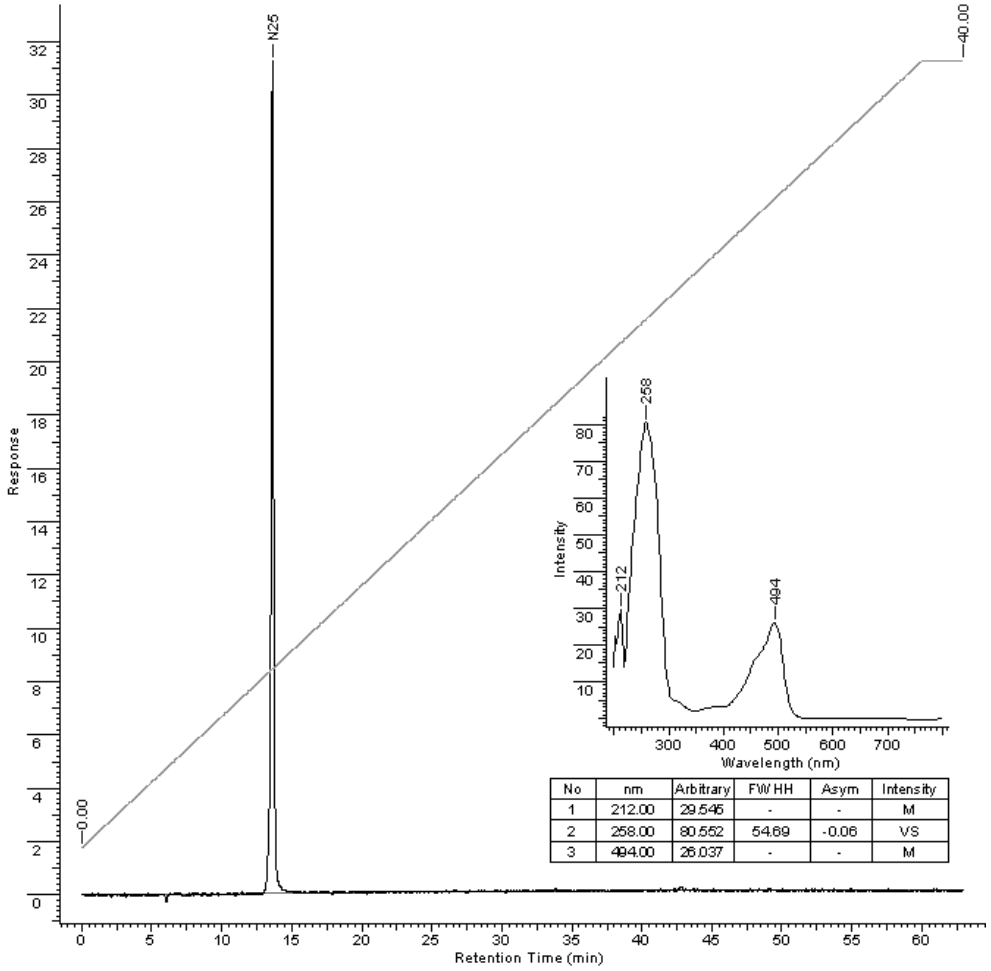
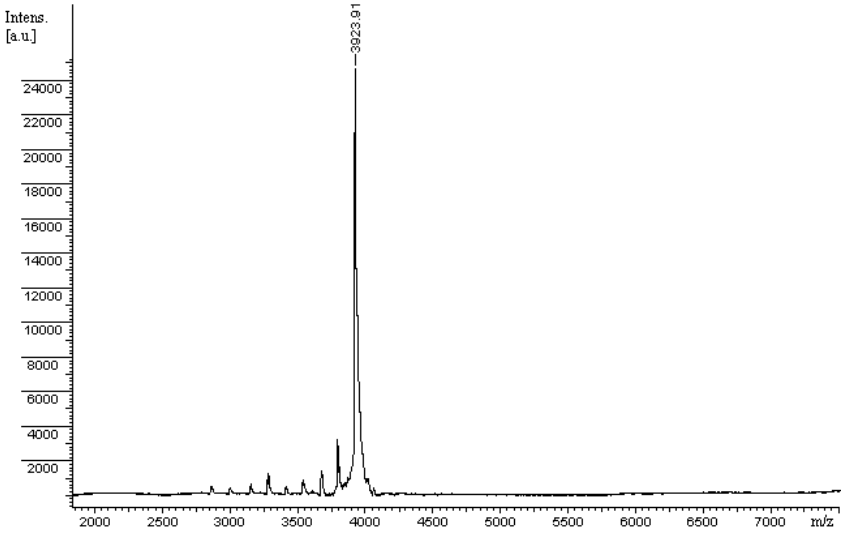
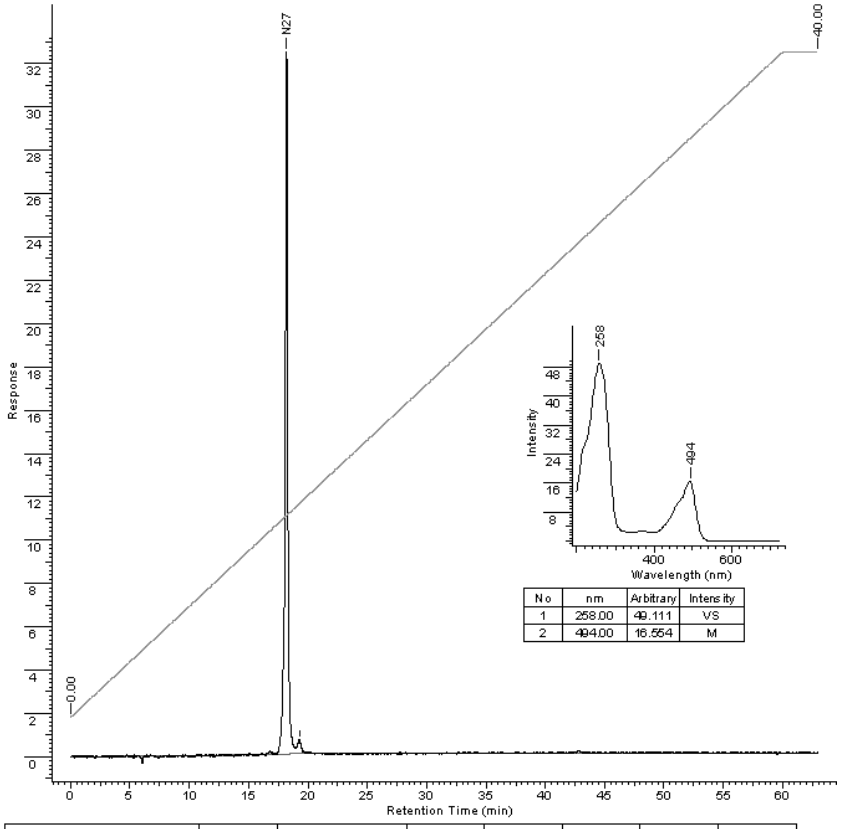


No	nm	Arbitrary	FWHH	Asym	Intensity
1	212.00	29.545	-	-	M
2	258.00	80.552	54.69	-0.06	VS
3	494.00	26.037	-	-	M

Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Plate Number
N25	13.613	374074.500	100.000	0.249	0.923	47913.387

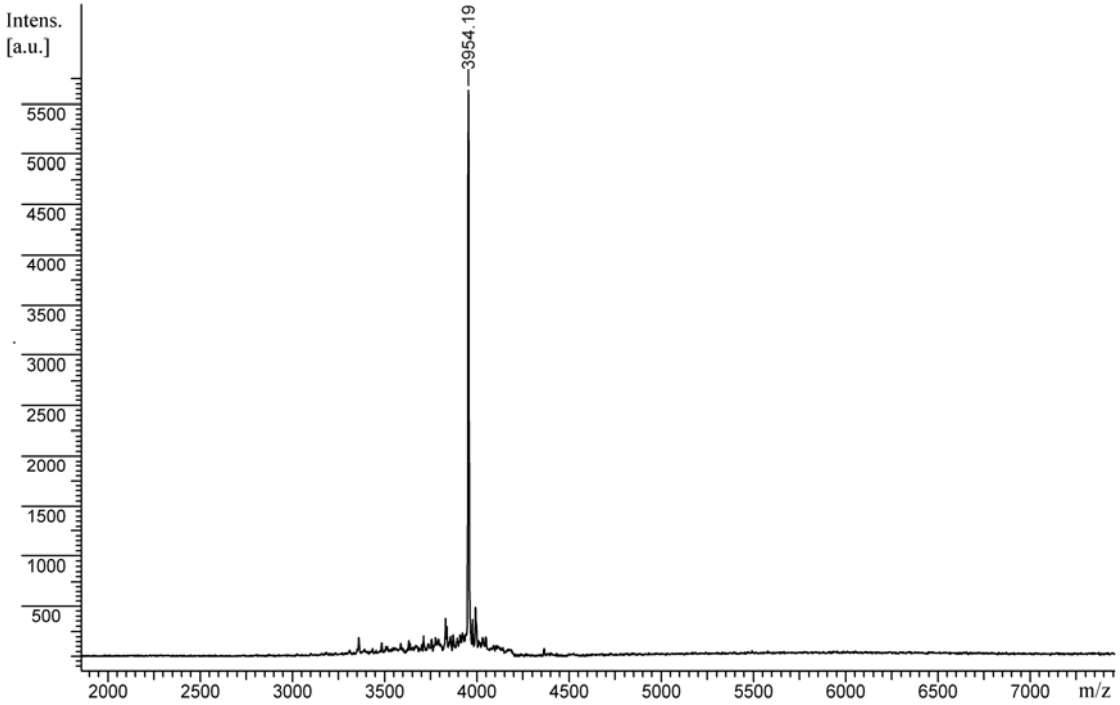


11D-FITC

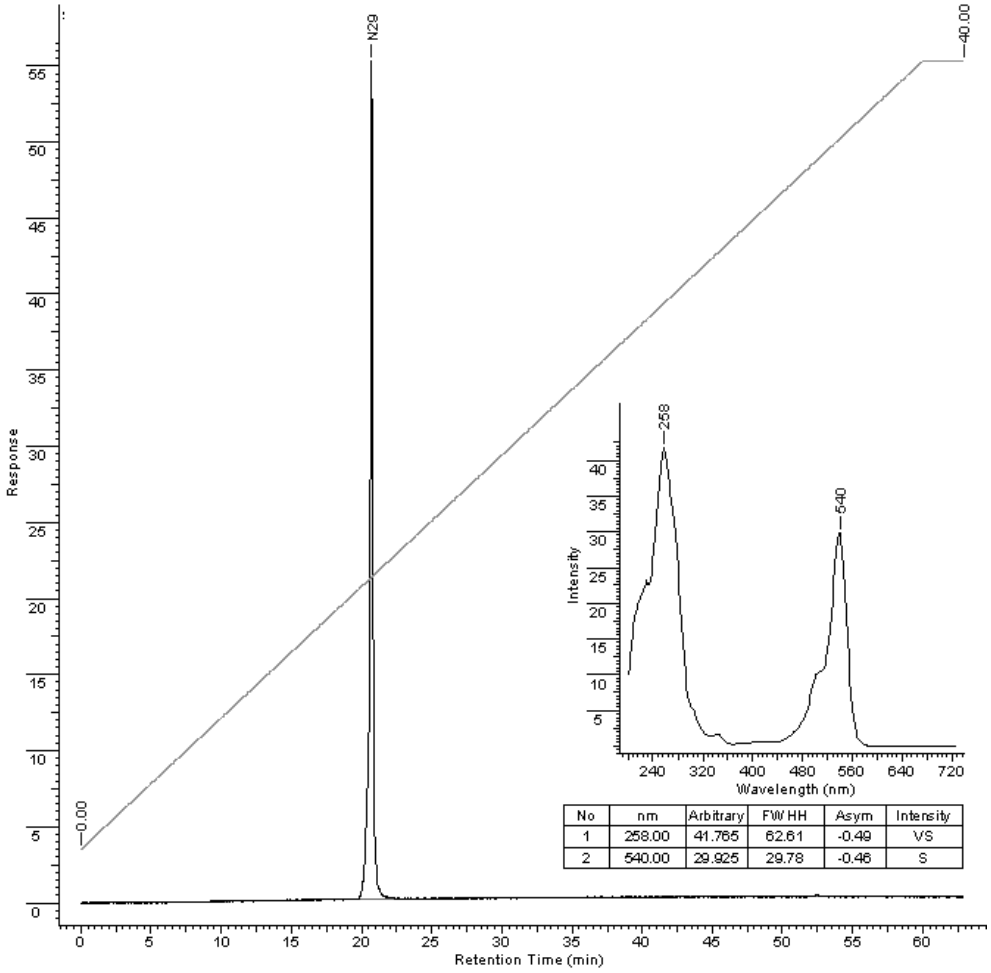


No	nm	Arbitrary	Intensity
1	258.00	49.111	VS
2	494.00	16.554	M

Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Resolution	Plate Number
N27	18.184	503382.969	97.323	0.308	1.067	2.632	55604.047
unknown	19.271	13844.640	2.677	0.517	0.733	-	22194.207



HEX-11D



No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	41.765	62.61	-0.49	VS
2	540.00	29.925	29.78	-0.46	S

Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Plate Number
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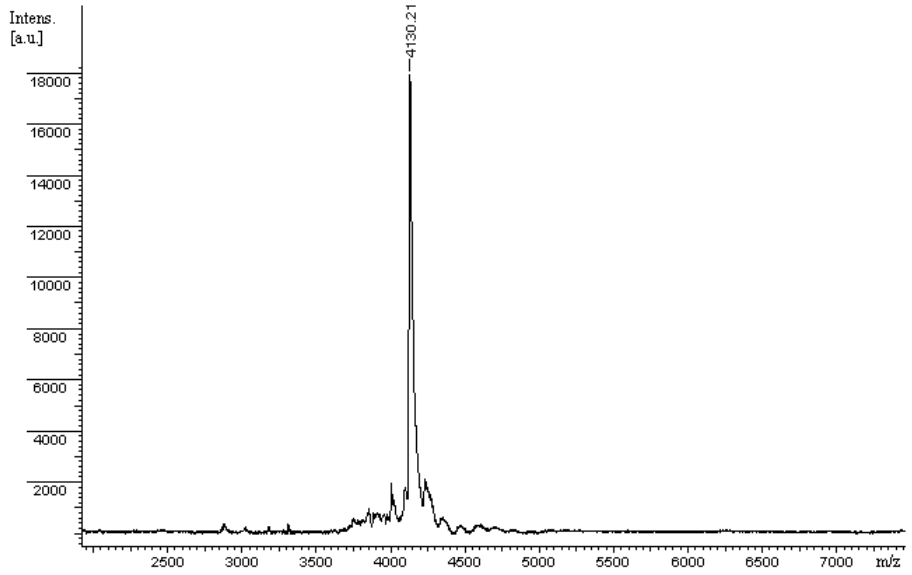
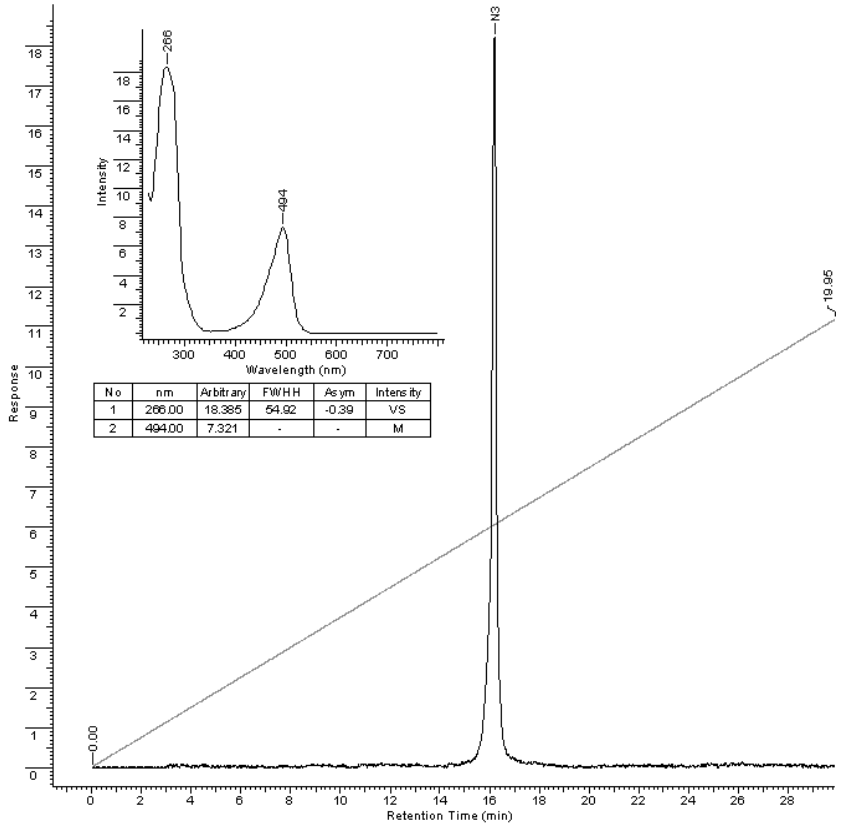
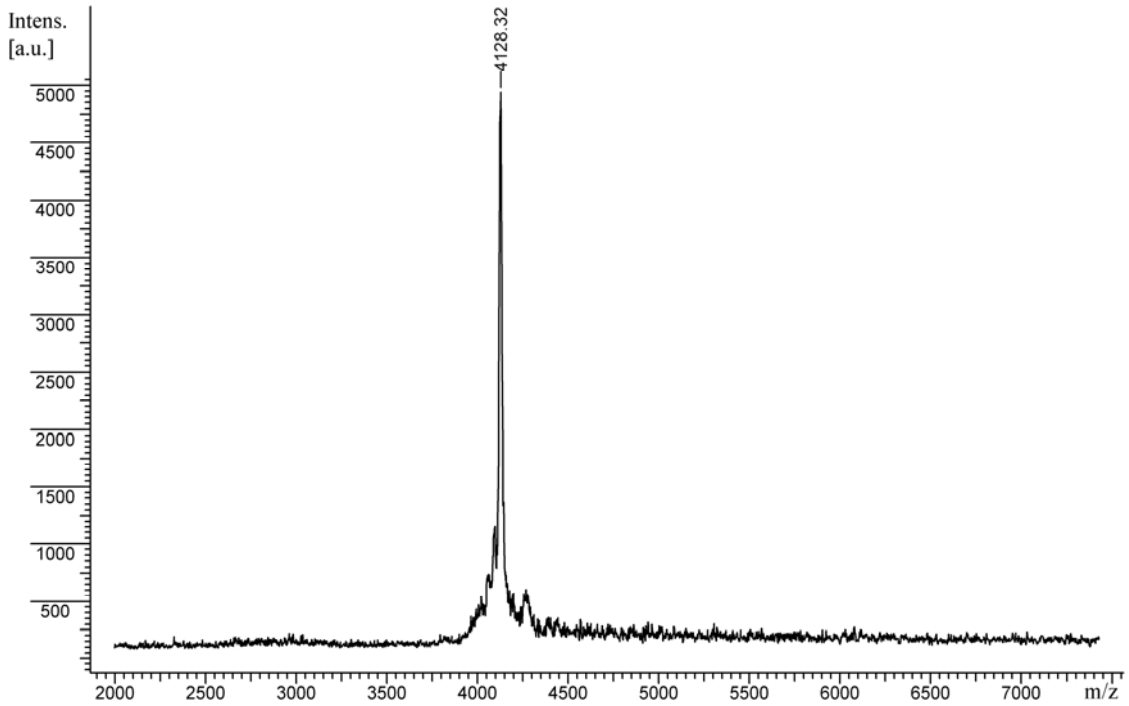


HEX_{Acr}-11D

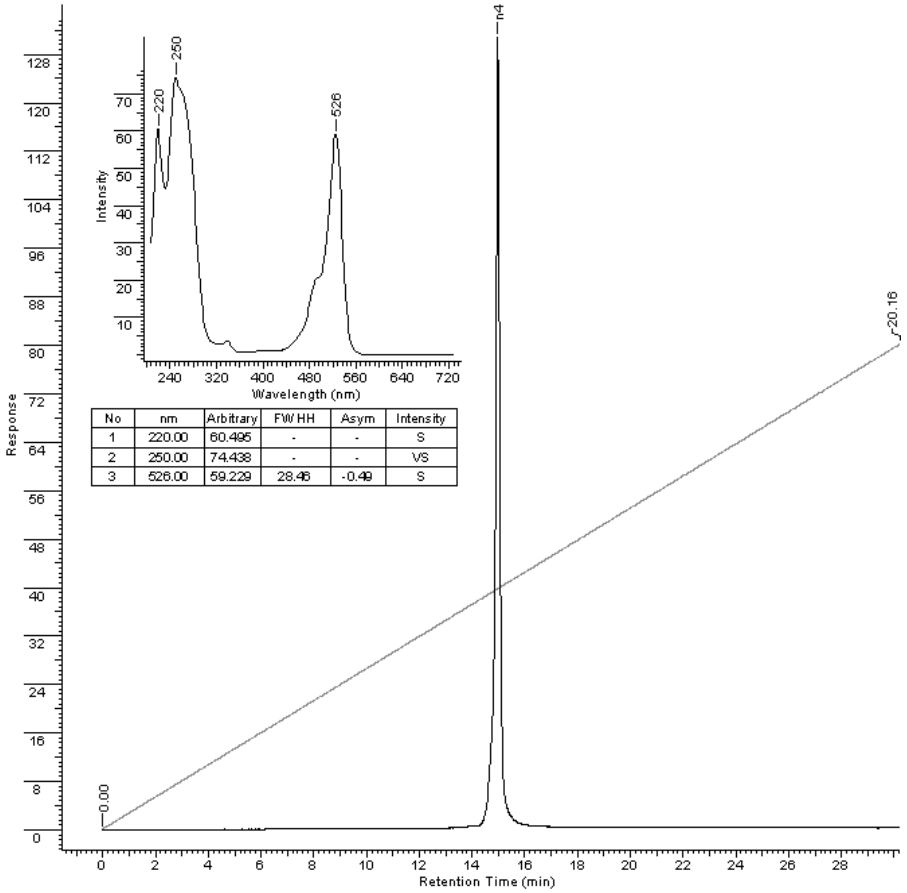


No	nm	Arbitrary	FWHH	Asym	Intensity
1	266.00	18.385	54.92	-0.39	VS
2	494.00	7.321	-	-	M

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N3	16.198	272590.375	100.000	0.279	0.867	54086.191

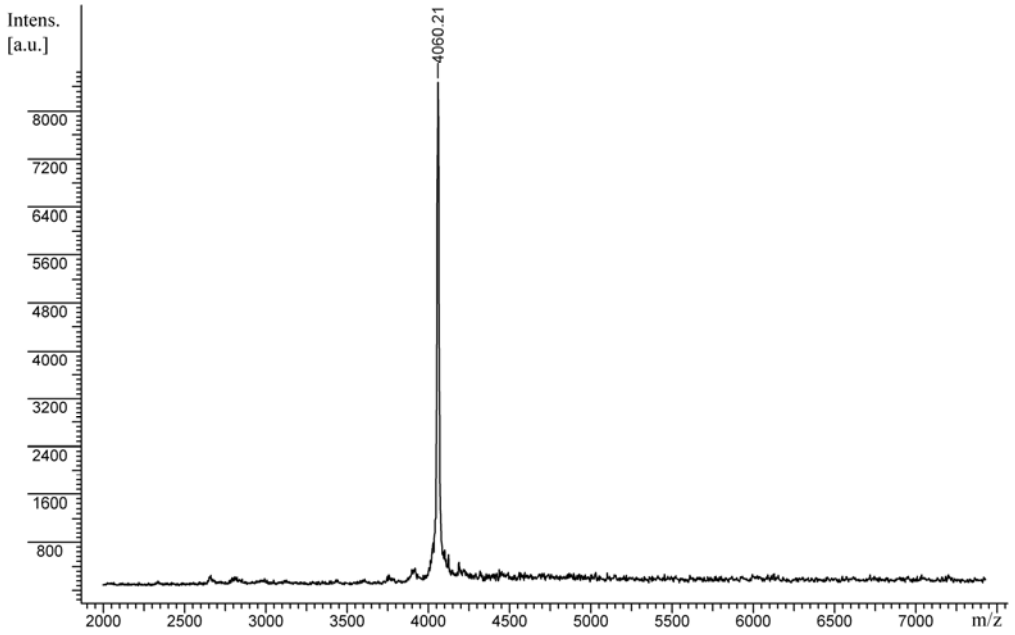


TET-11D

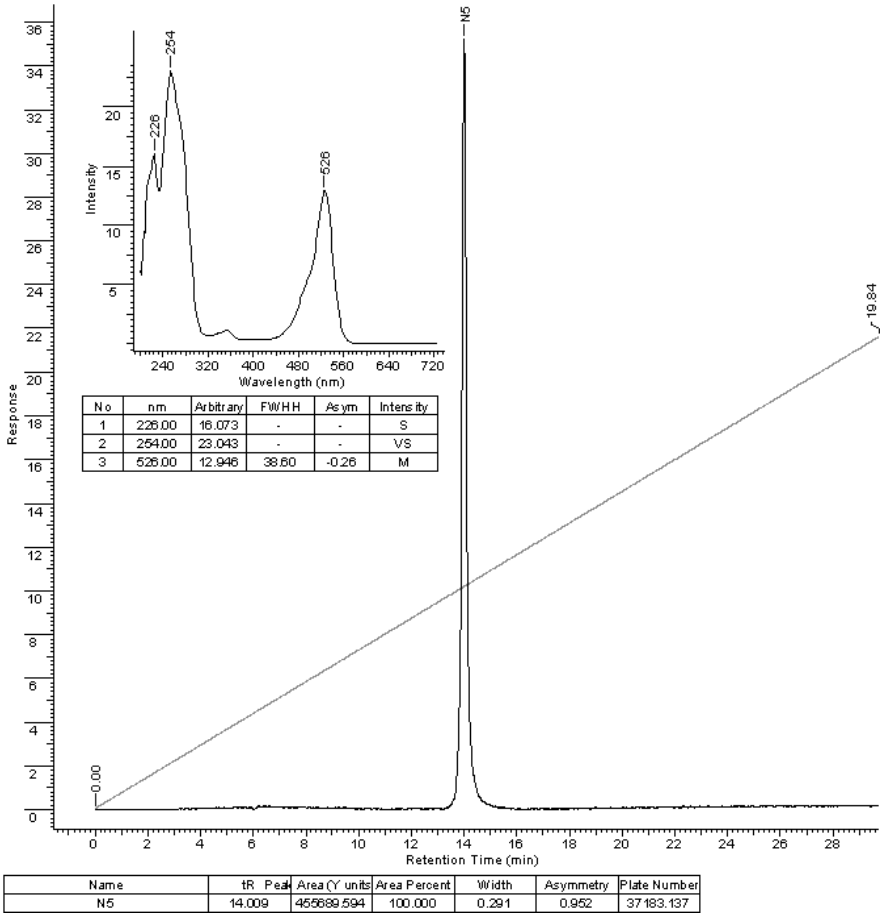


No	nm	Arbitrary	FWHH	Asym	Intensity
1	220.00	60.495	-	-	S
2	250.00	74.438	-	-	VS
3	526.00	59.229	28.46	-0.49	S

Name	tR Peak	Area (Y units)	Area Percent	Width	Asymmetry	Plate Number
n4	14.975	1579941.500	100.000	0.260	1.066	53220.902

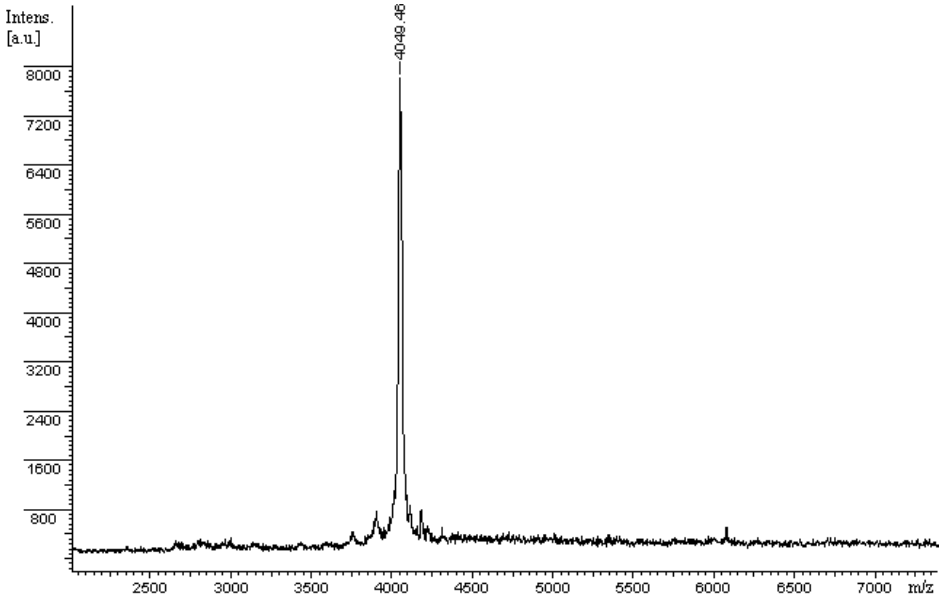


JOE-11D



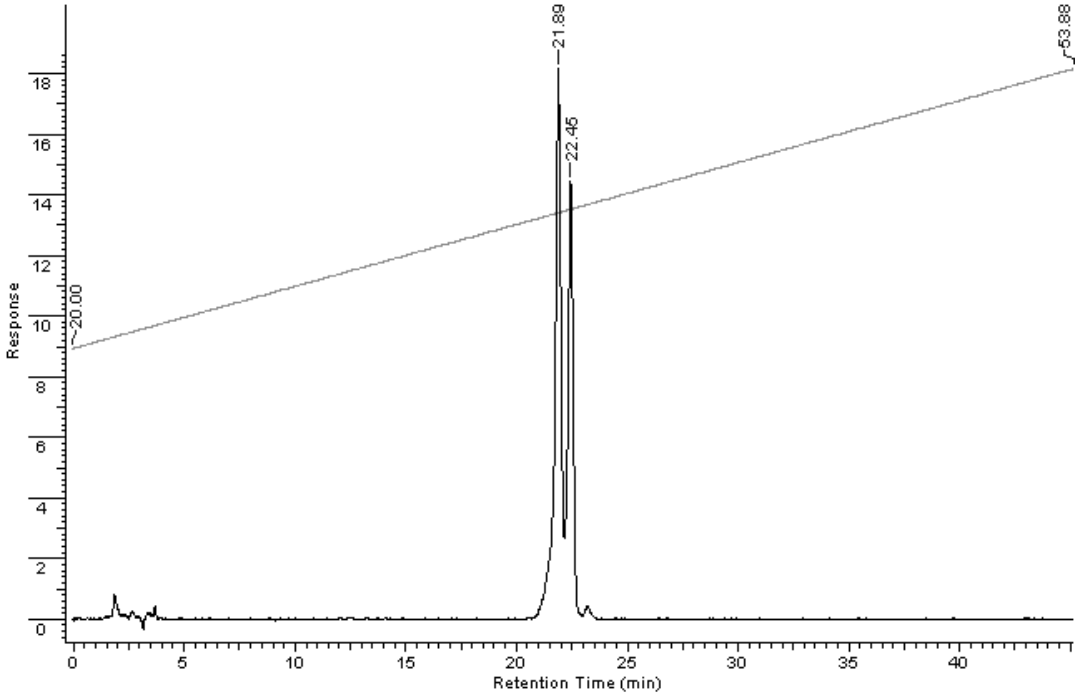
No	nm	Arbitrary	FWHH	Asym	Intensity
1	226.00	16.073	-	-	S
2	254.00	23.043	-	-	VS
3	526.00	12.946	38.60	-0.26	M

