Supporting information

Detection of active Granzyme A in NK92 cells with fluorescent activity-based probe

Sonia Kołt[1], Tomasz Janiszewski[1], Dion Kaiserman[2], Sylwia Modrzycka[1], Scott Snipas[3], Guy Salvesen[3], Marcin Drąg[1,3], Phillip I. Bird[2], Paulina Kasperkiewicz[1]

[1.] Wroclaw University of Science and Technology, Department of Chemical Biology and Bioimaging, Wyb. Wyspianskiego 29, 50-370 Wroclaw, Poland,

[2.] Monash University, Monash Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, 23 Innovation Walk, Clayton VIC 3800, Australia,

[3.] NCI-designated Cancer Center, Sanford-Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA,

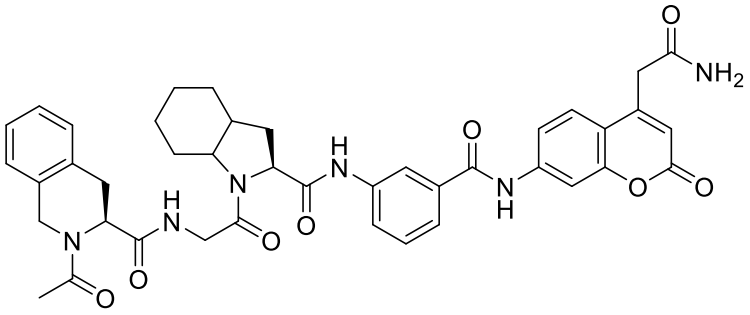
Corresponding: paulina.kasperkiewicz@pwr.edu.pl

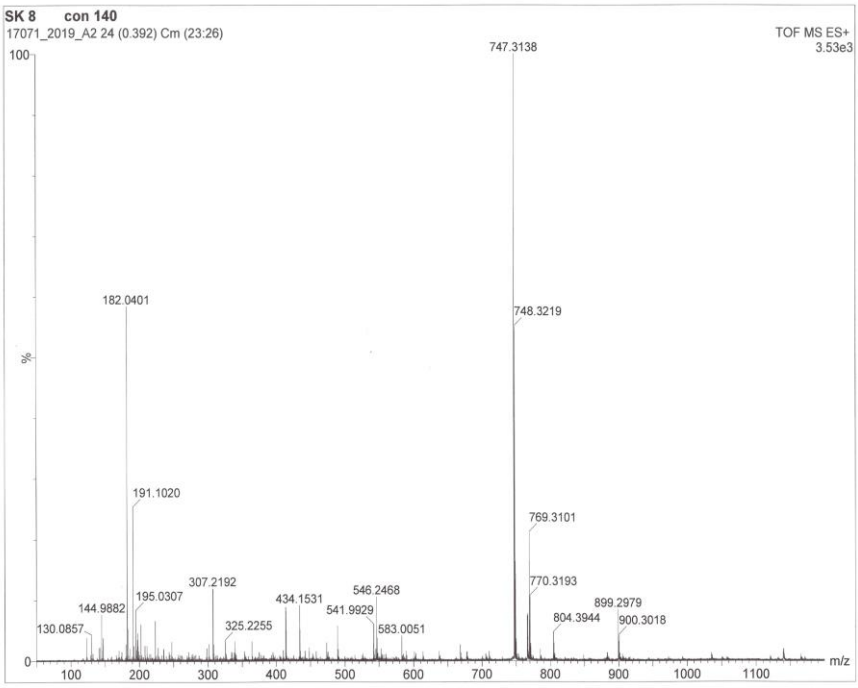
Contents of Supporting Information

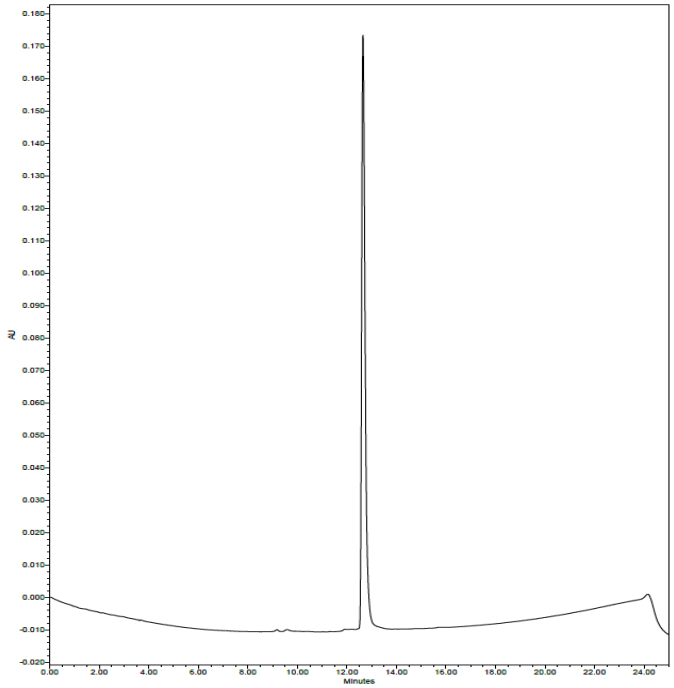
Table S1. Purity and MS analysis of synthesized compounds.

	Structure	$[M+H]^+$ calculated	$[M+H]^+$ measured	purity
SK8	Ac-Tic-Gly-Oic-3-Abz-ACC	747.3142	747.3138	≥99%
SK9	Ac-Tic-Abu(Bth)-Oic-3-Abz-ACC	908.3442	908.3450	≥98%
SK10	Ac-Ile-Gly-Oic-3-Abz-ACC	701.3299	701.3295	≥99%
SK12	Ac-Met(O)-Gly-Tyr(2,6-Cl_2-Bzl)-3-Abz-ACC	905.2139	905.2135	≥99%
SK13	Ac-Met(O)-Gly-Hyp(Bzl)-3-Abz-ACC	787.2761	787.2747	≥99%
SK14	Ac-Met(O)-Gly-Phe(4-F)-3-Abz-ACC	749.2405	749.2413	≥99%
SK15	Ac-Tic-Gly-Oic-Arg-ACC	784.3782	784.3790	≥97%
SK17	Ac-Ile-Gly-Oic-Arg-ACC	738.3939	738.3932	≥99%
SK18	Ac-Tic-Abu(Bth)-Oic-Arg-ACC	945.4081	945.4095	≥98%
SK19	Ac-Met(O)-Gly-Tyr(2,6-Cl_2-Bzl)-Arg-ACC	942.2778	942.2795	≥98%
SK20	Ac-Met(O)-Gly-Hyp(Bzl)-Arg-ACC	824.3401	824.3399	≥99%
SK21	Ac-Met(O)-Gly-Phe(4-F)-Arg-ACC	786.3045	786.3045	≥98%
SK22	Ac-Tic-Gly-Oic-Phe(guan)-ACC	832.3782	832.3781	≥97%
SK23	Ac-Tic-Abu(Bth)-Oic-Phe(guan)-ACC	993.4081	993.4070	≥97%
SK24	Ac-Ile-Gly-Oic-Phe(guan)-ACC	786.3939	786.3940	≥99%
SK26	Ac-Met(O)-Gly-Tyr(2,6-Cl_2-Bzl)-Phe(guan)-ACC	990.2778	990.2772	≥95%
SK27	Ac-Met(O)-Gly-Hyp(Bzl)-Phe(guan)-ACC	872.3401	872.3403	≥99%
SK28	Ac-Met(O)-Gly-Phe(4-F)-Phe(guan)-ACC	834.3045	834.3040	≥99%
SK7	Ac-Ile-Gly-Pro-Arg-ACC	684.3469	684.3463	≥95%
SK15I	MeOSuc-Tic-Gly-Oic-Arg^P$(OPh)_2$	844.3799	844.3809	≥95%
SK15.5	Cy5-Ahx-Tic-Gly-Oic-Arg^P$(OPh)_2$	654.3615	654.3613	≥95%

