

## Electronic Supplementary Information

# Replacing piperidine in Solid Phase Peptide Synthesis: effective Fmoc removal by alternative bases

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## Deprotection kinetic tests

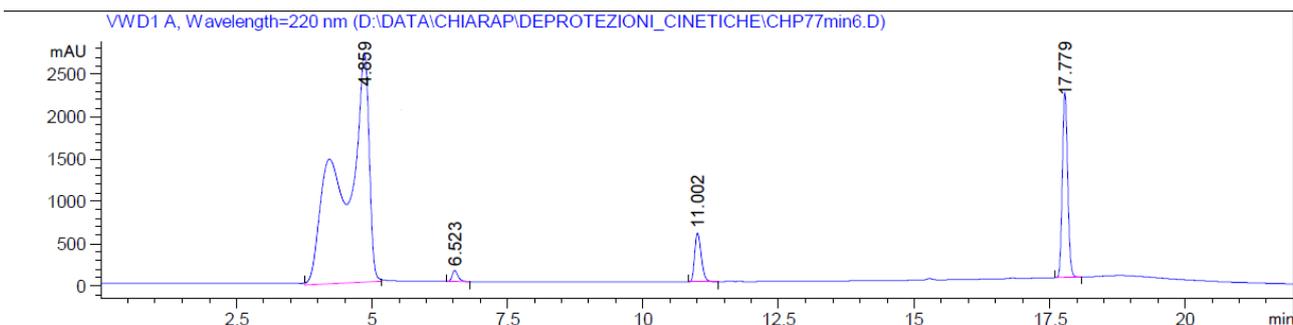
Selected examples of deprotection kinetic tests in all investigated pyrrolidones and bases are reported below. Only times (in minutes) revealing complete Fmoc removal were chosen.

**Figure S1.** Chromatogram of Fmoc-Phe-OH deprotection in DMF at t=0 (before base addition)



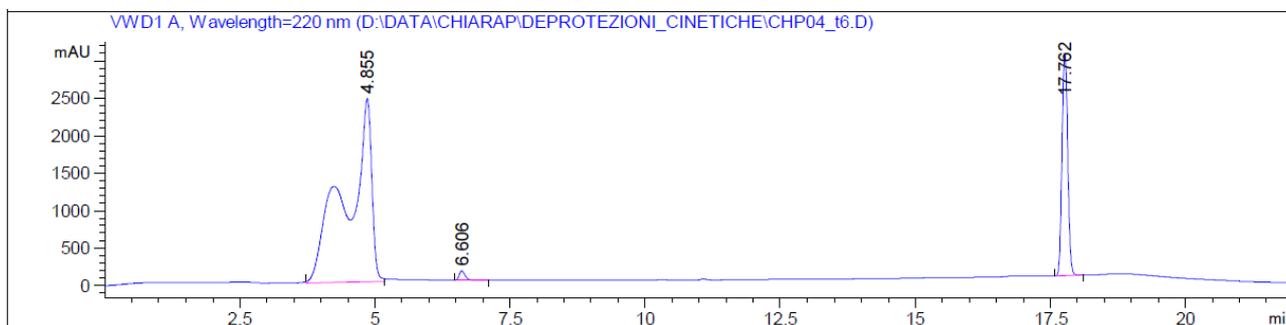
Legend: DMF = peak at 4.847 min; Fmoc-Phe-OH = peak at 15.248 min.

**Figure S2.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 5% piperidine at t=6 minutes



Legend: DMF = peak at 4.859 min; H-Phe-OH = peak at 6.523 min; DBF-piperidine adduct = peak at 11.002 min; dibenzofulvene (DBF) = peak at 17.779 min.

**Figure S3.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 20% TBA at t=6 minutes



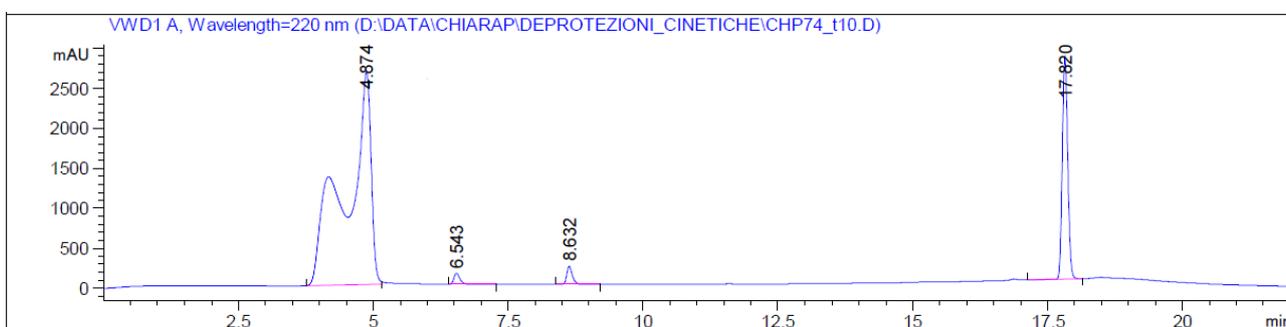
Legend: DMF = peak at 4.855 min; H-Phe-OH = peak at 6.606 min; dibenzofulvene (DBF) = peak at 17.762 min.

**Figure S4.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 5% TMG at t=8 minutes



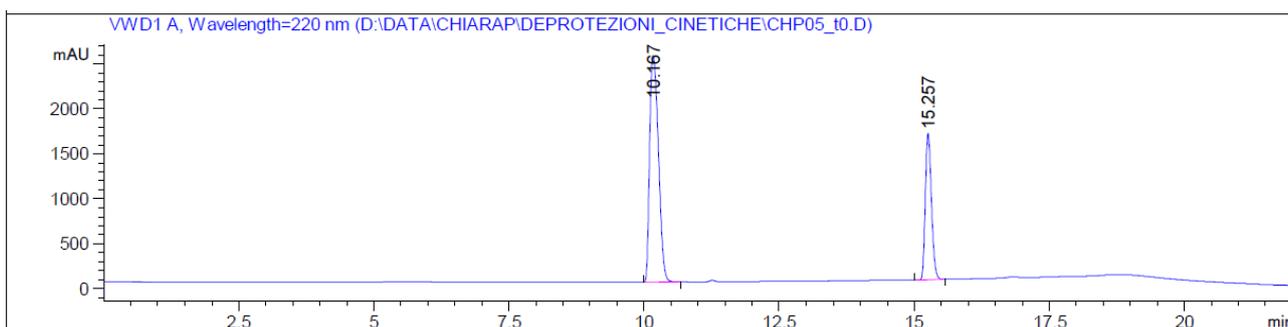
Legend: DMF = peak at 4.865 min; TMG = peak at 5.116 min; H-Phe-OH = peak at 6.531 min; dibenzofulvene (DBF) = peak at 17.797 min.

**Figure S5.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 10% DEAPA at t=10 minutes



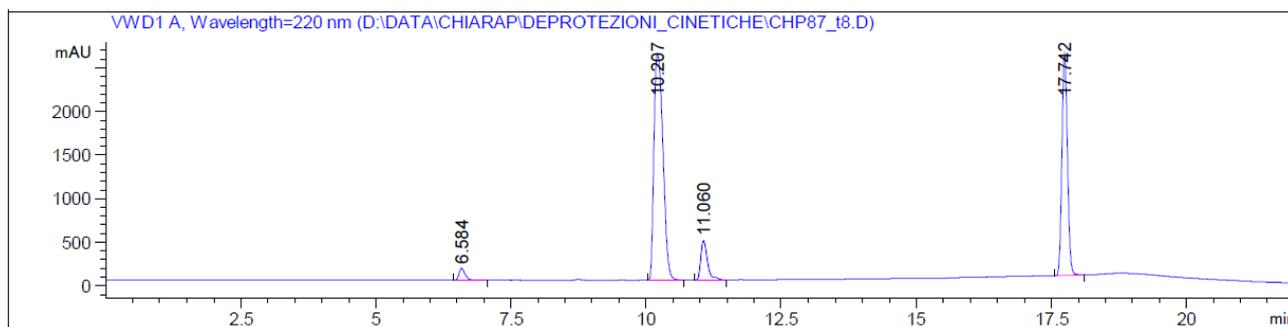
Legend: DMF = peak at 4.874 min; H-Phe-OH = peak at 6.543 min; DBF-DEAPA adduct = peak at 8.632 min; dibenzofulvene (DBF) = peak at 17.820 min.

**Figure S6.** Chromatogram of Fmoc-Phe-OH deprotection in NBP at t=0 (before base addition)



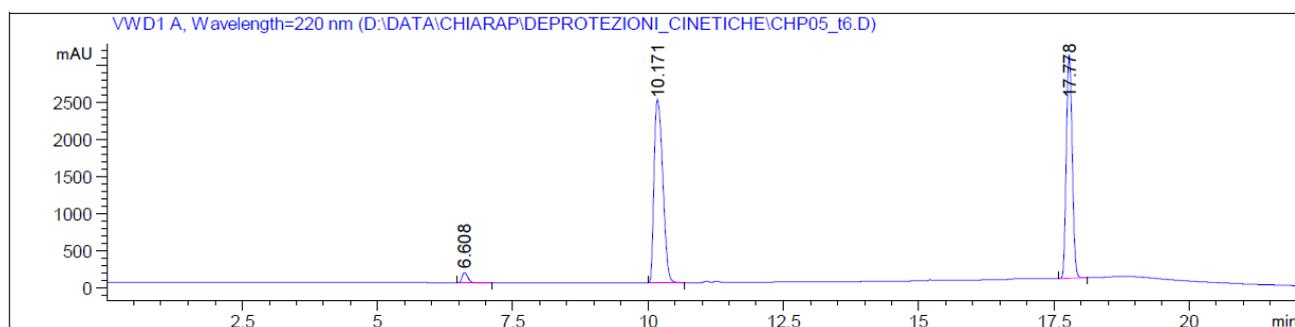
Legend: NBP = peak at 10.167 min; Fmoc-Phe-OH = peak at 15.257 min.