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N5	14.009	455689.594	100.000	0.291	0.952	37183.137

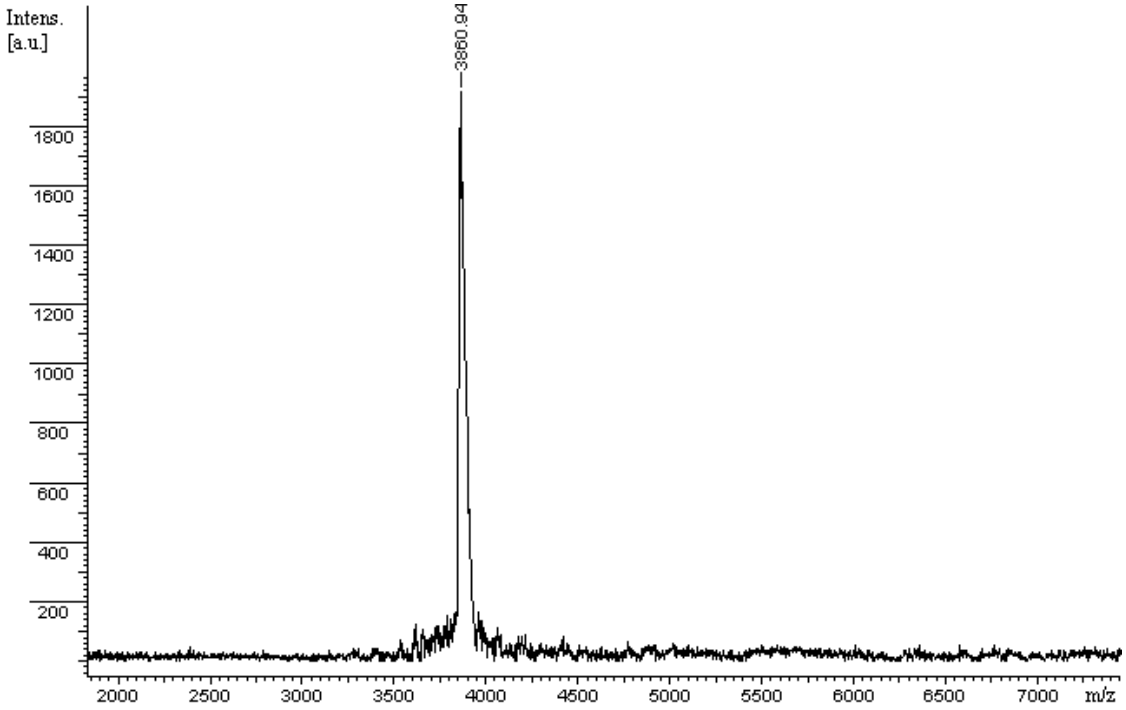


11D-Ole

Double peak on HPLC is a result of the presence of the stereocenter in glycerol moiety (Figure 1). Mass spectrum is recorded for a mixture of peaks.

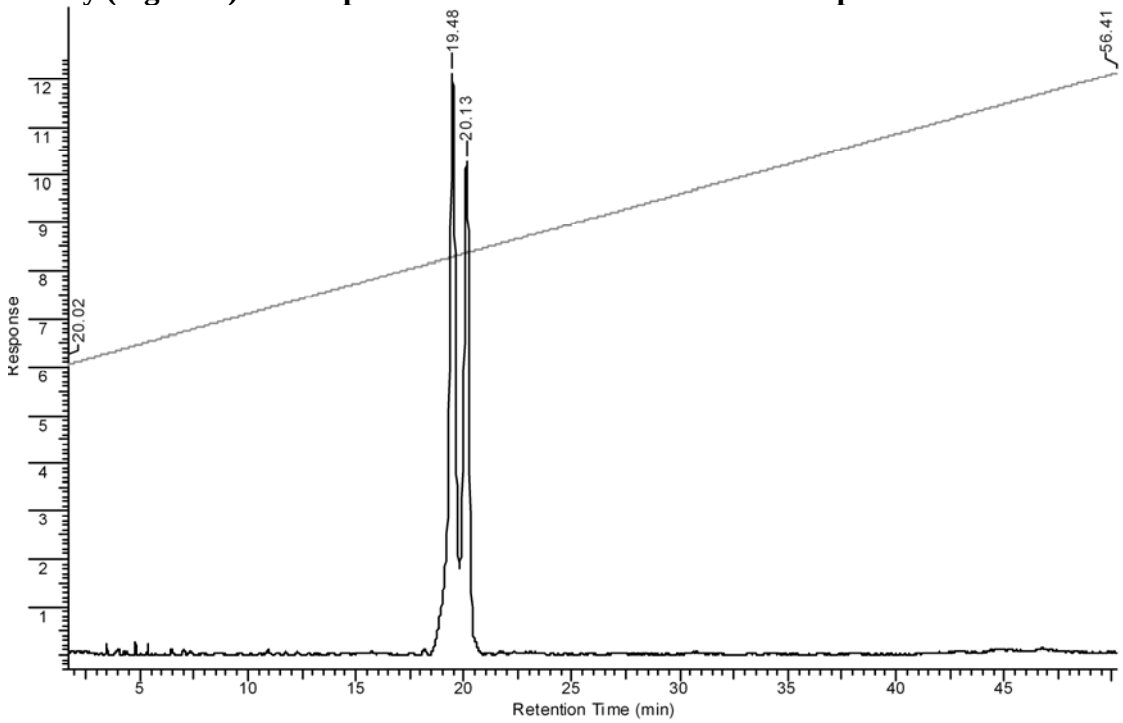


Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Resolution	Plate Number
N6	21.894	344811.125	56.923	0.388	0.950	1.463	50929.844
unknown	22.447	236705.625	39.076	0.368	0.947	1.896	59481.328

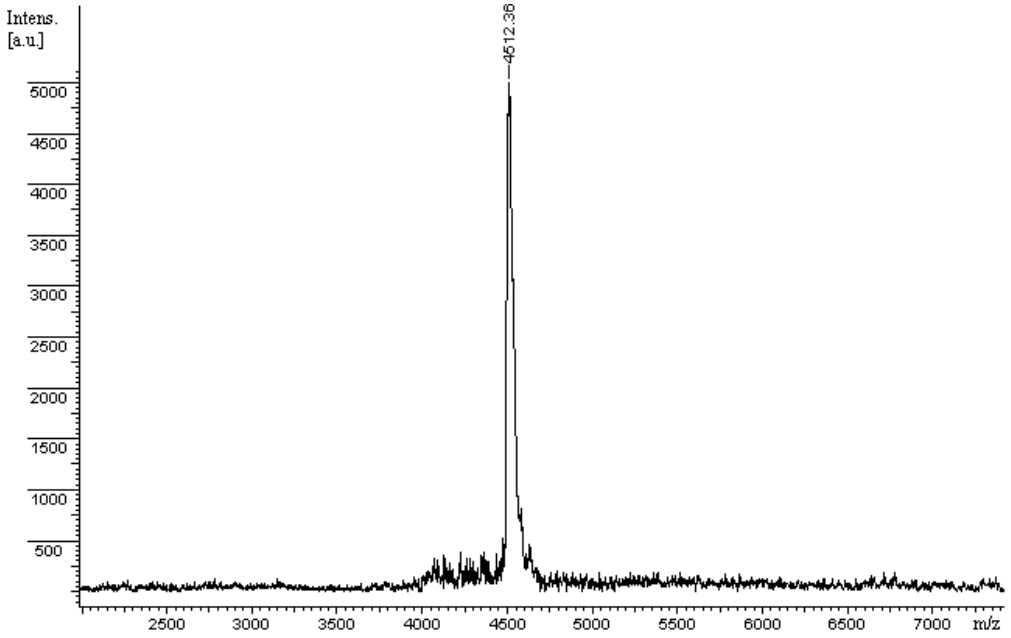


FAM-11D-Ole

Double peak on HPLC is a result of the presence of the stereocenter in glycerol moiety (Figure 1). Mass spectrum is recorded for a mixture of peaks.

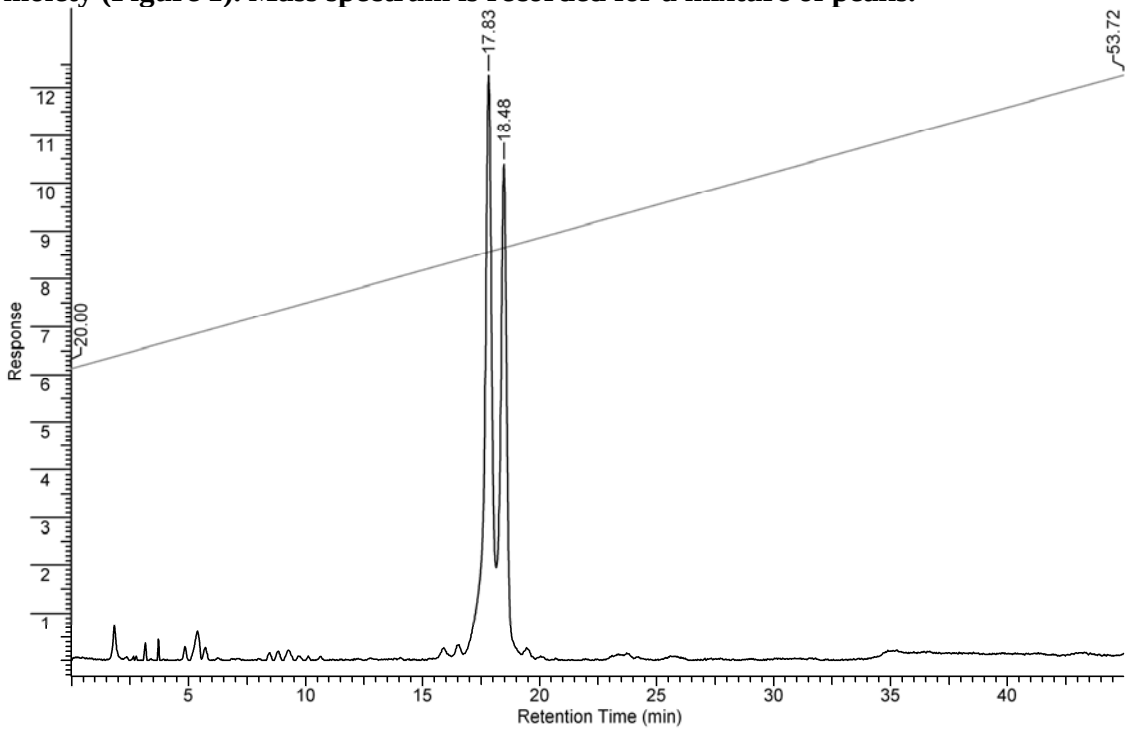


Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Assymetry	Resolution	Plate Number
N7	19.480	298653.354	54.870	0.435	0.952	1.357	39799.359
unknown	20.139	215639.571	39.618	0.424	0.818	-	44090.024

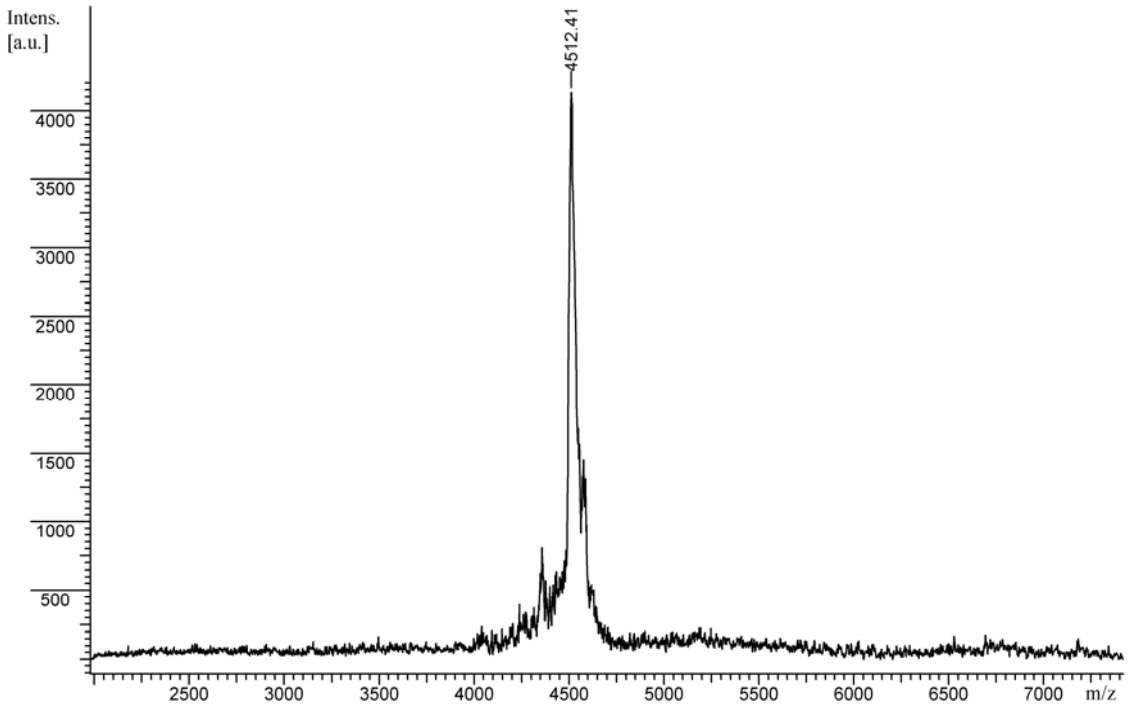


11D-FAM-Ole

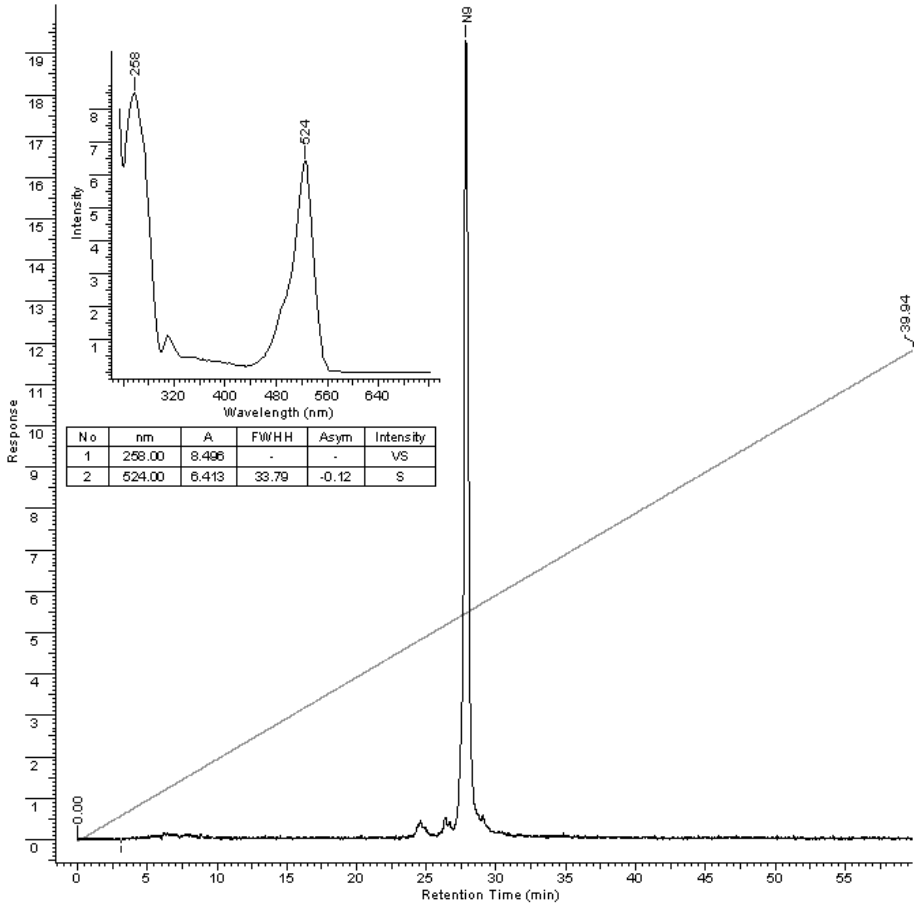
Double peak on HPLC is a result of the presence of the stereocenter in glycerol moiety (Figure 1). Mass spectrum is recorded for a mixture of peaks.