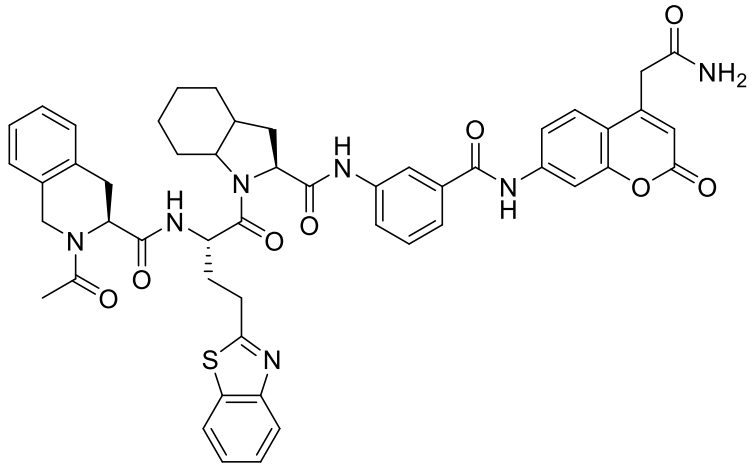
SK8, Ac-Tic-Gly-Oic-3-Abz-ACC

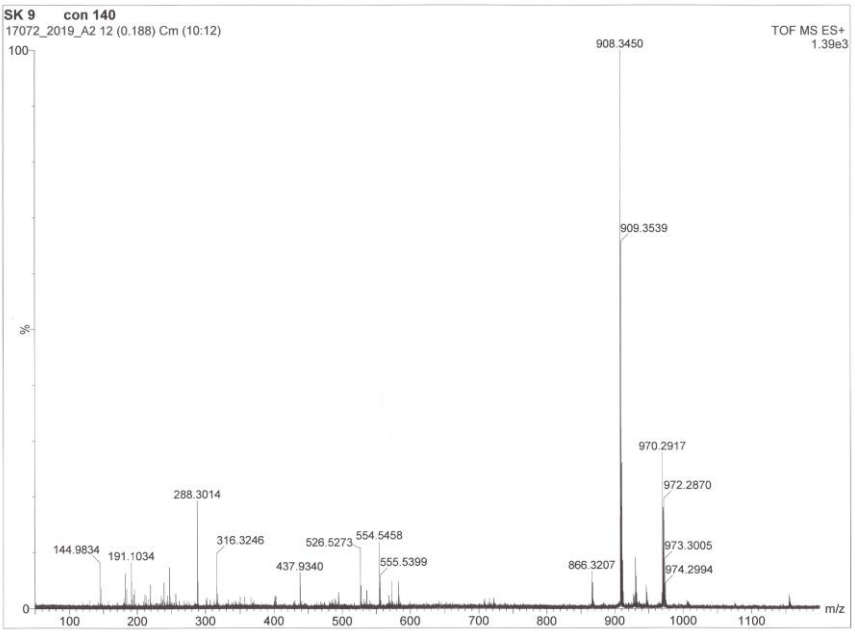


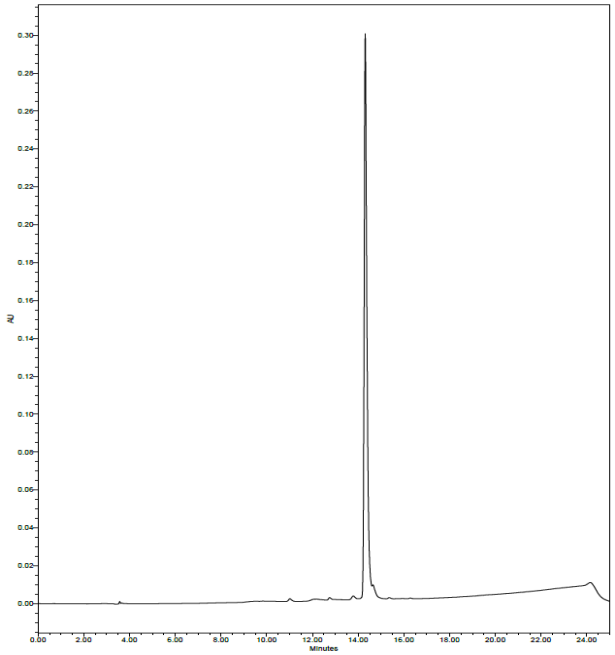




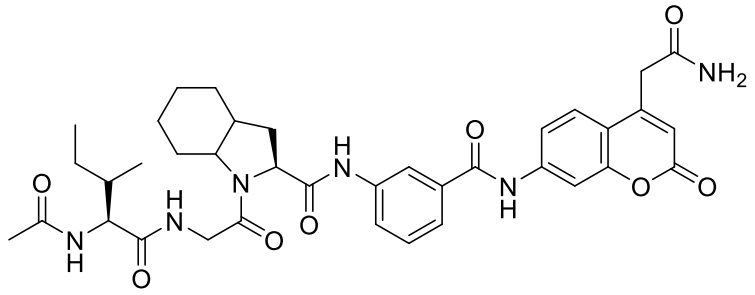
SK9, Ac-Tic-Abu(Bth)-Oic-3-Abz-ACC

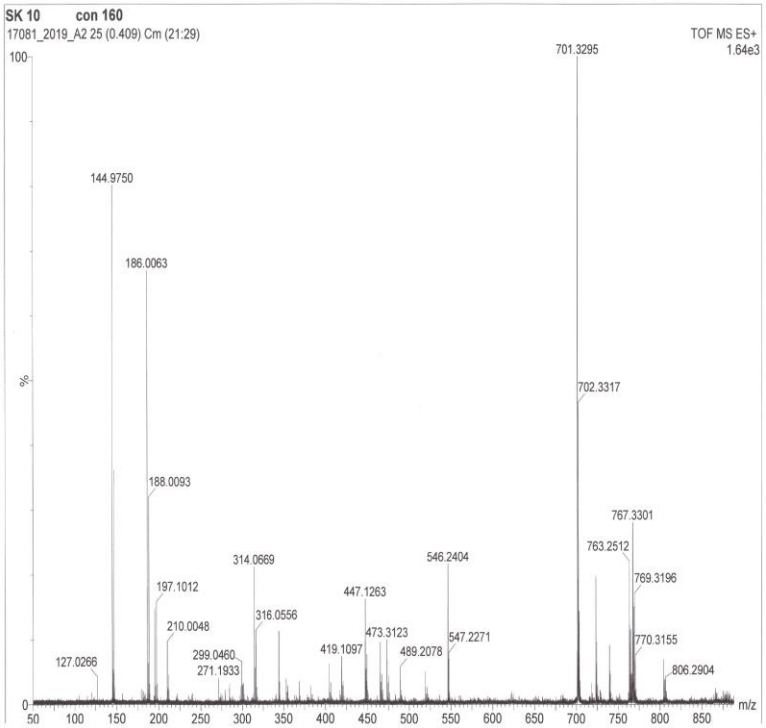


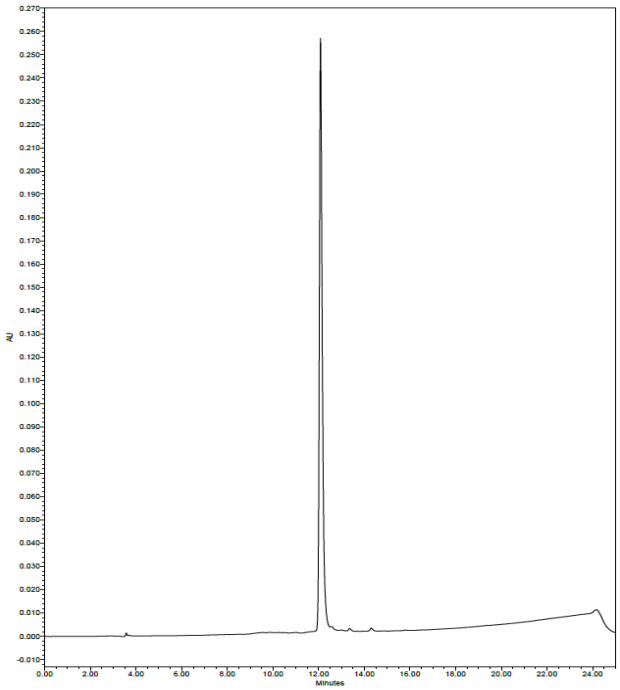




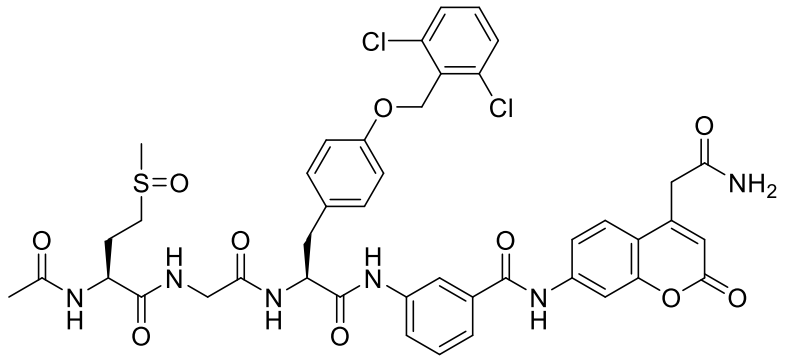
SK10, Ac-Ile-Gly-Oic-3-Abz-ACC

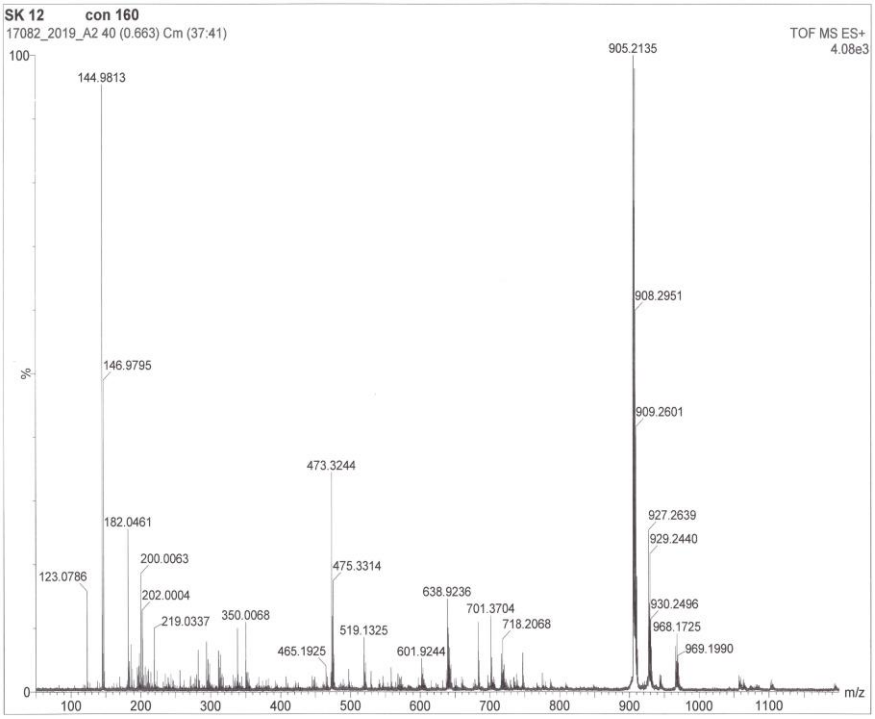


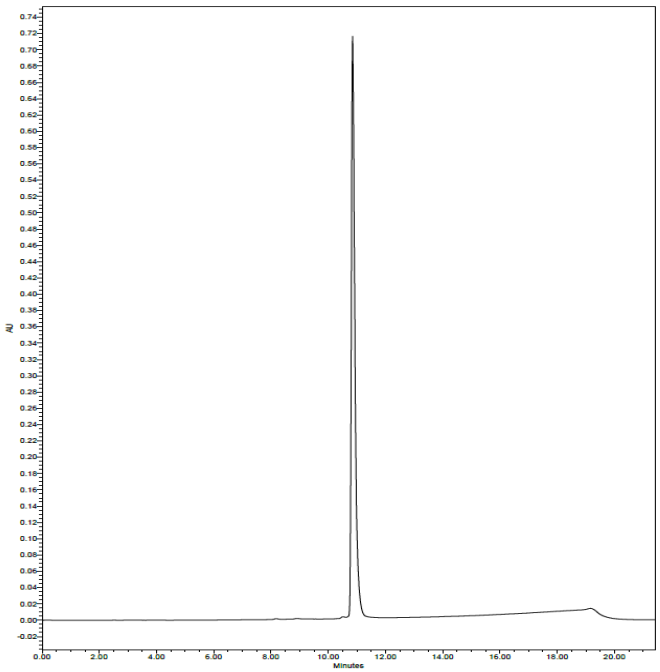




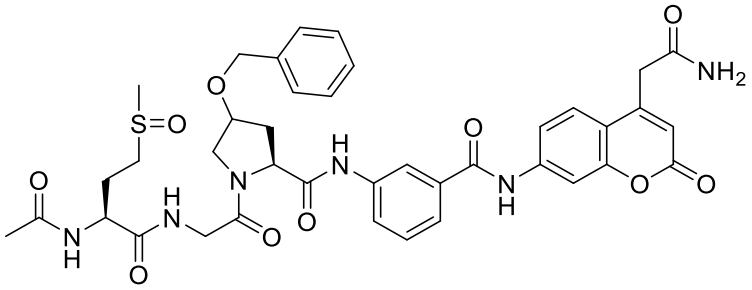
SK12, Ac-Met(O)-Gly-Tyr(2,6-Cl_2-Bzl)-3-Abz-ACC