**Figure S7.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 5% piperidine at t=8 minutes



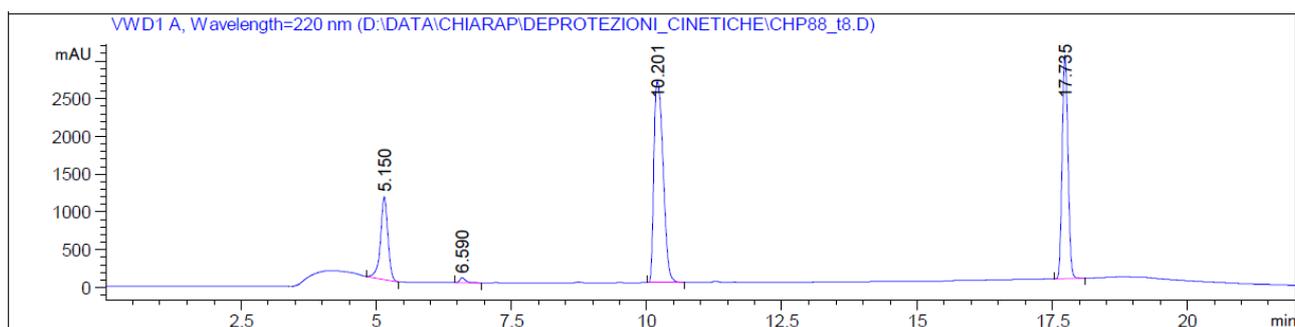
*Legend: H-Phe-OH = peak at 6.584 min; NBP = peak at 10.207 min; DBF-piperidine adduct = peak at 11.060 min; dibenzofulvene (DBF) = peak at 17.742 min.*

**Figure S8.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 20% TBA at t=6 minutes



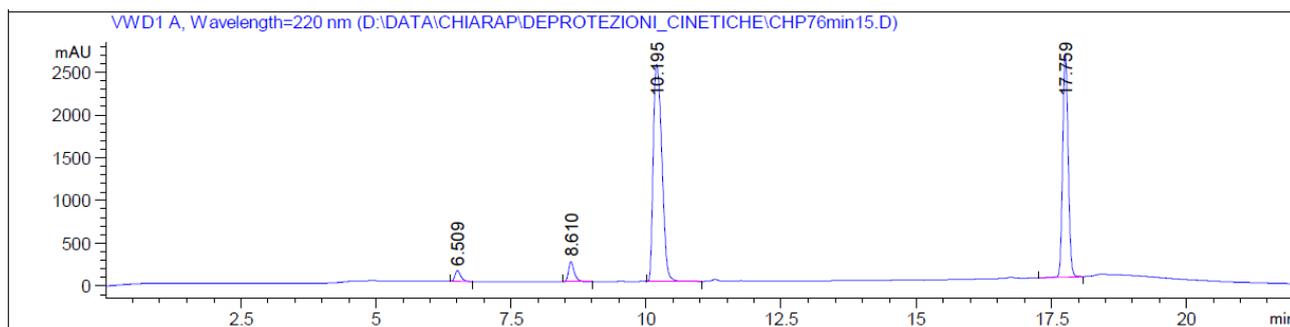
*Legend: H-Phe-OH = peak at 6.608 min; NBP = peak at 10.171 min; dibenzofulvene (DBF) = peak at 17.778 min.*

**Figure S9.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 5% TMG at t=8 minutes



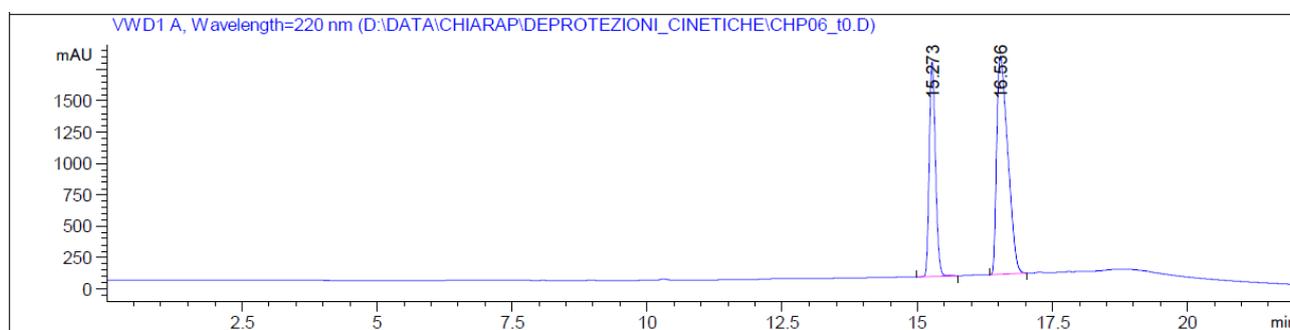
*Legend: TMG = peak at 5.150 min; H-Phe-OH = peak at 6.590 min; NBP = peak at 10.201 min; dibenzofulvene (DBF) = peak at 17.735 min.*

**Figure S10.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 10% DEAPA at t=15 minutes



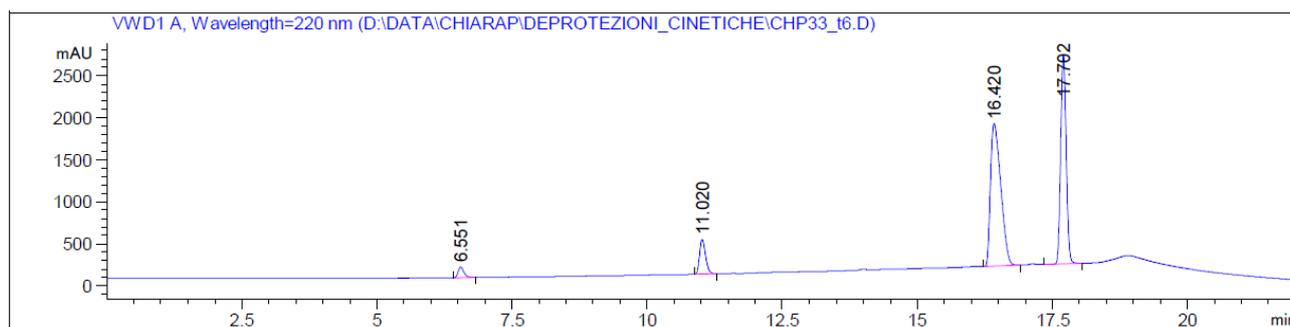
*Legend: H-Phe-OH = peak at 6.509 min; DBF-DEAPA adduct = peak at 8.610 min; NBP = peak at 10.195 min; dibenzofulvene (DBF) = peak at 17.759 min.*

**Figure S11.** Chromatogram of Fmoc-Phe-OH deprotection in NOP at t=0 (before base addition)



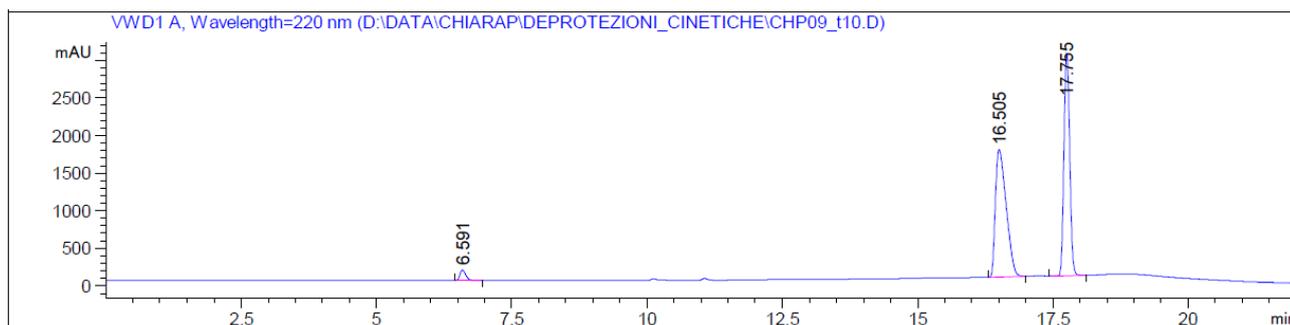
*Legend: Fmoc-Phe-OH = peak at 15.273 min; NOP = peak at 16.536 min.*

**Figure S12.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 5% piperidine at t=6 minutes



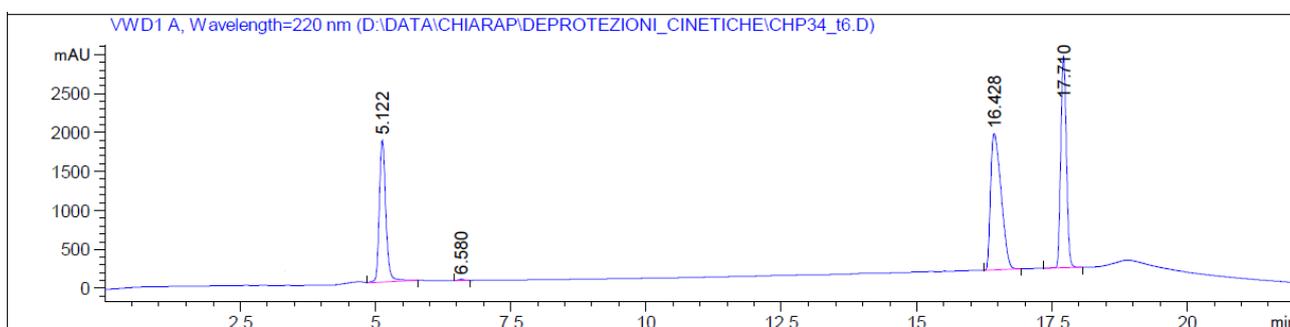
*Legend: H-Phe-OH = peak at 6.551 min; DBF-piperidine adduct = peak at 11.020 min; NOP = peak at 16.420 min; dibenzofulvene (DBF) = peak at 17.702 min.*