Name	tR	Peak Area (Y units/ms)	Area Percent	Width	Asymmetry	Resolution	Plate Number
N8	17.828	255549.922	45.452	0.428	0.955	1.545	27778.529
unknown	18.481	184714.344	32.853	0.418	0.909	-	31289.764

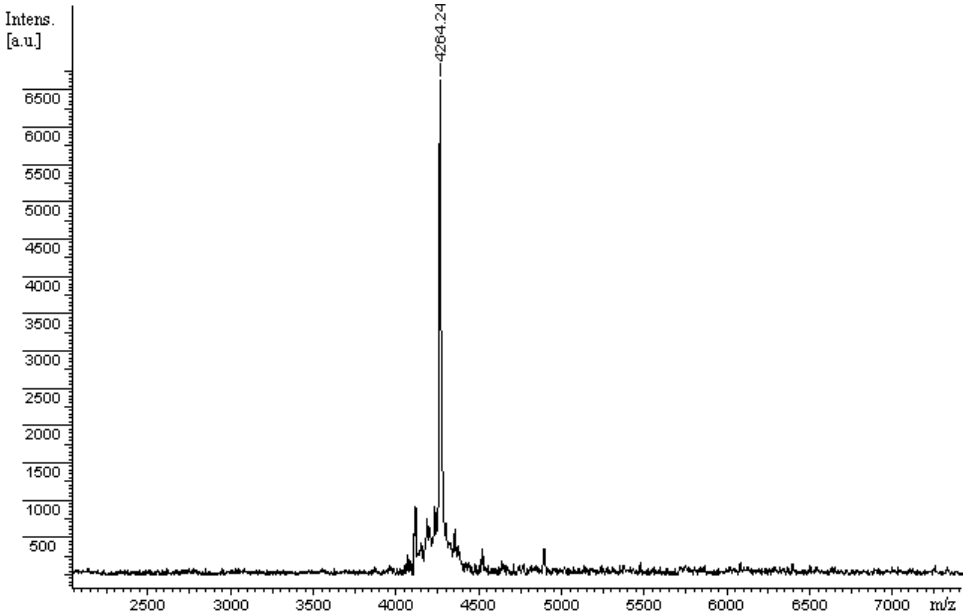


11DX-E-1

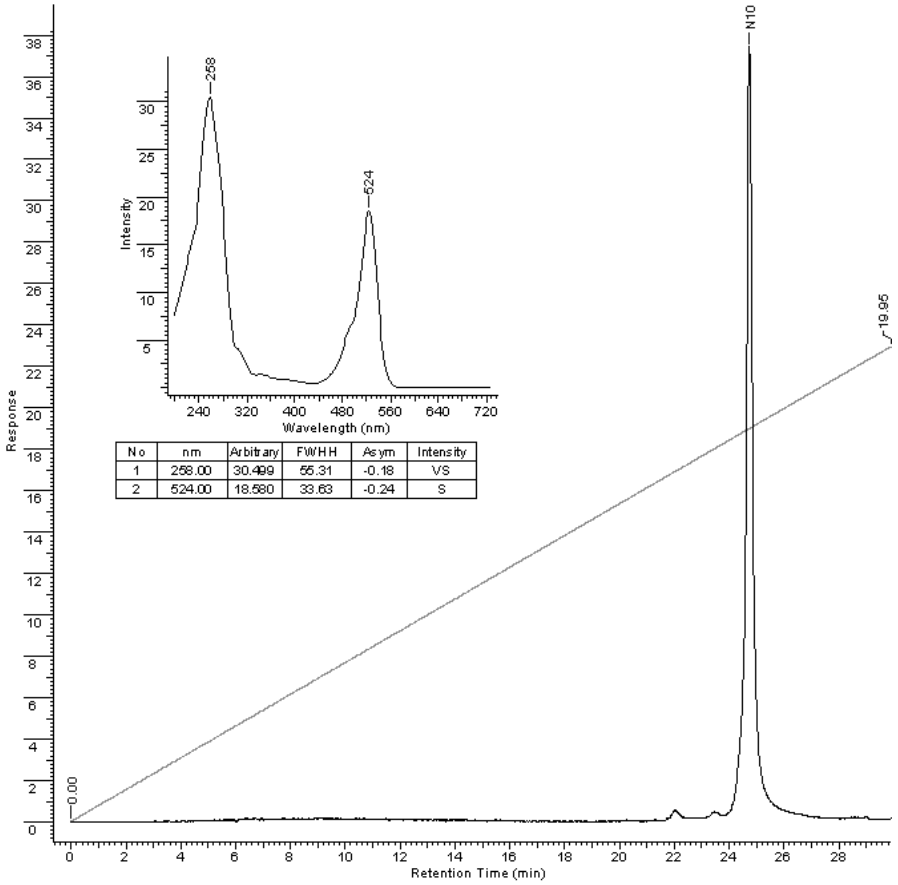


No	nm	A	FWHH	Asym	Intensity
1	258.00	8.498	-	-	VS
2	524.00	6.413	33.79	-0.12	S

Name	tR Peak	Area (Y units)	Area Percent	Width	Asymmetry	Plate Number
N9	27.842	450180.688	100.000	0.517	1.156	46373.379



11DX-E-2



No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	30.499	55.31	-0.18	VS
2	524.00	18.580	33.63	-0.24	S

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
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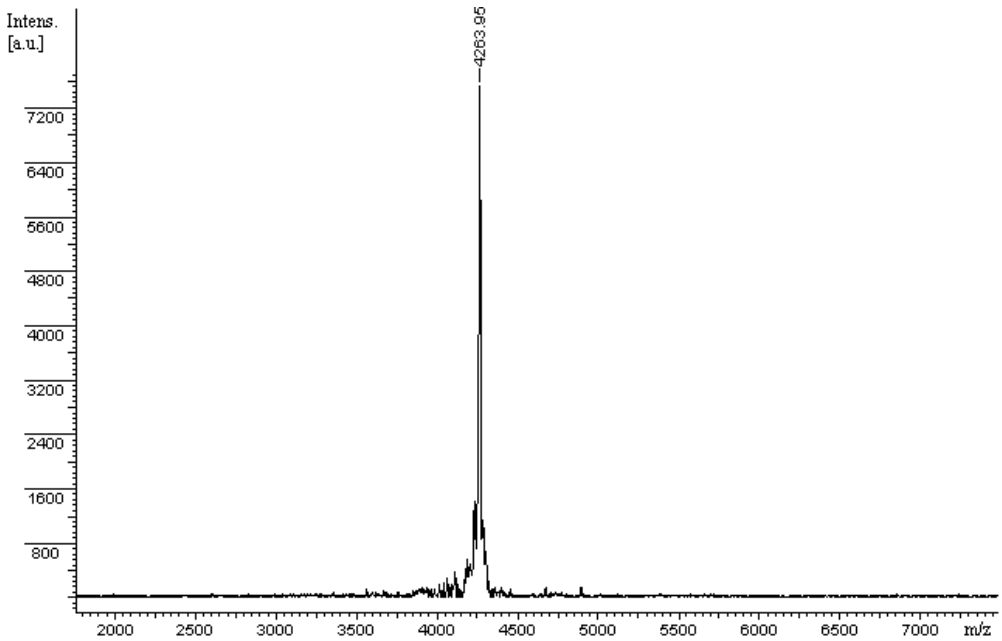

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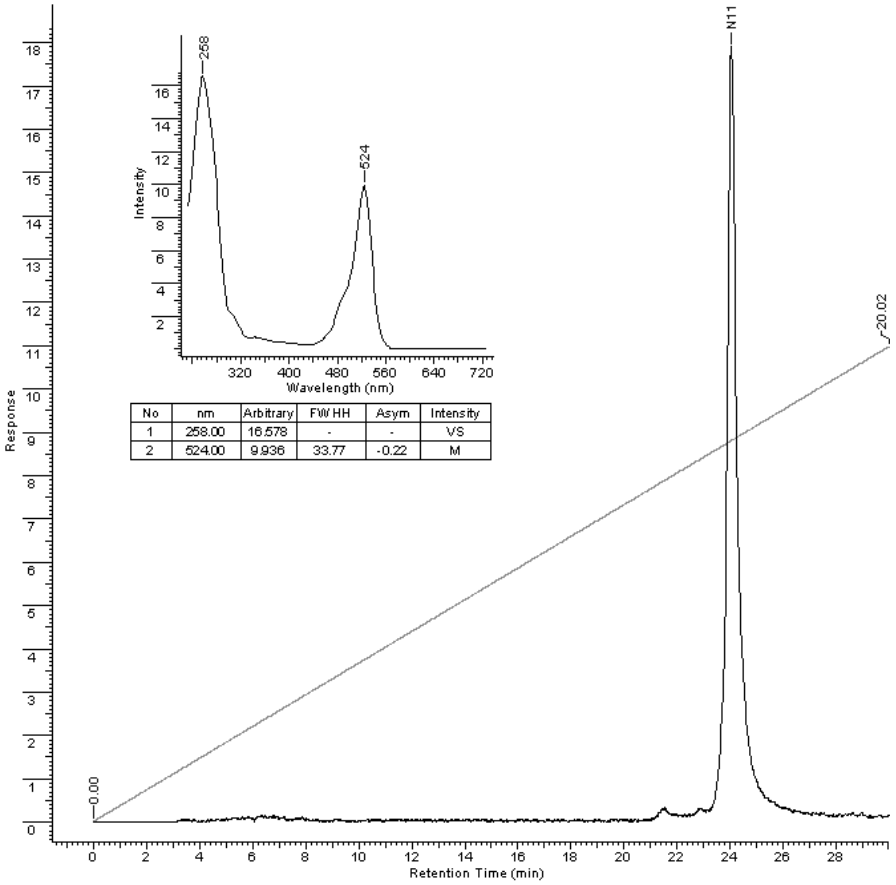
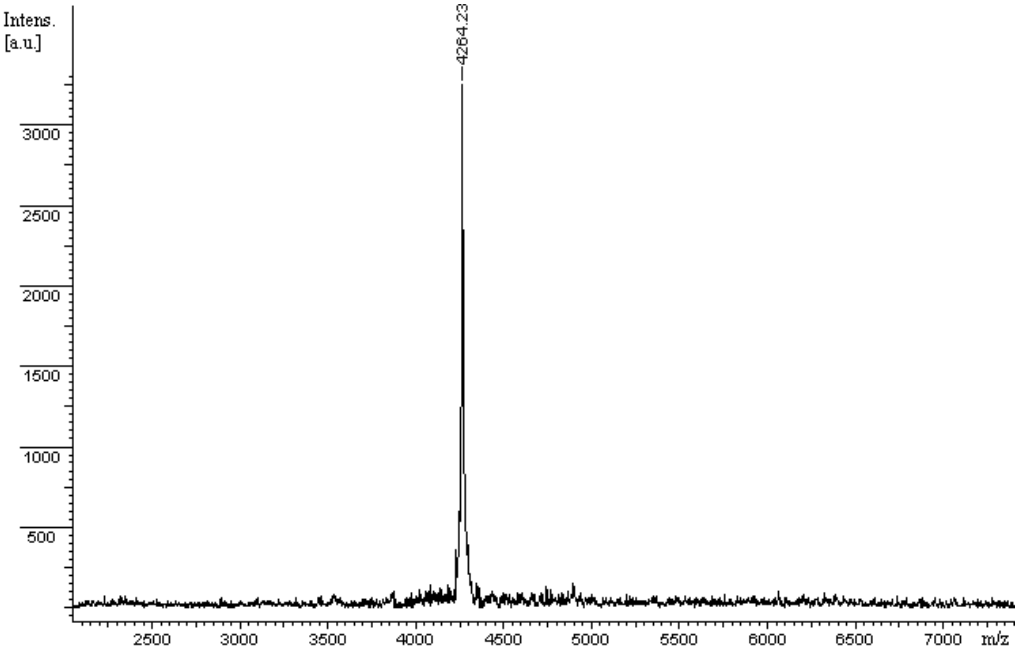
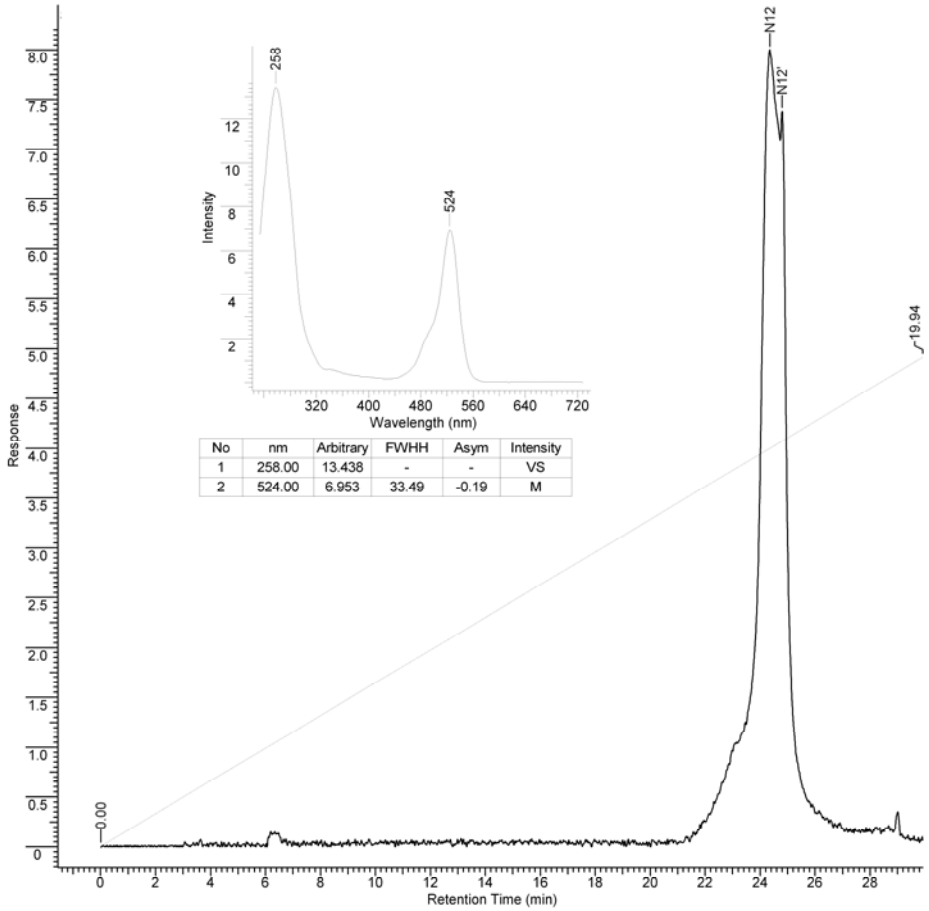