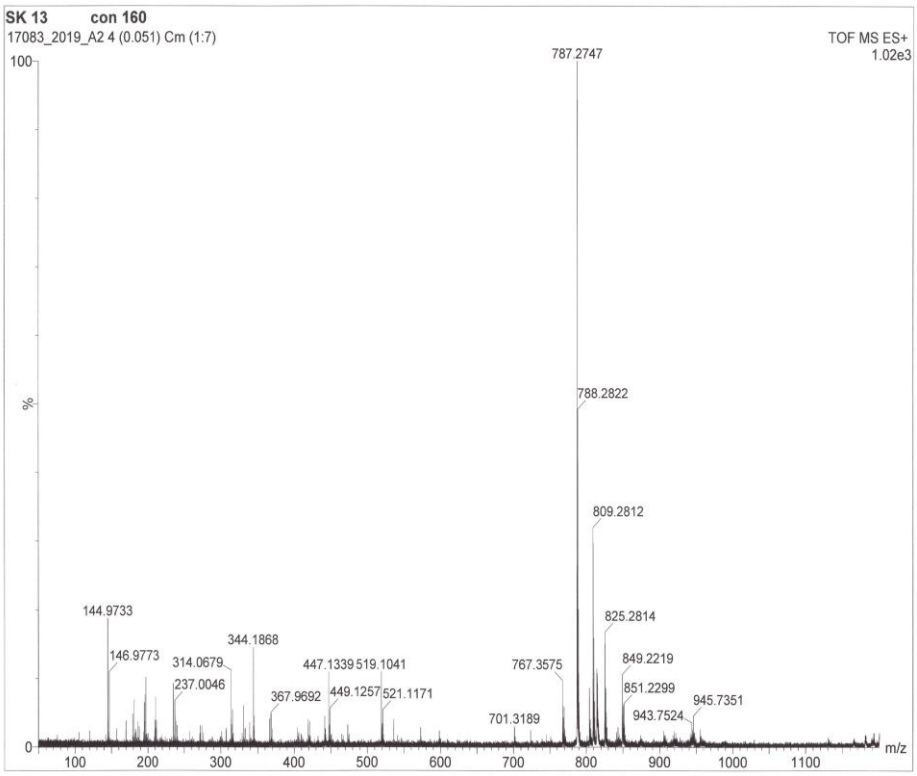


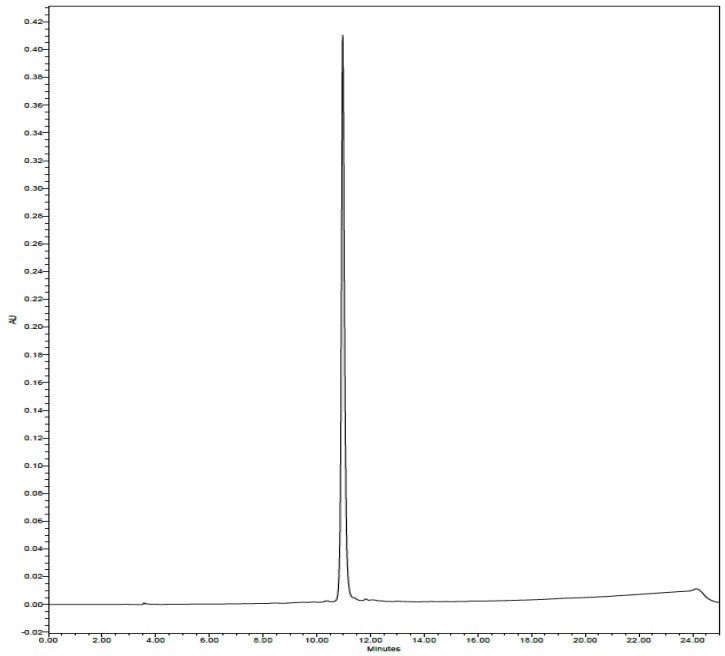




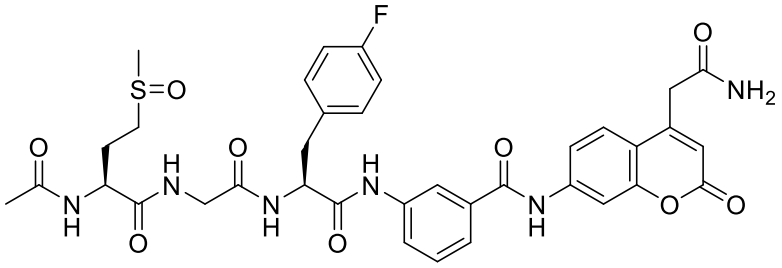
SK13, Ac-Met(O)-Gly-Hyp(Bzl)-3-Abz-ACC

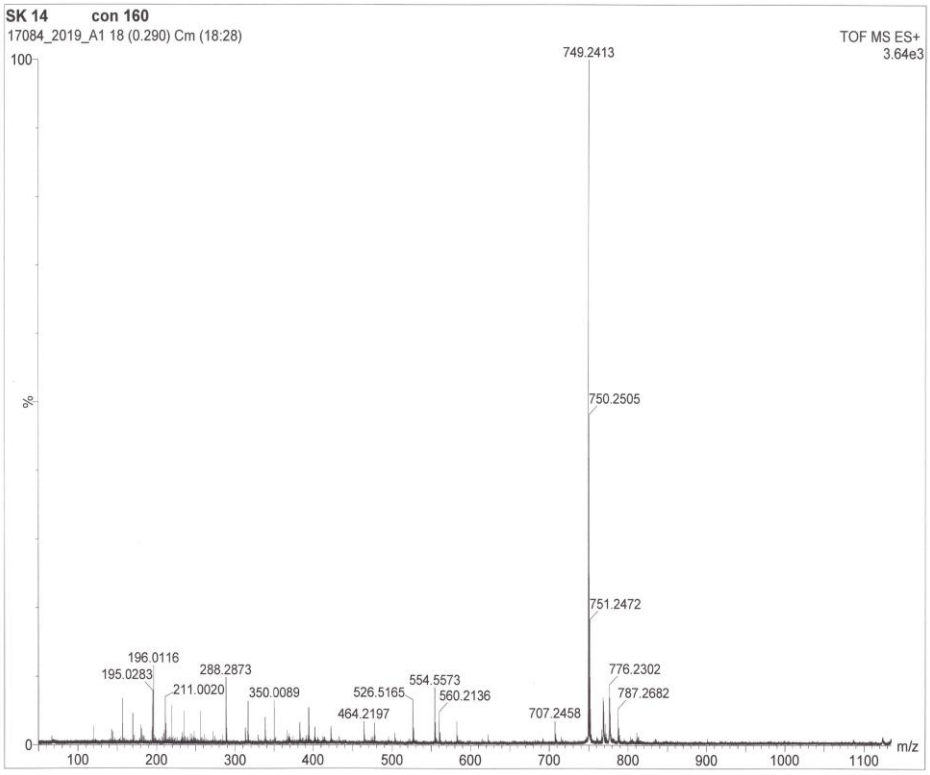


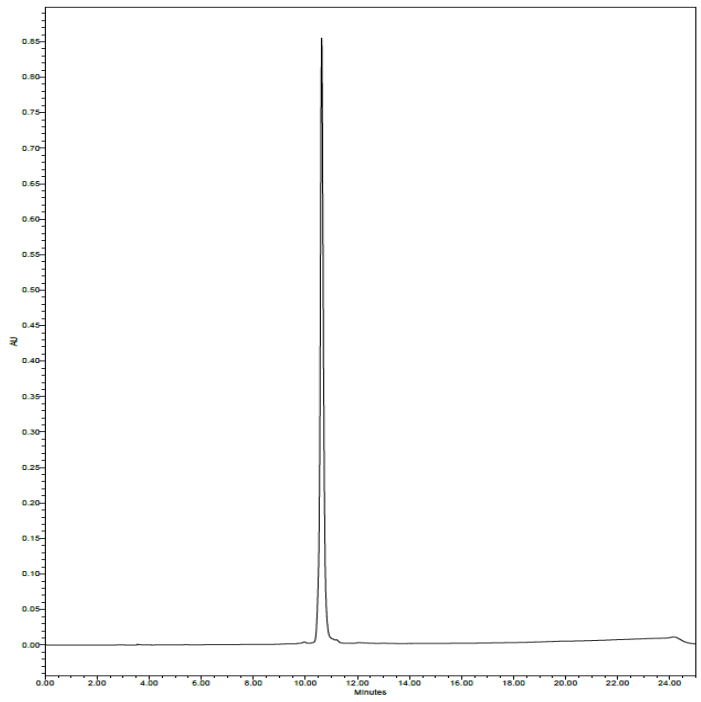




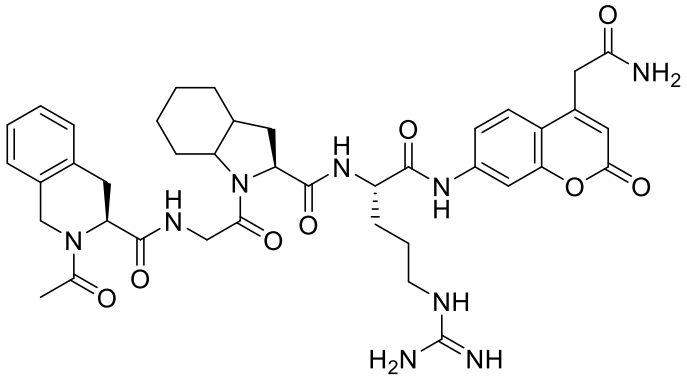
SK14, Ac-Met(O)-Gly-Phe(4-F)-3-Abz-ACC

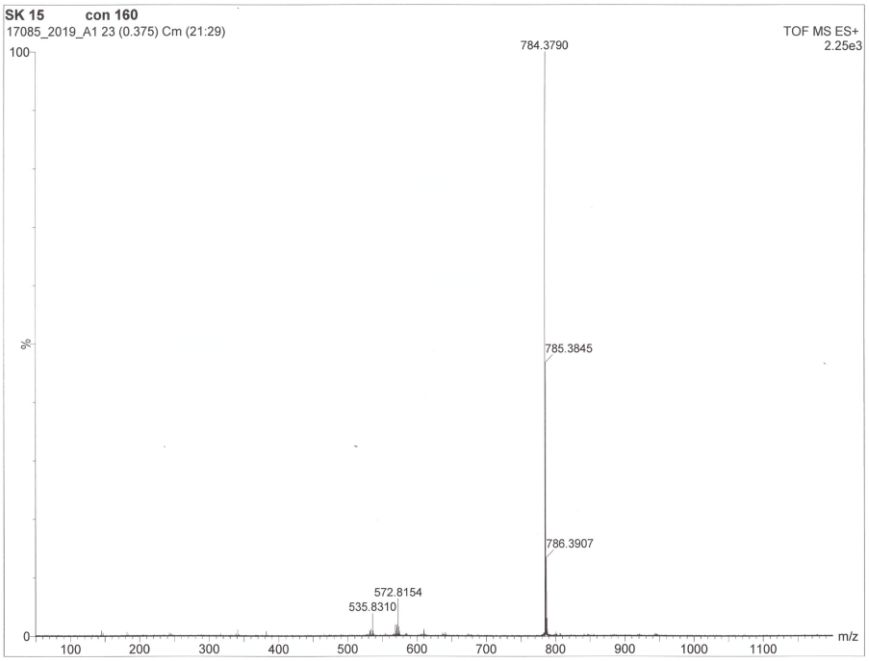


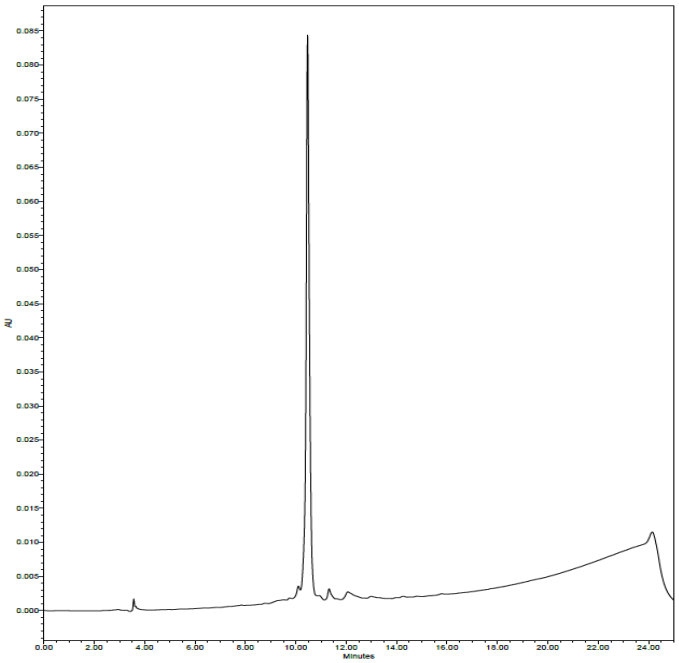




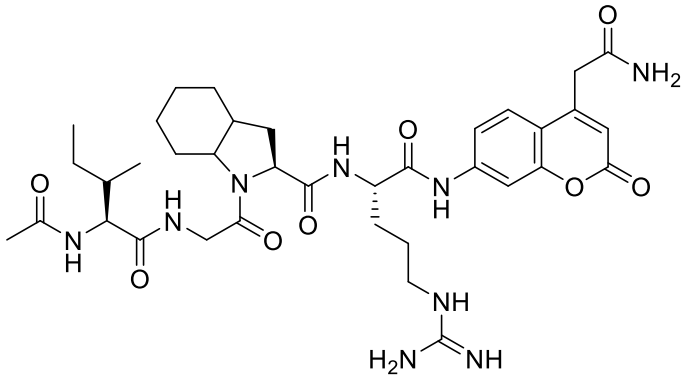
SK15, Ac-Tic-Gly-Oic-Arg-ACC

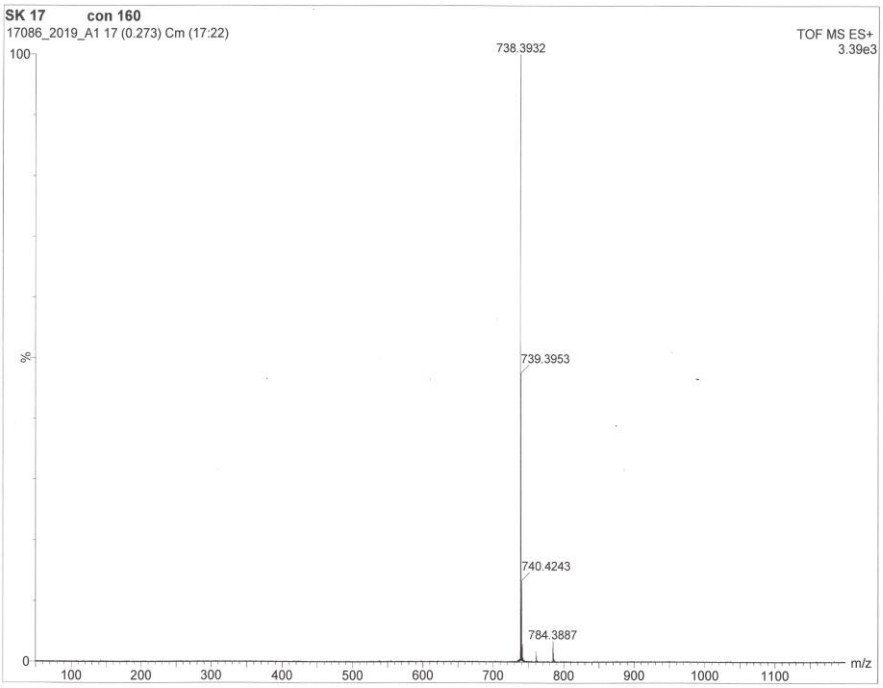


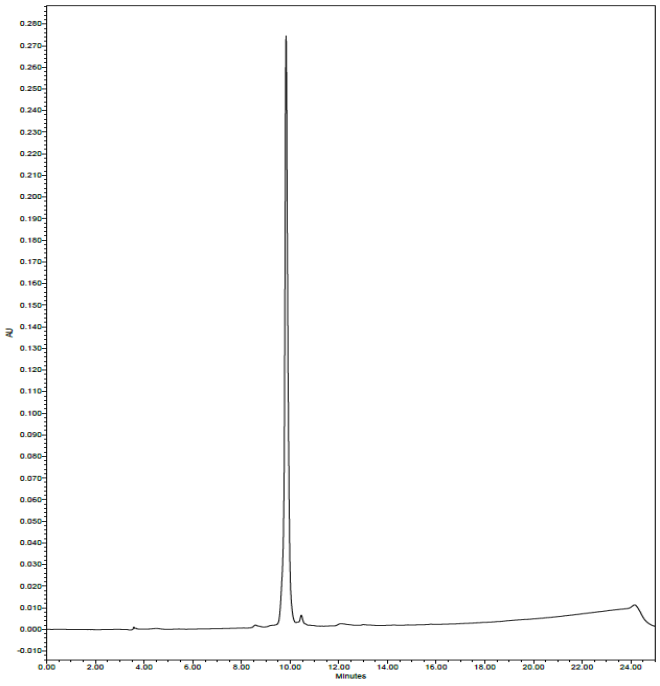




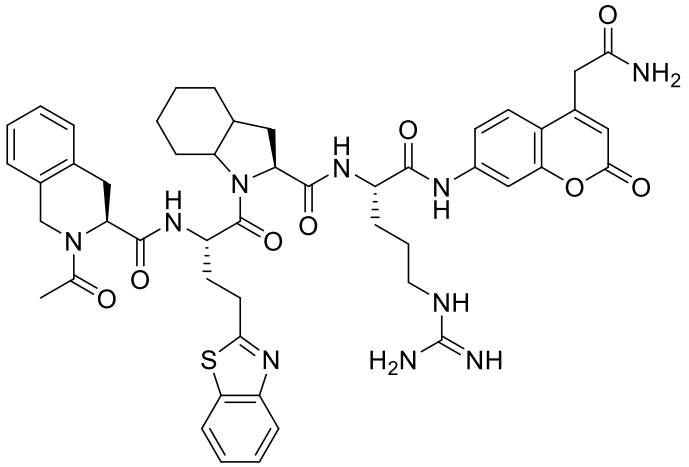
SK17, Ac-Ile-Gly-Oic-Arg-ACC

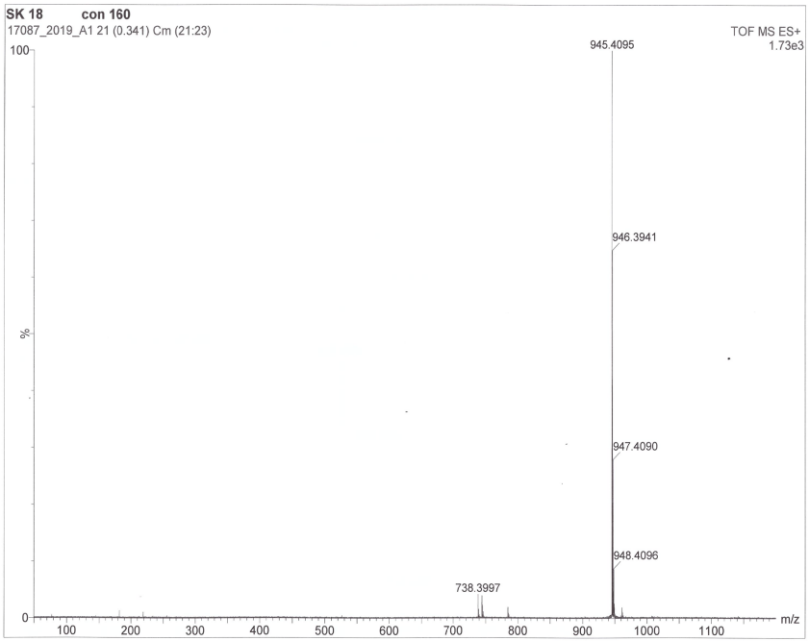


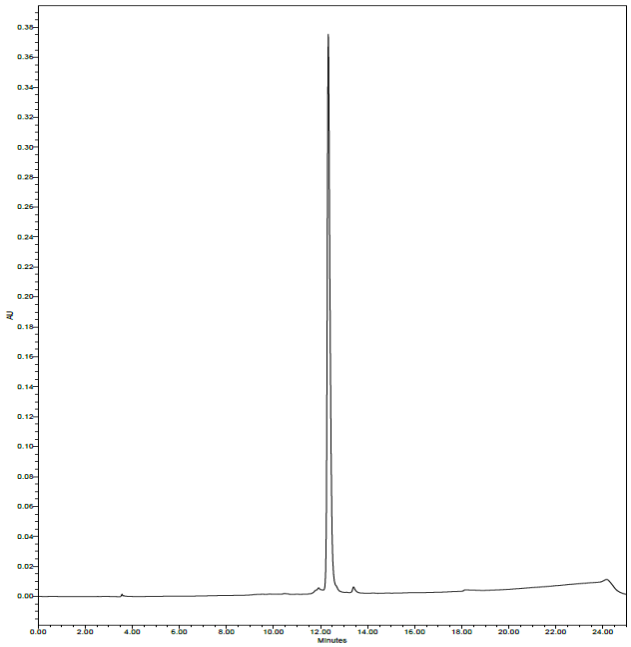




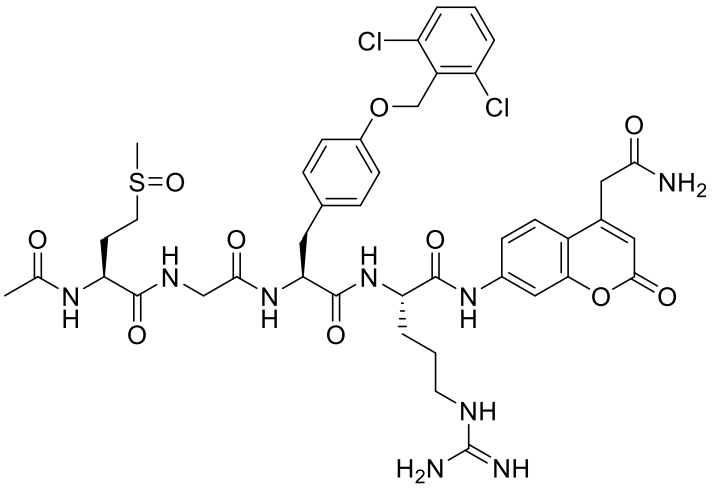
SK18, Ac-Tic-Abu(Bth)-Oic-Arg-ACC

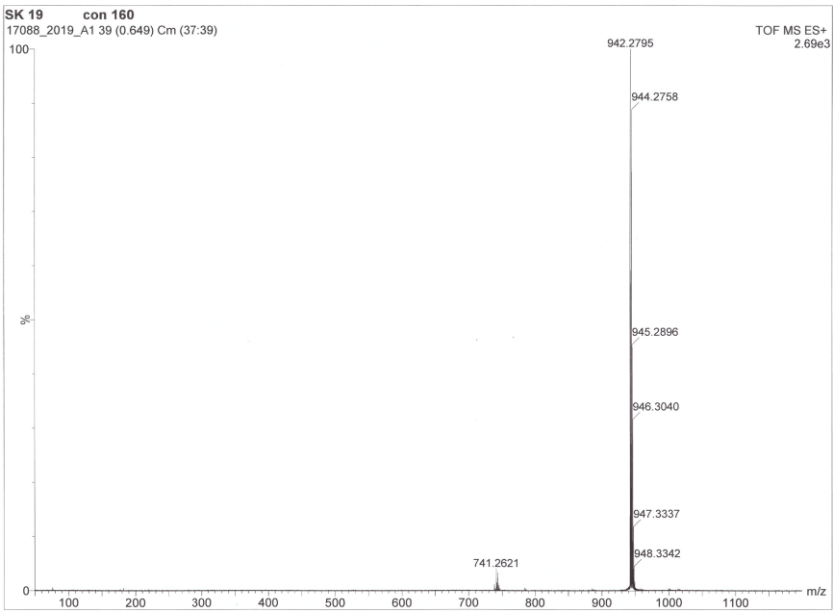


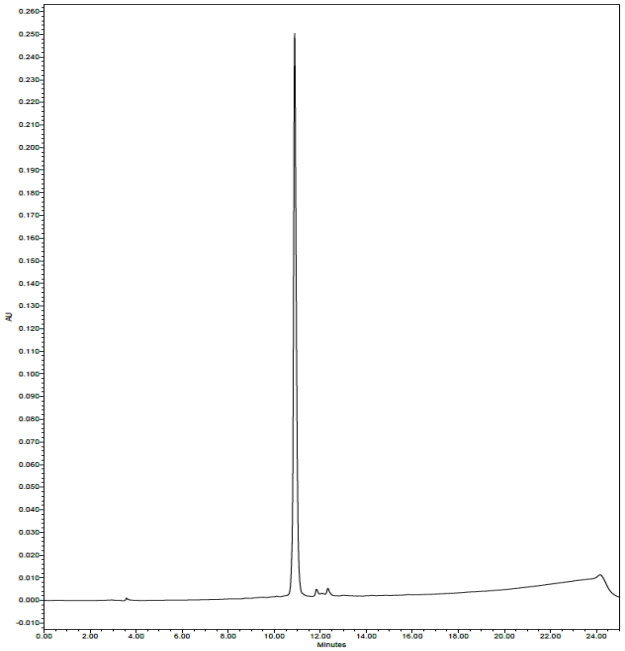




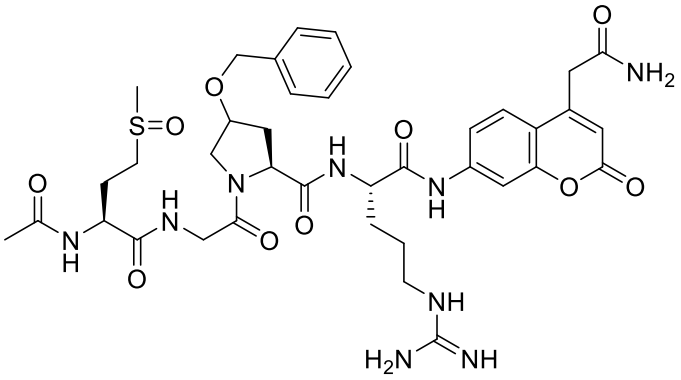
SK19, Ac-Met(O)-Gly-Tyr(2,6-Cl_2-Bzl)-Arg-ACC