**Figure S13.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 20% TBA at t=10 minutes



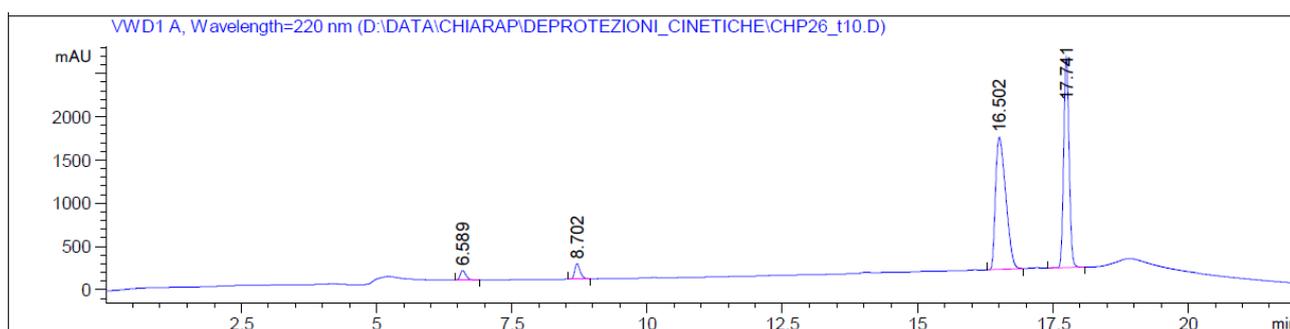
*Legend: H-Phe-OH = peak at 6.591 min; NOP = peak at 16.505 min; dibenzofulvene (DBF) = peak at 17.755 min.*

**Figure S14.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 5% TMG at t=6 minutes



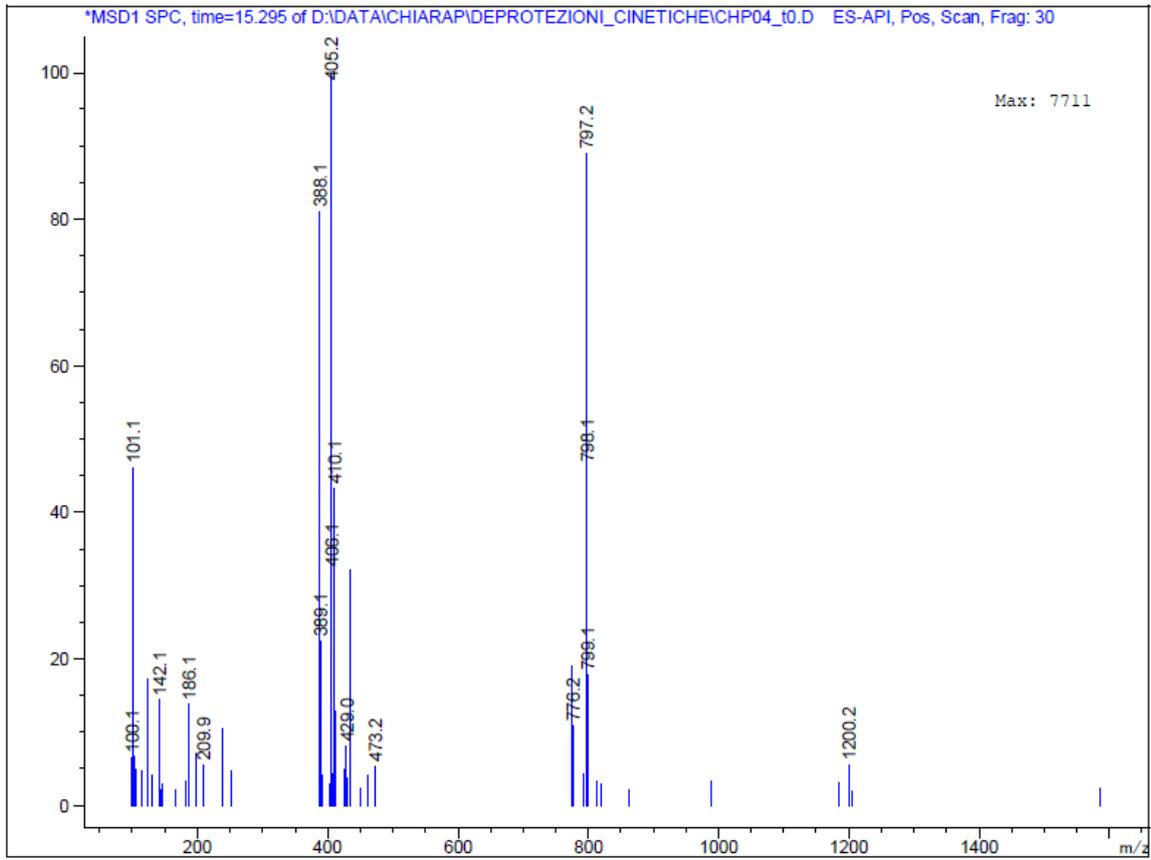
*Legend: TMG = peak at 5.122 min; H-Phe-OH = peak at 6.580 min; NOP = peak at 16.428 min; dibenzofulvene (DBF) = peak at 17.710 min.*

**Figure S15.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 10% DEAPA at t=10 minutes

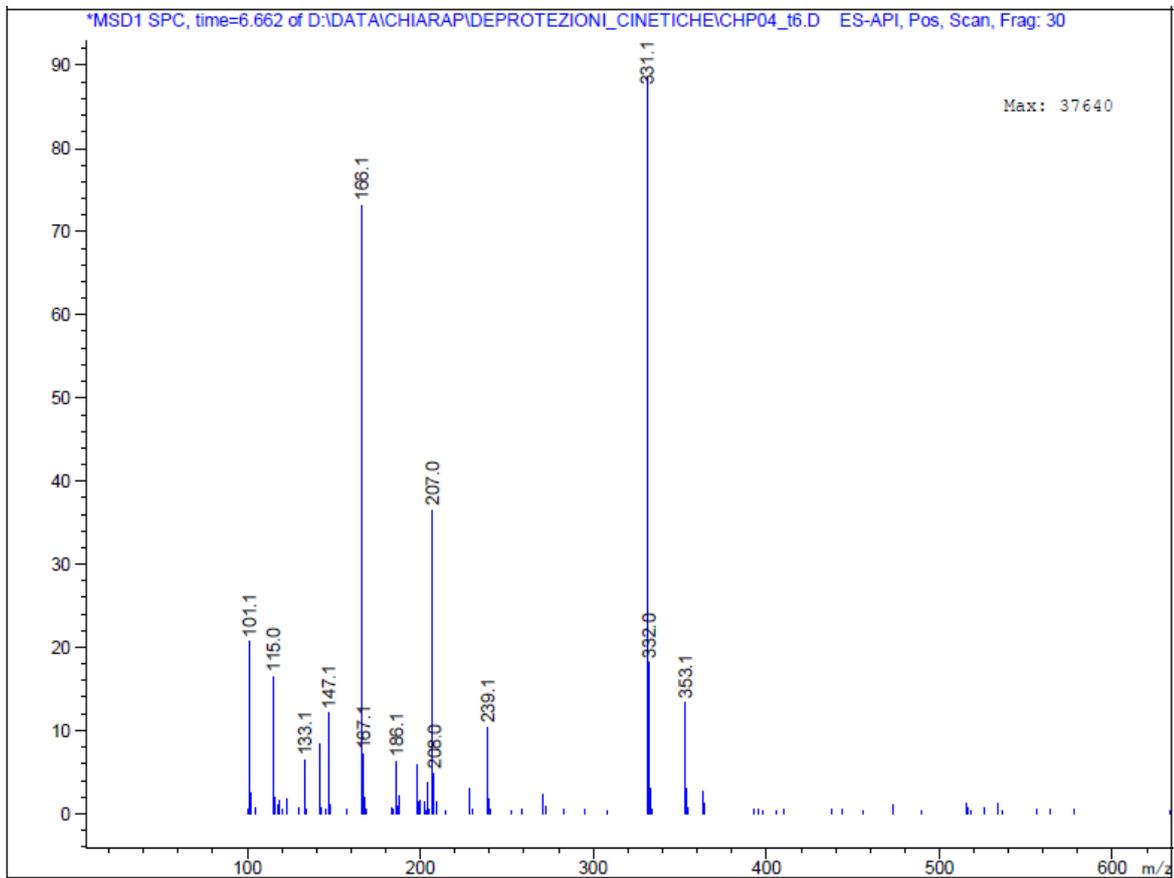


*Legend: H-Phe-OH = peak at 6.589 min; DBF-DEAPA adduct = peak at 8.702 min; NOP = peak at 16.502 min; dibenzofulvene (DBF) = peak at 17.741 min.*

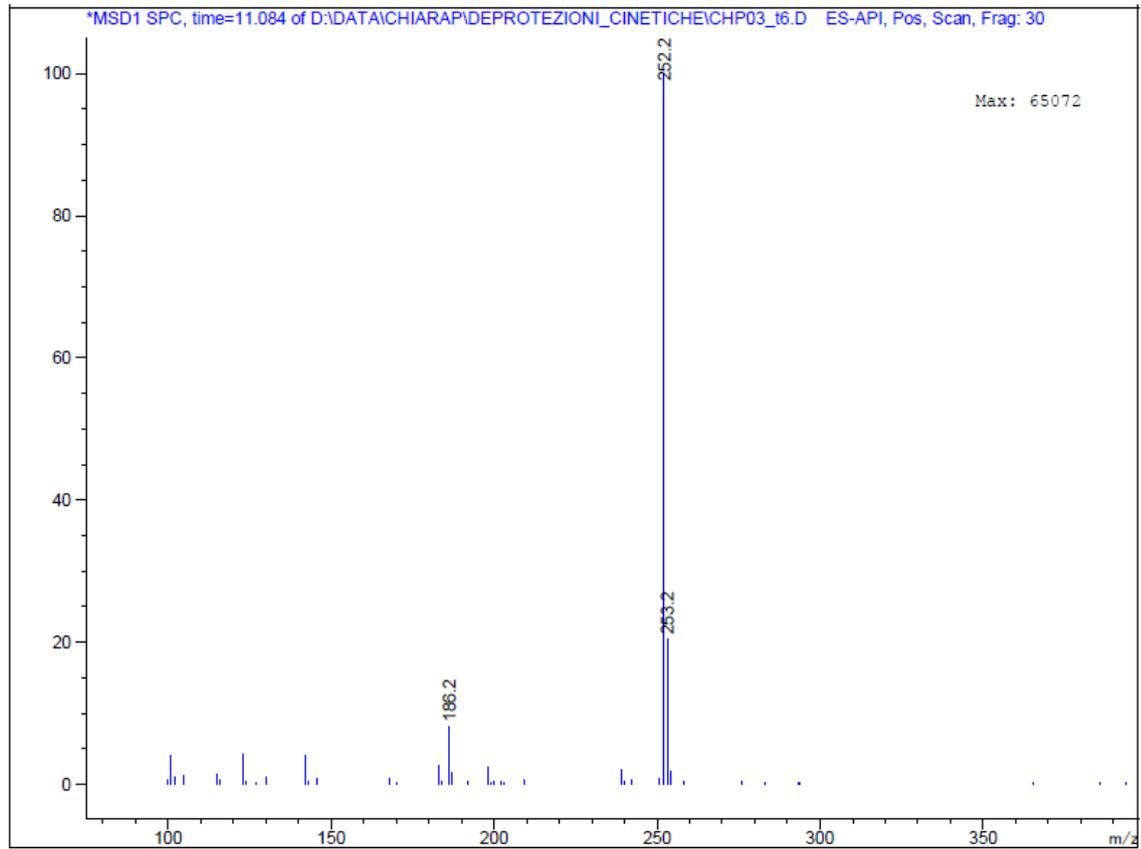
**Figure S16.** Mass spectrum of Fmoc-Phe-OH