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	16.578	-	-	VS
2	524.00	9.936	33.77	-0.22	M

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N11	24.063	516031.781	100.000	0.584	1.110	27199.270

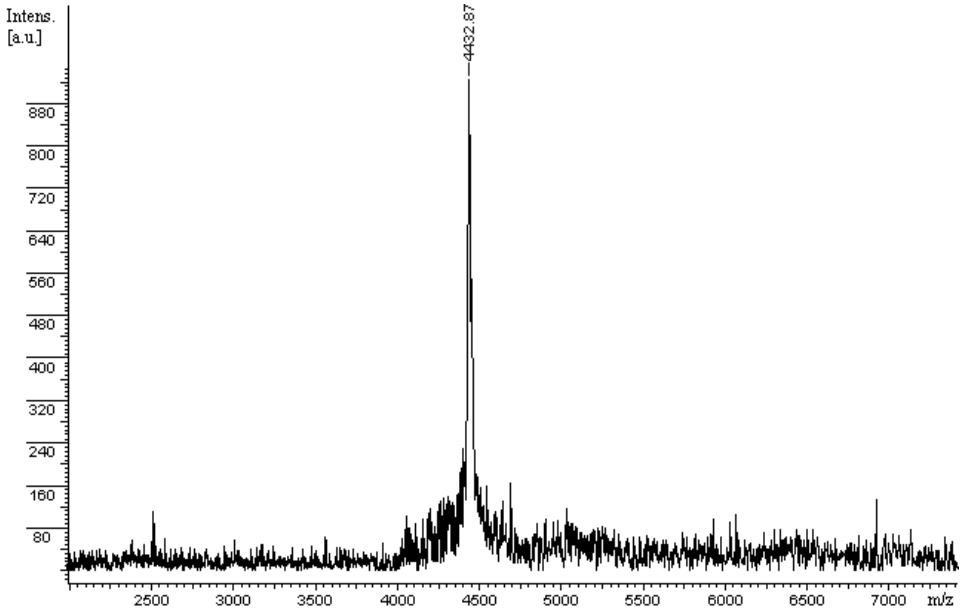


11DS-E-1

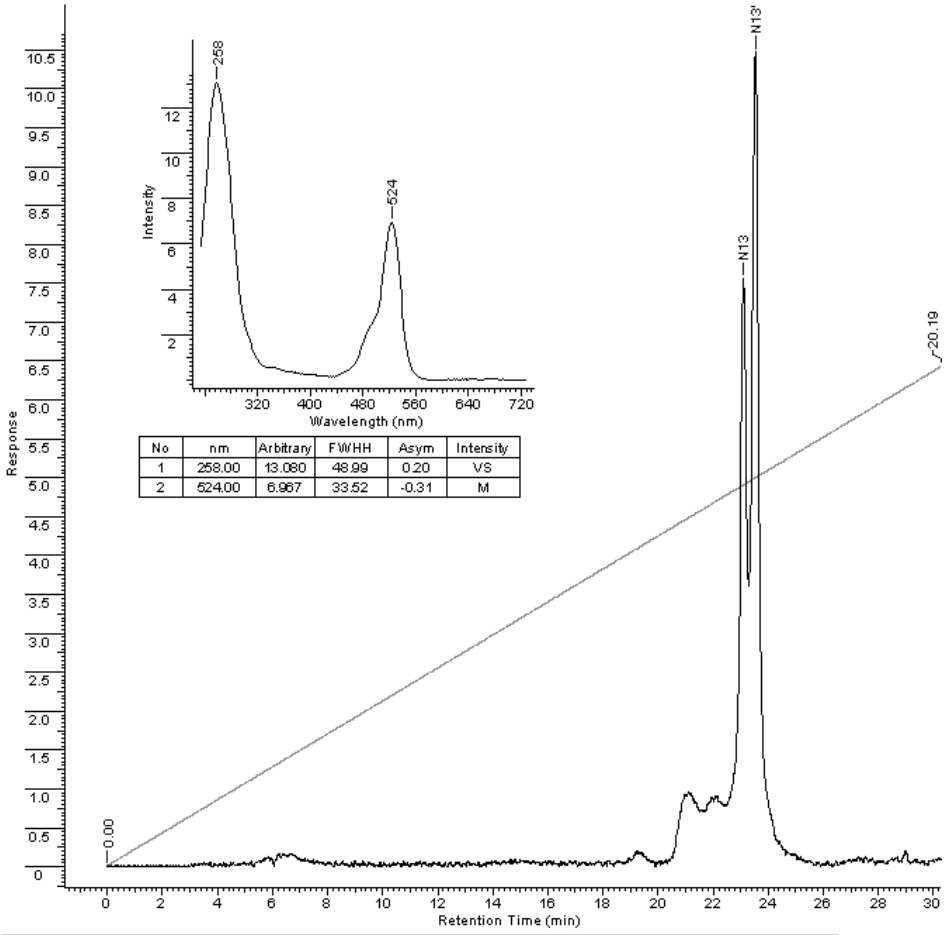


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	13.438	-	-	VS
2	524.00	6.953	33.49	-0.19	M

Name	tR	Peak Area (Y units	Area Percent	Width	Asymmetry	Resolution	Plate Number
N12	24.357	411262.563	74.932	1.035	1.000	0.567	8863.962
N12'	24.797	137583.938	25.068	0.517	1.000	-	36748.016

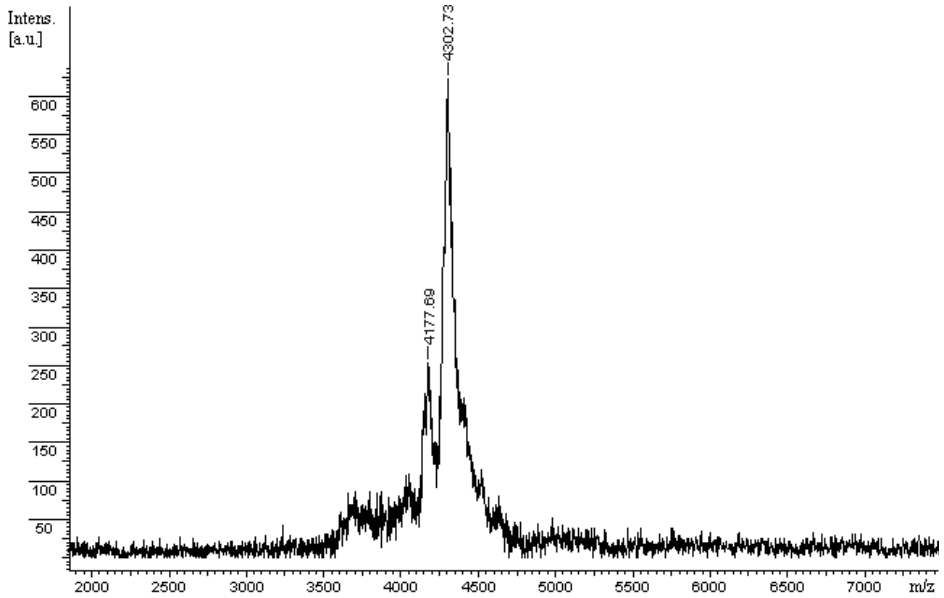


11DS-E-2

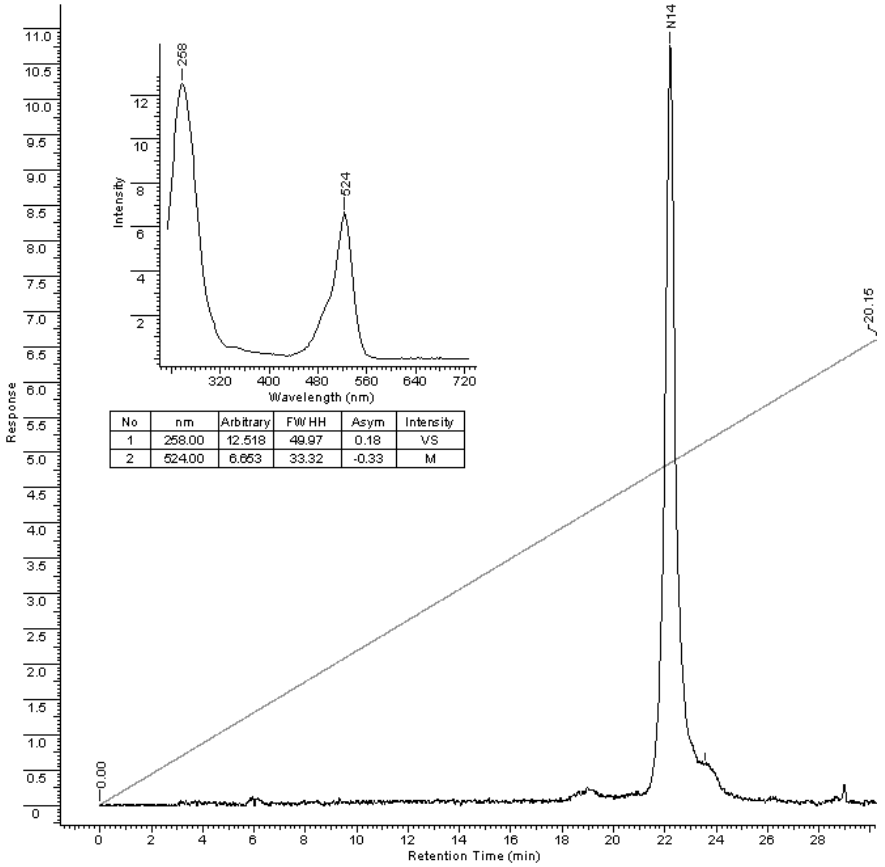


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	13.080	48.99	0.20	VS
2	524.00	6.967	33.52	-0.31	M

Name	tR Peak	Area (Y units)	Area Percent	Width	Asymmetry	Resolution	Plate Number
N13	23.103	148400.844	41.727	0.430	1.094	0.978	46187.953
N13'	23.536	207242.859	58.273	0.456	0.891	-	42667.832

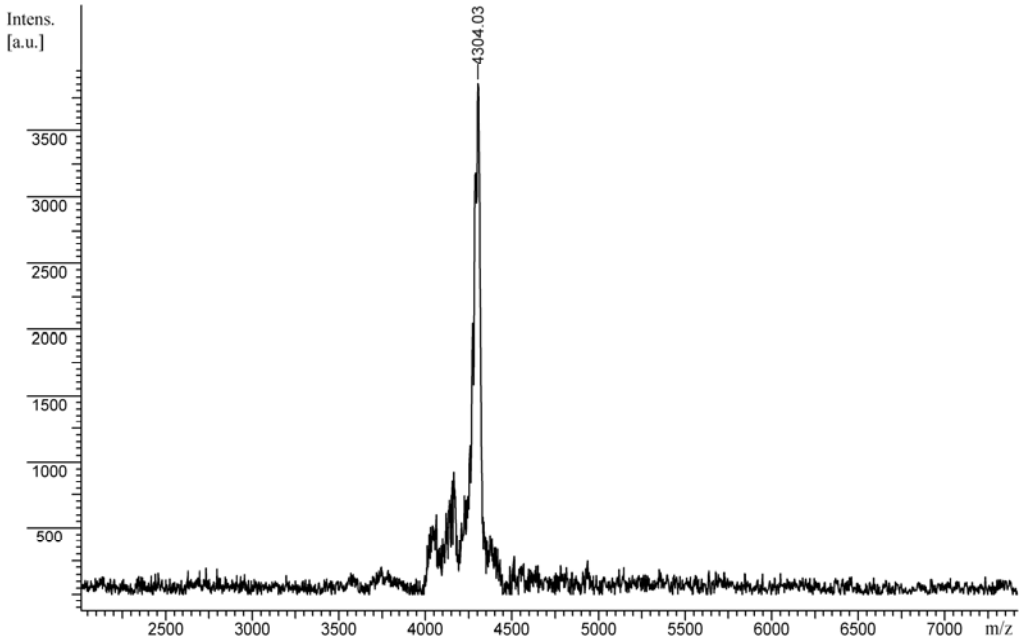


11DS-E-3

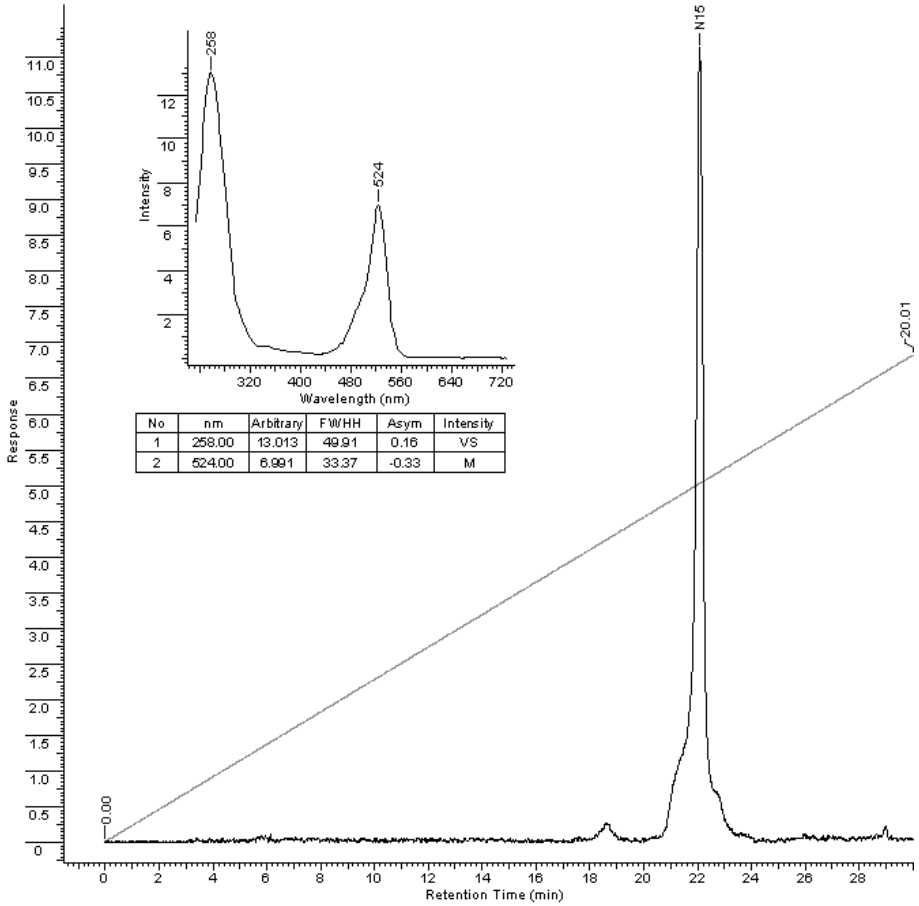


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	12.518	49.97	0.18	VS
2	524.00	6.653	33.32	-0.33	M

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Resolution	Plate Number
N14	22.200	345420.156	94.443	0.607	0.968	1.216	21404.902
unknown	23.574	20323.539	5.557	1.652	1.000	-	3259.045