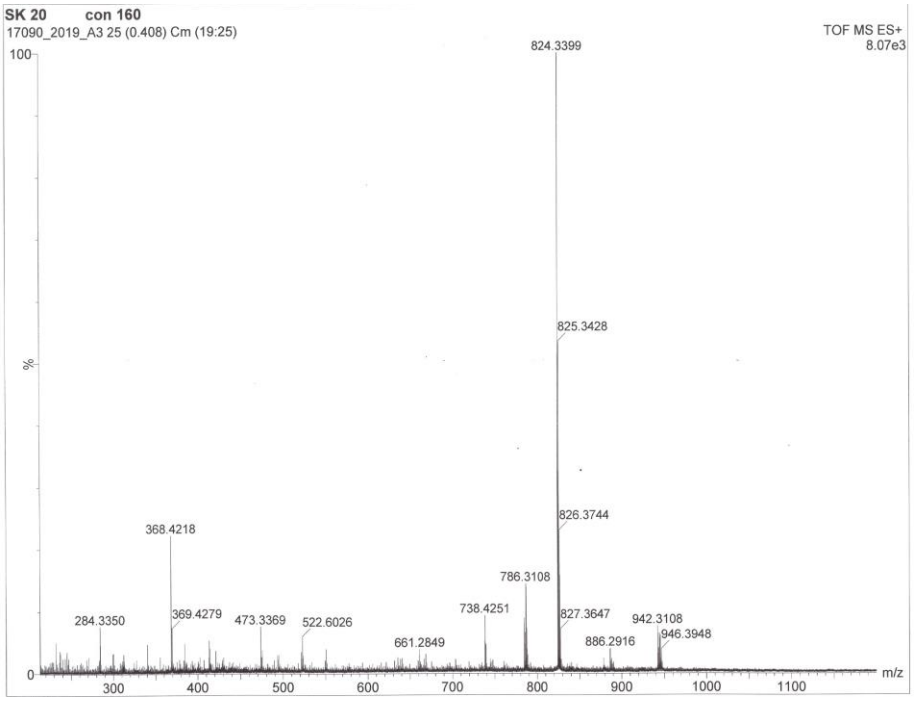


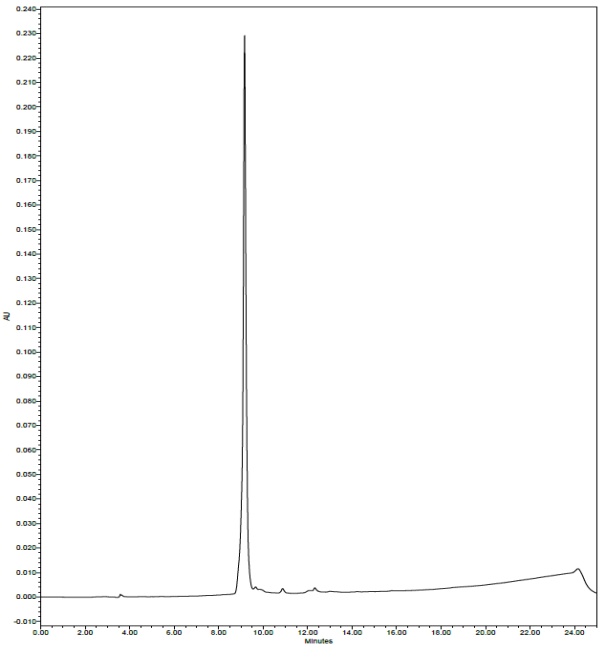




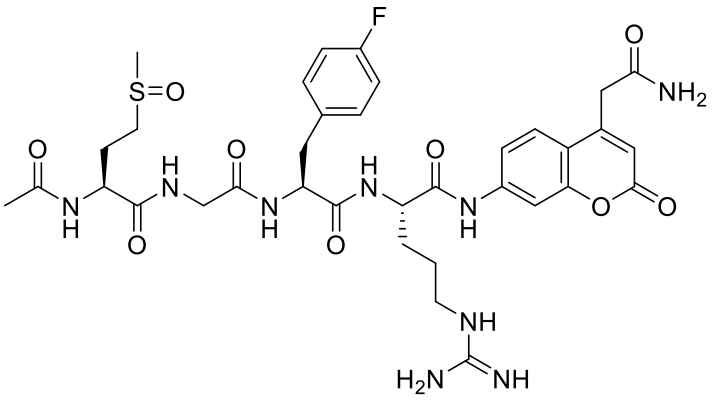
SK20, Ac-Met(O)-Gly-Hyp(Bzl)-Arg-ACC

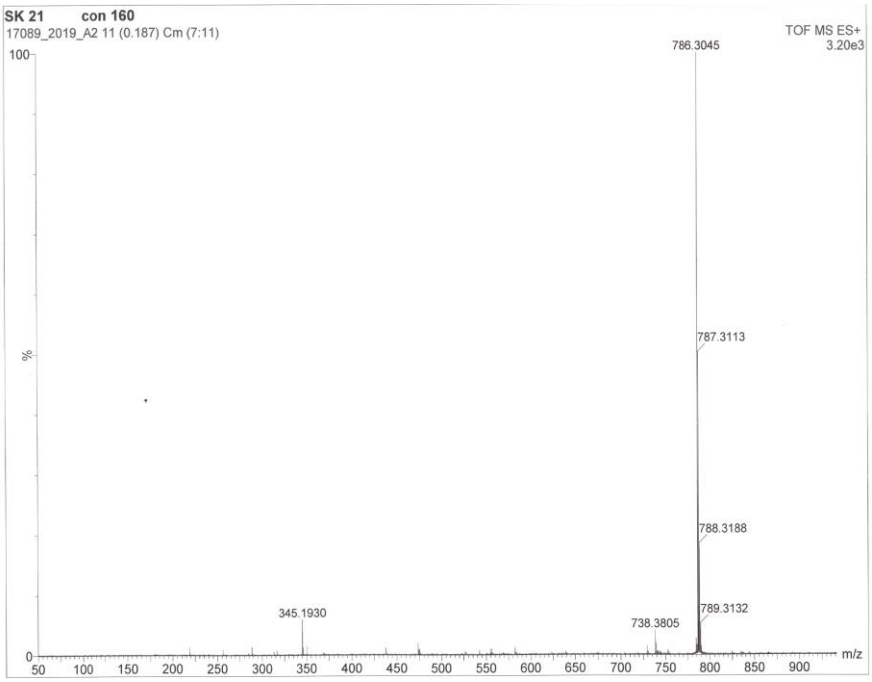


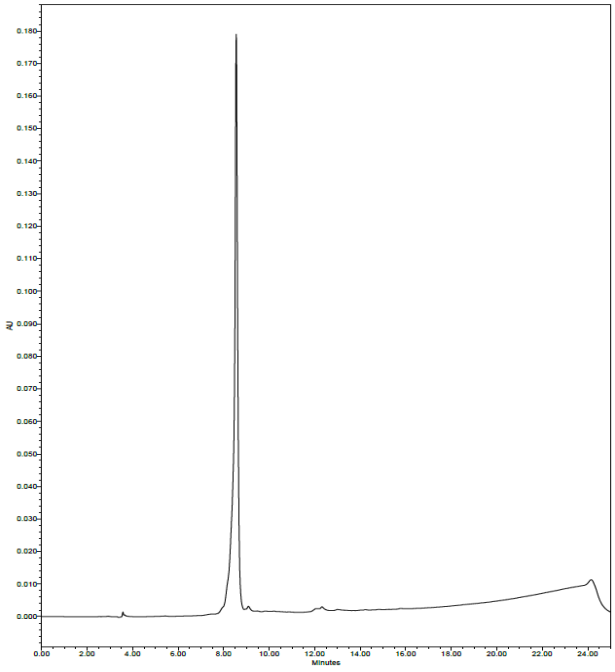




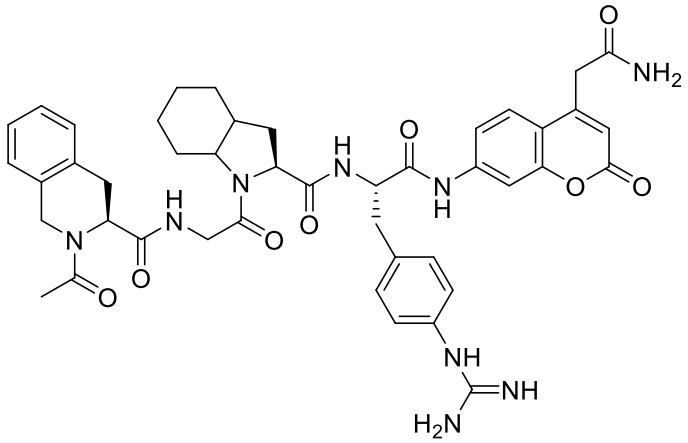
SK21, Ac-Met(O)-Gly-Phe(4-F)-Arg-ACC

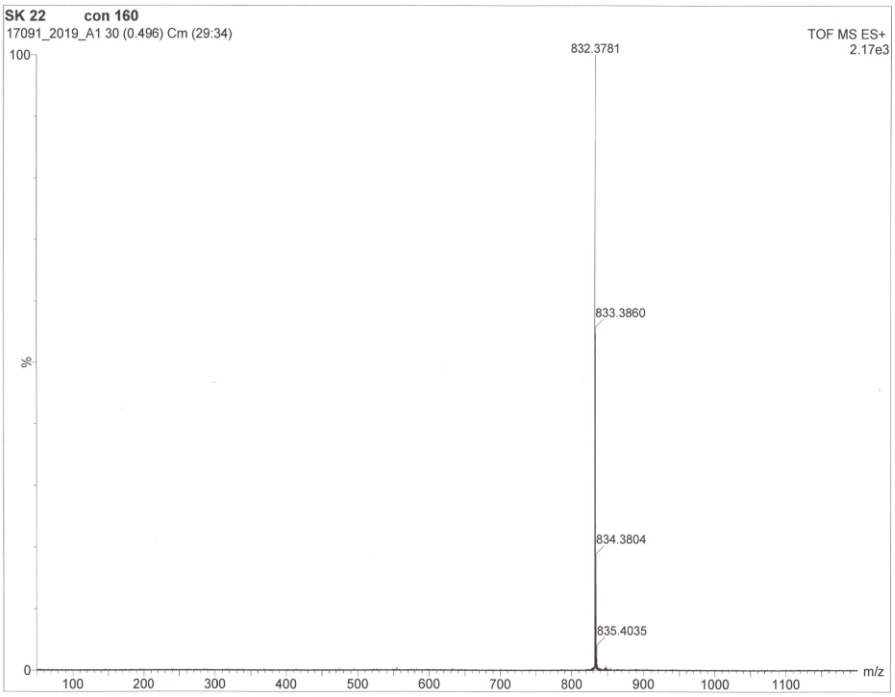


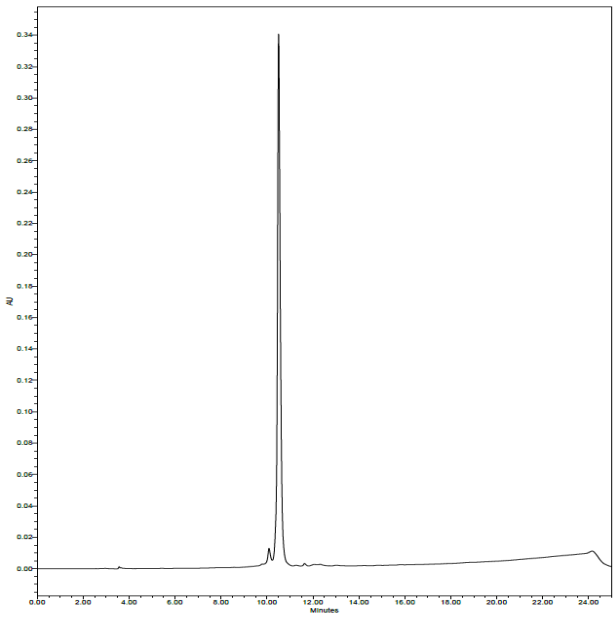




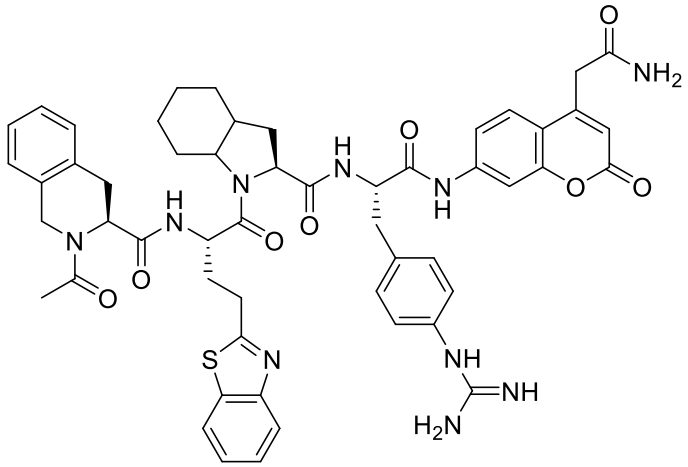
SK22, Ac-Tic-Gly-Oic-Phe(guan)-ACC

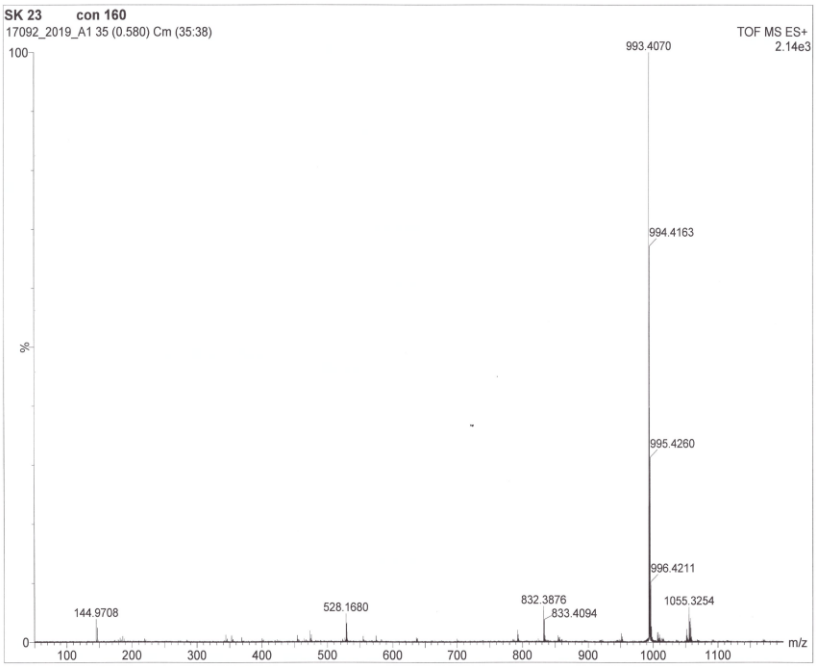


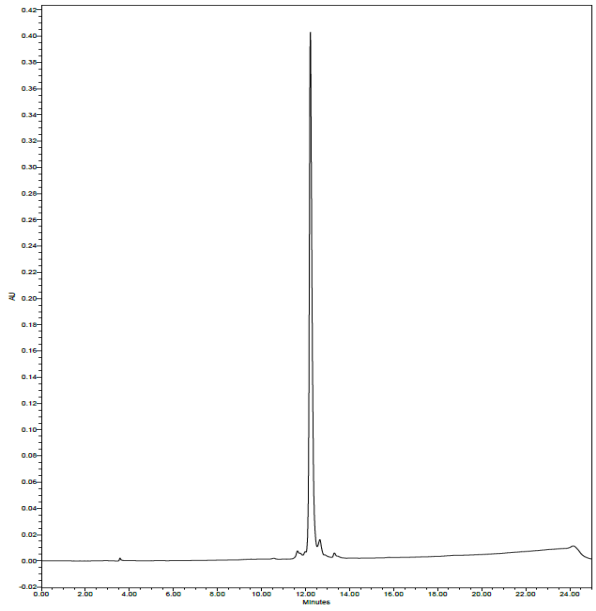




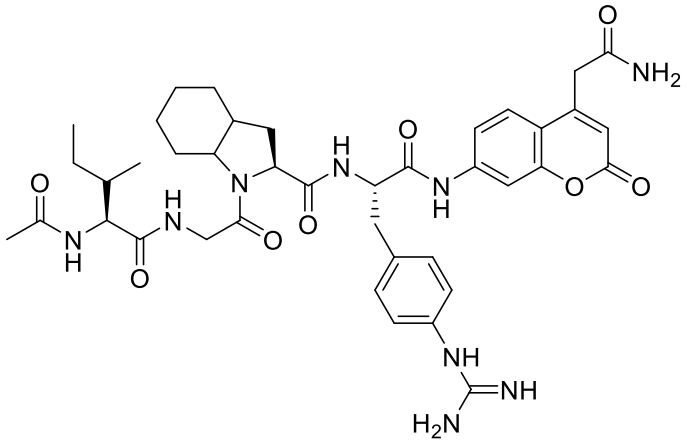
SK23, Ac-Tic-Abu(Bth)-Oic-Phe(guan)-ACC

