

Supporting Information

Toward once-monthly insulin therapy via synergy in two pharmacokinetic protractors: Fc-conjugation and fatty acid acylation.

Alexander N. Zaykov,^a Vasily M. Gelfanov,^a Tina M. Tagmose,^b Damien Demozey,^b Valentina Manfè,^b Rebecca Rohlfes,^a Marita Rivir,^c Diego Perez-Tilve,^c Brian Finan,^a and Richard D. DiMarchi^{a,d}

^a Novo Nordisk Research Center Indianapolis, Indianapolis, IN, 46241, USA

^b Novo Nordisk, Global Research Technologies, DK-2760 Maaloev, Denmark

^c Department of Pharmacology and Systems Physiology, University of Cincinnati-College of Medicine, Cincinnati, OH, 45267, USA

^d Department of Chemistry, Indiana University, Bloomington, IN, 47405, USA

† Corresponding Author: Alexander N. Zaykov – Novo Nordisk Research Center Indianapolis, Indianapolis, IN, 46241, USA. E-mail:

alexander.n.zaykov@gmail.com

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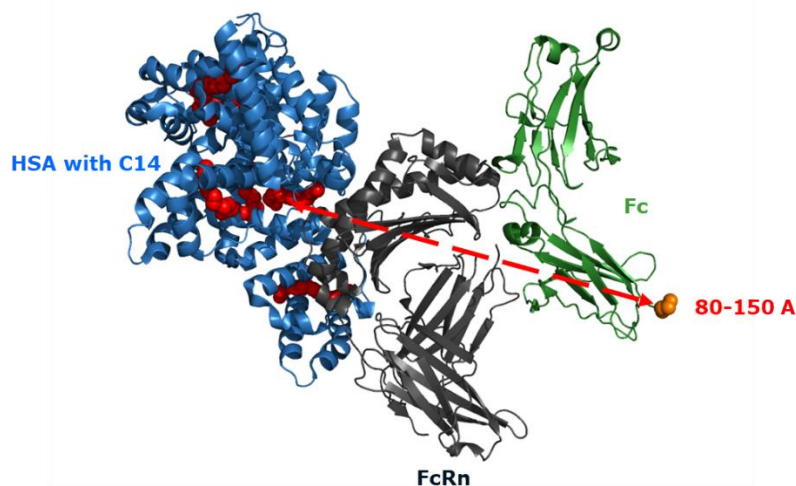


Figure S1. Human albumin and Fc in complex with FcRn receptor (PDB 4N0U). The distance from fatty acids binding sites to the N-terminus of Fc protein varies from 80 to 150Å. We hypothesized that a spacer with 10 Ado (OEG) units, which approximately 110Å in length, will allow simultaneous binding of albumin and Fc, and formation of quasi-quaternary complex.

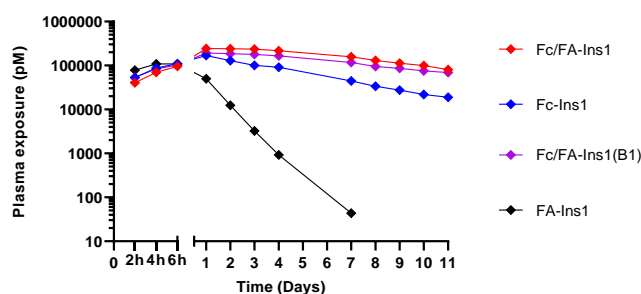


Figure S2. Pharmacokinetic profiles compounds Fc/FA-Ins1, Fc-Ins1, Fc/FA-Ins1(B1) and FA-Ins1 from s.c. PK study in rats.

Compound	Stat	Dose (nmol)	# of animals	HL_Lambda_z (hr)	Cmax (pmol/L)	AUCINF_pred (hr*pmol/L)	AUC_%Extrap_pred (%)	Vz_pred (L/kg)	Cl_pred (L/hr/kg)
Fc-Ins1	Mean	30	5	124	2.52E+05	6.09E+07	26	0.087	4.97E-04
	SD			29	1.48E+04	6.14E+06	7	0.013	5.26E-05
Fc/FA-Ins1	Mean	30	5	76	1.69E+05	2.12E+07	9	0.155	1.42E-03
	SD			6	1.68E+04	1.28E+06	2	0.009	9.10E-05
FA-Ins1	Mean	30	5	135	1.98E+05	4.79E+07	28	0.122	6.29E-04
	SD			16	1.39E+04	3.87E+06	4	0.007	5.07E-05
Fc/FA-Ins1(B1)	Mean	30	5	13	1.11E+05	2.92E+06	0.3	0.188	1.03E-02
	SD			1	1.26E+04	1.51E+05	0.2	0.021	5.58E-04

Table S1. PK parameters for compounds Fc/FA-Ins1, Fc-Ins1, Fc/FA-Ins1(B1) and FA-Ins1 from s.c. PK study in rats.

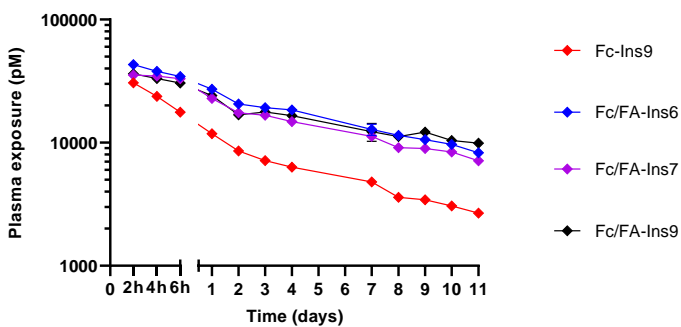


Figure S3. Pharmacokinetic profiles of compounds Fc-Ins9, Fc/FA-Ins6, Fc/FA-Ins7, and Fc/FA-Ins9 from i.v. PK study in rats.

Compound	Stat	Dose (nmol/kg)	# of animals	HL_Lambda_z (hr)	Cmax (pmol/L)	AUCINF_pred (hr*pmol/L)	AUC_%Extrap_pred (%)	Vz_pred (L/kg)	CL_pred (L/hr/kg)
Fc-Ins9	Mean	2	5	132	30620	2.26E+06	23	0.169	8.88E-04
	SD			4	2520	1.64E+05	2	0.011	6.16E-05
Fc/FA-Ins9	Mean	2	5	239	36460	7.58E+06	44	0.09	2.65E-04
	SD			64	2971	6.16E+05	7	0.016	3.07E-05
Fc/FA-Ins6	Mean	2	5	153	43300	6.29E+06	29	0.071	3.21E-04
	SD			8	1900	6.36E+05	2	0.008	2.33E-05
Fc/FA-Ins7	Mean	2	5	164	30620	5.39E+06	32	0.088	3.72E-04
	SD			19	2520	3.64E+05	5	0.012	2.01E-05

Table S2. PK parameters of compounds Fc-Ins9, Fc/FA-Ins6, Fc/FA-Ins7, and Fc/FA-Ins9 from i.v. PK study in rats.

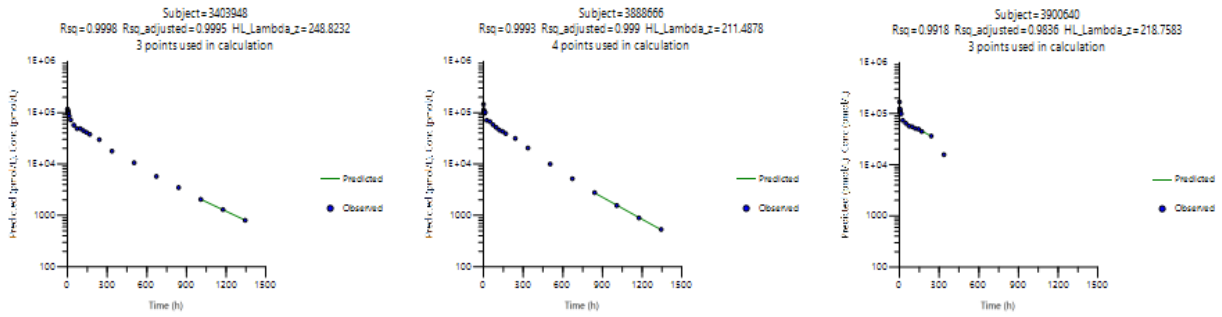


Figure S4. Pharmacokinetic profiles from individual animals in dog PK study, Fc/FA-Ins1

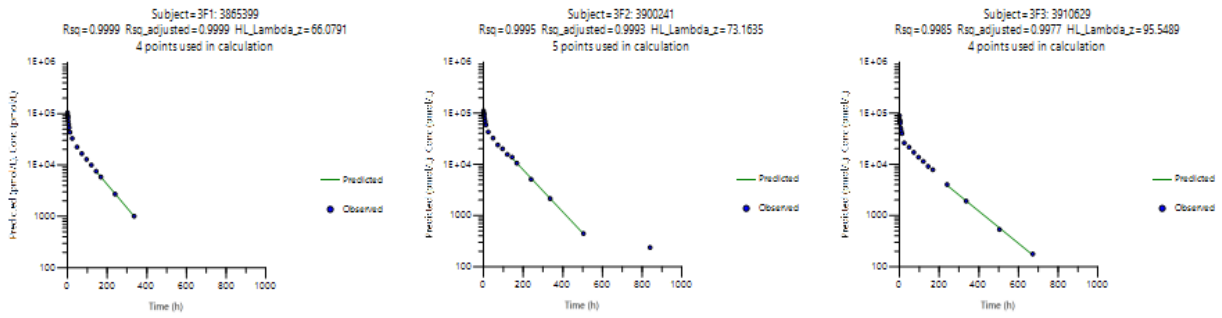


Figure S5. Pharmacokinetic profiles from individual animals in dog PK study, Fc-Ins1

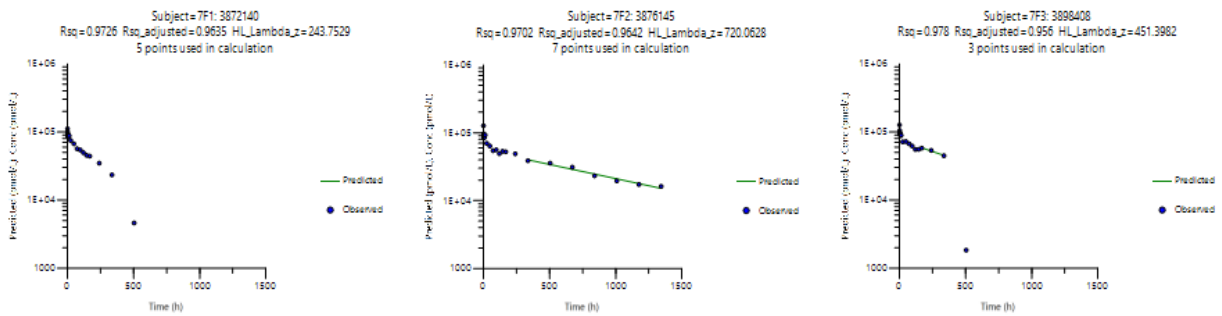


Figure S6. Pharmacokinetic profiles from individual animals in dog PK study, Fc/FA-Ins9