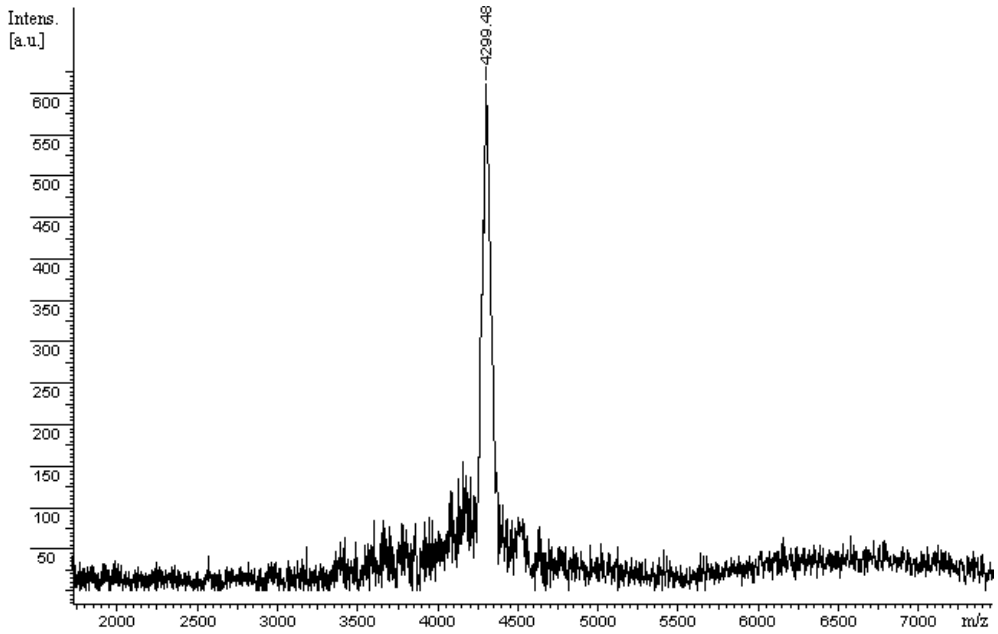


11DS-E-4

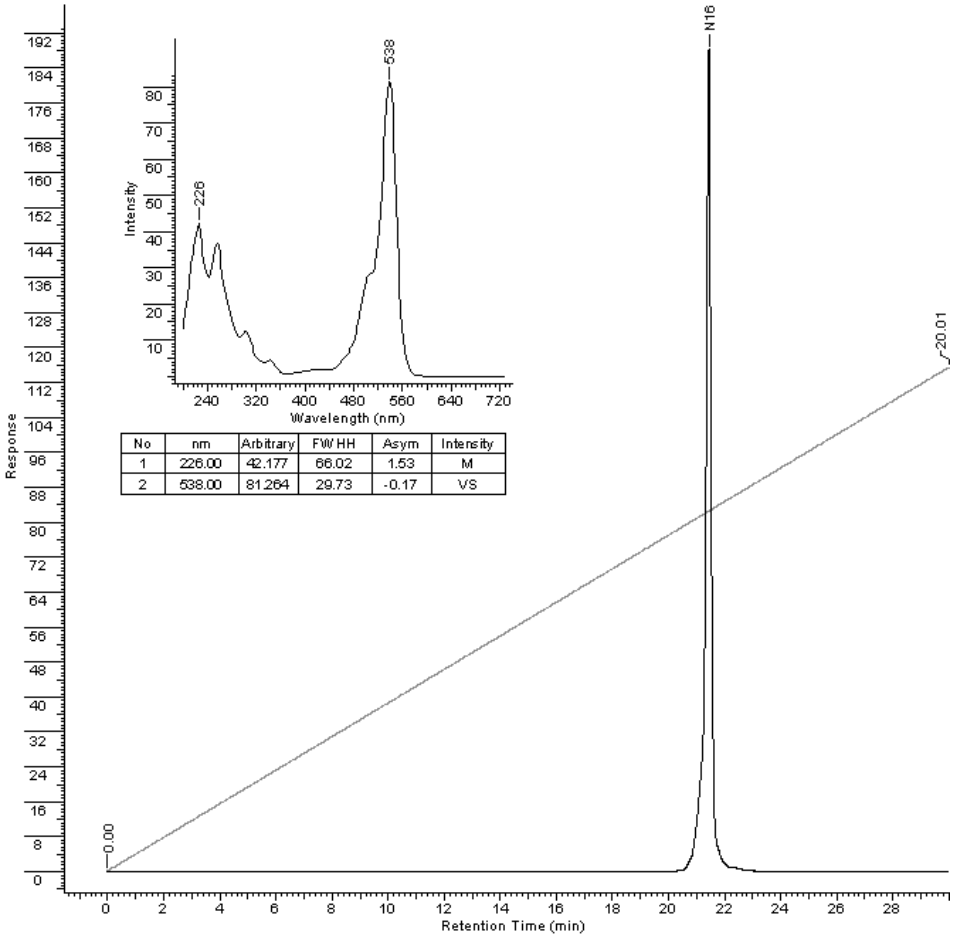


No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	13.013	49.91	0.16	VS
2	524.00	6.991	33.37	-0.33	M

Name	tR Peak	Area (Y units)	Area Percent	Width	Asymmetry	Plate Number
N15	22.085	255721.203	100.000	0.418	0.909	44682.055

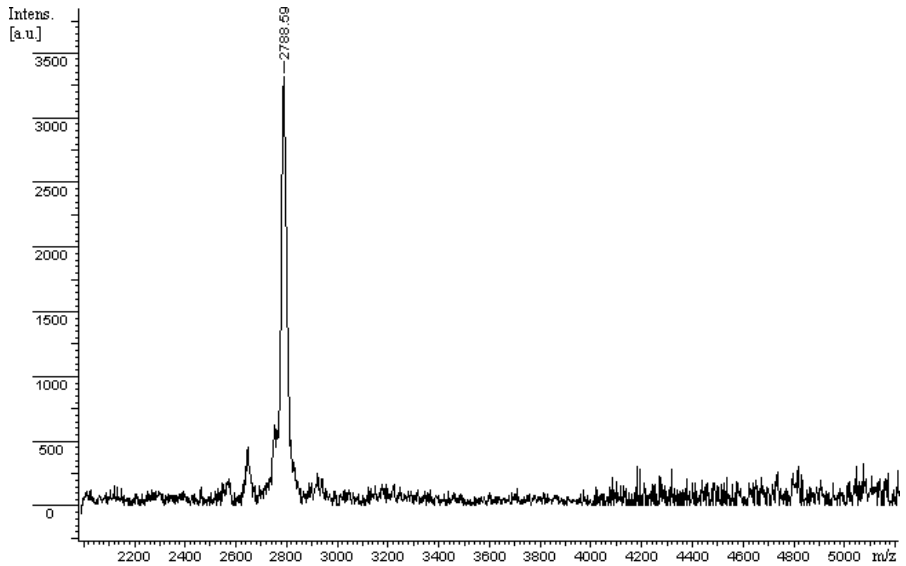


HEX-11D-ddR

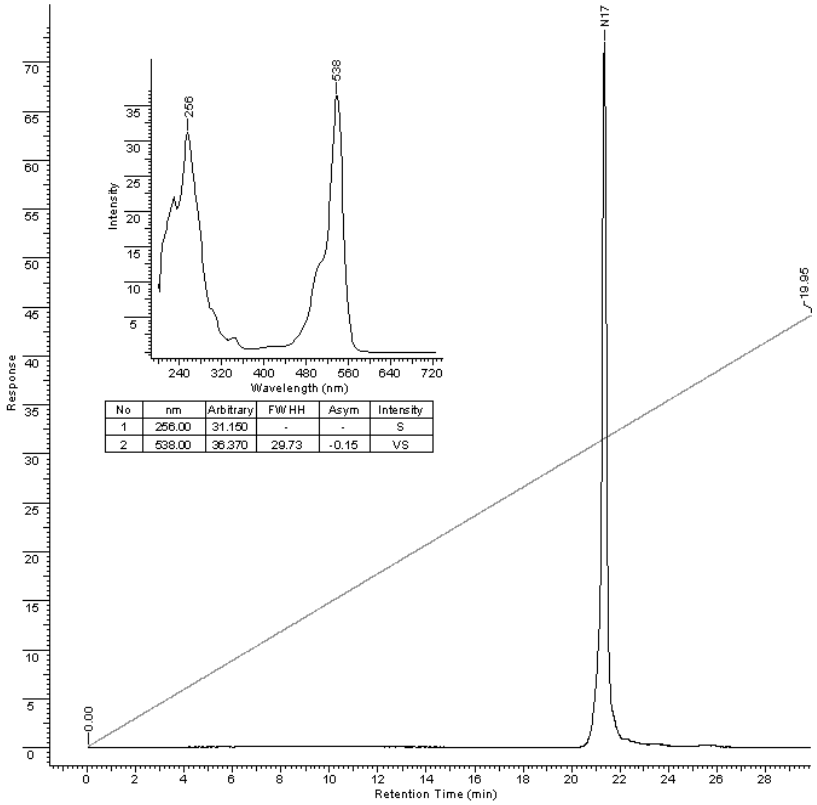


No	nm	Arbitrary	FWHH	Asym	Intensity
1	226.00	42.177	66.02	1.53	M
2	538.00	81.264	29.73	-0.17	VS

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N16	21.437	2627330.250	100.000	0.309	0.945	77207.094

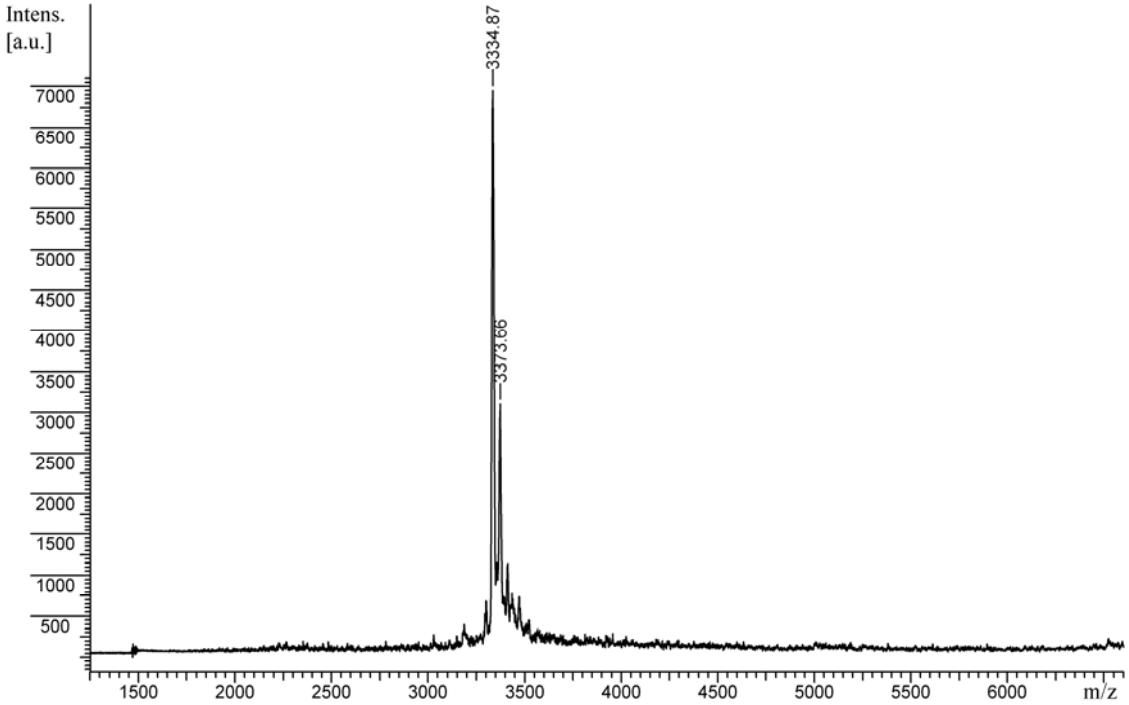


HEX-11D-ddR1

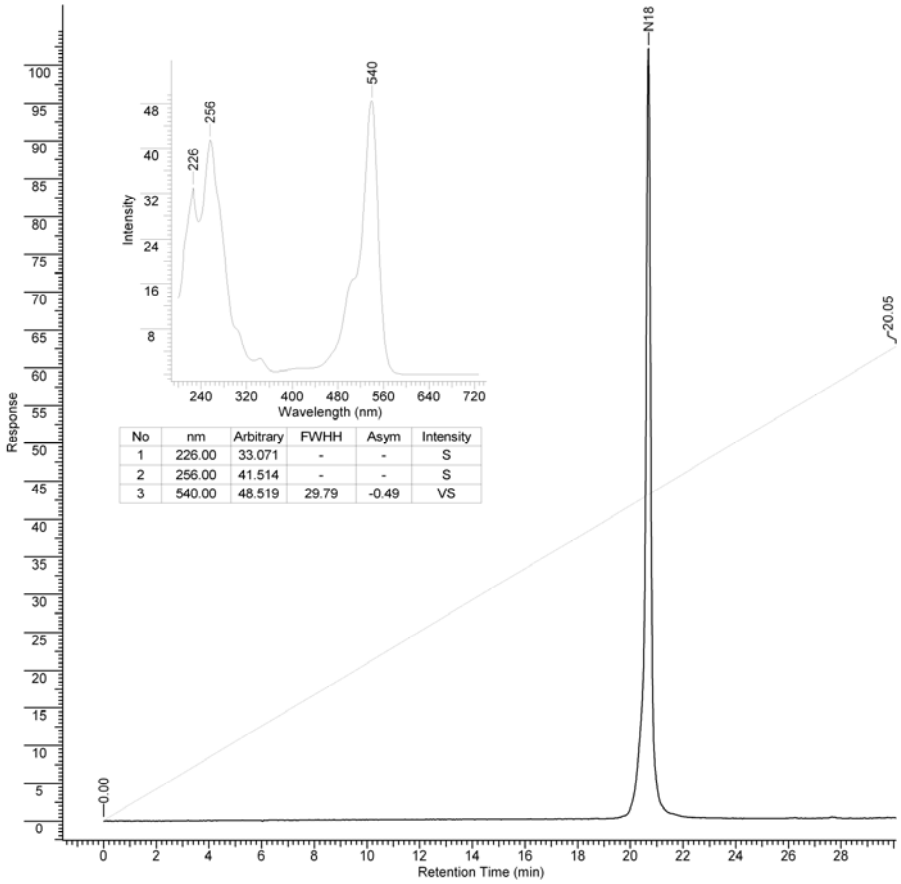


No	nm	Arbitrary	FWHH	Asym	Intensity
1	256.00	31.150	-	-	S
2	538.00	36.370	29.73	-0.15	VS

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N17	21.359	1075851.125	100.000	0.316	1.080	72872.289