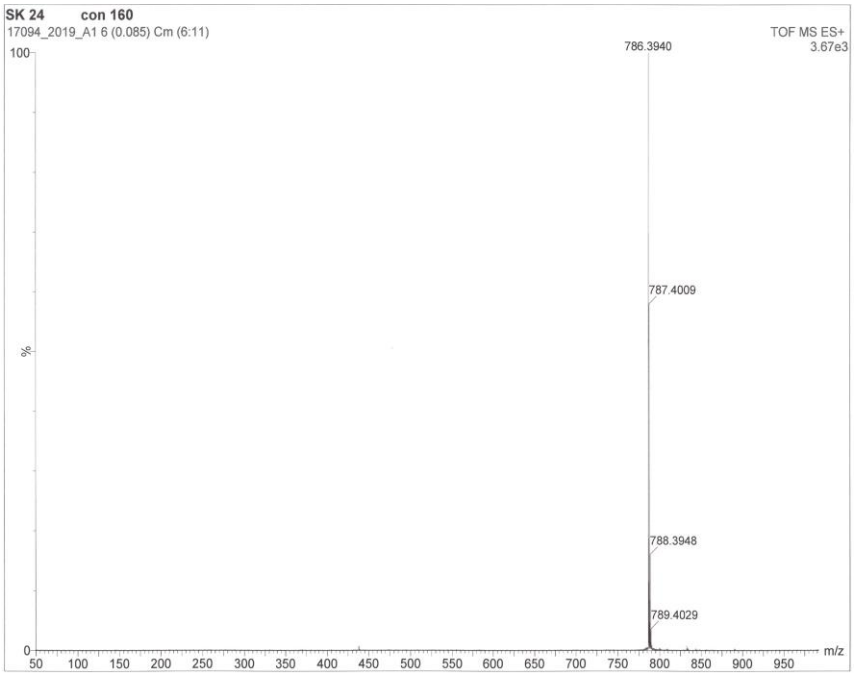


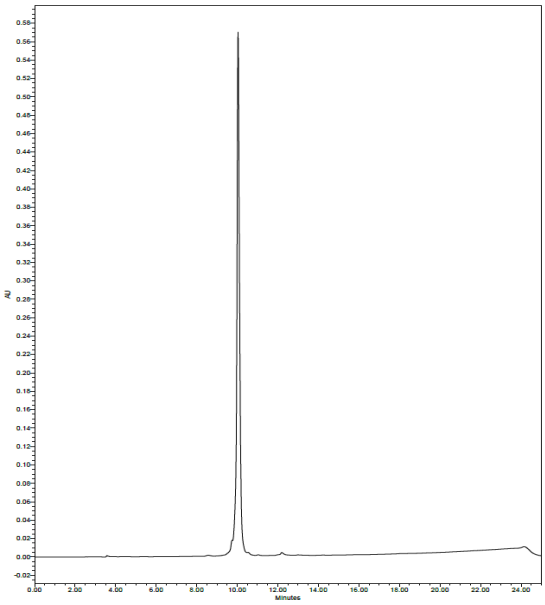




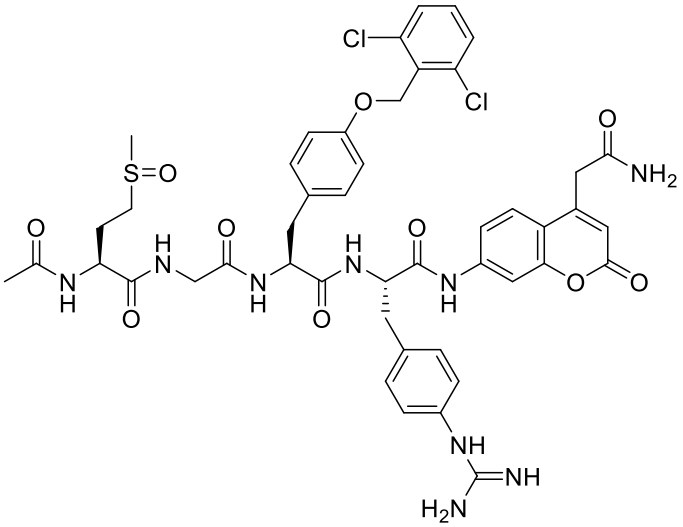
SK24, Ac-Ile-Gly-Oic-Phe(guan)-ACC

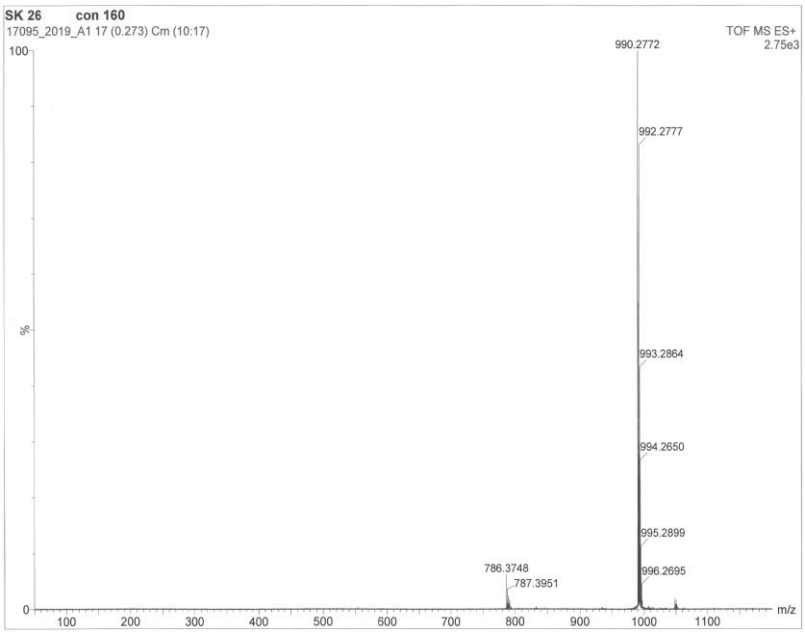


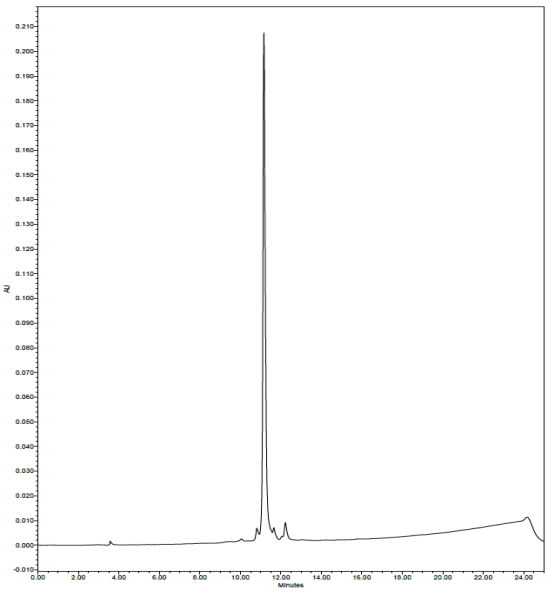




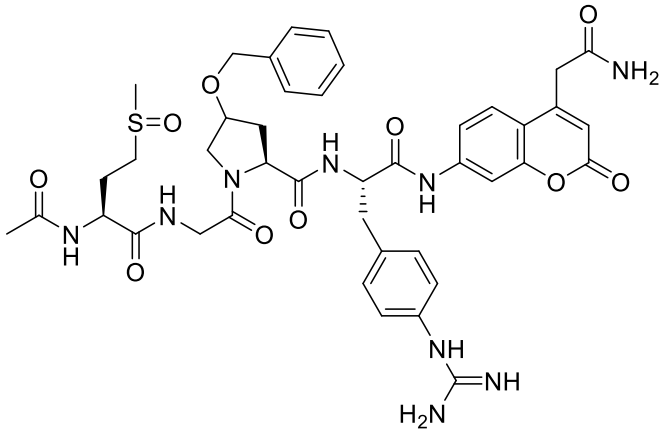
SK26, Ac-Met(O)-Gly-Tyr(2,6-Cl_2-BzI)-Phe(guan)-ACC

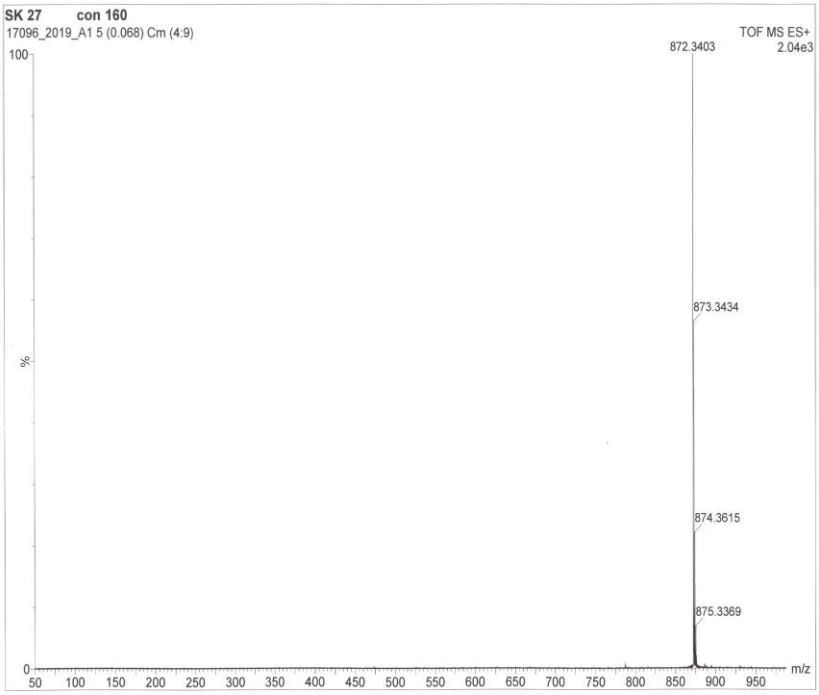


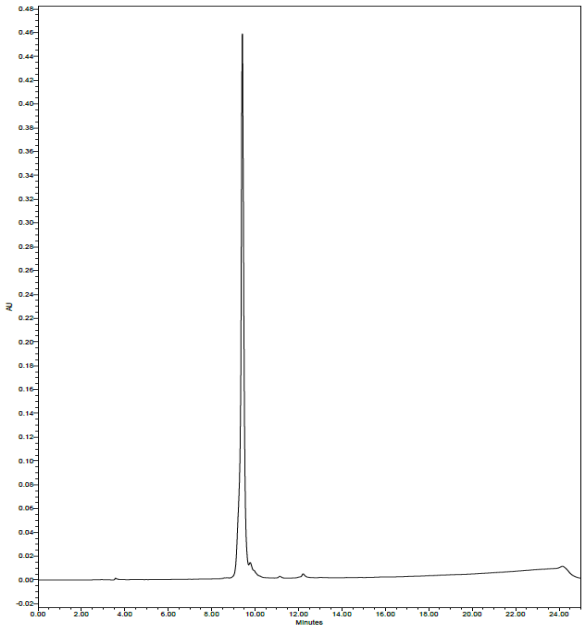




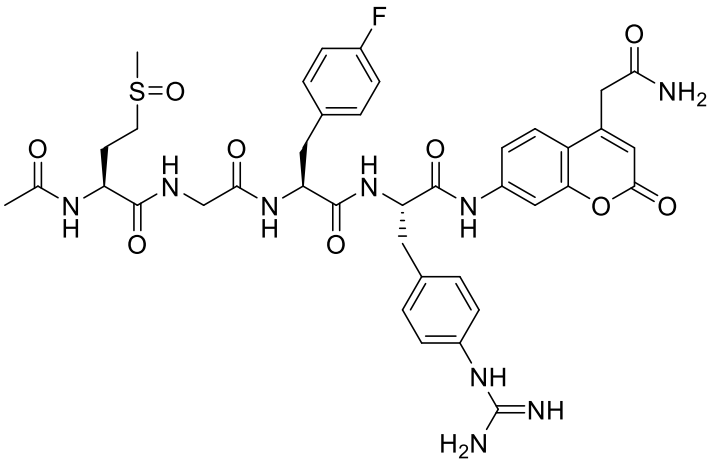
SK27, Ac-Met(O)-Gly-Hyp(Bzl)-Phe(guan)-ACC

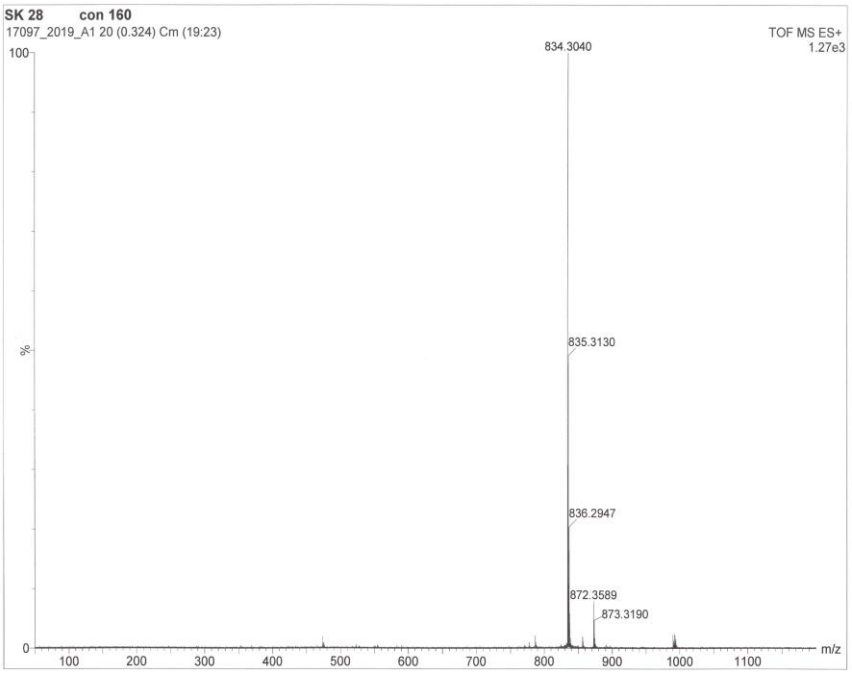


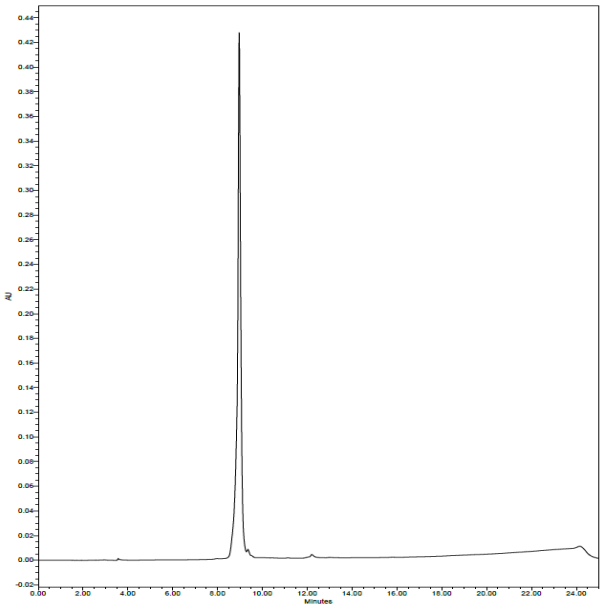




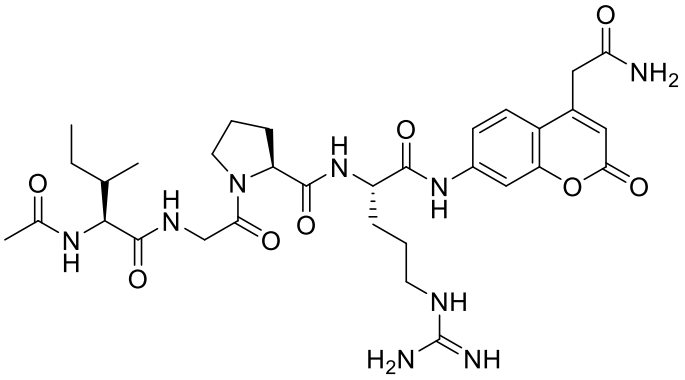
SK28, Ac-Met(O)-Gly-Phe(4-F)-Phe(guan)-ACC