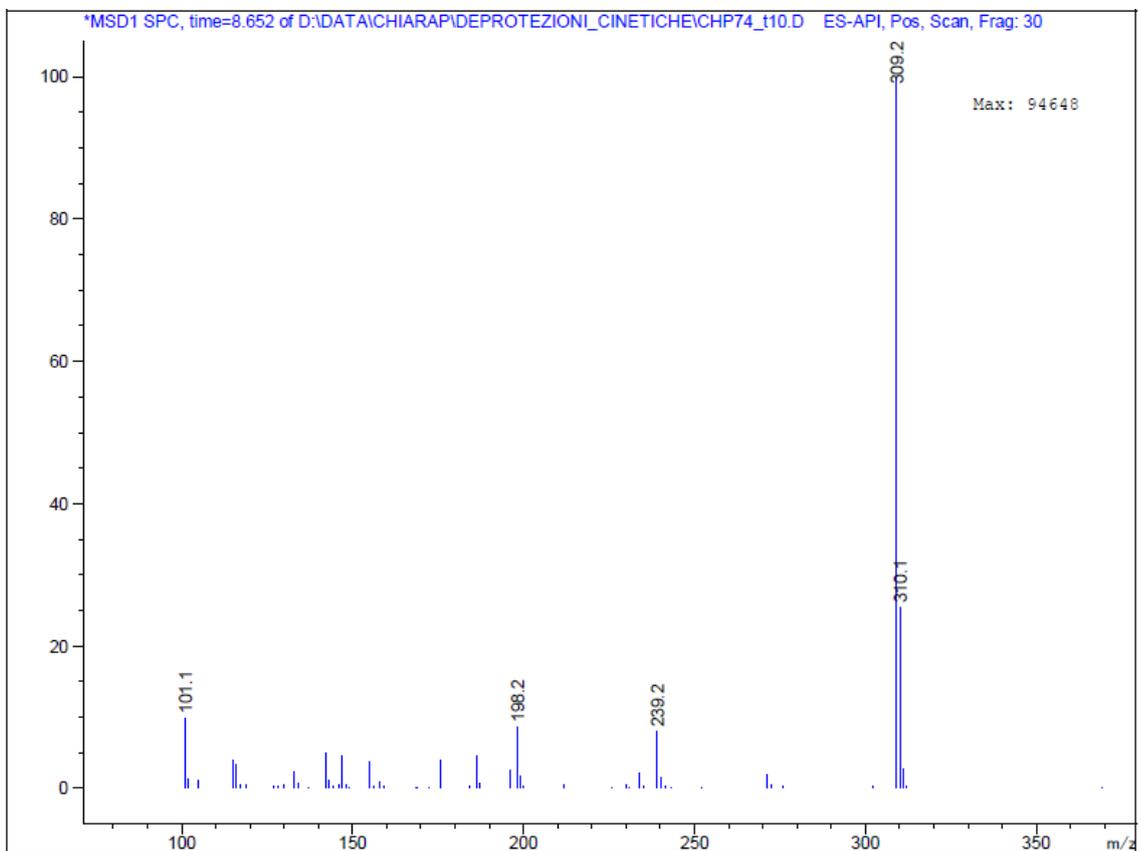
**Figure S17.** Mass spectrum of H-Phe-OH