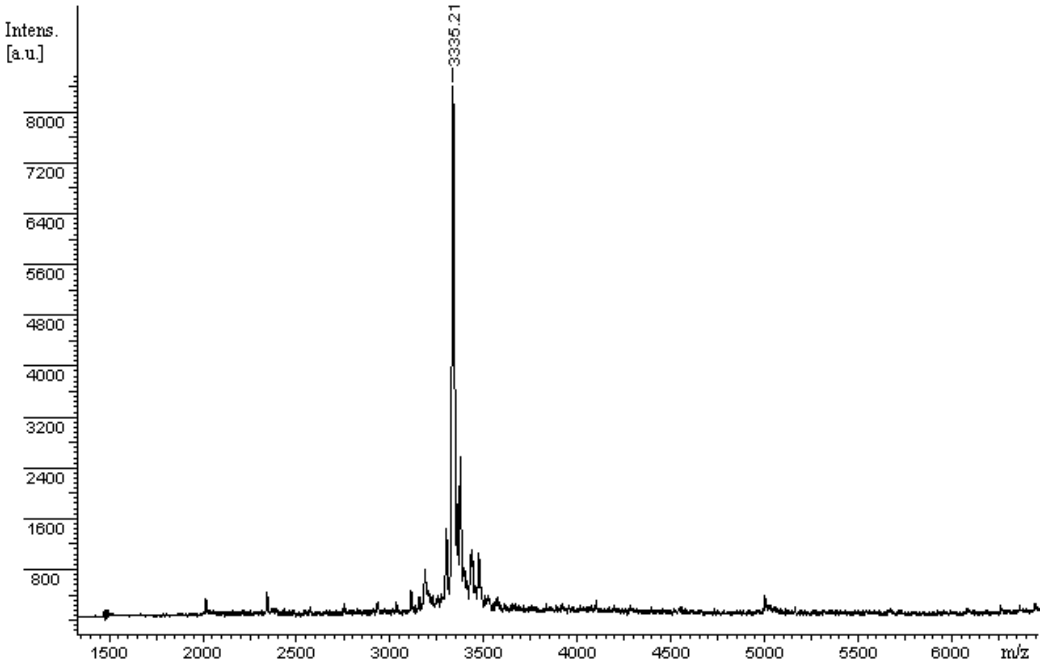


HEX-11D-ddR2



No	nm	Arbitrary	FWHH	Asym	Intensity
1	226.00	33.071	-	-	S
2	256.00	41.514	-	-	S
3	540.00	48.519	29.79	-0.49	VS

Name	tR	Peak Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N18	20.686	1517555.250	100.000	29.944	180.374	7.636



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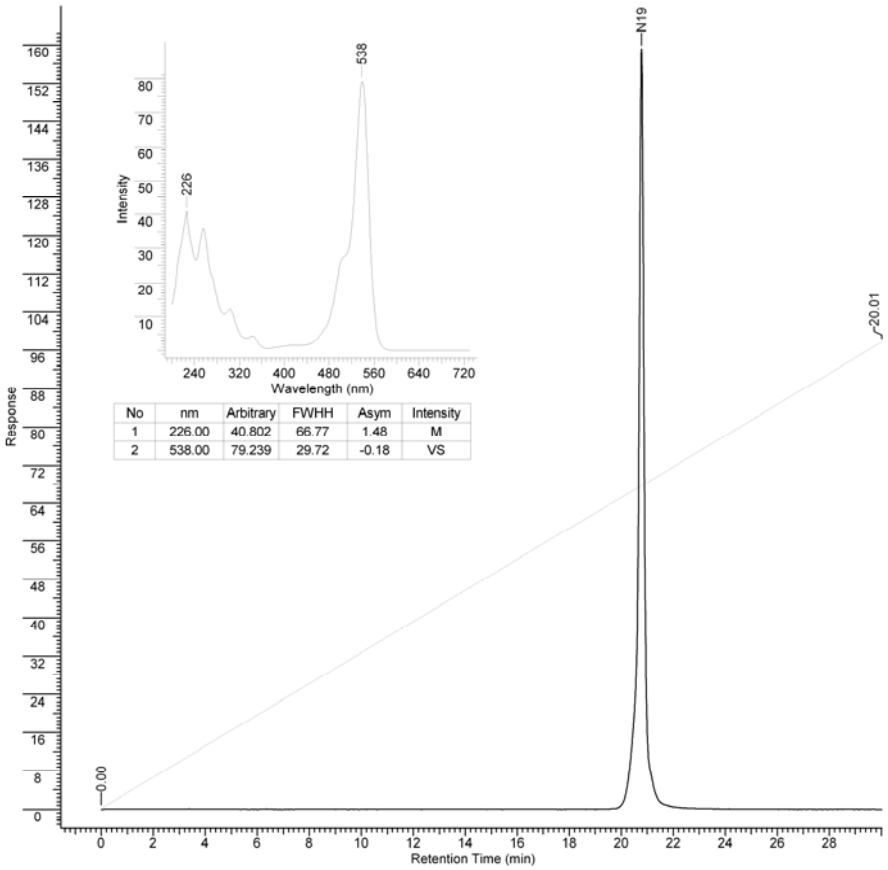
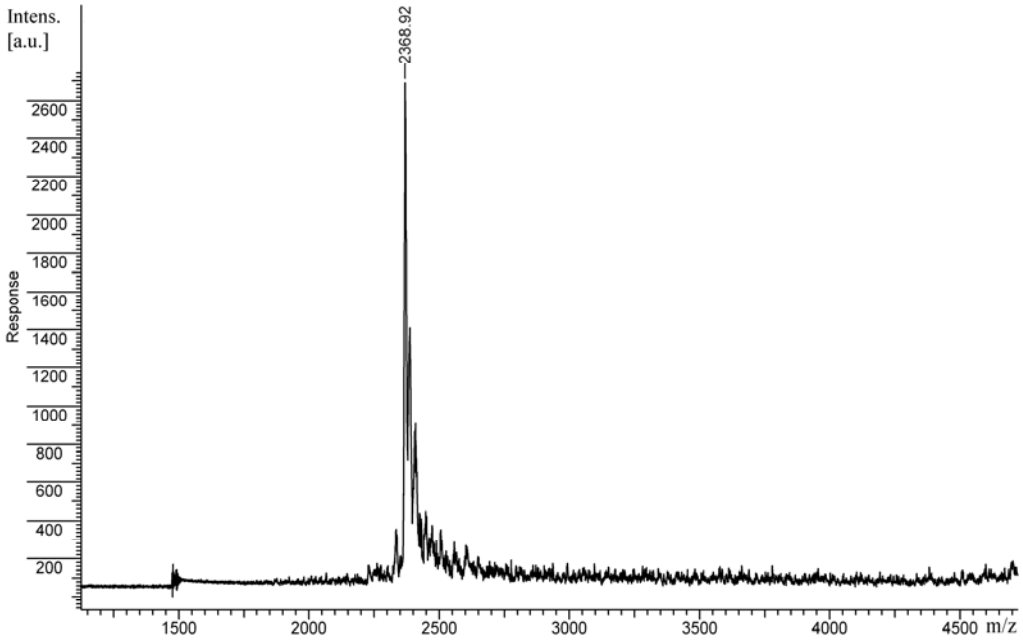


No	nm	Arbitrary	FWHH	Asym	Intensity
1	226.00	40.802	66.77	1.48	M
2	538.00	79.239	29.72	-0.18	VS

Name	tR	Peak Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N19	20.773	2426446.500	100.000	0.308	1.067	72568.461



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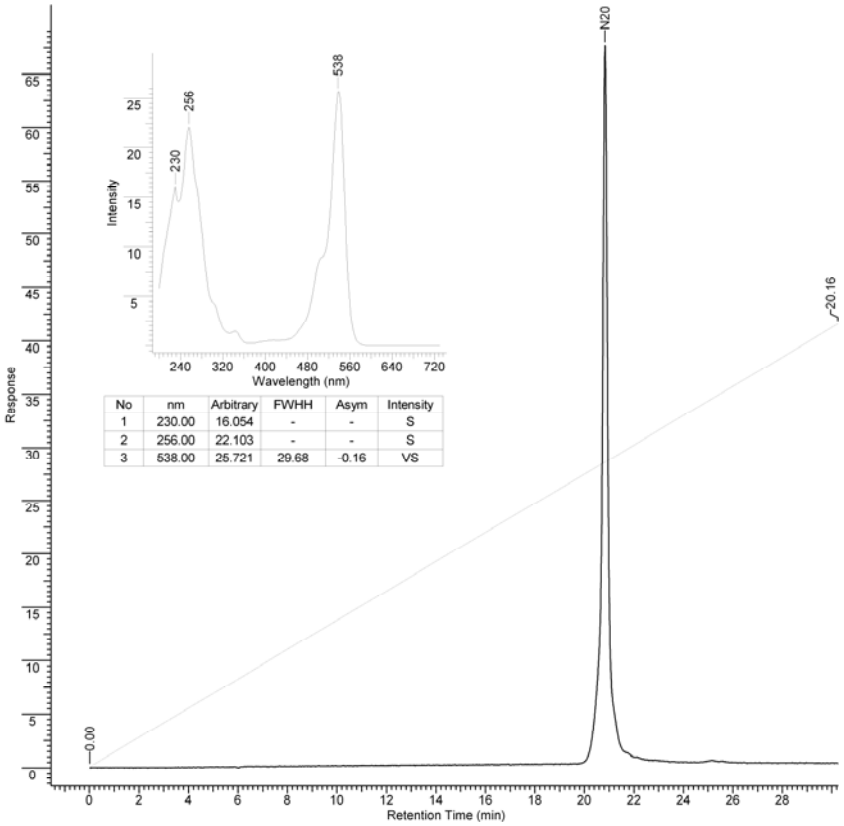
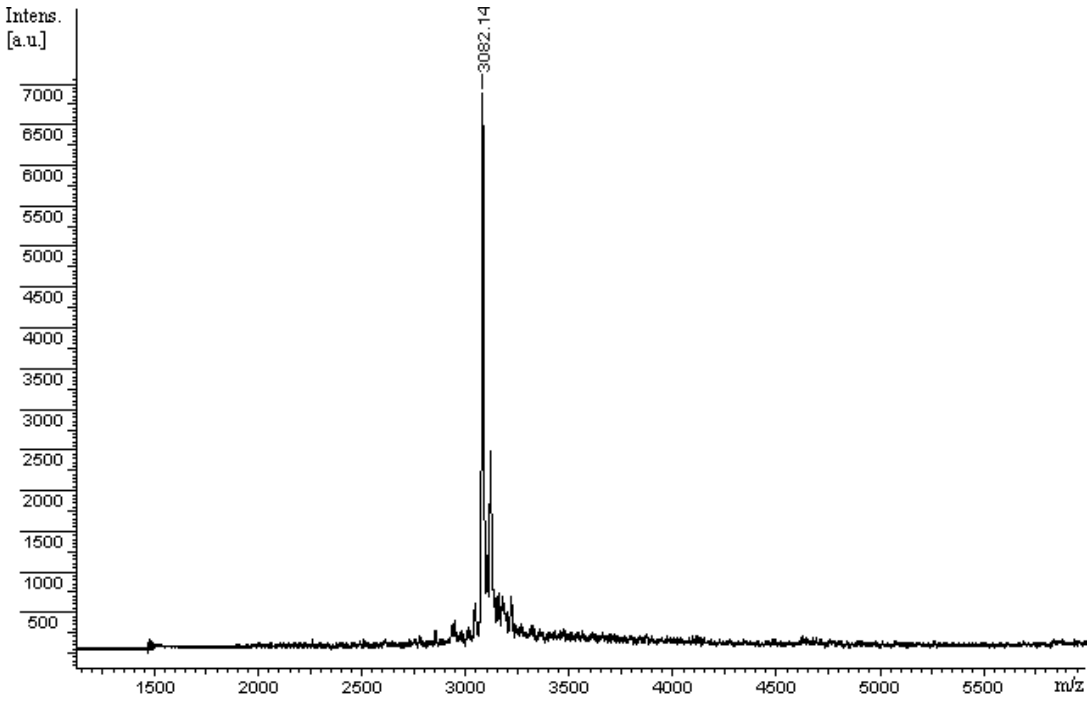


No	nm	Arbitrary	FWHH	Asym	Intensity
1	230.00	16.054	-	-	S
2	256.00	22.103	-	-	S
3	538.00	25.721	29.68	-0.16	VS

Name	tR	Peak Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N20	20.831	1156564.000	100.000	0.342	1.073	59188.613



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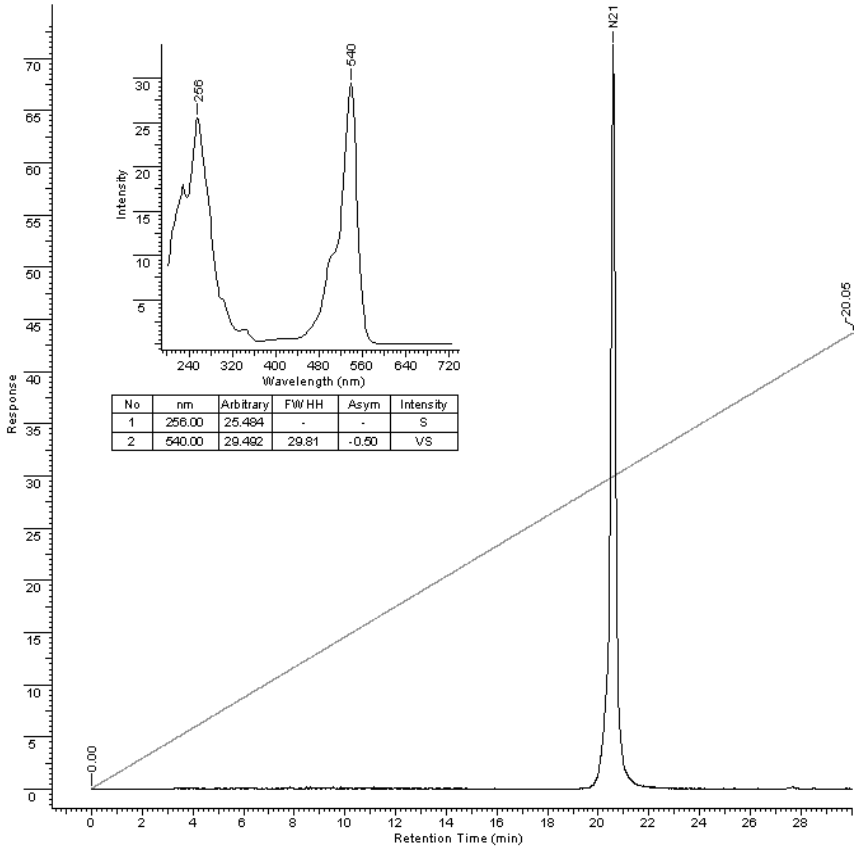
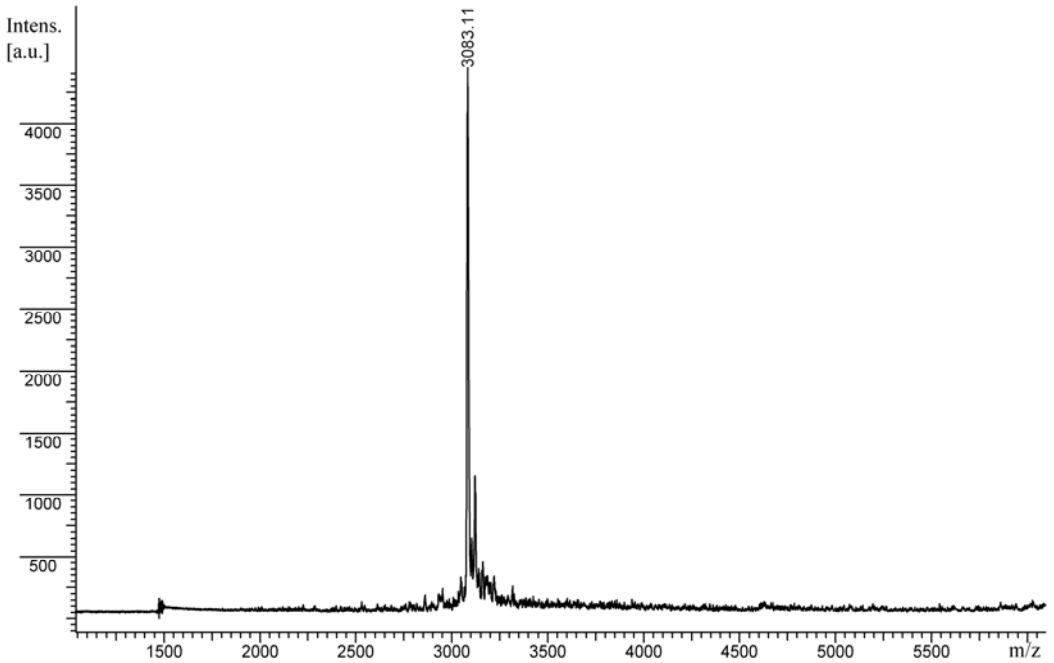
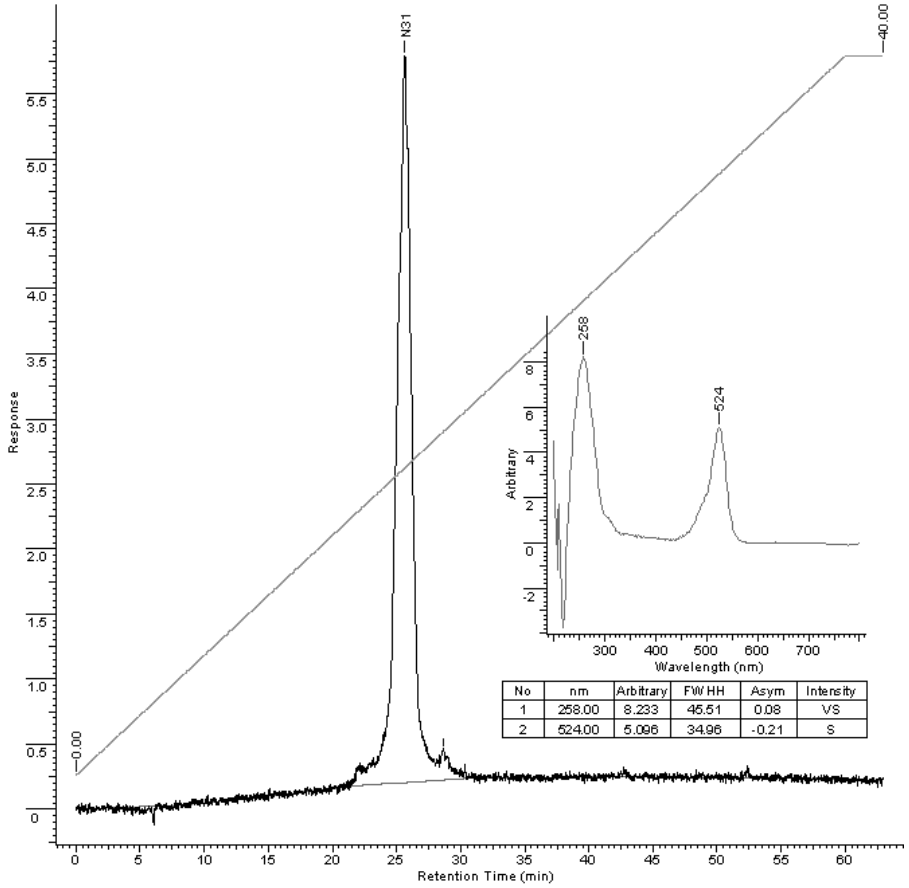