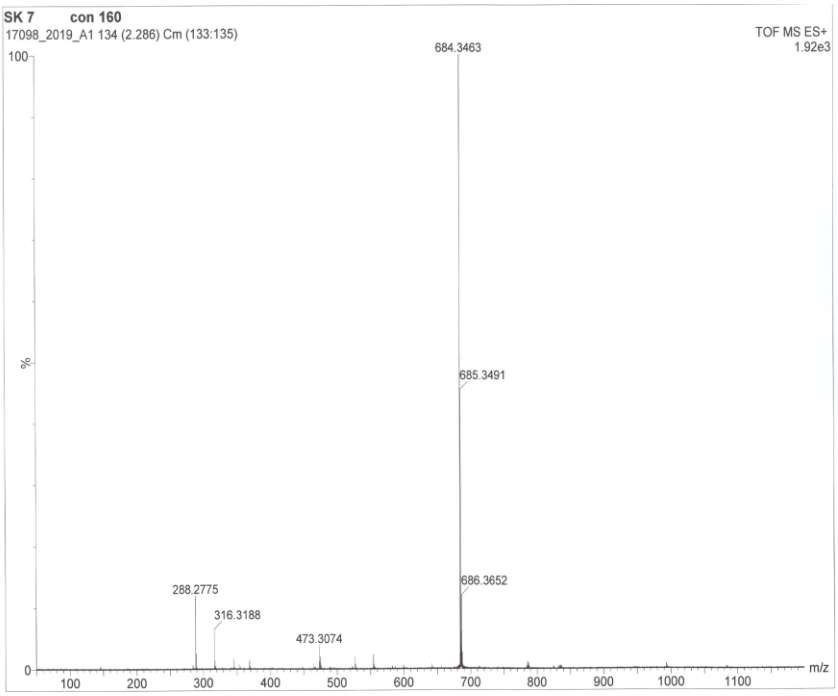


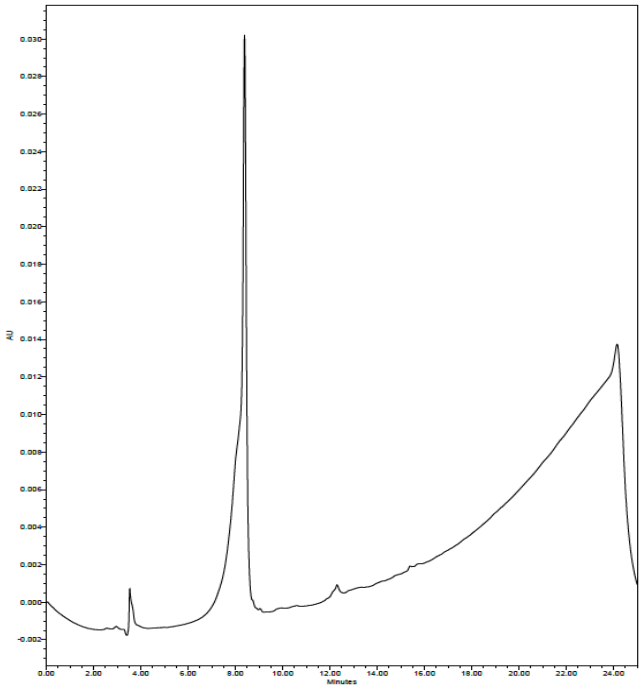




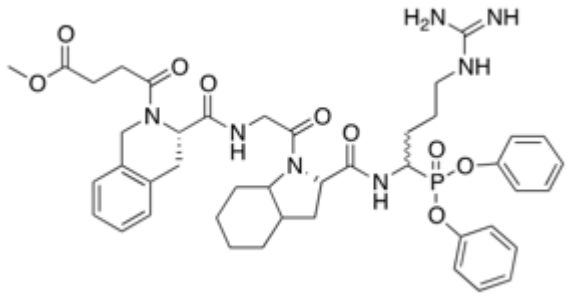
SK7, Ac-Ile-Gly-Pro-Arg-ACC

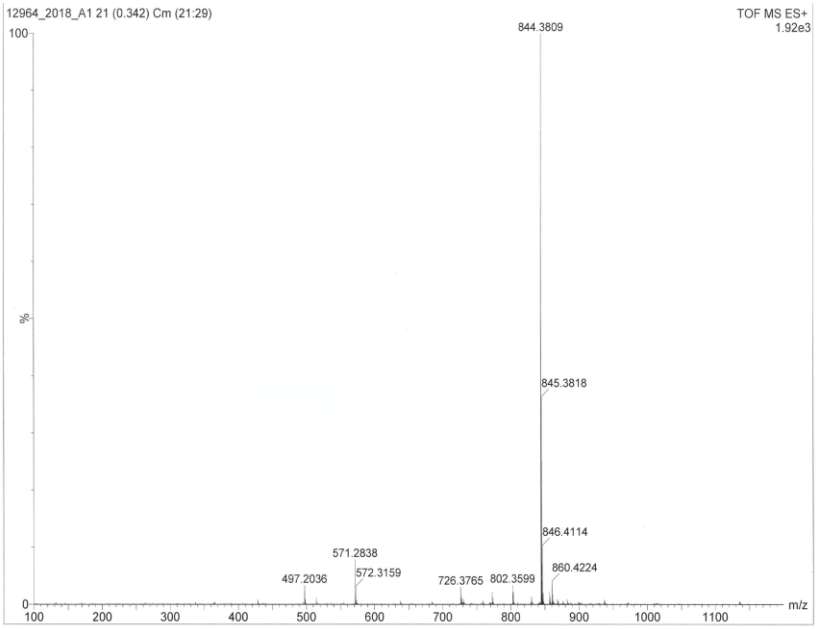


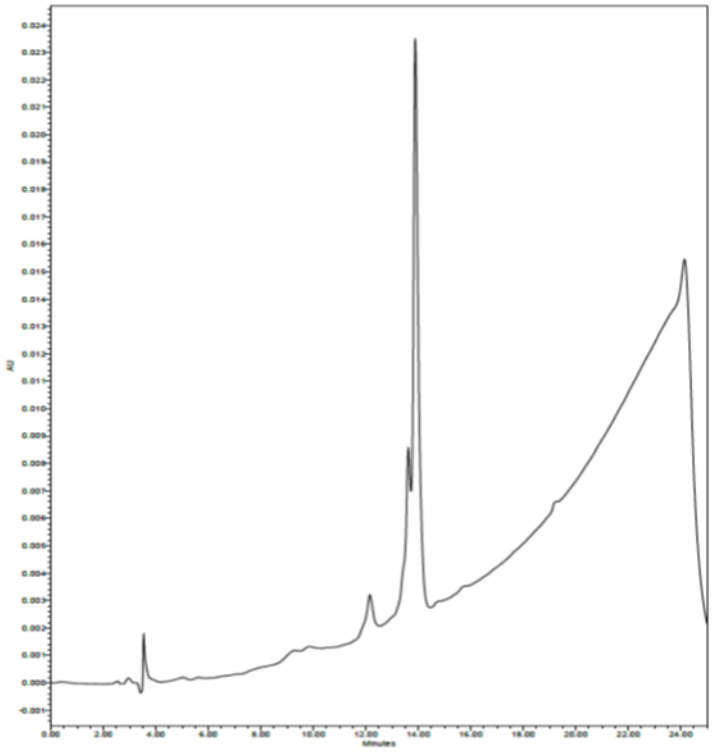




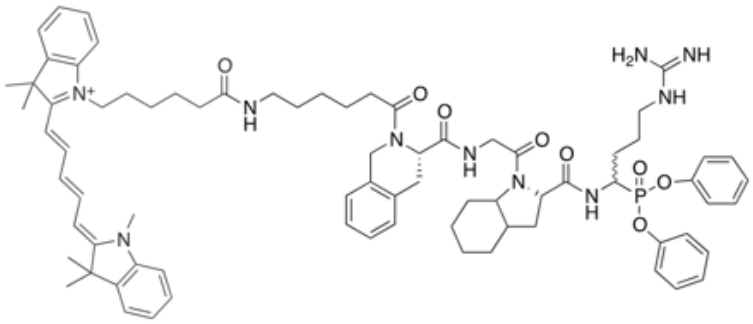
SK15I, MeOSuc-Tic-Gly-Oic-Arg^{P}(OPh)$_2$

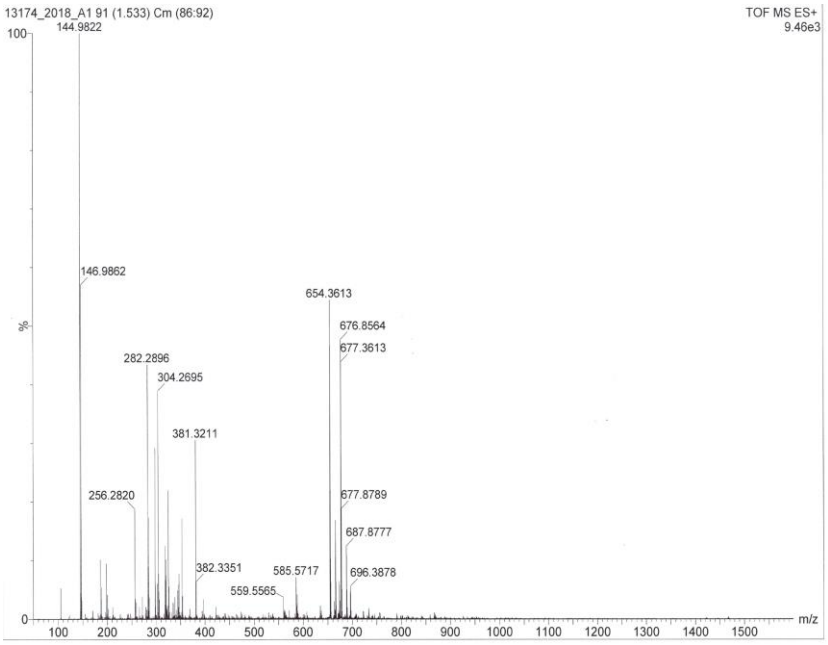






SK15.5, Cy5-Ahx-Tic-Gly-Oic-Arg^{P}(OPh)$_2$





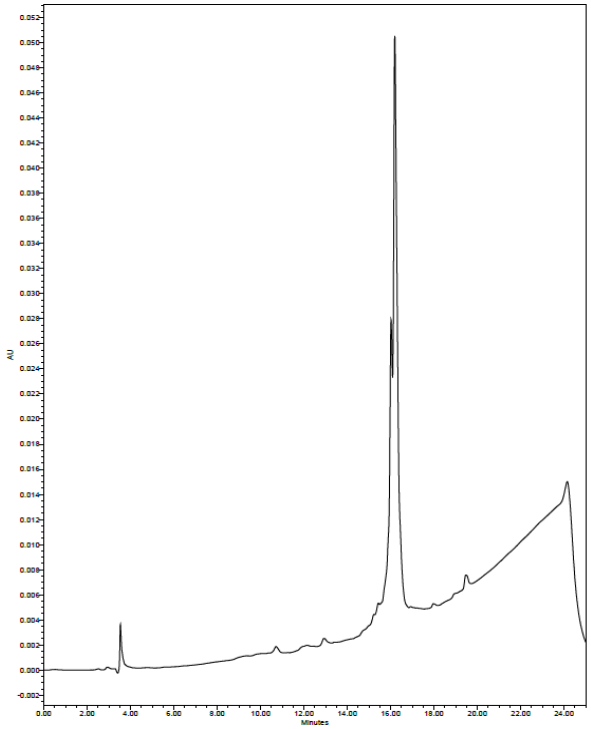


Figure S1. GrA substrate specificity profiles presented as heat maps.

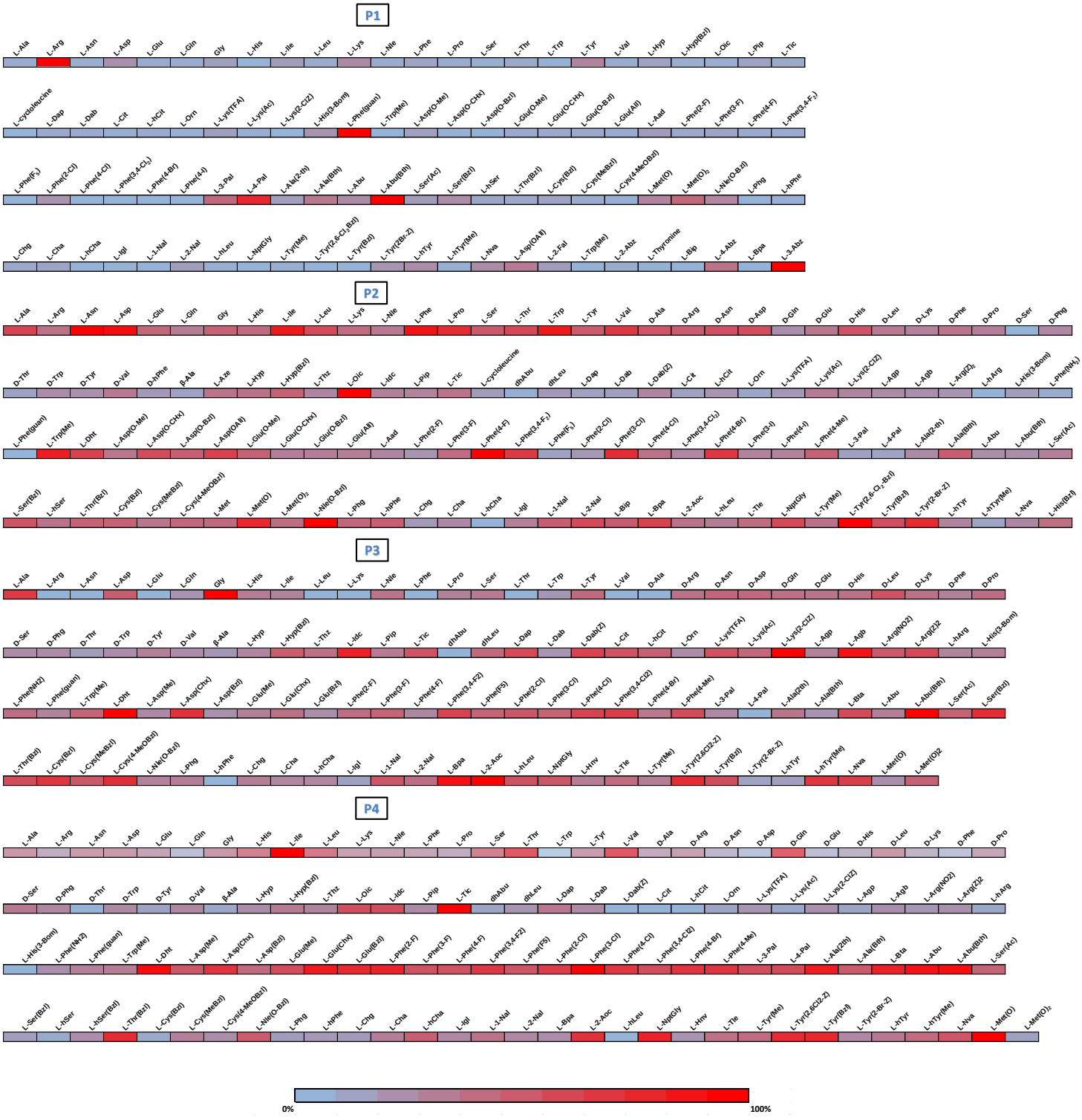
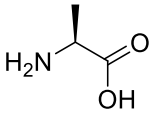
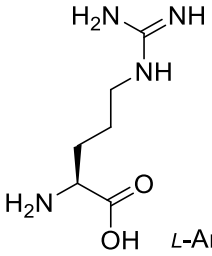
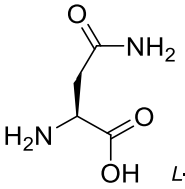
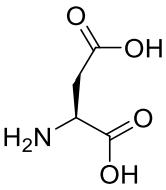
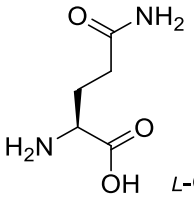
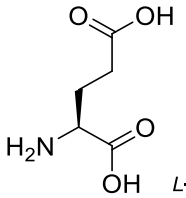
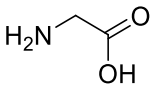
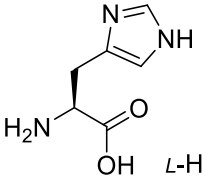
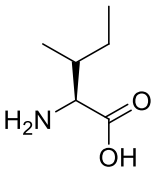
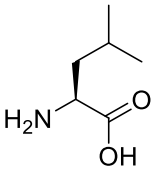
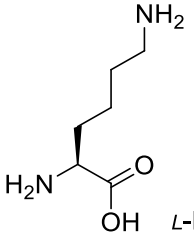
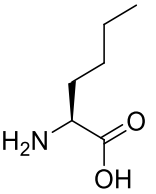
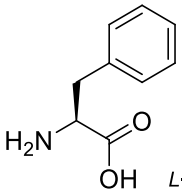
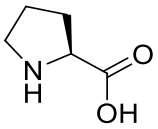
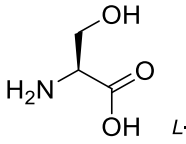
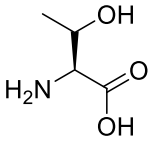
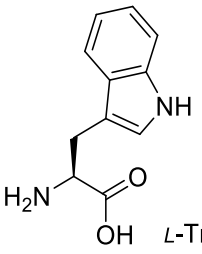
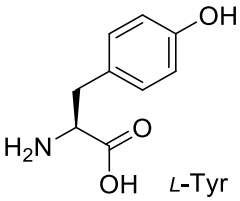
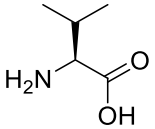
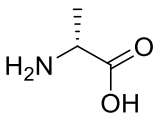
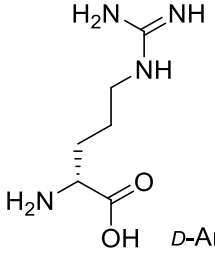
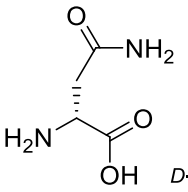
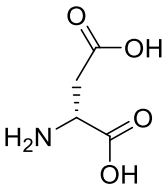
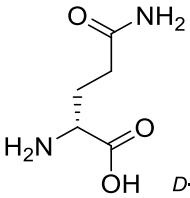