**Figure S18.** Mass spectrum of DBF-piperidine adduct

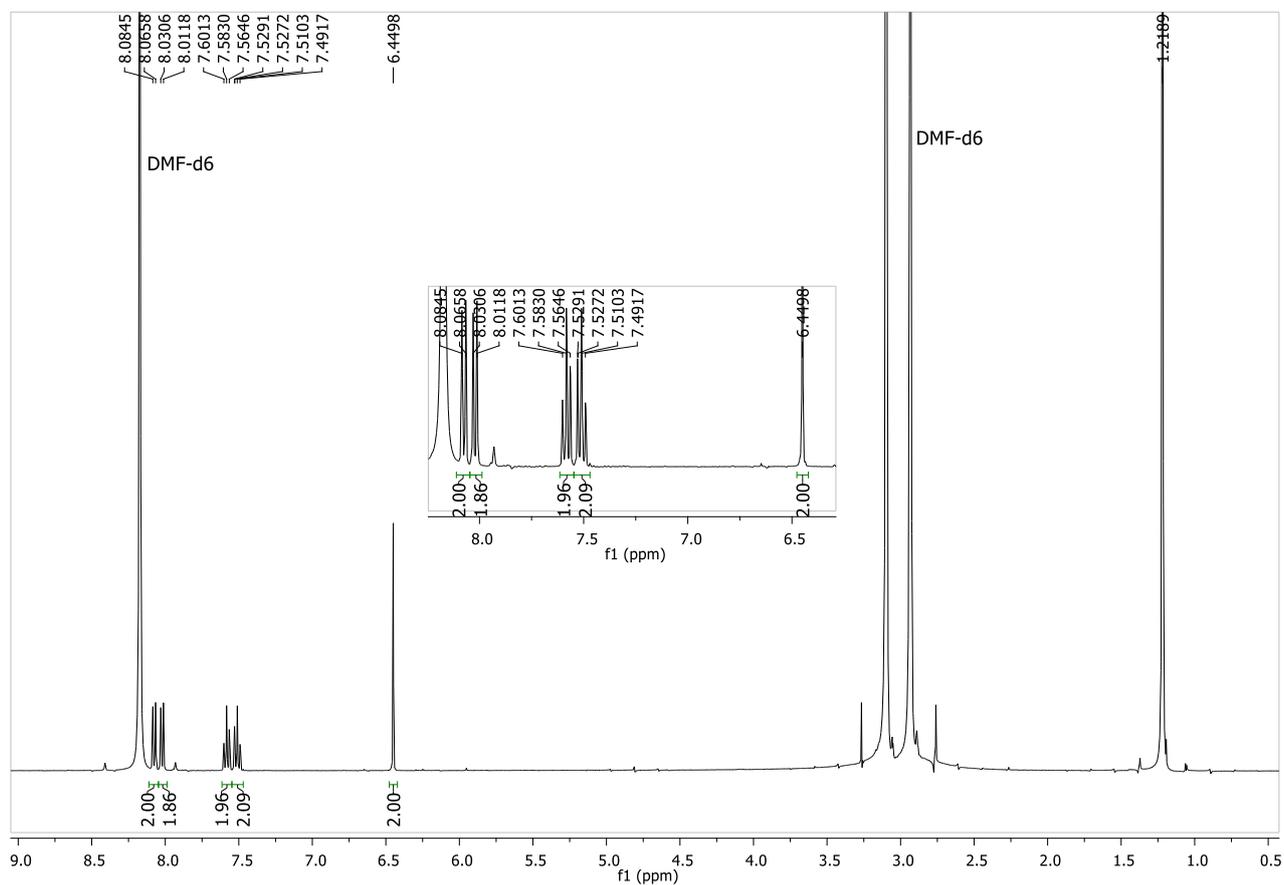


**Figure S19.** Mass spectrum of DBF-DEAPA adduct

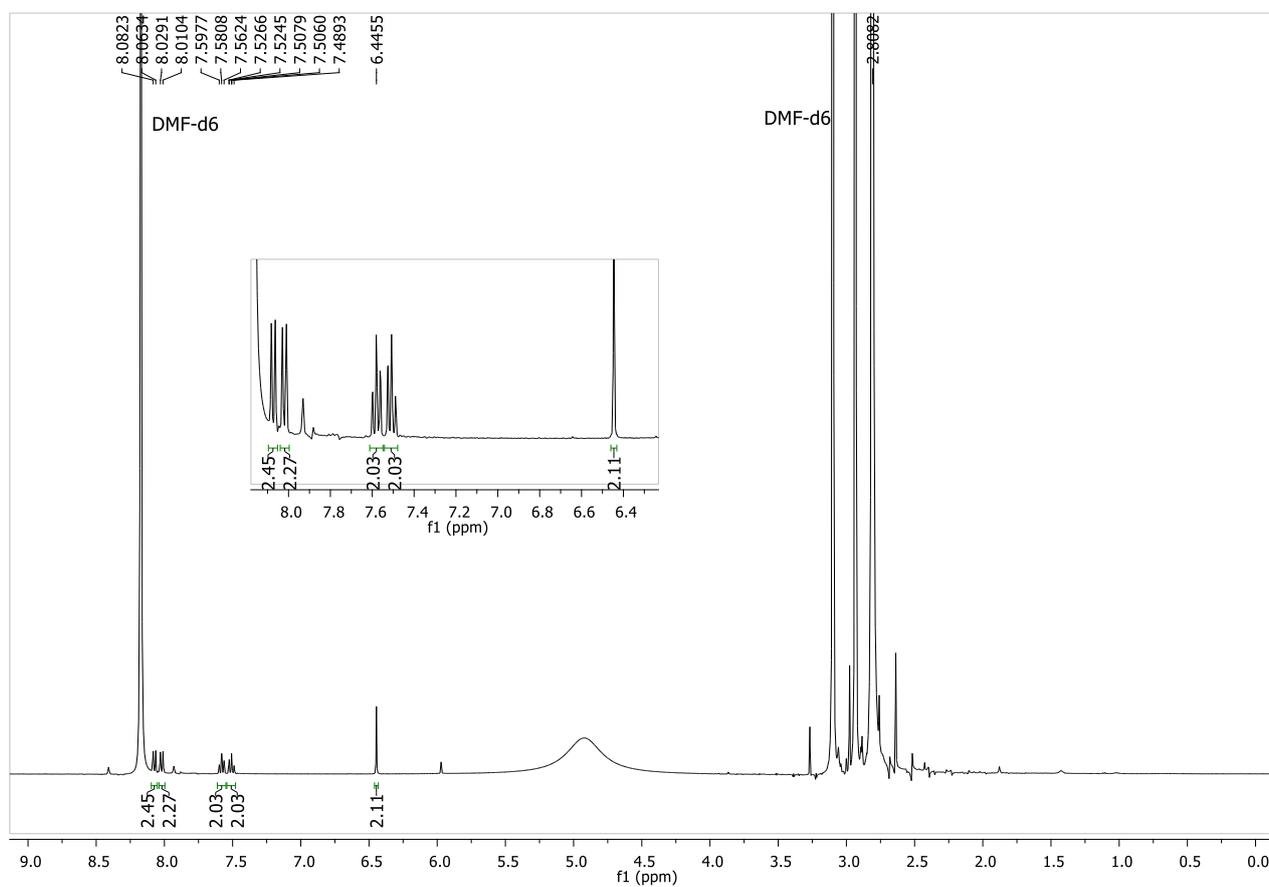


## Deprotection mechanism

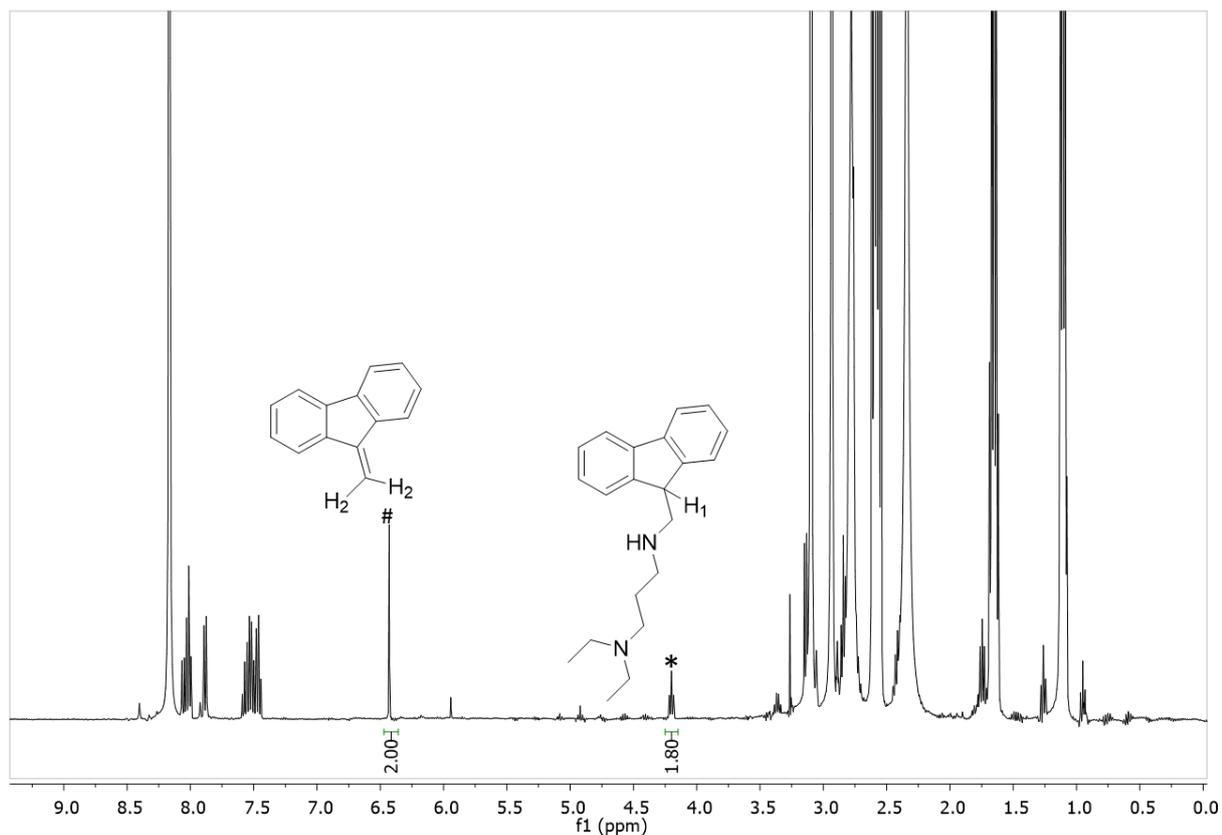
**Figure S20.**  $^1\text{H}$  NMR spectrum (400 MHz, DMF-d<sub>6</sub>) after deprotection with 20% TBA in DMF-d<sub>6</sub> of Fmoc-Gly-Trt-PS resin; the peak at 1.21 ppm corresponds to *t*Bu group of *t*BuNH<sub>2</sub>; the peaks at 6.45 ppm and aromatic signals between 7.5 and 8.5 ppm correspond to DBF



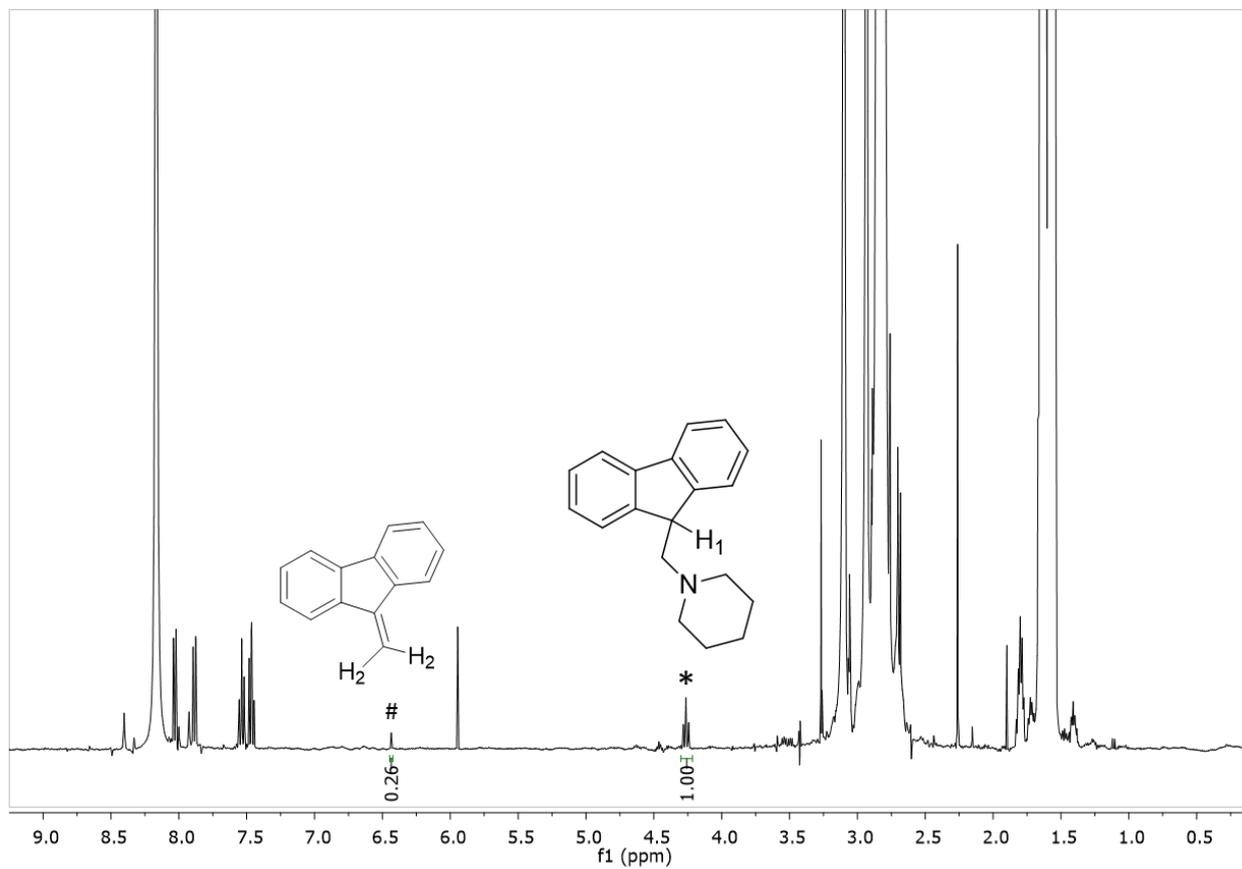
**Figure S21.**  $^1\text{H}$  NMR spectrum (400 MHz, DMF-d<sub>6</sub>) after deprotection with 20% TMG in DMF-d<sub>6</sub> of Fmoc-Gly-Trt-PS resin; peak at 2.81 ppm corresponds to CH<sub>3</sub> groups of TMG; peaks at 6.45 ppm and aromatic signals between 7.5 and 8.5 ppm correspond to DBF



**Figure S22.**  $^1\text{H}$  NMR spectrum (400 MHz, DMF- $d_6$ ) after deprotection with 20% DEAPA in DMF- $d_6$  of Fmoc-Gly-Trt-PS resin; the peak indicated with \* corresponds to  $\text{H}_1$  signal of DBF-DEAPA adduct; the peak indicated with # corresponds to  $\text{H}_2$  signals of DBF; signals between 7.5 and 8.5 ppm are referred to a mixture of DBF and DBF-DEAPA aromatic protons. Calculated ratio of DBF/DBF-DEAPA adduct is 1/1.8.



**Figure S23.**  $^1\text{H}$  NMR spectrum (400 MHz, DMF-d<sub>6</sub>) after deprotection with 20% piperidine in DMF-d<sub>6</sub> of Fmoc-Gly-Trt-PS resin; the peak indicated with \* corresponds to H<sub>1</sub> signal of DBF-piperidine adduct; the peak indicated with # corresponds to H<sub>2</sub> signals of DBF; signals between 7.5 and 8.5 ppm are referred to a mixture of DBF and DBF-piperidine aromatic protons. Calculated ratio of DBF/DBF-piperidine adduct is 1/7.7.