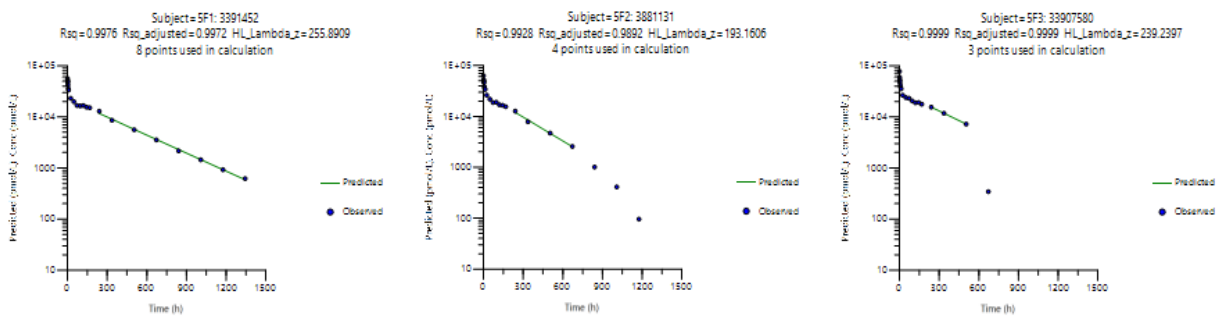
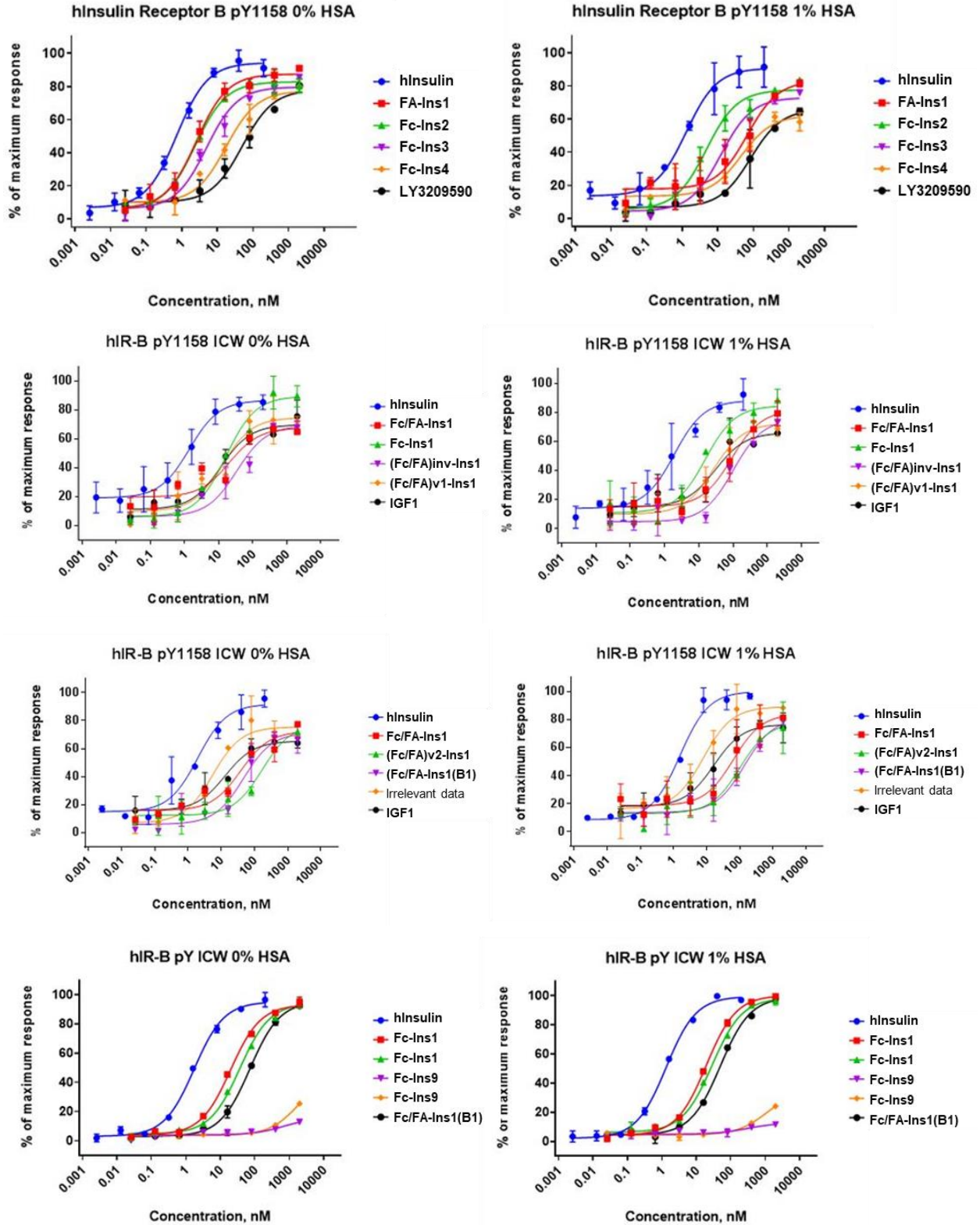


Figure S7. Pharmacokinetic profiles from individual animals in dog PK study, Fc-Ins9

Table S3. Pharmacokinetic parameters from individual animals in dog PK study

Analyte	Subject	Dose (pmol)	Rsq	No_points_lambda_z	HL_Lambda_z (h)	AUC_%Extrap_pred (%)	Tmax (h)	Cmax (pmol/L)	C0 (pmol/L)	AUCINF_pred (h*pmol/L)	Vz_pred (L/kg)	Cl_Pred (L/h/kg)	Vss_pred (L/kg)
Fc/FA-Ins1	3400000	5000	1	3	249	1.47	1	111000	117000	19700000	0.0912	0.000254	0.0709
	3890000	5000	0.999	4	211	0.787	1	114000	145000	20400000	0.0749	0.000246	0.0612
	3900000	5000	0.992	3	219	34.7	3	123000	168000	24300000	0.065	0.000206	0.0636
	Mean	5000	0.997	3.33	226	12.3	1.67	116000	143000	21400000	0.077	0.000235	0.0652
	SD	0	0.00449	0.577	19.8	19.4	1.15	6240	25400	2470000	0.0132	2.56E-05	0.00508
	CV%	0	0.45	17.3	8.74	157	69.3	5.38	17.7	11.5	17.1	10.9	7.79
	Median	5000	0.999	3	219	1.47	1	114000	145000	20400000	0.0749	0.000246	0.0636
	Geometric Mean	5000	0.997	3.3	226	34.2	1.44	116000	142000	21300000	0.0763	0.000234	0.0651
	Geometric SD	1	1	1.18	1.09	7.61	1.89	1.05	1.2	1.12	1.12	1.12	1.08
	Geometric CV%	0	0.451	16.7	8.59	777	70.4	5.33	18.2	11.3	17.1	11.3	7.68
Fc-Ins1	3F1:3865399	5000	1	4	661	2.43	1	92300	104000	3950000	0.121	0.00127	0.103
	3F2:3900241	5000	0.999	5	73.2	0.0324	1	100000	111000	5910000	0.0892	0.000845	0.0834
	3F3:3910629	5000	0.998	4	95.5	0.553	1	73800	89000	4210000	0.164	0.00119	0.133
	Mean	5000	0.999	4.33	78.3	1	1	88700	101000	4690000	0.125	0.0011	0.106
	SD	0	0.000731	0.577	15.4	1.26	0	13500	11400	1070000	0.0373	0.000224	0.0248
	CV%	0	0.0732	13.3	19.7	125	0	15.2	11.2	22.7	30	20.3	23.4
	Median	5000	0.999	4	73.2	0.553	1	92300	104000	4210000	0.121	0.00119	0.103
	Geometric Mean	5000	0.999	4.31	77.3	0.351	1	88000	101000	4620000	0.121	0.00108	0.104
	Geometric SD	1	1	1.14	1.21	8.97	1	1.17	1.12	1.24	1.35	1.24	1.26
	Geometric CV%	0	0.0732	12.9	19.2	1110	0	15.8	11.5	22	31	22	23.6
Fc-Ins9	5F1:3391452	5000	0.998	8	256	2.51	1	51900	56600	8620000	0.214	0.00058	0.203
	5F2:3881131	5000	0.993	4	193	1.48	1	52900	63200	7830000	0.178	0.000639	0.166
	5F3:33907580	5000	1	3	239	15	1	59800	77900	10200000	0.169	0.000488	0.163
	Mean	5000	0.997	5	229	6.32	1	54900	65900	8900000	0.187	0.000569	0.177
	SD	0	0.00365	2.65	32.5	7.51	0	4300	10900	1230000	0.024	7.58E-05	0.0224
	CV%	0	0.366	52.9	14.2	119	0	7.84	16.5	13.8	12.9	13.3	12.6
	Median	5000	0.998	4	239	2.51	1	52900	63200	8620000	0.178	0.00058	0.166
	Geometric Mean	5000	0.997	4.58	228	3.82	1	54800	65300	8840000	0.186	0.000565	0.176
	Geometric SD	1	1	1.66	1.16	3.36	1	1.08	1.18	1.15	1.13	1.15	1.13
	Geometric CV%	0	0.367	53.8	14.8	183	0	7.7	16.3	13.7	12.6	13.7	12.3
Fc/FA-Ins9	7F1:3872140	5000	0.973	5	244	24.6	1	99300	111000	23900000	0.0736	0.000209	0.0698
	7F2:3876145	5000	0.97	7	720	26.4	1	96700	127000	59700000	0.0871	8.38E-05	0.0838
	7F3:3898408	5000	0.978	3	451	48.9	1	105000	127000	46500000	0.07	0.000107	0.0707
	Mean	5000	0.974	5	472	33.3	1	100000	122000	43400000	0.0769	0.000133	0.0748
	SD	0	0.00398	2	239	13.5	0	4250	8870	18100000	0.00901	6.66E-05	0.00782
	CV%	0	0.409	40	50.6	40.7	0	4.23	7.3	41.7	11.7	49.9	10.5
	Median	5000	0.973	5	451	26.4	1	99300	127000	46500000	0.0736	0.000107	0.0707
	Geometric Mean	5000	0.974	4.72	429	31.7	1	100000	121000	40500000	0.0765	0.000124	0.0745
	Geometric SD	1	1	1.53	1.72	1.46	1	1.04	1.08	1.6	1.12	1.6	1.11
	Geometric CV%	0	0.409	44.7	58.6	39.2	0	4.21	7.48	50.1	11.5	50.1	10.2

In-vitro insulin receptor activity assays



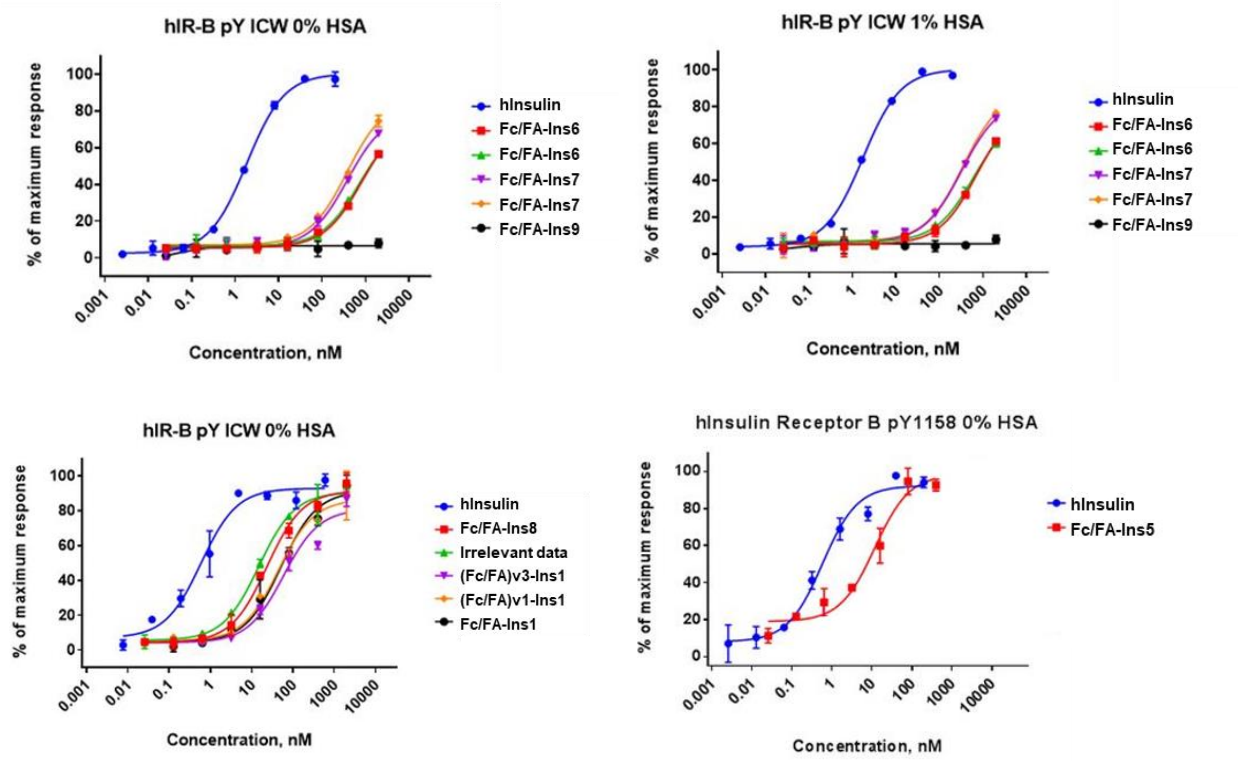


Figure S8. Representative dose-response curves from in-vitro insulin receptor activity assays. Insulin receptor phosphorylation was measured in an indirect ELISA assay using HEK293 cells overexpressing B-isoform of human insulin receptor (hIR-B). The EC50 values and potencies relative to native insulin used in the same assay are reported in the Figure 1.