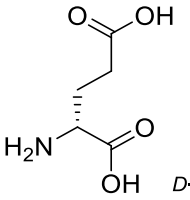
Table S2. Structures of natural and unnatural amino acids used in P1-Arg HyCoSuL library and Ac-ISPX-ACC library.

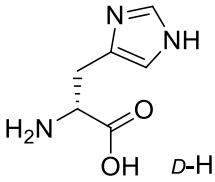
Lp	Structure + code	Lp	Structure + code	Lp	Structure + code
1	 *L*-Ala	2	 *L*-Arg	3	 *L*-Asn
4	 *L*-Asp	5	 *L*-Gln	6	 *L*-Glu
7	 Gly	8	 *L*-His	9	 *L*-Ile
10	 *L*-Leu	11	 *L*-Lys	12	 *L*-Nle
13	 *L*-Phe	14	 *L*-Pro	15	 *L*-Ser
16	 *L*-Thr	17	 *L*-Trp	18	 *L*-Tyr
19	 *L*-Val	20	 *D*-Ala	21	 *D*-Arg

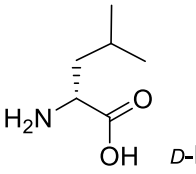
22  *D*-Asn

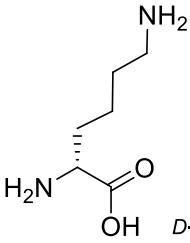
23  *D*-Asp

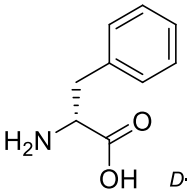
24  *D*-Gln

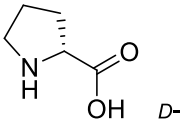
25  *D*-Glu

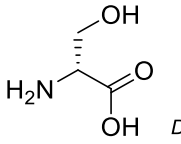
26  *D*-His

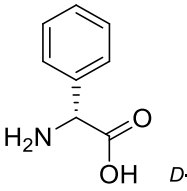
27  *D*-Leu

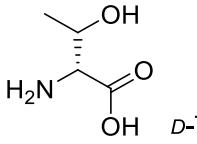
28  *D*-Lys

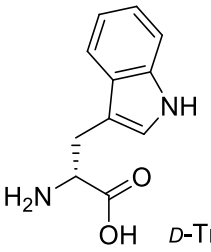
29  *D*-Phe

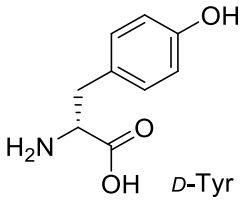
30  *D*-Pro

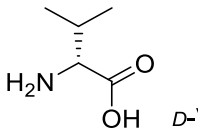
31  *D*-Ser

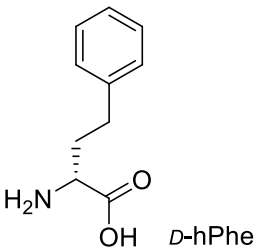
32  *D*-Phg

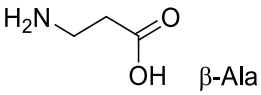
33  *D*-Thr

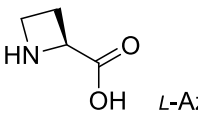
34  *D*-Trp

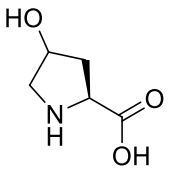
35  *D*-Tyr

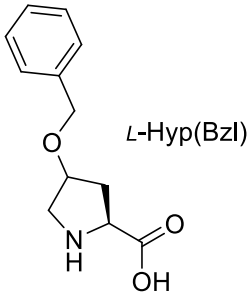
36  *D*-Val

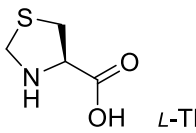
37  *D*-hPhe

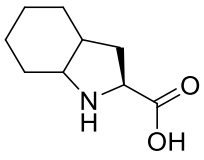
38  β-Ala

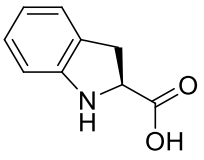
39  *L*-Aze

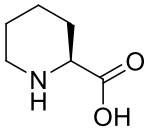
40  *L*-Hyp

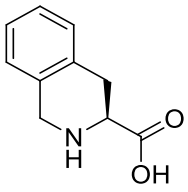
41  *L*-Hyp(Bzl)

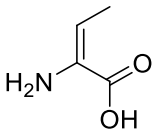
42  *L*-Thz

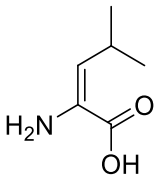
43  *L*-Oic

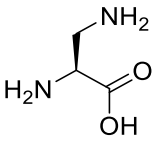
44  *L*-Idc

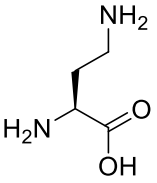
45  *L*-Pip

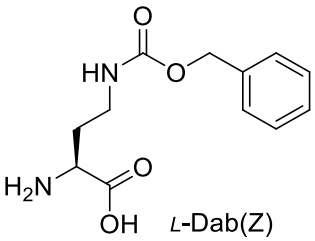
46  *L*-Tic

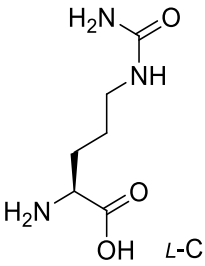
47  dhAbu

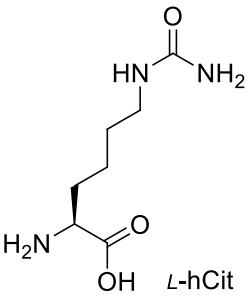
48  dhLeu

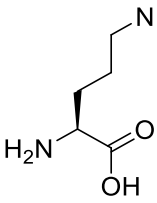
49  *L*-Dap

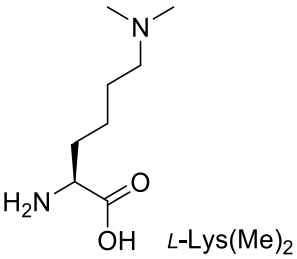
50  *L*-Dab

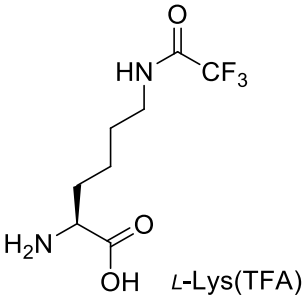
51  *L*-Dab(Z)

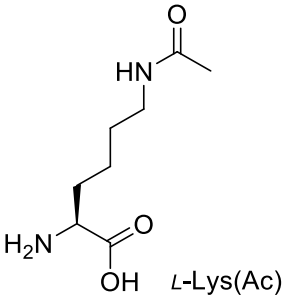
52  *L*-Cit

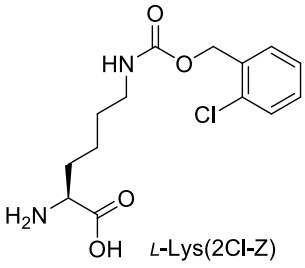
53  *L*-hCit

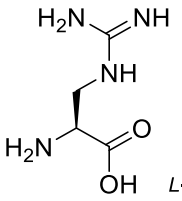
54  *L*-Orn

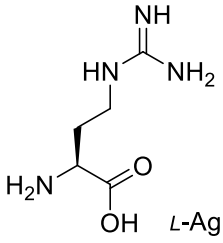
55  *L*-Lys(Me)$_2$

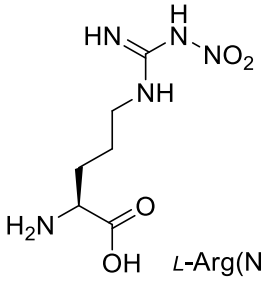
56  *L*-Lys(TFA)

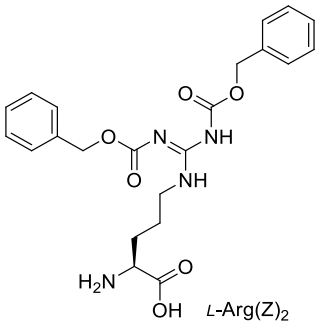
57  *L*-Lys(Ac)

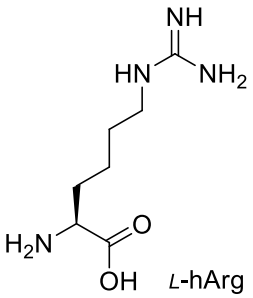
58  *L*-Lys(2Cl-Z)

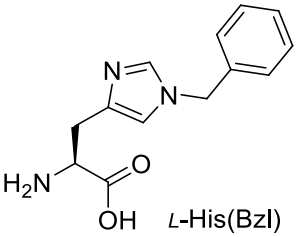
59  *L*-Agp

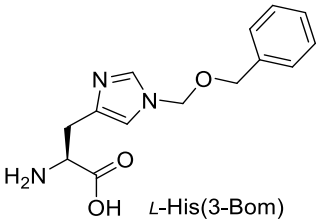
60  *L*-Agb

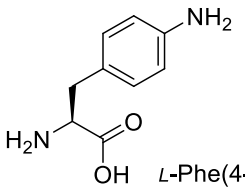
61  *L*-Arg(NO_2)

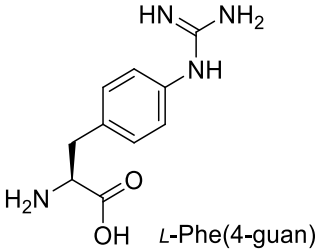
62  *L*-Arg(Z)$_2$

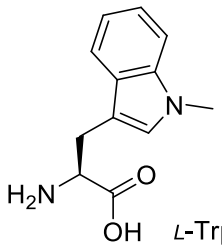
63  *L*-hArg

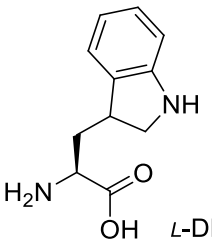
64  *L*-His(Bzl)

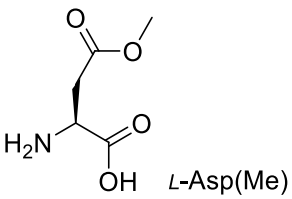
65  *L*-His(3-Bom)

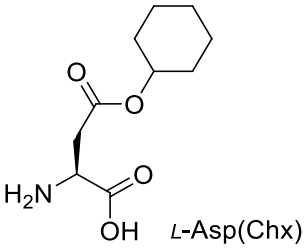
66  *L*-Phe(4-NH_2)

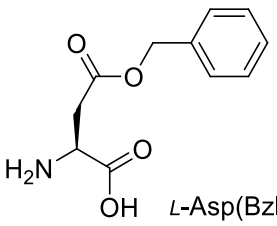
67  *L*-Phe(4-guan)

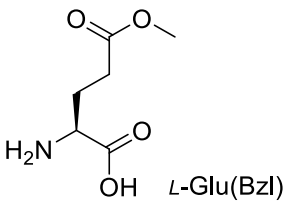
68  *L*-Trp(Me)

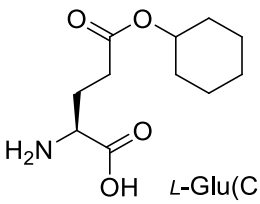
69  *L*-Dht

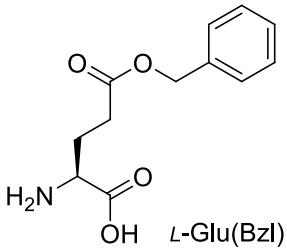
70  *L*-Asp(Me)

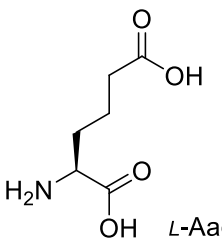
71  *L*-Asp(Chx)

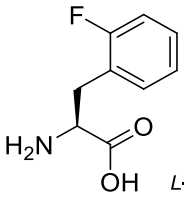
72  *L*-Asp(Bzl)

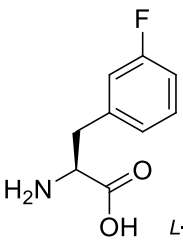
73  *L*-Glu(Bzl)

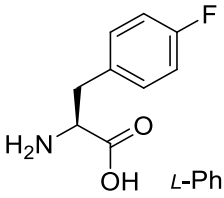
74  *L*-Glu(Chx)

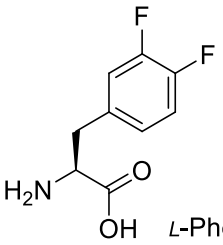
75  *L*-Glu(Bzl)

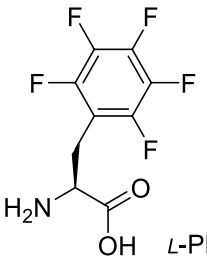
76  *L*-Aad

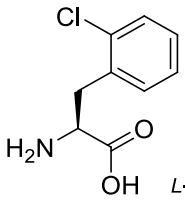
77  *L*-Phe(2-F)

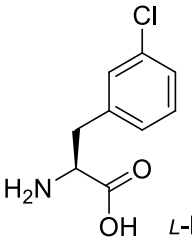
78  *L*-Phe(3-F)

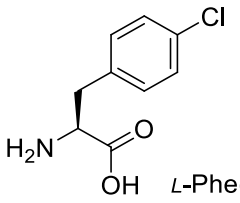
79  *L*-Phe(4-F)

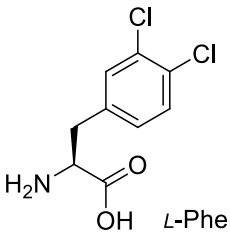
80  *L*-Phe(4,3-F_2)