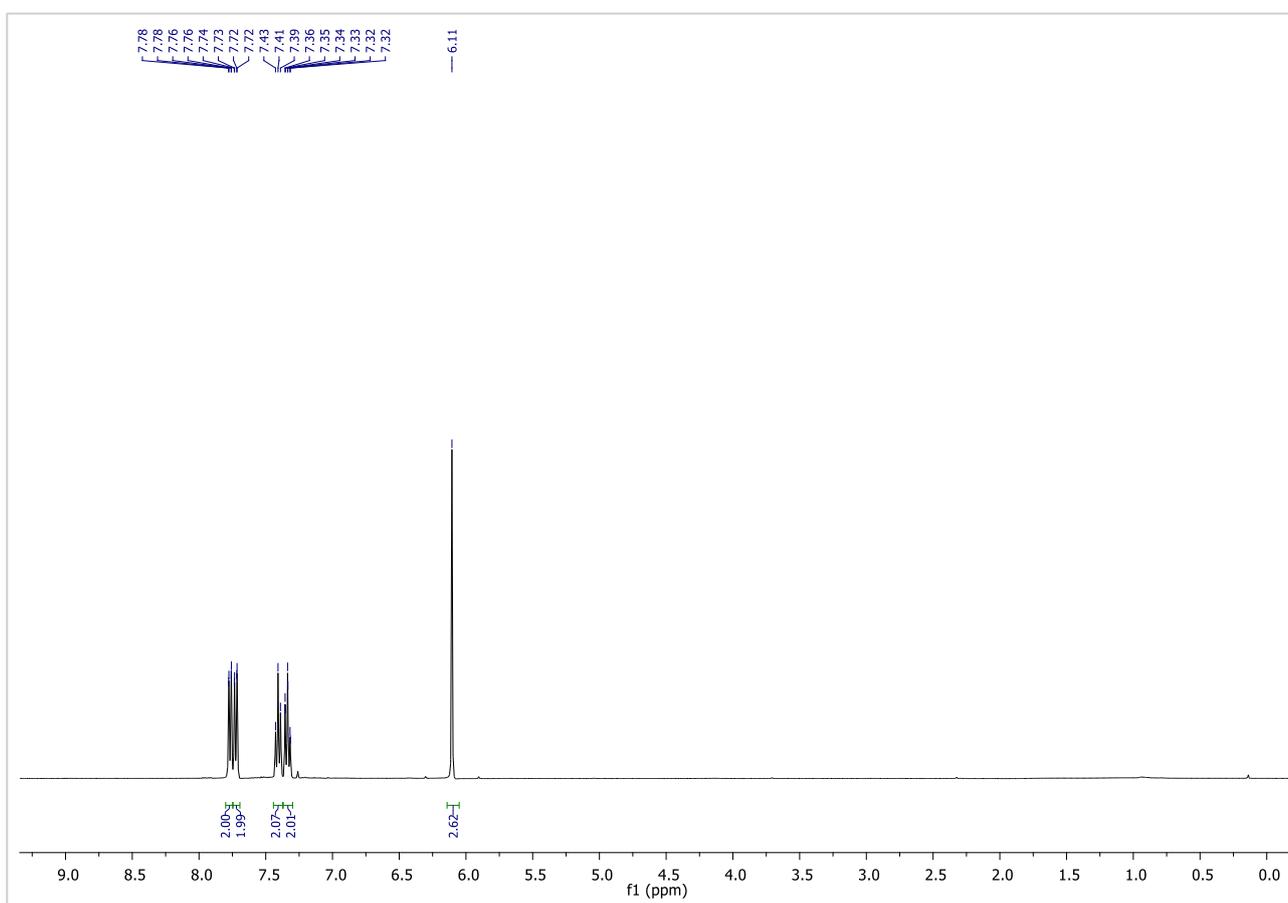


## Relative response factor (RRF) calculation

Relative Response Factor between dibenzofulvene (DBF) and DBF-base adduct (piperidine or DEAPA) was estimated calculating RRF between dibenzofulvene and 9-fluorenemethanol (9-FM) by HPLC-UV analysis. 9-fluorenemethanol was purchased from Merck and used as such; dibenzofulvene was obtained from deprotection of Fmoc-Phe-OH with *tert*-butylamine (30% in THF) followed by solvent evaporation (at reaction completion), acidic aqueous work-up, extraction with DCM and isolation of the organic fractions, which were purified by column chromatography (cyclohexane 100%). Purity of isolated dibenzofulvene was assessed by HPLC and  $^1\text{H}$  NMR as >95%; DBF was used immediately for RRF calculation.

**Figure S24.**  $^1\text{H}$  NMR spectrum of isolated dibenzofulvene

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 7.77 (d,  $J = 7.5$ , 2H), 7.73 (d,  $J = 7.5$ , 2H), 7.41 (t,  $J = 7.5$  Hz, 2H), 7.34 (t,  $J = 7.5$ , 2H), 6.11 (s, 2H).

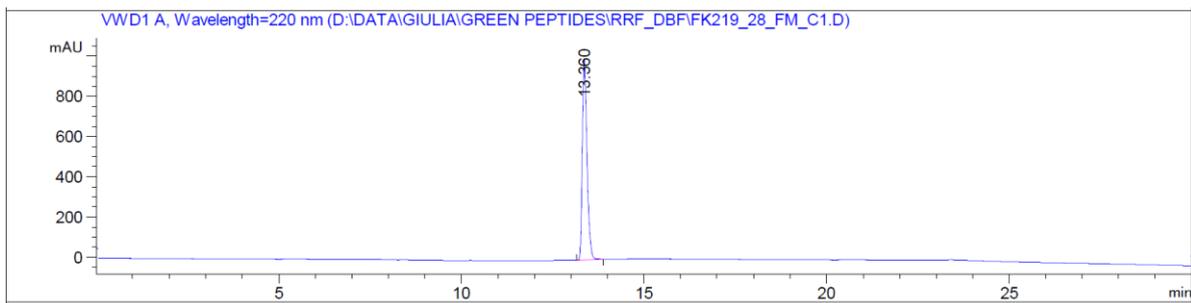


Relative Response Factor between 9-fluorenemethanol and dibenzofulvene was calculated considering the peak absorption areas of equimolar concentrations of both compounds at 220 nm (mean of analyses at three different concentrations).

A representative example of chromatograms of 9-FM and DBF at 0.0005 M concentration is reported below.

Conc (M)	Area DBF (milliAu)	Area 9-FM (milliAu)	RRF <sub>(9-FM/DBF)</sub>	Mean value RRF
0.0005	11132.7	8833.5	0.79	<b>0.80</b>
0.00025	5652.9	4533.7	0.80	
0.00005	2823.9	2240.3	0.79	

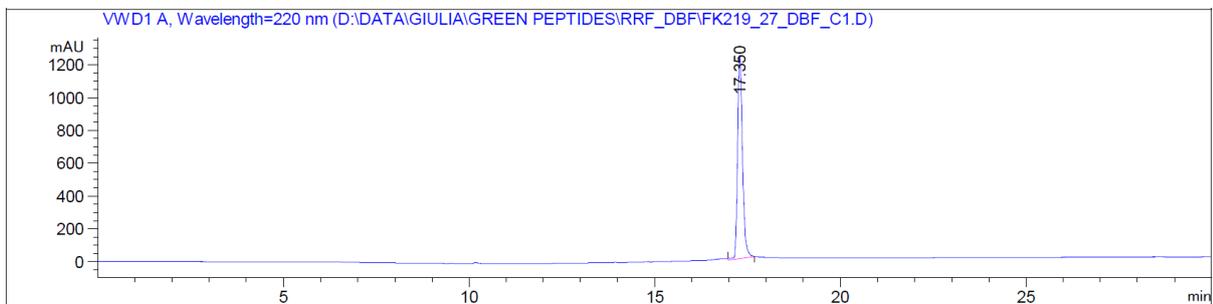
**Figure S25.** Chromatogram of 9-fluorenemethanol at 0.005 M concentration



Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.360	BB	0.1331	8833.49316	1000.05835	100.0000

**Figure S26.** Chromatogram of dibenzofulvene at 0.005 M concentration



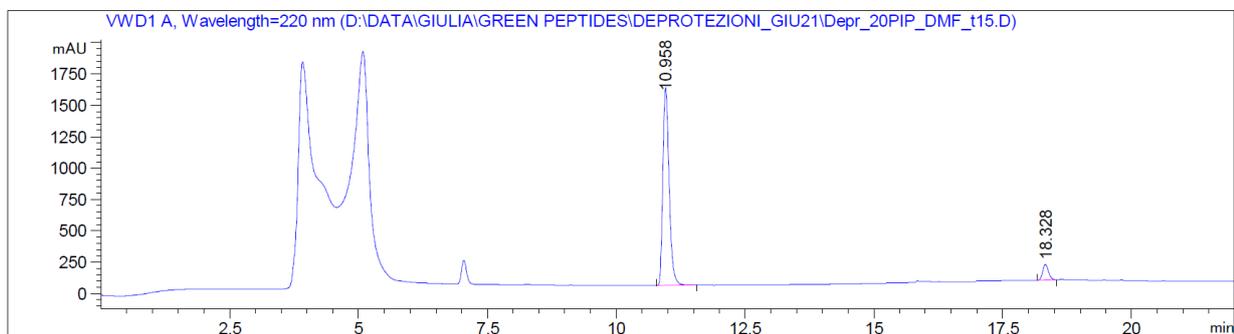
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.350	MM	0.1499	1.11327e4	1238.07373	100.0000

Totals :                      1.11327e4   1238.07373

Relative Response Factor was used in the correction of peak areas for monitoring the conversion between DBF and DBF-base adduct (See Table 3 in the main text).