Representative synthesis

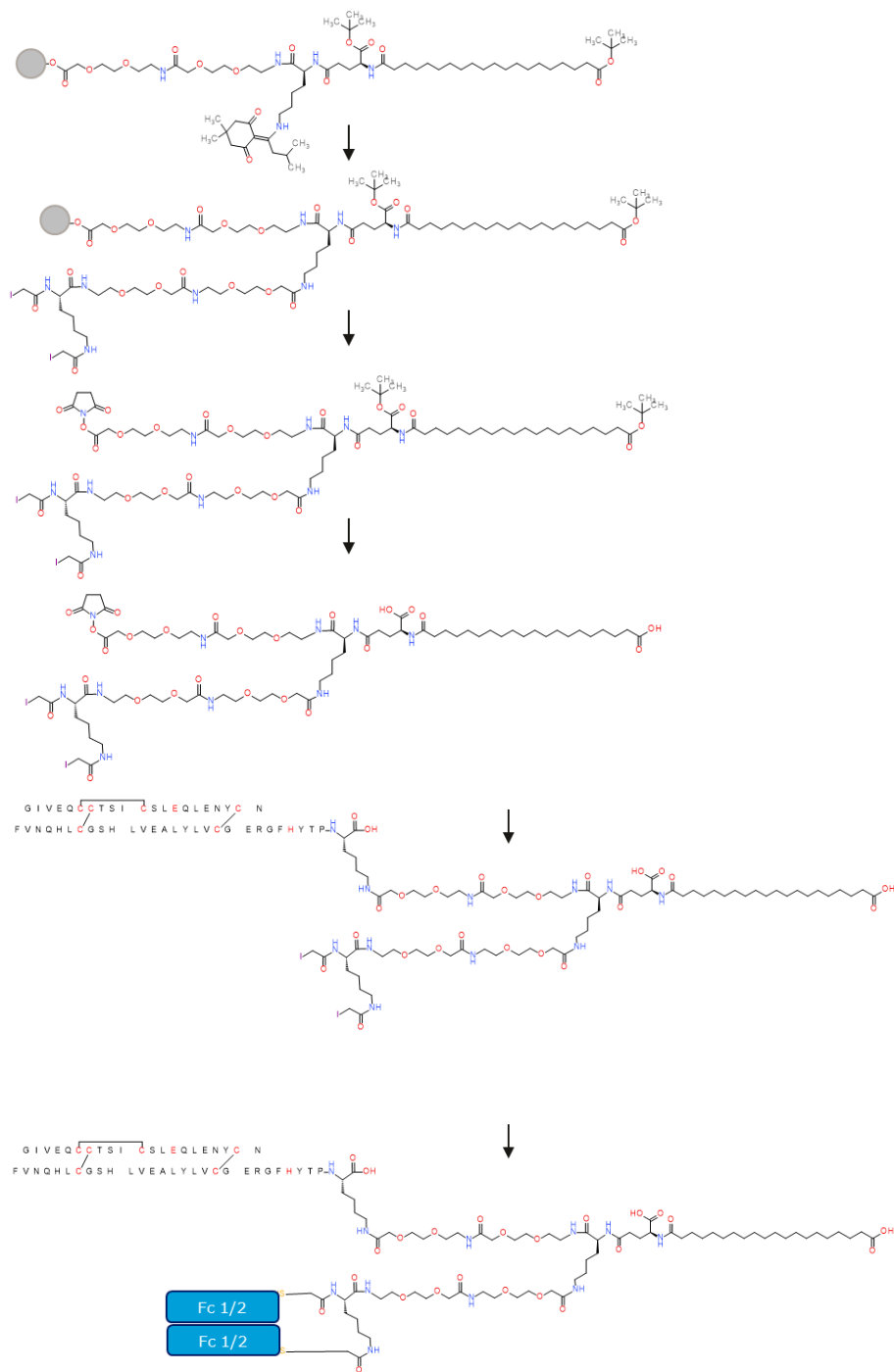


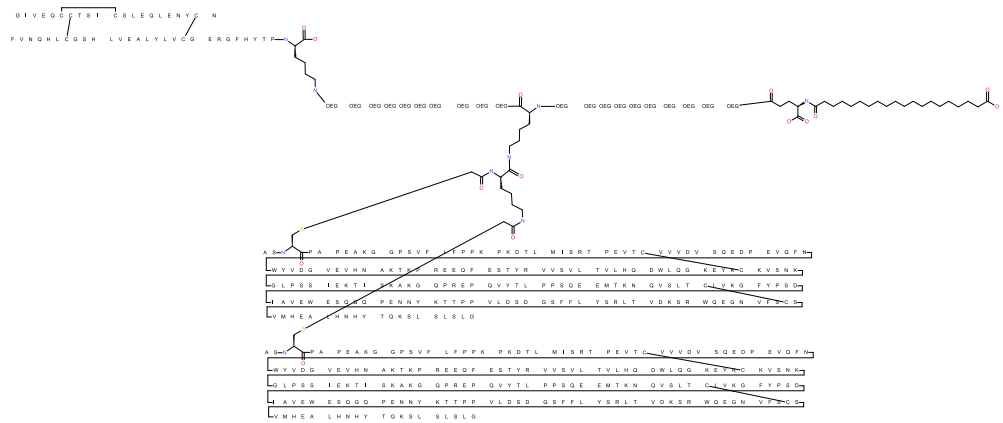
Figure S9. A generalized synthetic scheme for preparation of Fc/FA-Ins1.

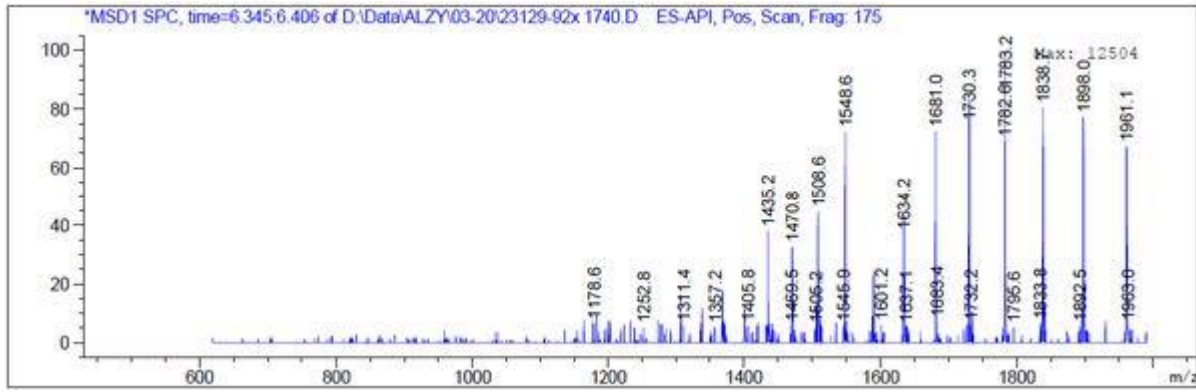
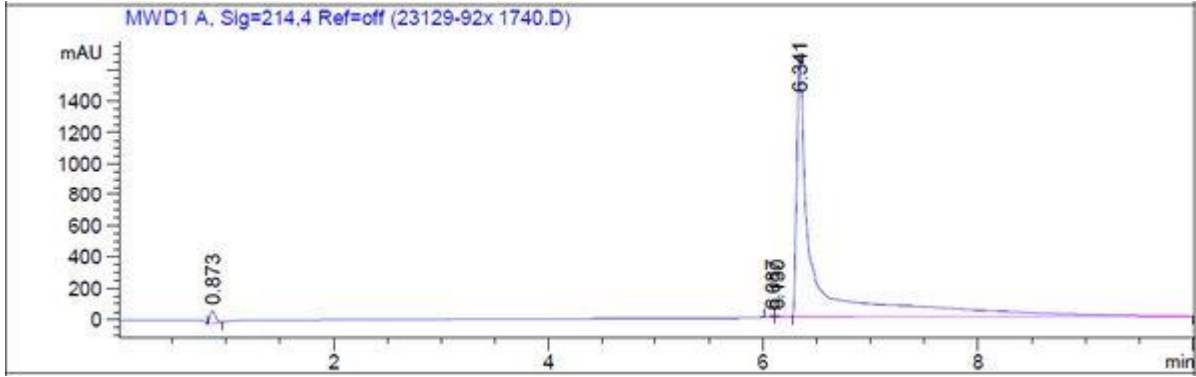
Structures and characterizations

Table S4. LCMS characterization of the insulin compounds prepared in this study.

Compound	Calc mass	Obs. Mass
Fc/FA-Ins1	58807	58807
Fc-Ins1	56771	56774
Fc/FA-Ins1(B1)	58807	58809
Fc/FA-Ins5	58964	58962
Fc/FA-Ins6	58758	58760
Fc/FA-Ins7	58782	58782
Fc/FA-Ins8	58851	58851
(Fc/FA)inv-Ins1	58801	58809
(Fc/FA)v1-Ins1	57936	57933
(Fc/FA)v2-Ins1	57936	57939
(Fc/FA)v3-Ins1	57645	57645
(Fc/FA)half-Ins1	34099	34101
Fc-Ins9	58736	58740
Fc-Ins9	56701	56700

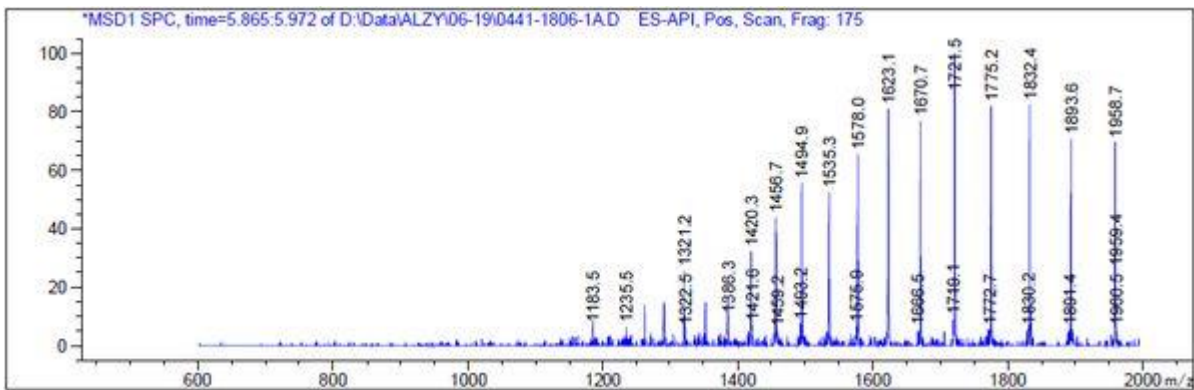
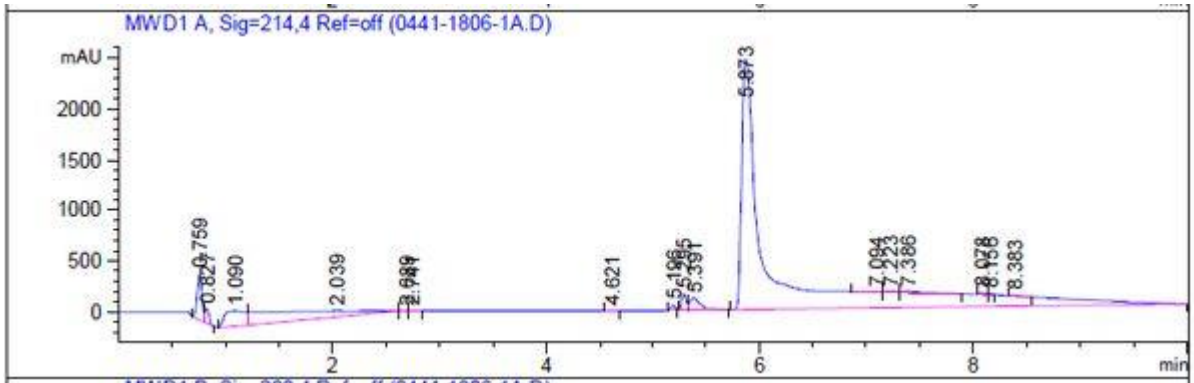
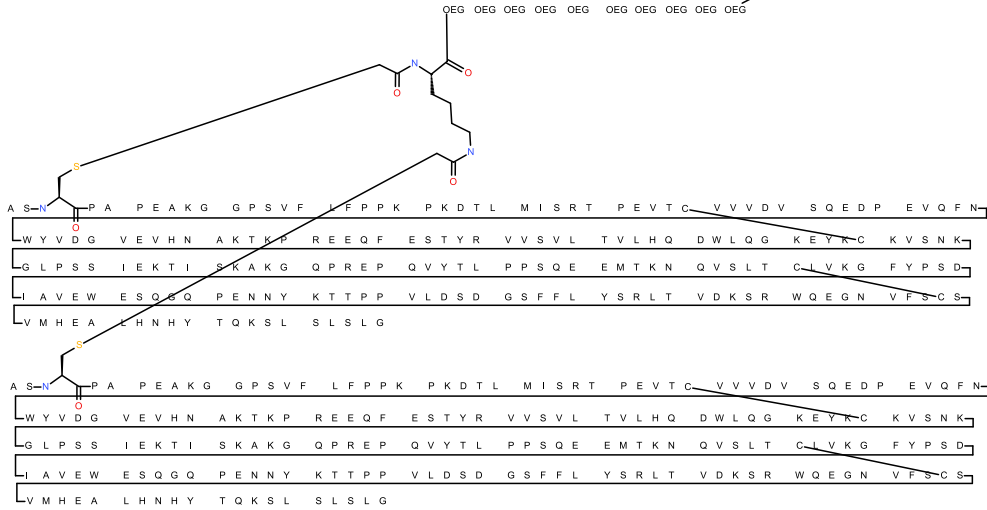
Fc/FA-Ins1