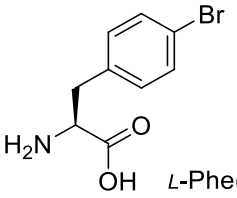
81  *L*-Phe(F_5)

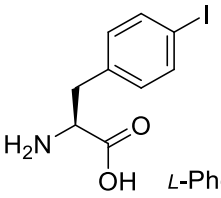
82  *L*-Phe(2-Cl)

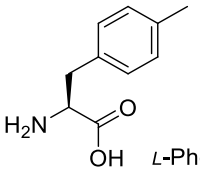
83  *L*-Phe(3-Cl)

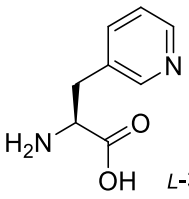
84  *L*-Phe(4-Cl)

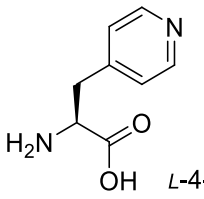
85  *L*-Phe(3,4-Cl_2)

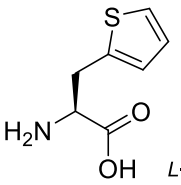
86  *L*-Phe(4-Br)

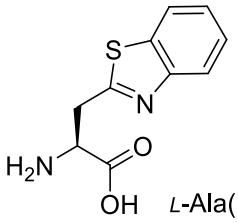
87  *L*-Phe(4-I)

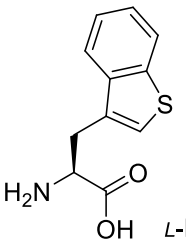
88  *L*-Phe(4-Me)

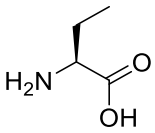
89  *L*-3-Pal

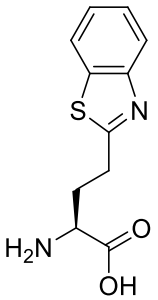
90  *L*-4-Pal

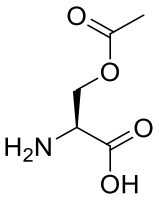
91  *L*-Ala(2-th)

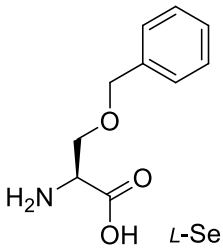
92  *L*-Ala(Bth)

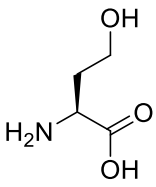
93  *L*-Bta

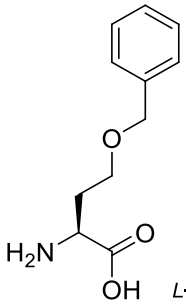
94  *L*-Abu

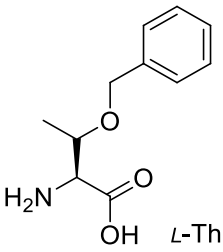
95  *L*-Abu(Bth)

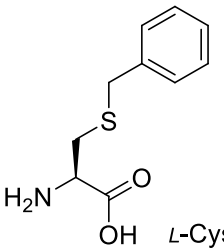
96  *L*-Ser(Ac)

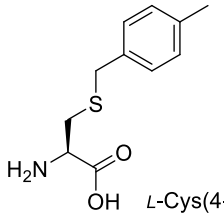
97  *L*-Ser(Bzl)

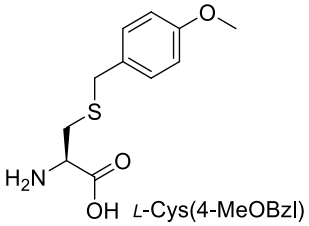
98  *L*-hSer

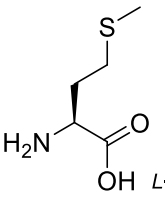
99  *L*-hSer(Bzl)

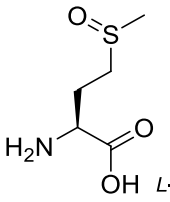
100  *L*-Thr(Bzl)

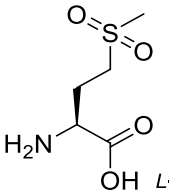
101  *L*-Cys(Bzl)

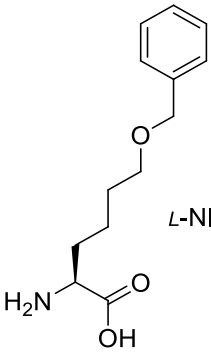
102  *L*-Cys(4-MeBzl)

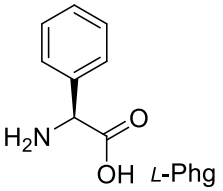
103  *L*-Cys(4-MeOBzl)

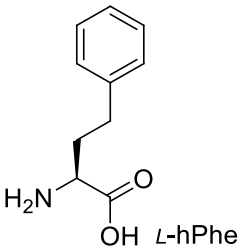
104  *L*-Met

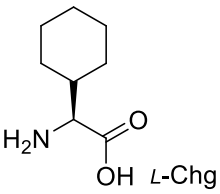
105  *L*-Met(O)

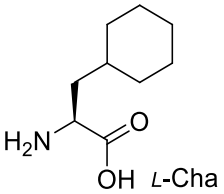
106  *L*-Met(O_2)

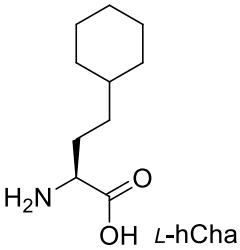
107  *L*-Nle(*O*-Bzl)

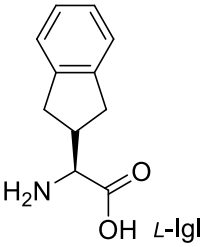
108  *L*-Phg

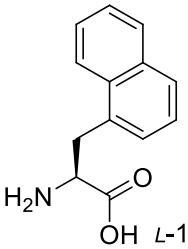
109  *L*-hPhe

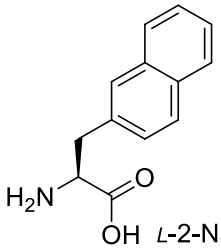
110  *L*-Chg

111  *L*-Cha

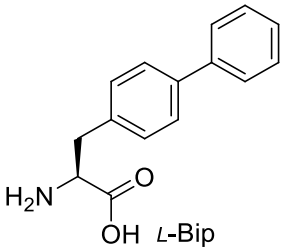
112  *L*-hCha

113  *L*-Igl

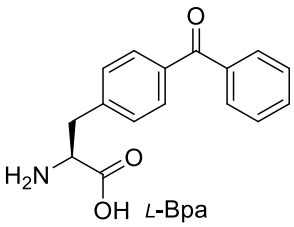
114  *L*-1-Nal

115 

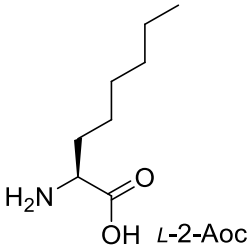
L-2-Nal

116 

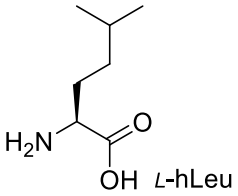
L-Bip

117 

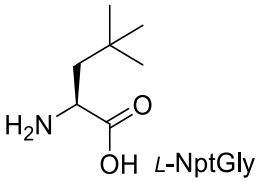
L-Bpa

118 

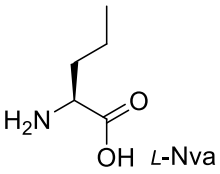
L-2-Aoc

119 

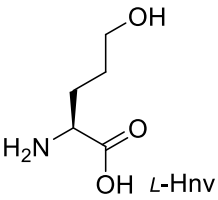
L-hLeu

120 

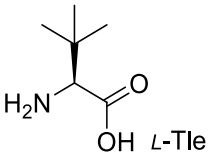
L-NptGly

121 

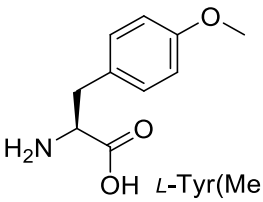
L-Nva

122 

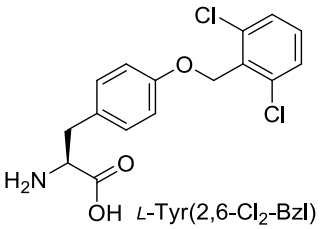
L-Hnv

123 

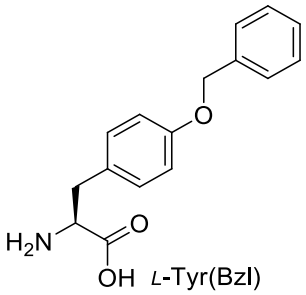
L-Tle

124 

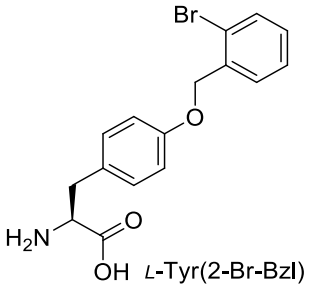
L-Tyr(Me)

125 

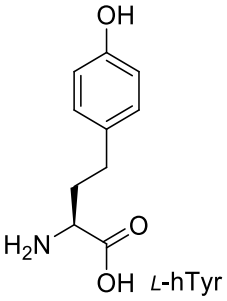
L-Tyr(2,6-Cl_2-Bzl)

126 

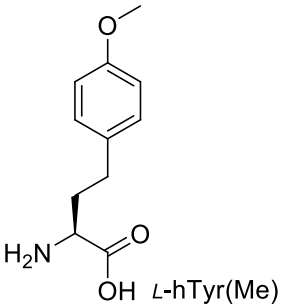
L-Tyr(Bzl)

127 

L-Tyr(2-Br-Bzl)

128 

L-hTyr

129 

L-hTyr(Me)

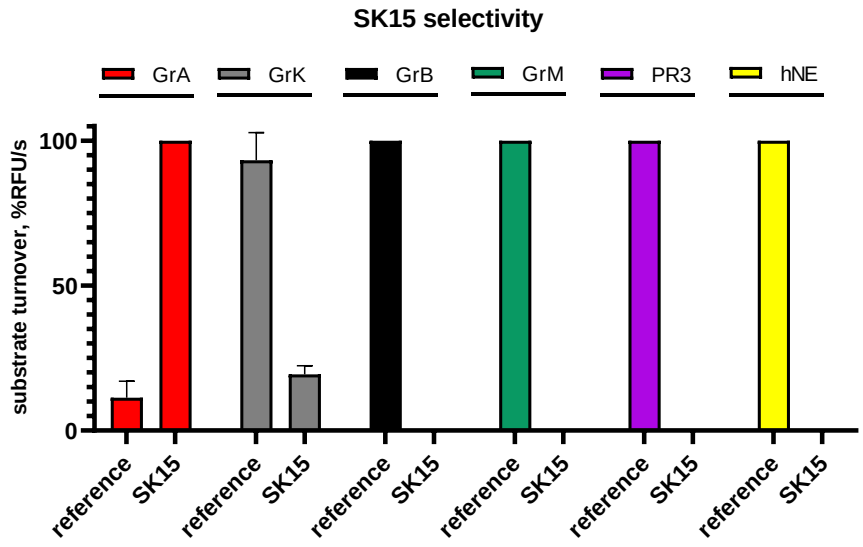


Figure S2. Selectivity of SK15 substrate toward selected serine proteases (GrA, GrB, GrM, GrK, PR3, and hNE). The reference substrates were as follows: Ac-Ile-Gly-Pro-Arg-ACC for GrA, Ac-Tyr-Arg-Phe-Lys-ACC for GrK, Ac-Ile-Glu-Pro-Asp-ACC for GrB, Ac-Lys-Val-Pro-Leu-ACC for GrM, Ac-Glu(O-Bzl)-Lys(Ac)-Hyp(Bzl)-Nva-ACC for PR3 and Ac-Arg$(NO)_2$-Glu(Bzl)-Oic-Abu-ACC for hNE. Substrates were placed in a 96-well plate at a concentration of 67 µM and treated with optimal concentration of each enzyme (89 nM of GrA, 488 nM of GrK, 18 nM of GrB, 74 nM of GrM, 3.3 nM of hNE and 3.4 nM of PR3). The fluorescence increase was measured with 355 nm/460 nm excitation and emission wavelengths. Data present the average of 2 replicates as adjusted RFU/s.

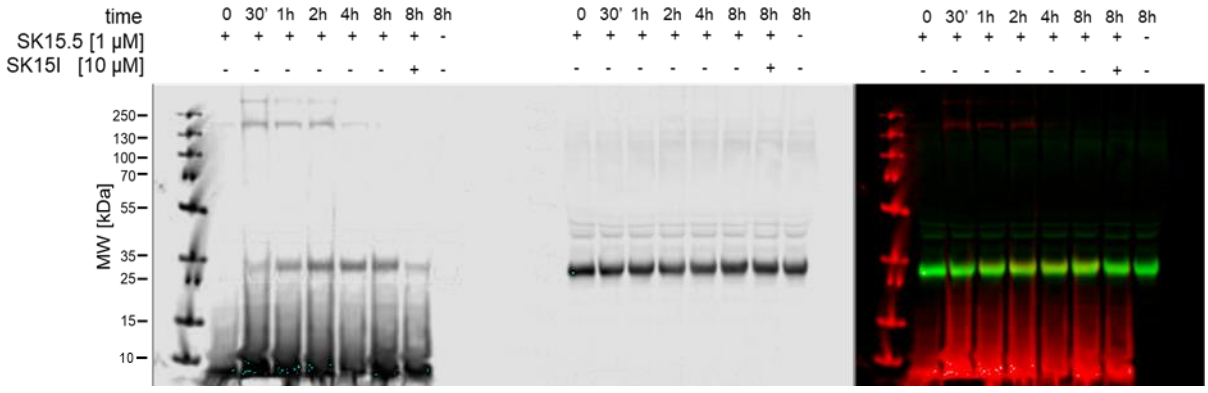


Figure S3. Detection of active GrA in NK92 cells.

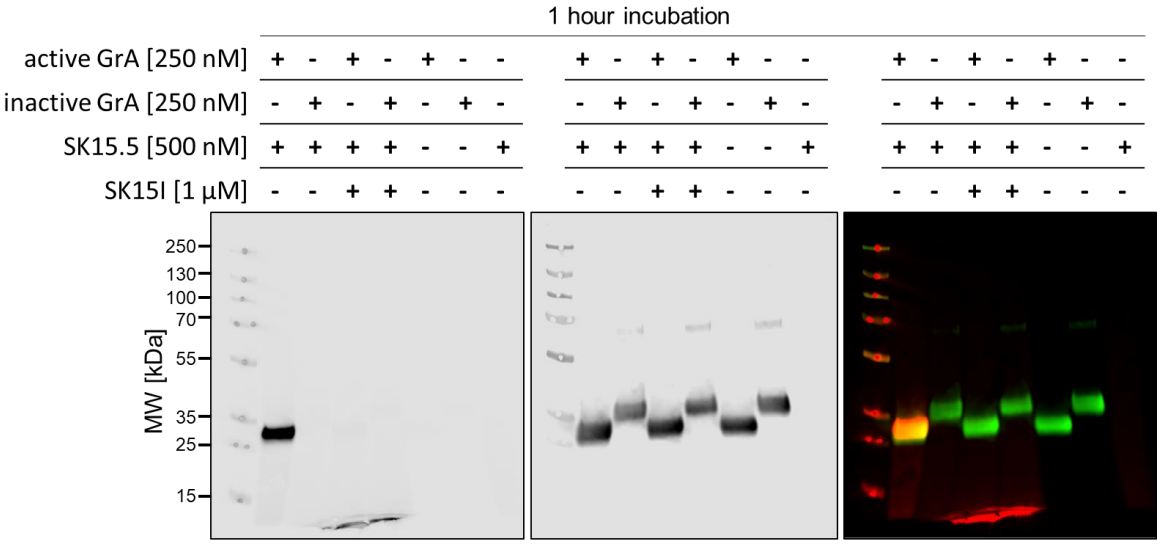


Figure S4. SK15.5 binds exclusively with the active GrA.