**Figure S27.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 20% piperidine at t=15 minutes



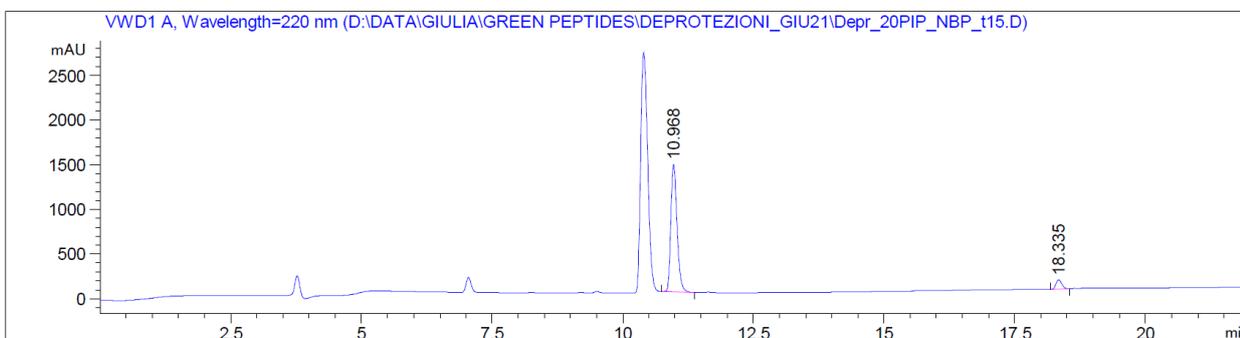
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.958	BB	0.1306	1.32837e4	1571.63379	94.9046
2	18.328	MM	0.1104	713.19806	107.64330	5.0954

Totals : 1.39969e4 1679.27708

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
Piperidine-DBF adduct	10.958	94.9	96
DBF	18.328	5.1	4

**Figure S28.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 20% piperidine at t=15 minutes



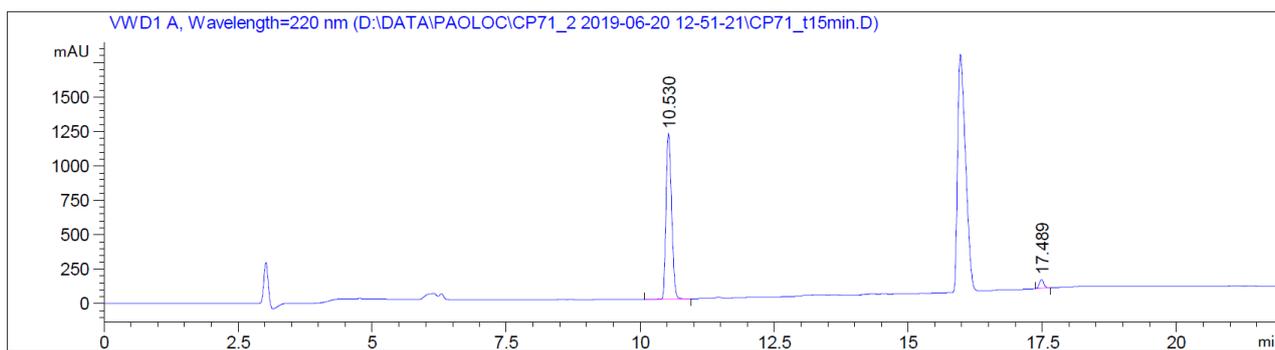
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.968	BB	0.1281	1.17705e4	1428.96436	95.2675
2	18.335	MM	0.1071	584.70221	91.00556	4.7325

Totals : 1.23552e4 1519.96992

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
Piperidine-DBF adduct	10.968	95.3	96
DBF	18.335	4.7	4

**Figure S29.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 20% piperidine at t=15 minutes



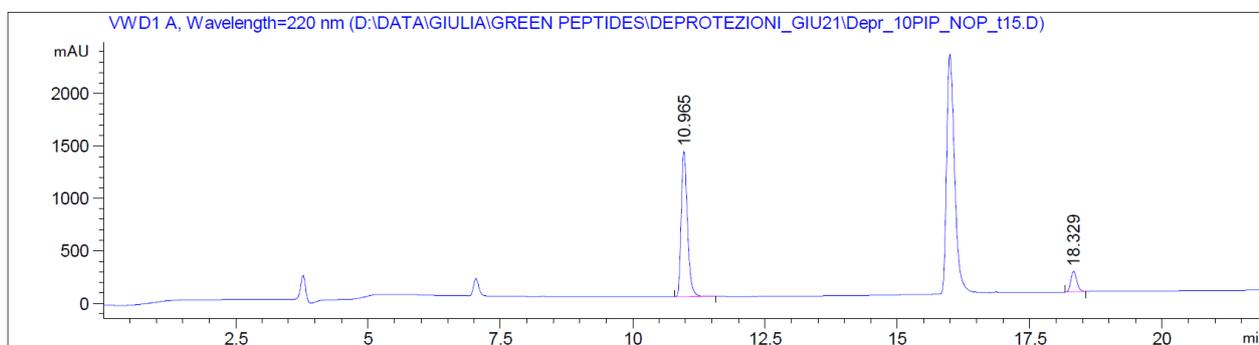
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.530	BB	0.1120	8661.23438	1202.82922	95.3461
2	17.489	MM	0.1062	422.76453	66.37790	4.6539

Totals : 9083.99890 1269.20712

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
Piperidine-DBF adduct	10.530	95.3	96
DBF	17.489	4.7	4

**Figure S30.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 10% piperidine at t=15 minutes



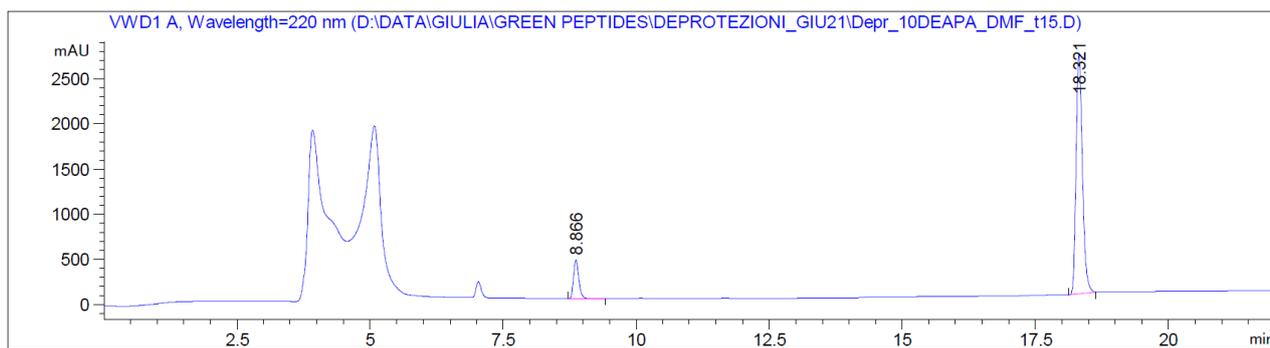
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.965	BB	0.1256	1.13672e4	1387.41321	88.0628
2	18.329	BB	0.1209	1540.86584	197.72040	11.9372

Totals : 1.29081e4 1585.13361

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
Piperidine-DBF adduct	10.965	88.1	90
DBF	18.329	11.9	10

**Figure S31.** Chromatogram of Fmoc-Phe-OH deprotection in DMF with 10% DEAPA at t=15 minutes



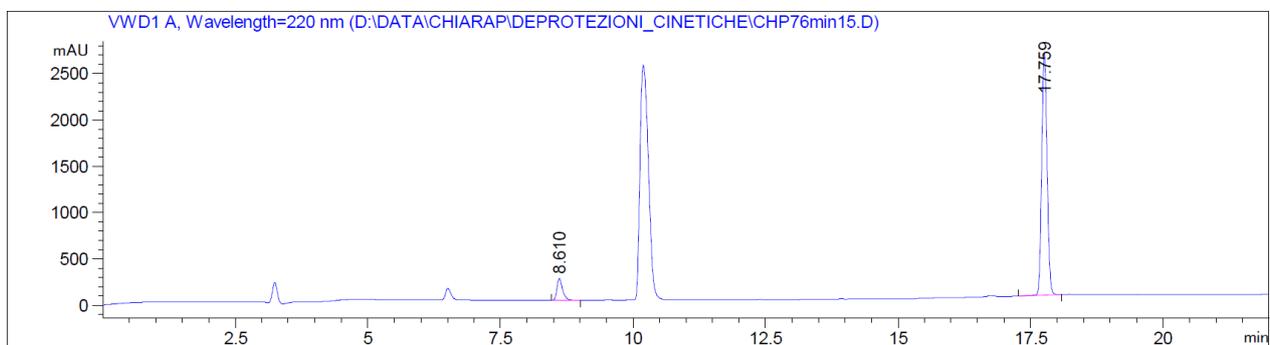
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.866	BB	0.1036	2916.41187	427.69070	11.7024
2	18.321	BB	0.1286	2.20051e4	2658.70679	88.2976

Totals : 2.49215e4 3086.39749

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
DEAPA-DBF adduct	8.866	11.7	14
DBF	18.321	88.3	86

**Figure S32.** Chromatogram of Fmoc-Phe-OH deprotection in NBP with 10% DEAPA at t=15 minutes



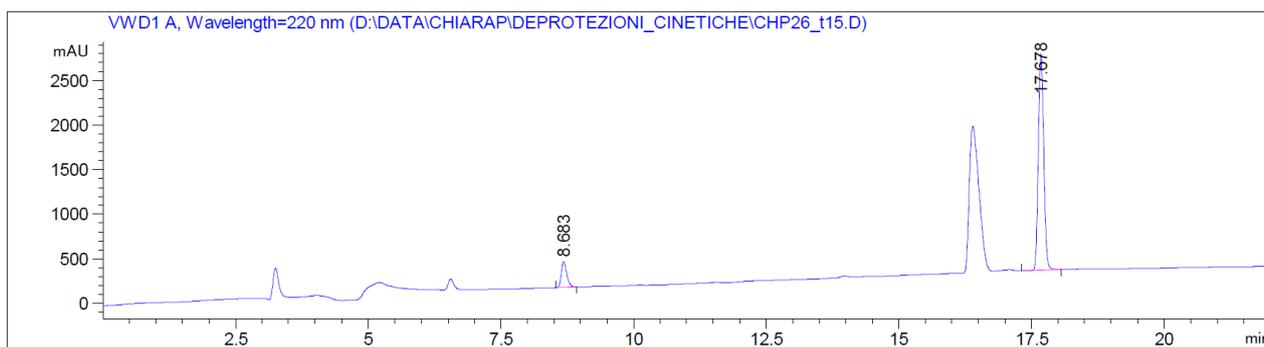
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.610	BB	0.1106	1699.32837	234.23108	8.4328
2	17.759	BB	0.1103	1.84520e4	2616.63135	91.5672

Totals : 2.01513e4 2850.86243

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
DEAPA-DBF adduct	8.810	8.4	10
DBF	17.759	91.6	90