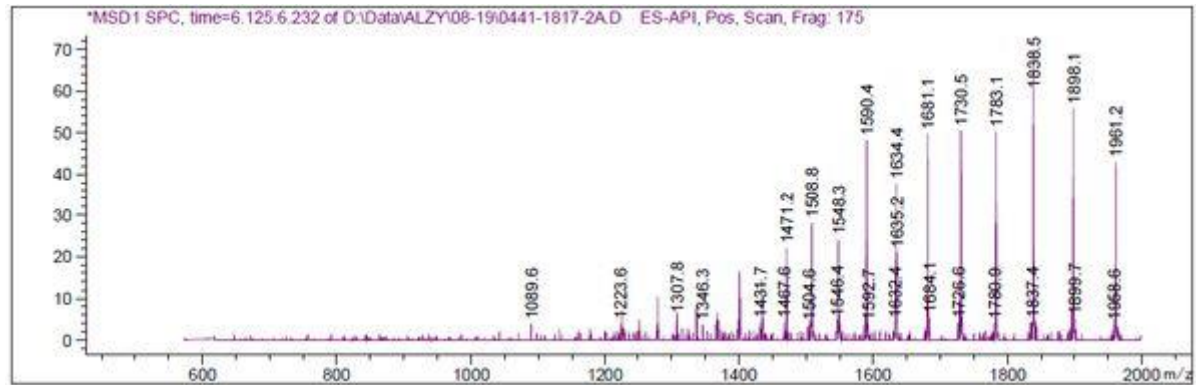
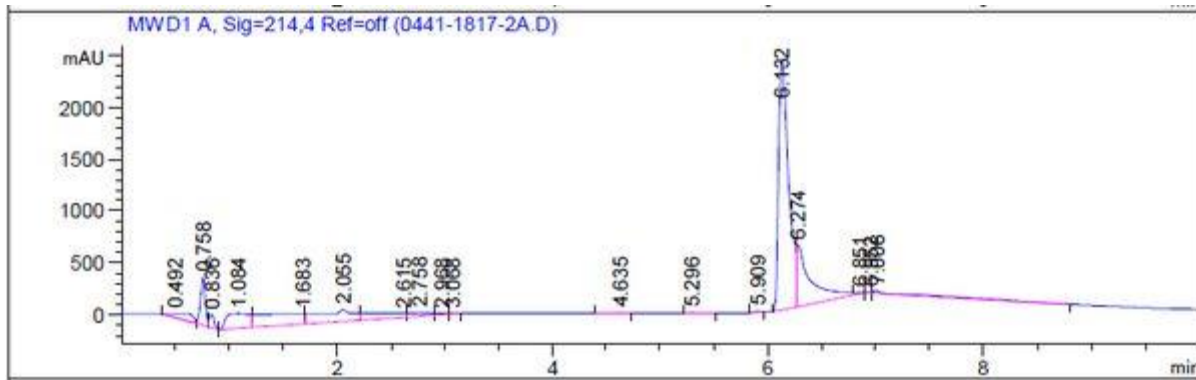
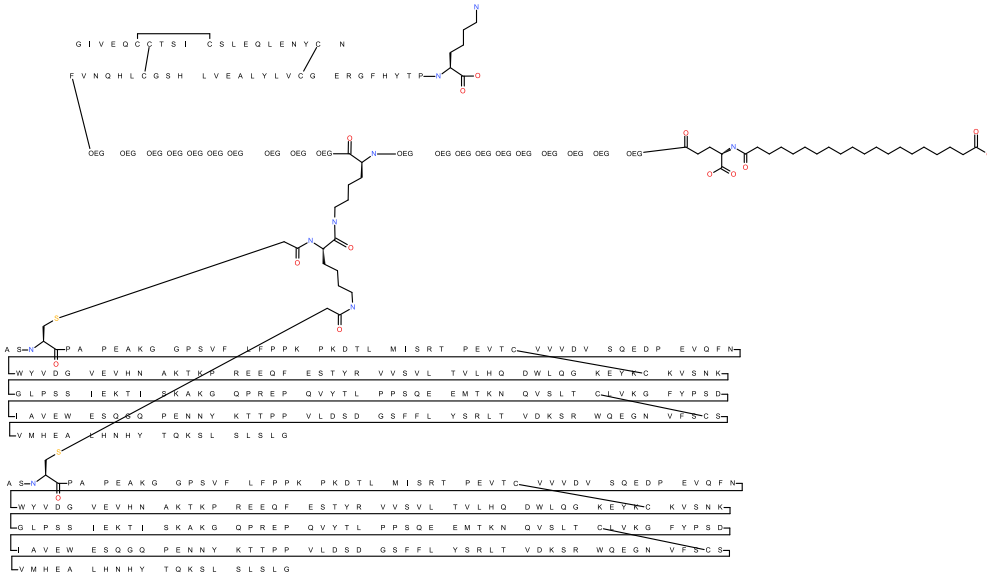
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Fc-Ins1



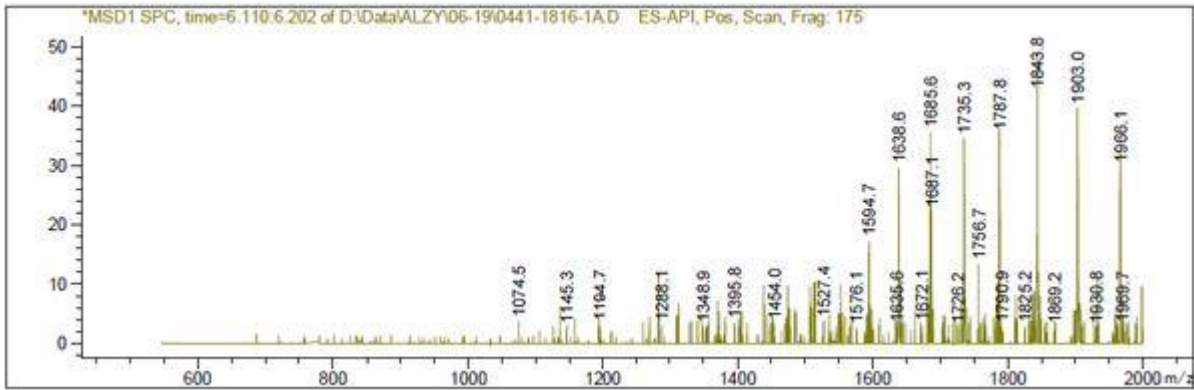
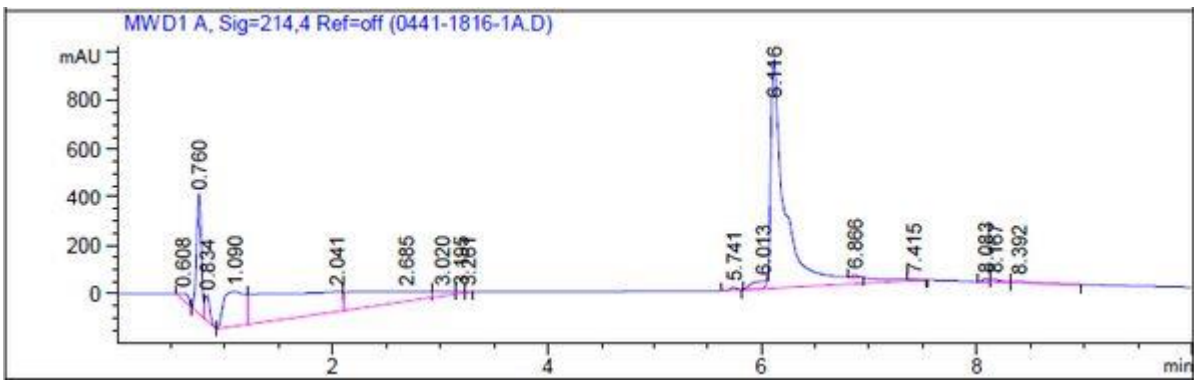
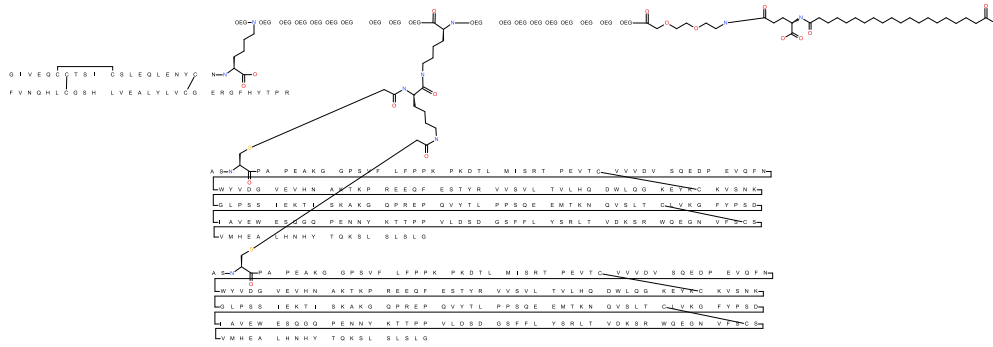
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Fc/FA-Ins1(B1)