No	nm	Arbitrary	FWHH	Asym	Intensity
1	256.00	25.484	-	-	S
2	540.00	29.492	29.81	-0.50	VS

Name	tR Peak	Area (Y units	Area Percent	Width	Asymmetry	Plate Number
N21	20.593	1095541.500	100.000	0.322	1.037	65270.320

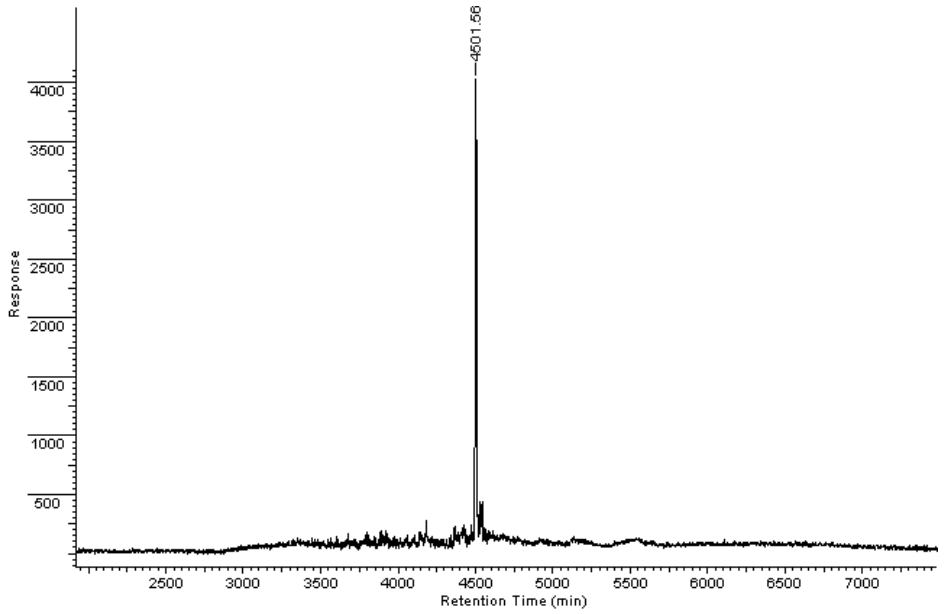


11M-E



No	nm	Arbitrary	FWHH	Asym	Intensity
1	258.00	8.233	45.51	0.08	VS
2	524.00	5.096	34.96	-0.21	S

No.	Name	tR	Peak Area (Y units/ms)	Area Percent
1	N31	25.633	438455.000	98.671
2	unknown	28.633	5905.575	1.329



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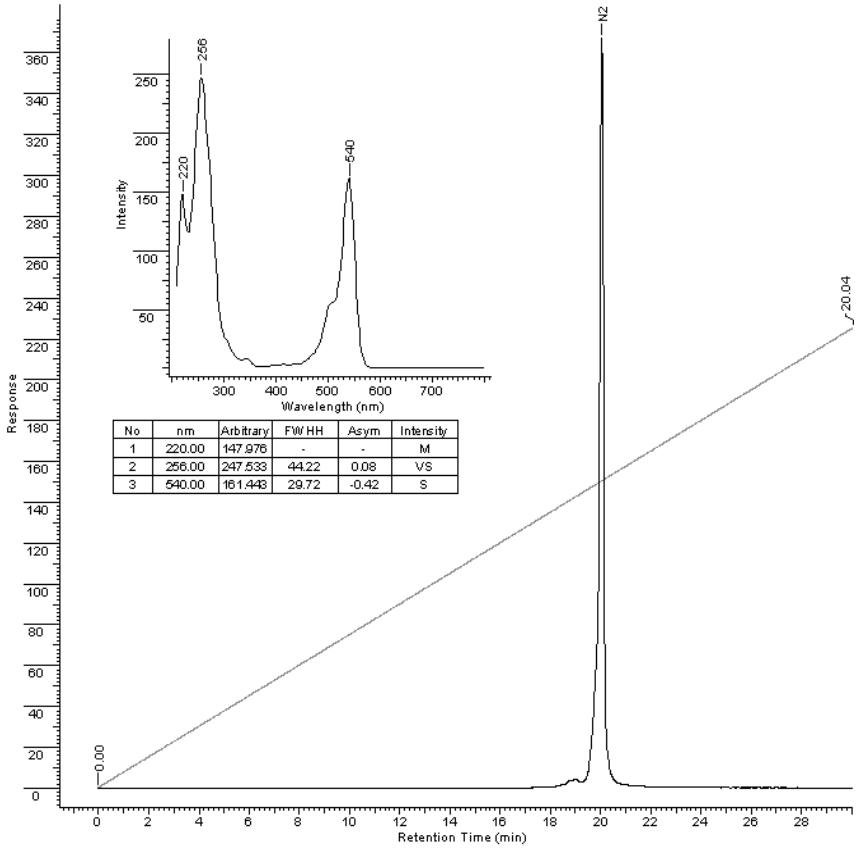
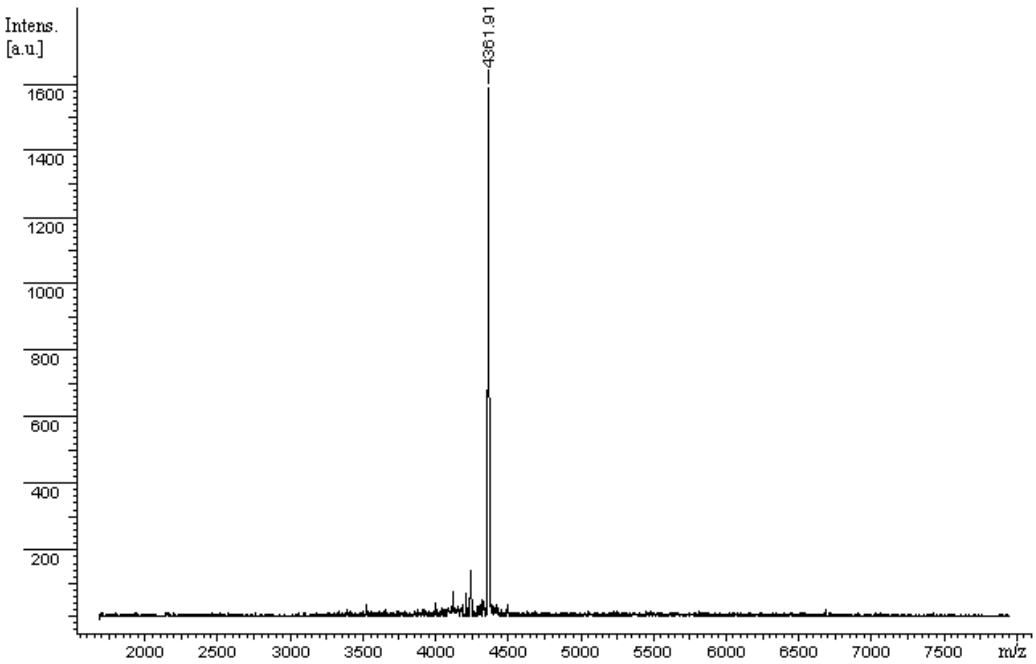


No	nm	Arbitrary	FWHH	Asym	Intensity
1	220.00	147.976	-	-	M
2	256.00	247.533	44.22	0.08	VS
3	540.00	161.443	29.72	-0.42	S

Name	tR Peak	Area (Y units)	Area Percent	Width	Asymmetry	Plate Number
N2	20.047	4743072.500	100.000	0.277	0.891	83983.758



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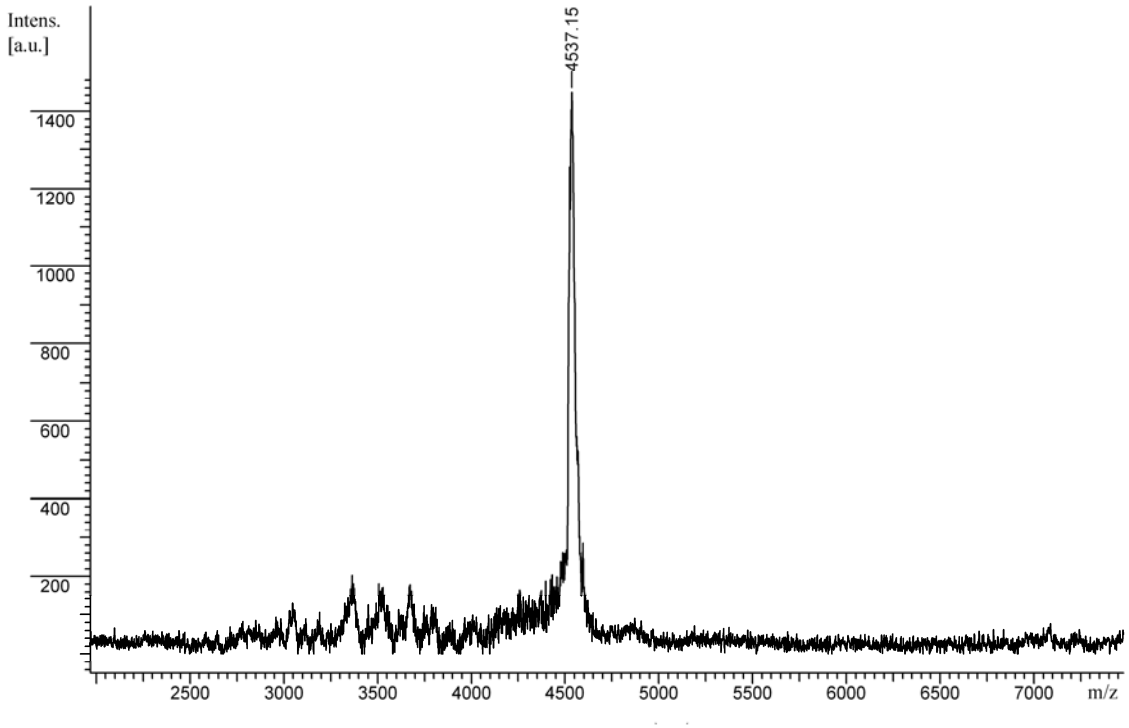
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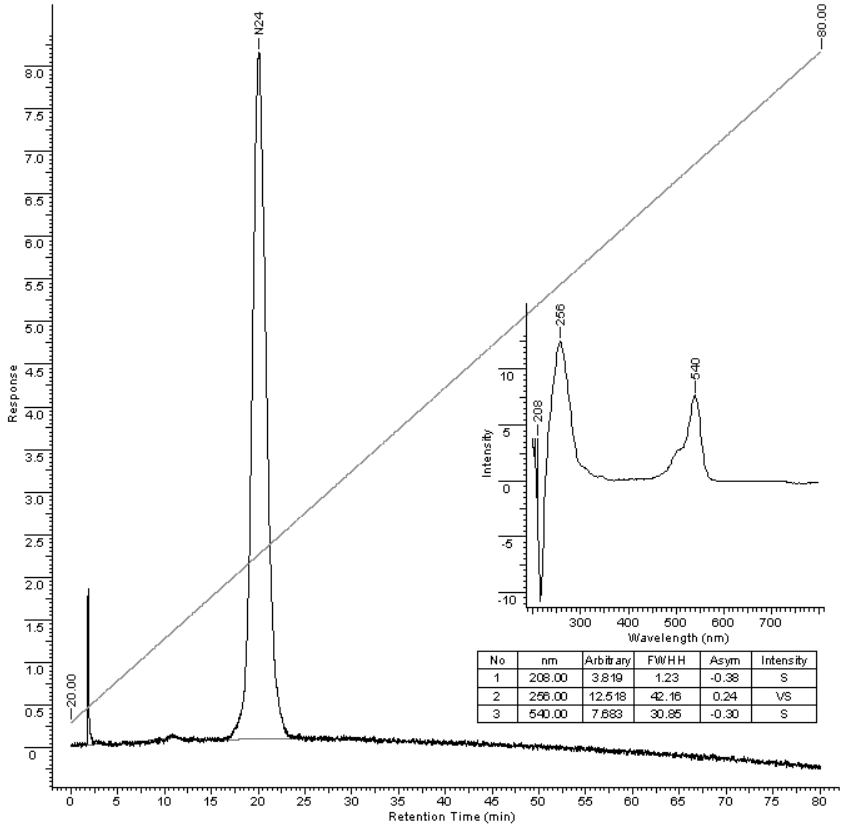

No	nm	Arbitrary	FWHH	Asym	Intensity
1	208.00	3.819	1.23	-0.38	S
2	256.00	12.518	42.16	0.24	VS
3	540.00	7.683	30.85	-0.30	S

Name	tR	Peak Area (Y units*ms)	Area Percent	Width	Asymmetry	Plate Number
N24	20.064	836683.625	100.000	2.378	1.173	1138.895