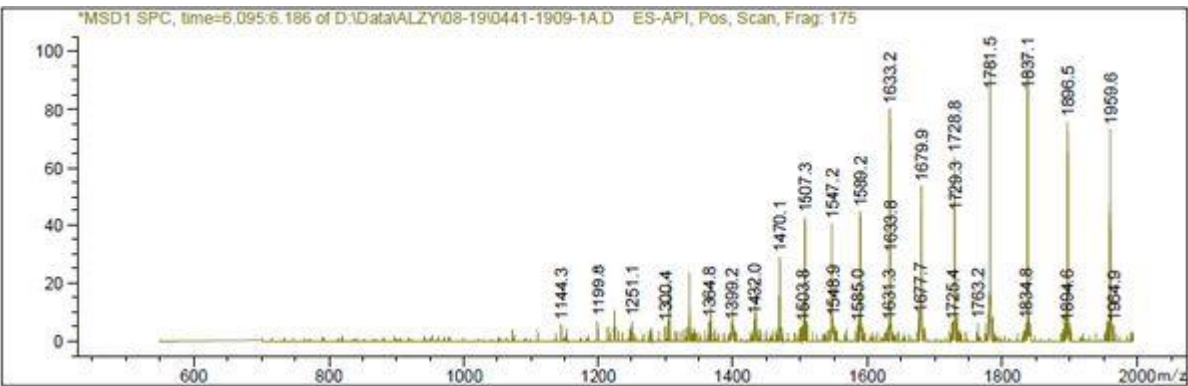
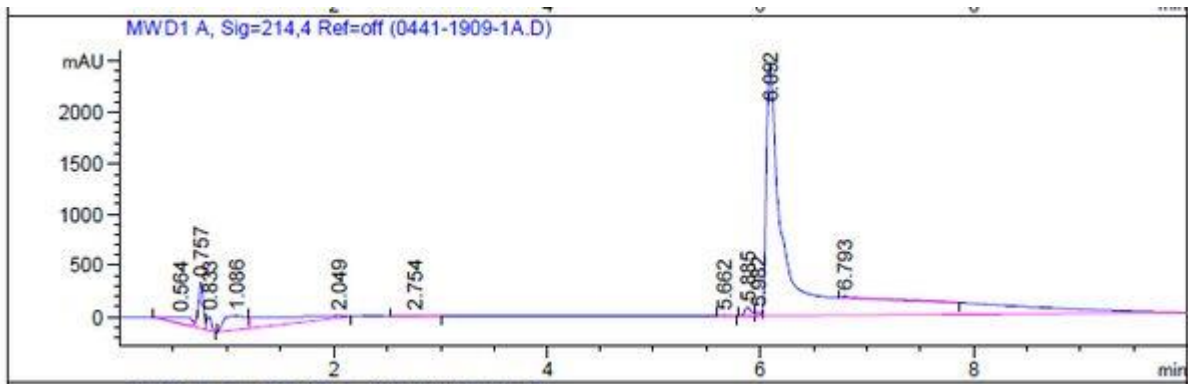
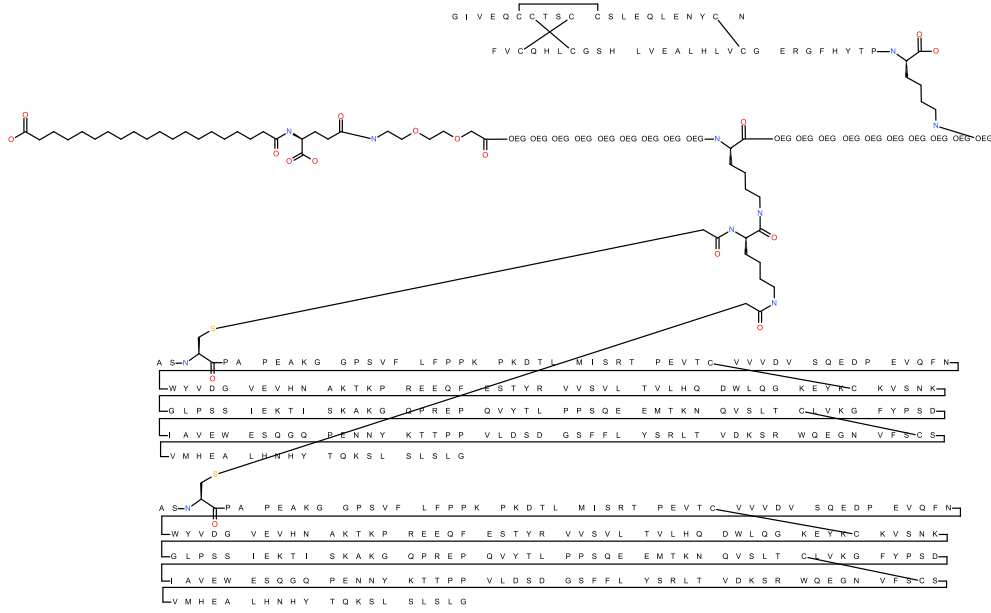
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Fc/FA-Ins5



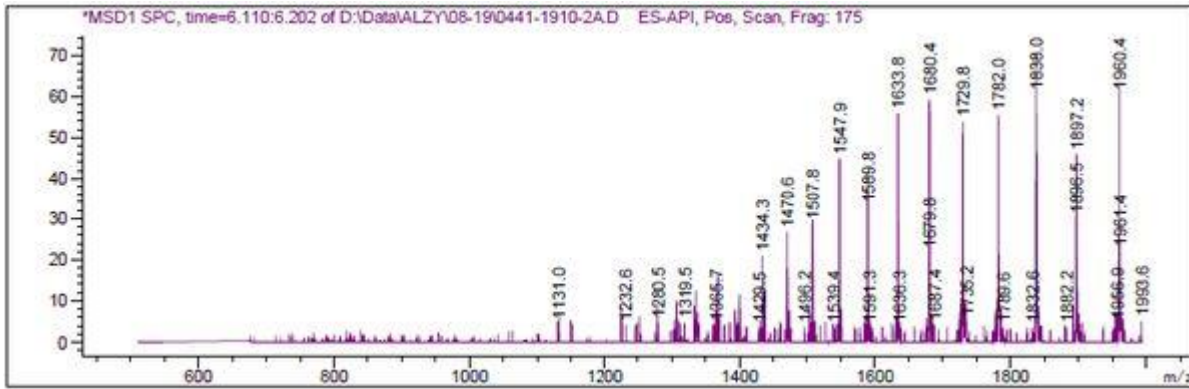
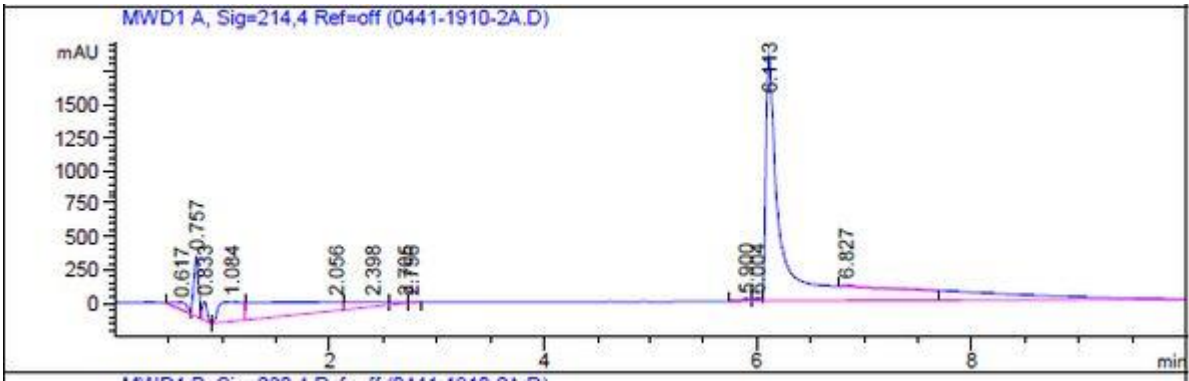
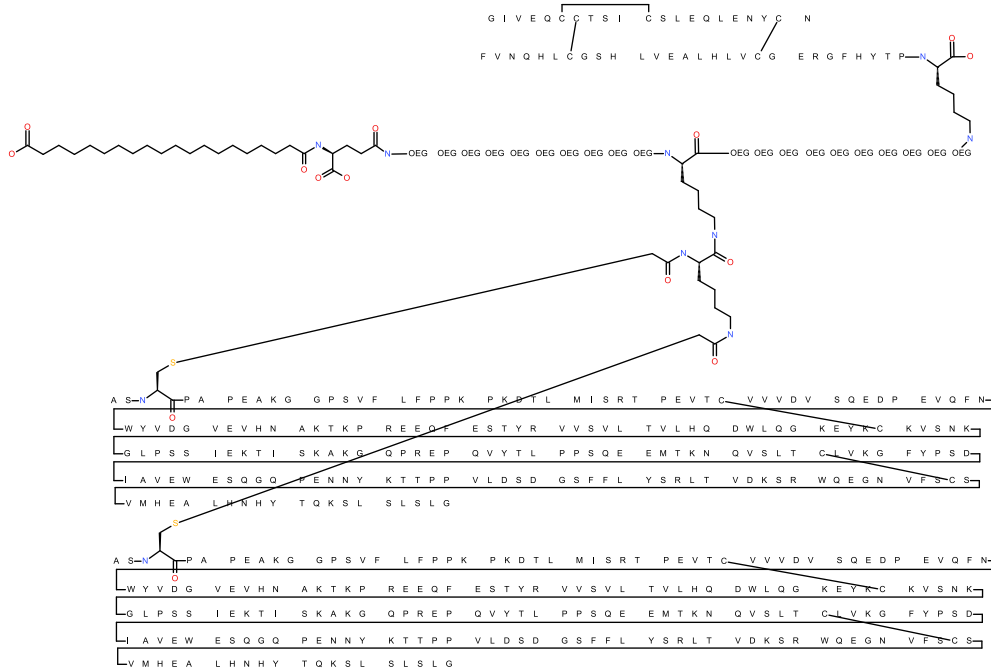
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Fc/FA-Ins6



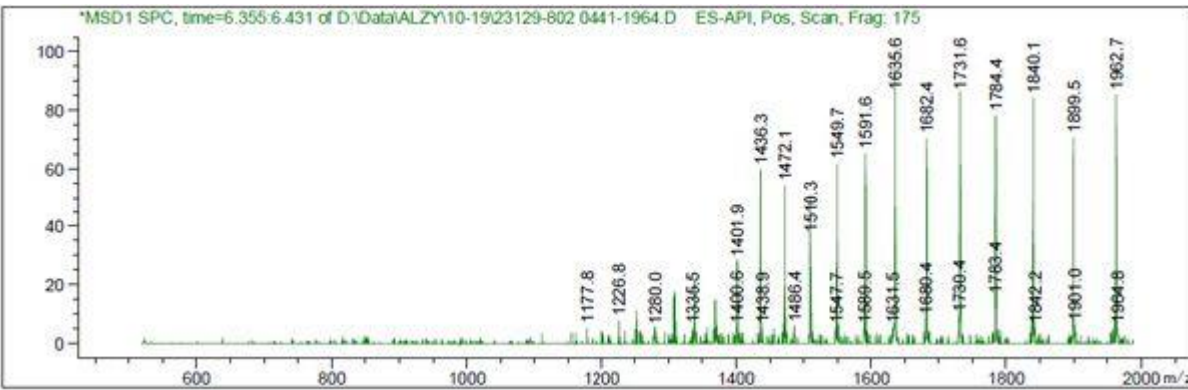
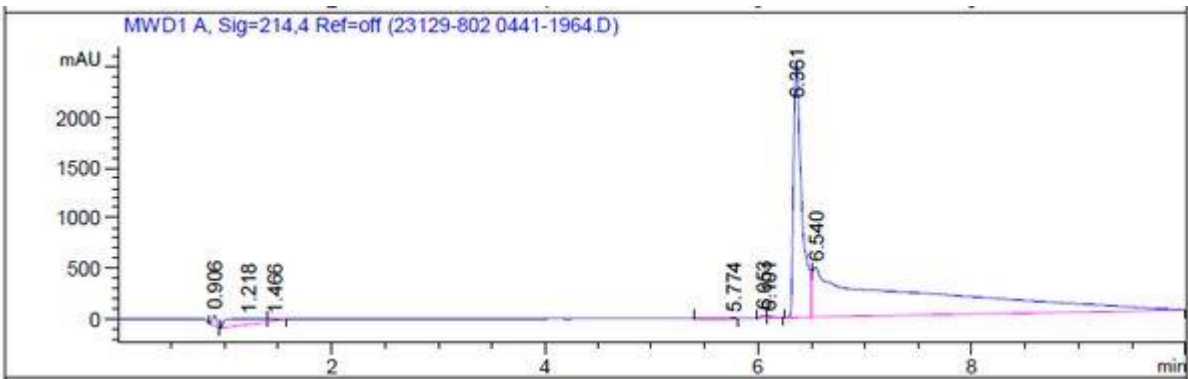
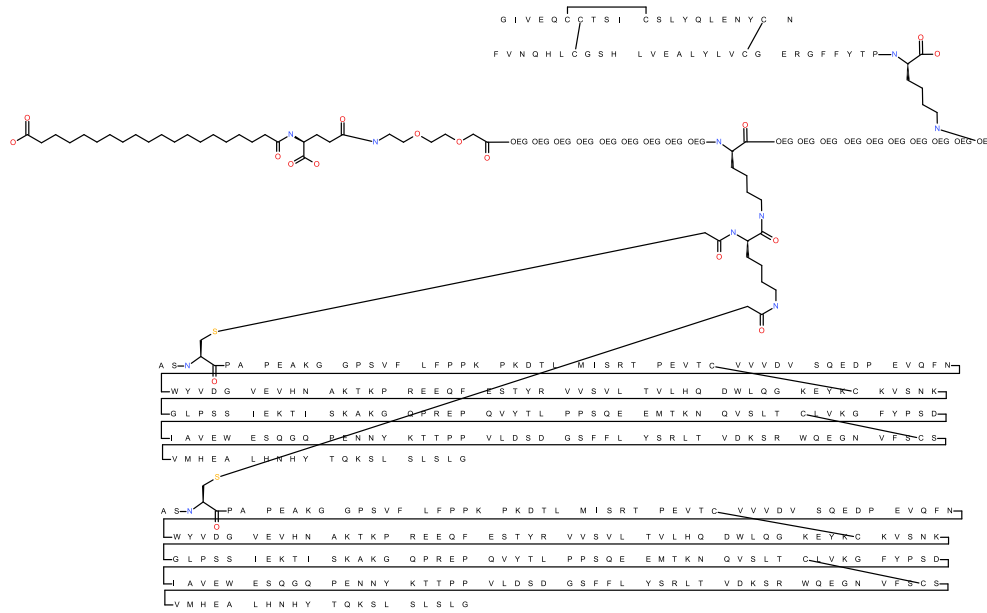
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Fc/FA-Ins7



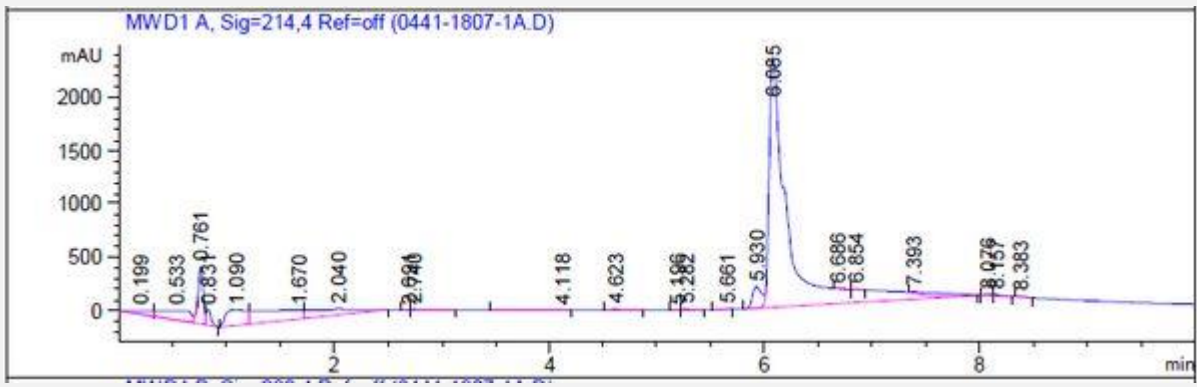
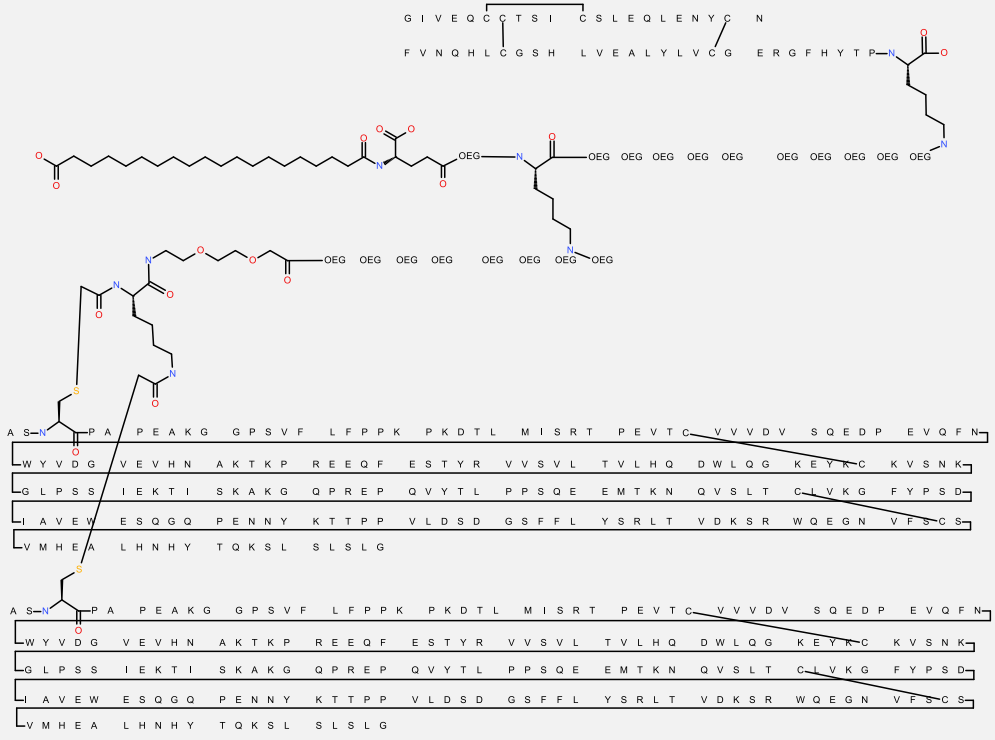
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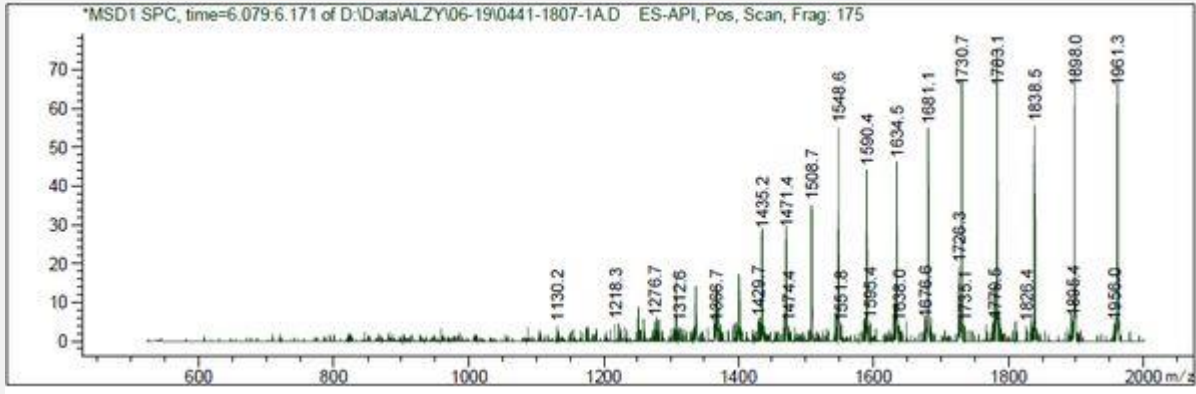
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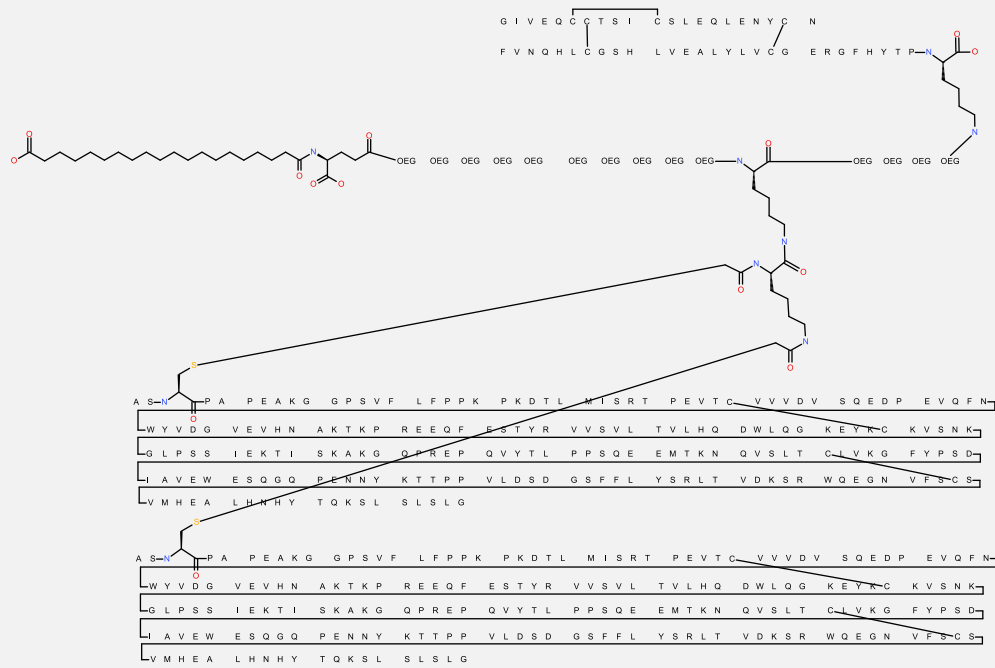
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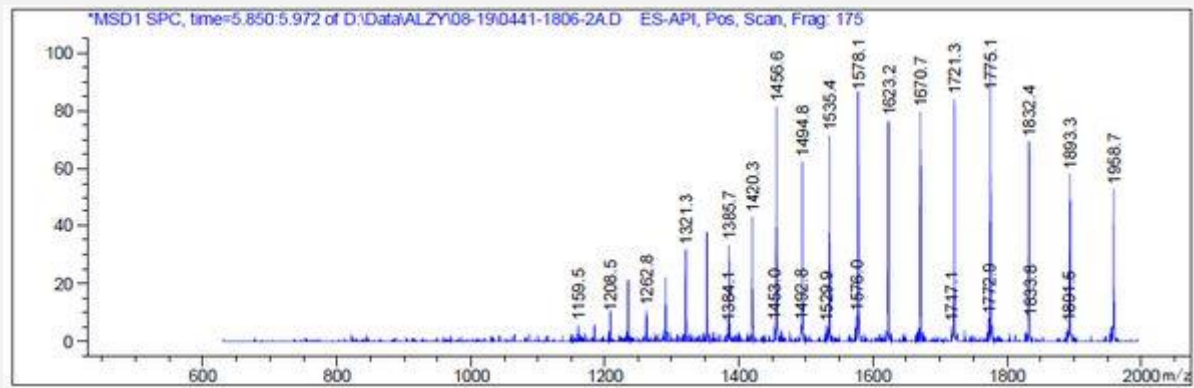
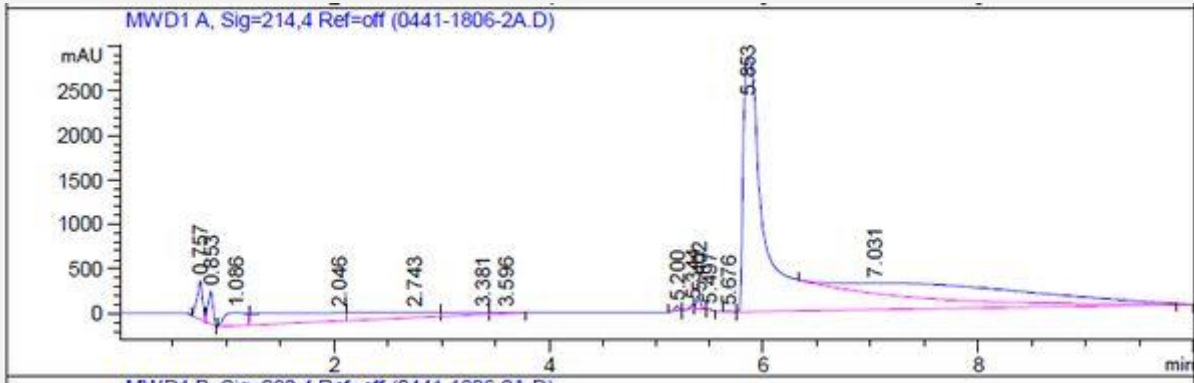




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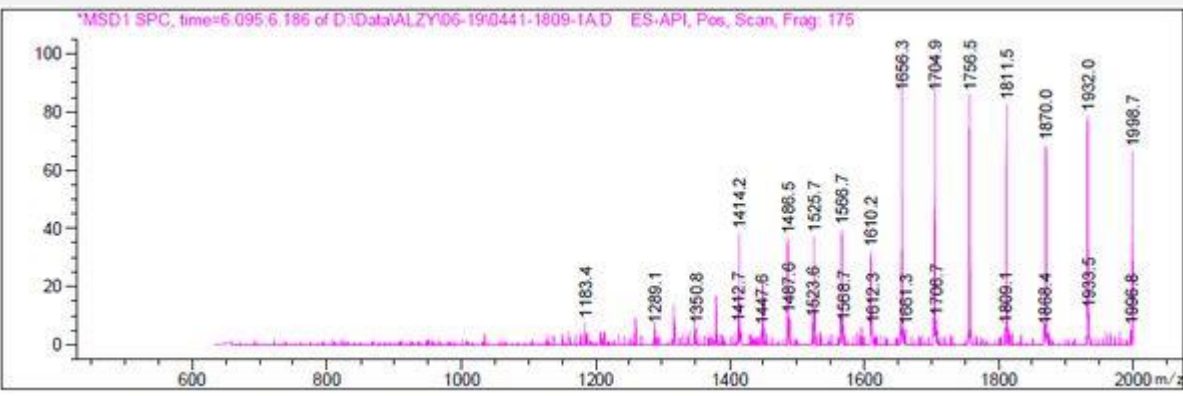
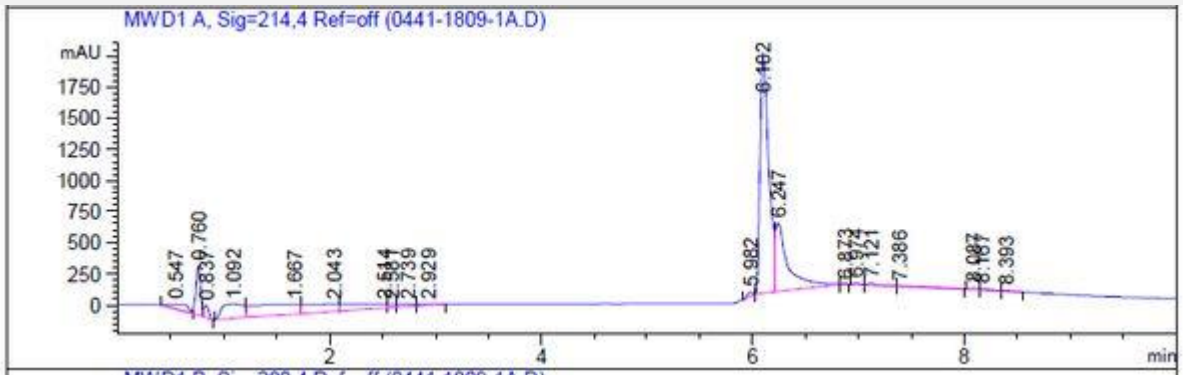
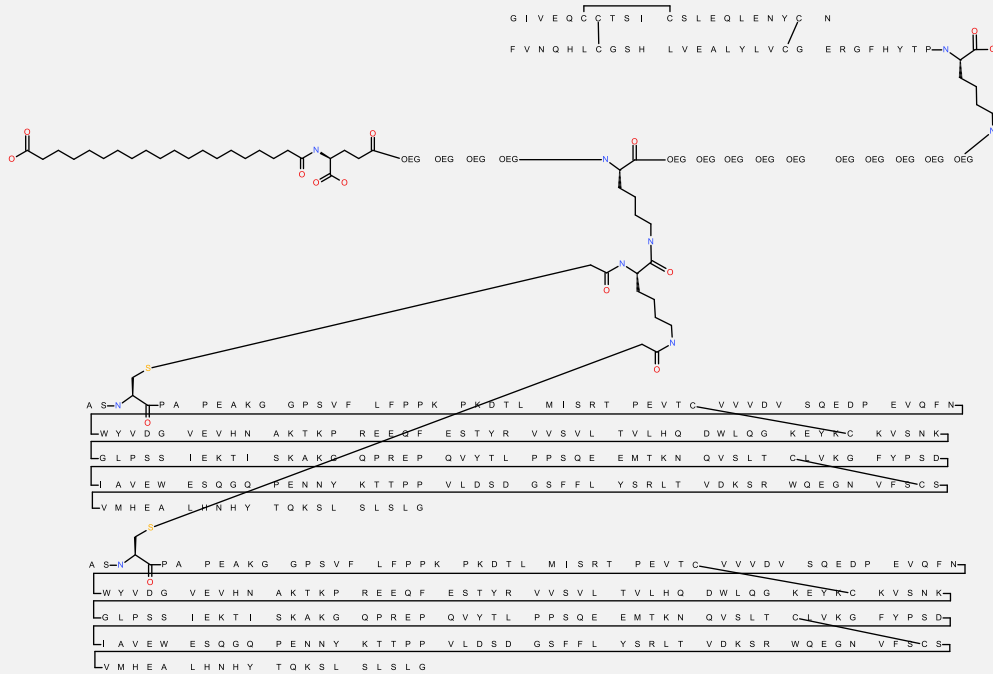
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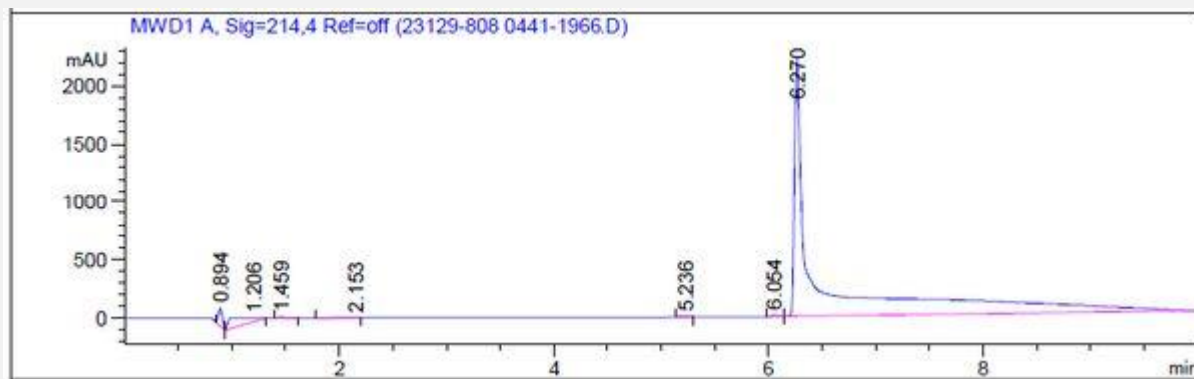
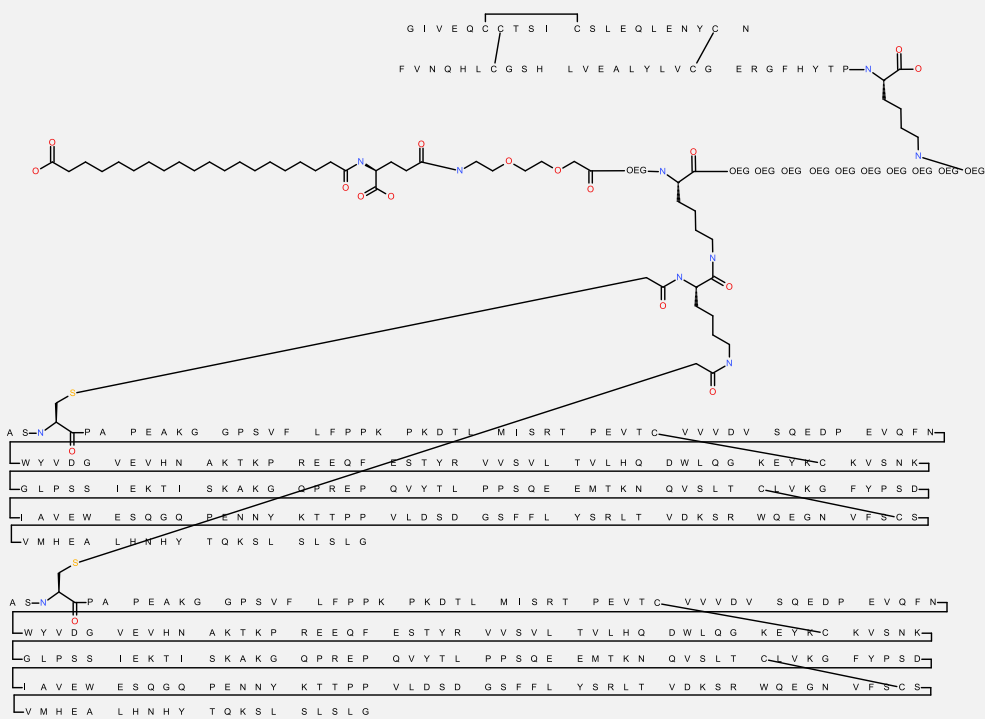
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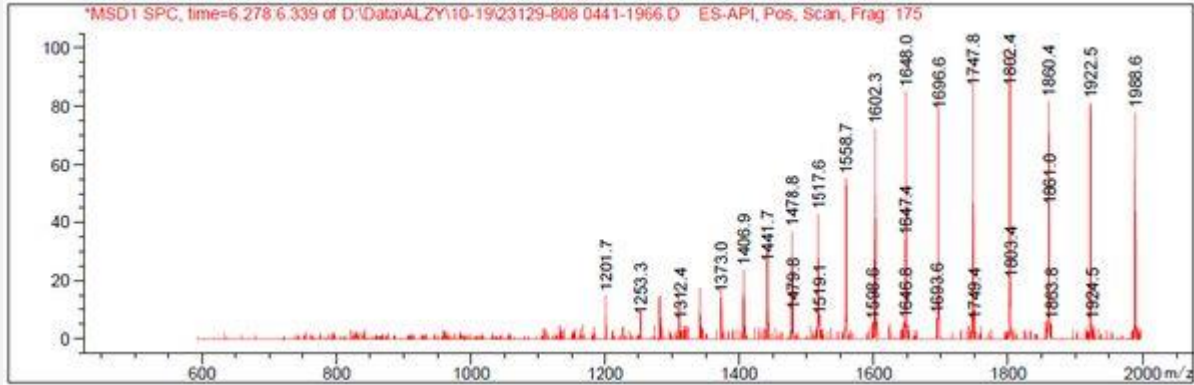
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MW 57936, obs 57939

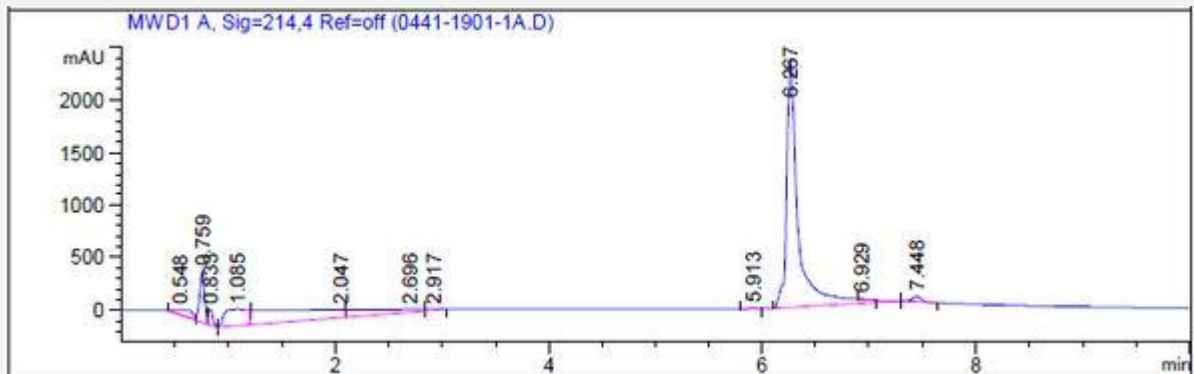
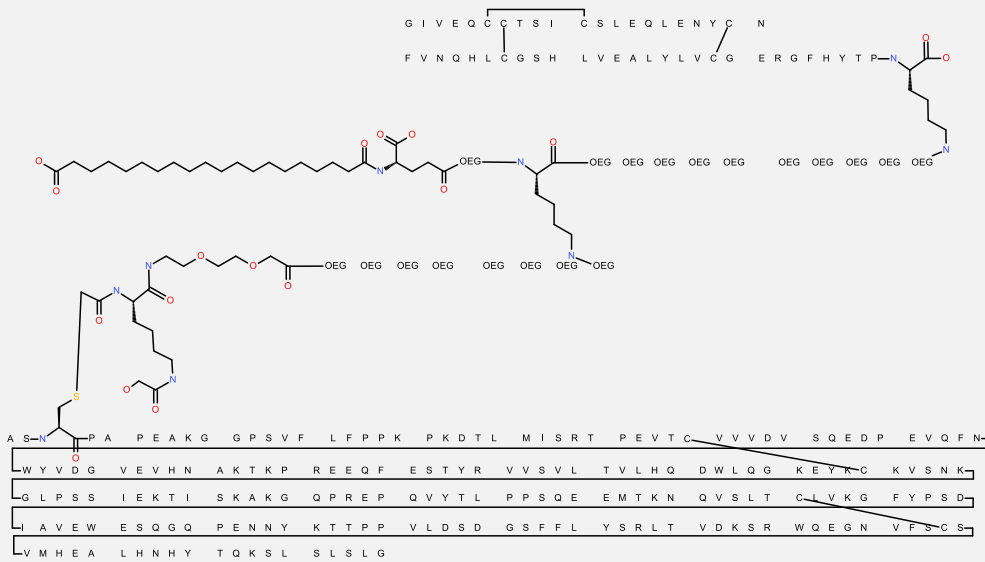
(Fc/FA)v3-Ins1

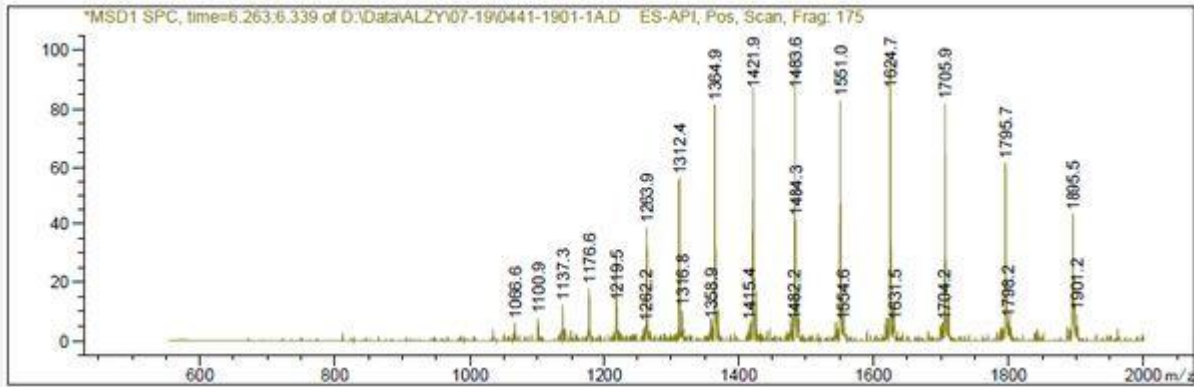




MW exp 57645, obs 57645

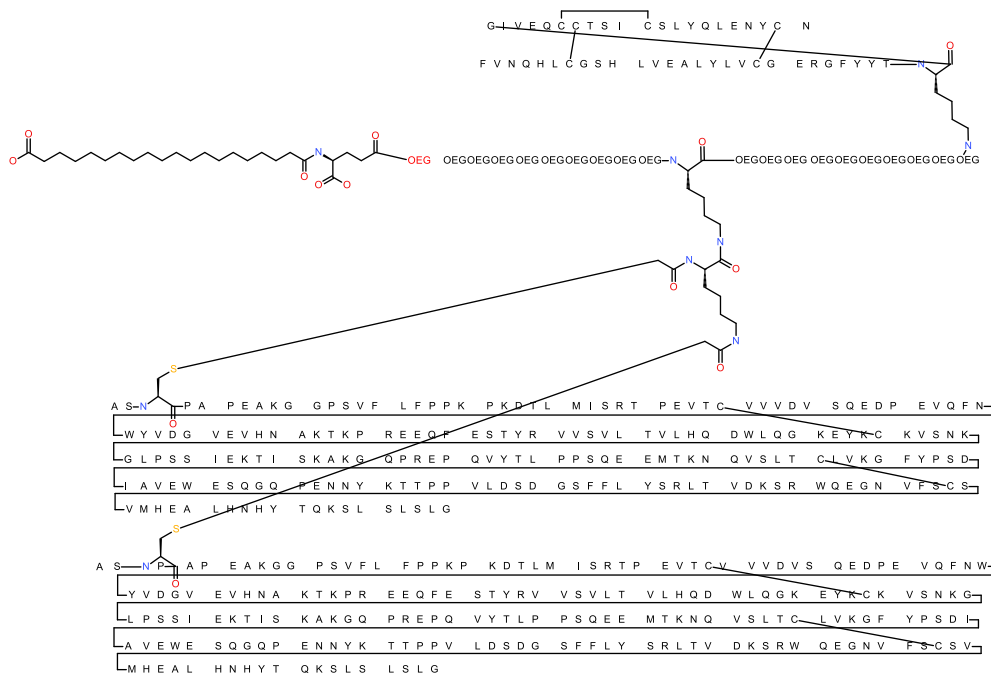
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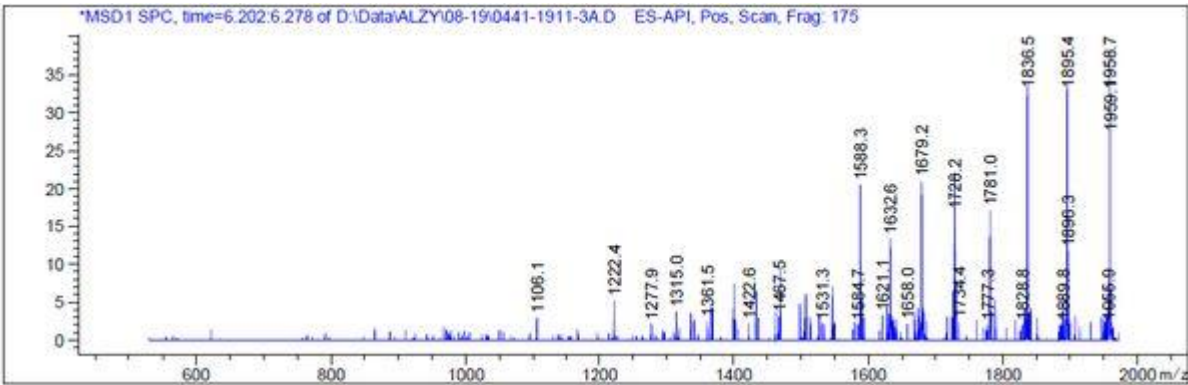
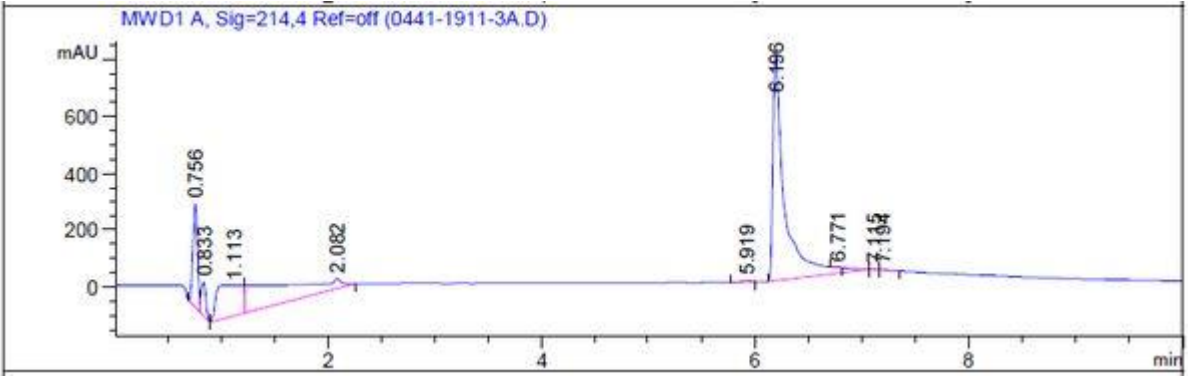




MW 34099, obs 34101

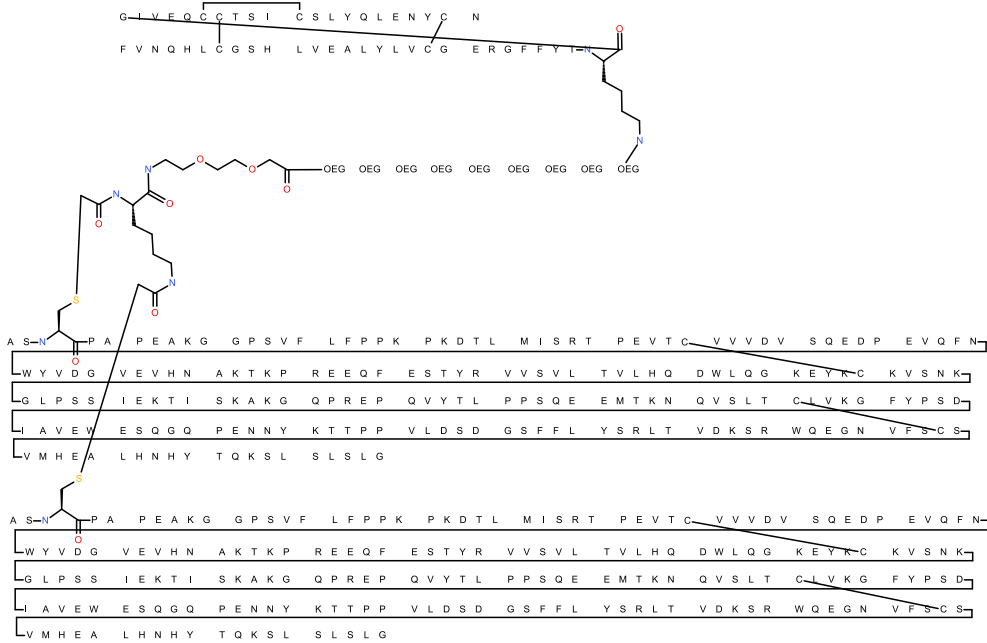
Fc/FA-Ins9

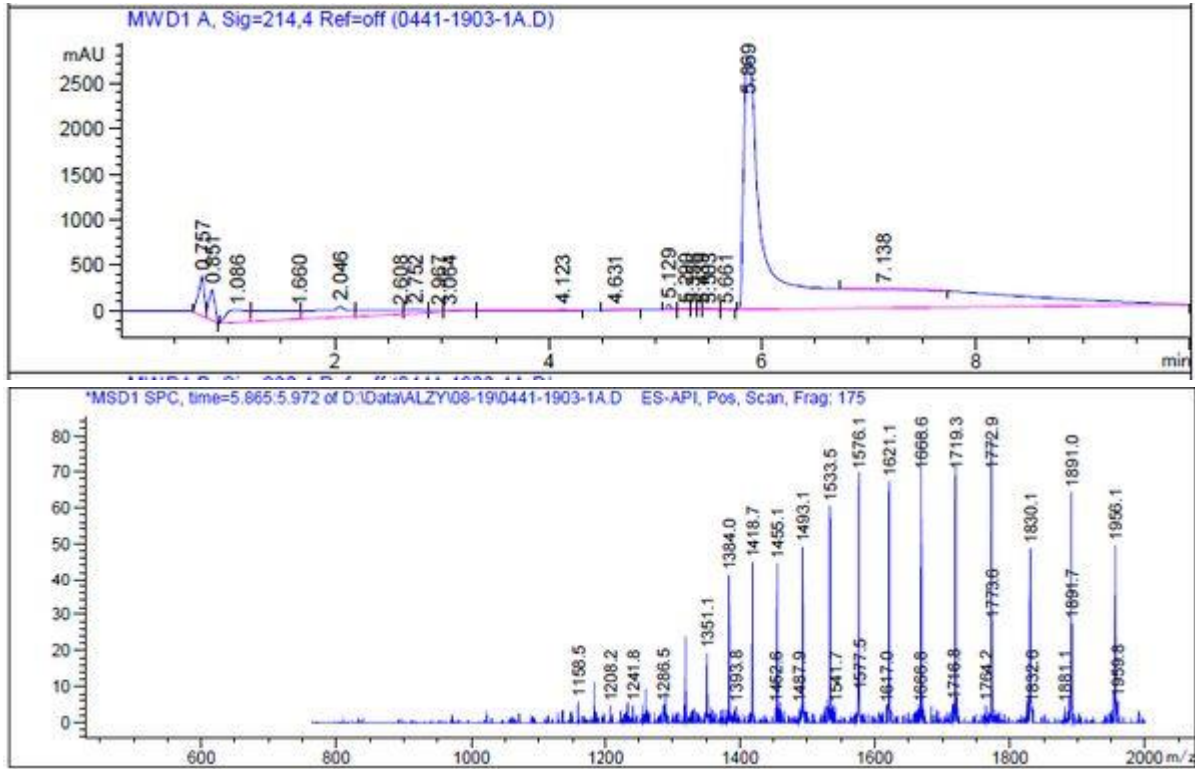




MW exp 58736, obs 58740

Fc-Ins9





MW exp 56701, obs 56700