**Figure S33.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 10% DEAPA at t=15 minutes



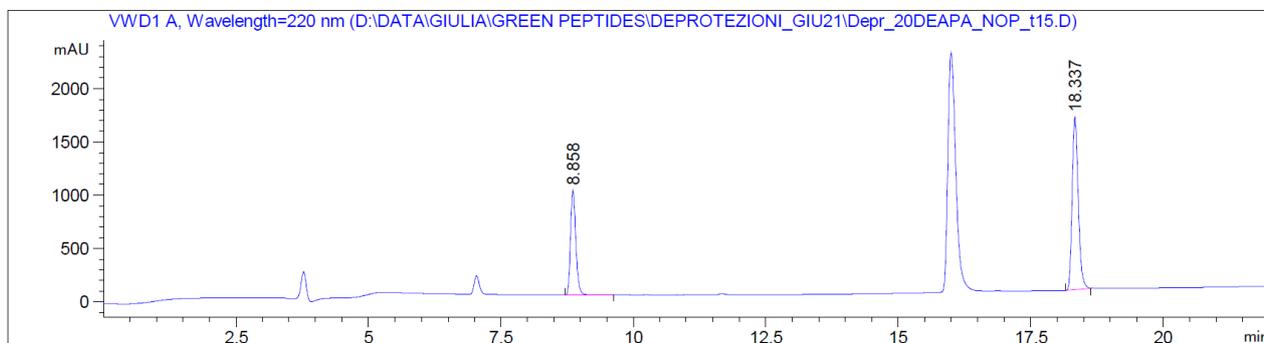
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.683	BB	0.1080	2046.08594	291.06232	10.6296
2	17.678	BB	0.1108	1.72029e4	2423.63745	89.3704

Totals : 1.92490e4 2714.69977

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
DEAPA-DBF adduct	8.683	10.6	13
DBF	17.678	89.4	87

**Figure S34.** Chromatogram of Fmoc-Phe-OH deprotection in NOP with 20% DEAPA at t=15 minutes



Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.858	BB	0.1074	6852.90625	982.75287	34.5512
2	18.337	BB	0.1235	1.29812e4	1619.73462	65.4488

Totals : 1.98341e4 2602.48749

Product	Rt (min)	Area (%)	Area corrected with RRF (%)
DEAPA-DBF adduct	8.858	34.6	40
DBF	18.337	65.4	60

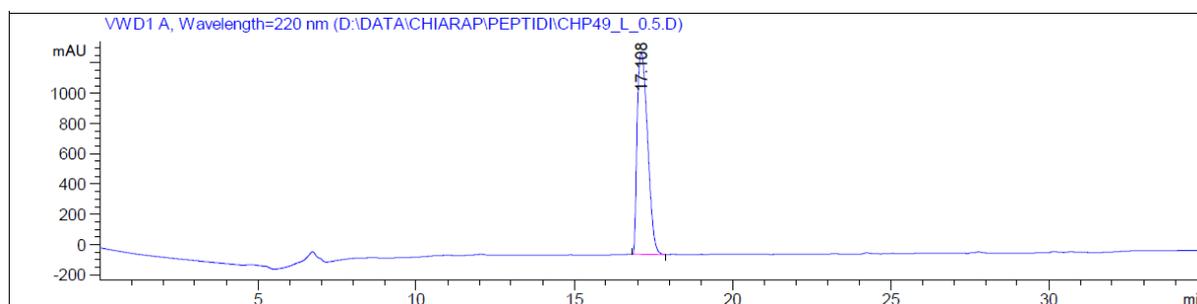
**Table S1.** Formation of base-DBF adduct in the studied solvents with deprotection times at 6 minutes

Entry	Base (%)	Solvent	Base-DBF adduct:DBF <sup>a</sup>
1	Piperidine (5)	DMF	29:71
2	Piperidine (10)	DMF	89:11
3	Piperidine (20)	DMF	95:5
4	Piperidine (20)	NBP	95:5
5	Piperidine (20)	NOP	78:22
6	DEAPA (10)	DMF	5:95
7	DEAPA (20)	DMF	23:77
8	DEAPA (30)	DMF	43:57
9	DEAPA (10)	NBP	5:95
10	DEAPA (20)	NBP	23:77
11	DEAPA (30)	NBP	43:57
12	DEAPA (10)	NOP	5:95
13	DEAPA (20)	NOP	22:78
14	DEAPA (30)	NOP	38:62

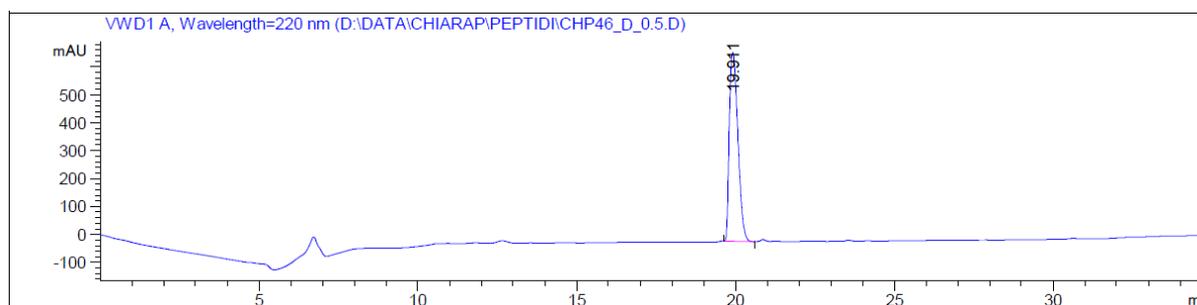
<sup>a</sup> Calculated on the basis on HPLC signal integration of crude samples after t=6 min deprotection after correction with RRF=0.8 (see ESI)

## Racemization tests

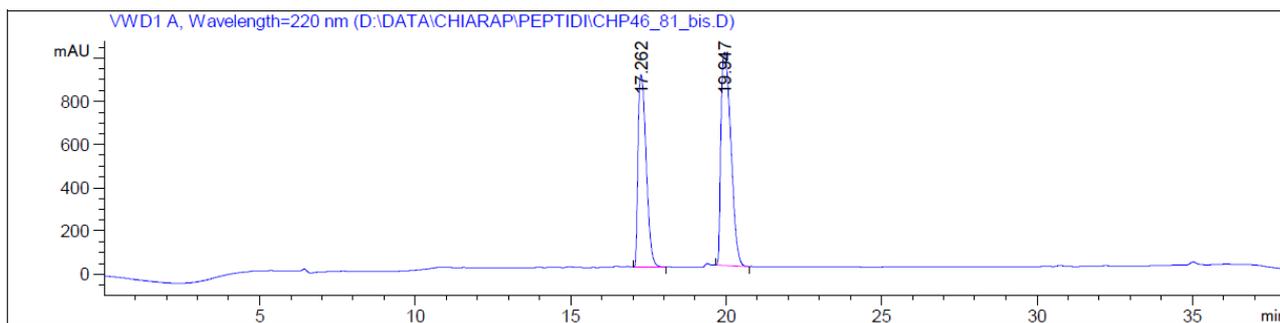
**Figure S35.** Chromatogram of H-Phe-L-Cys-Gly-OH, manual SPPS in DMF in standard conditions for reference



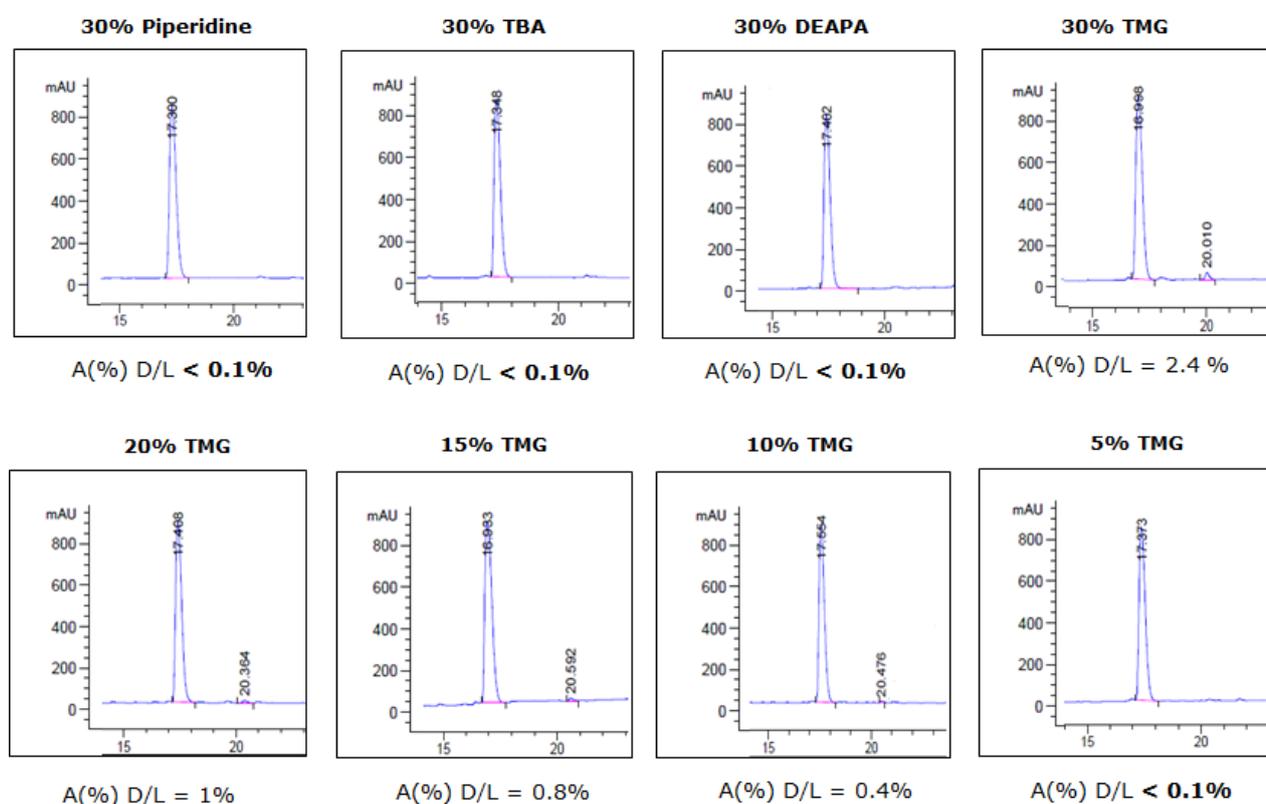
**Figure S36.** Chromatogram of H-Phe-D-Cys-Gly-OH, manual SPPS in DMF in standard conditions for reference



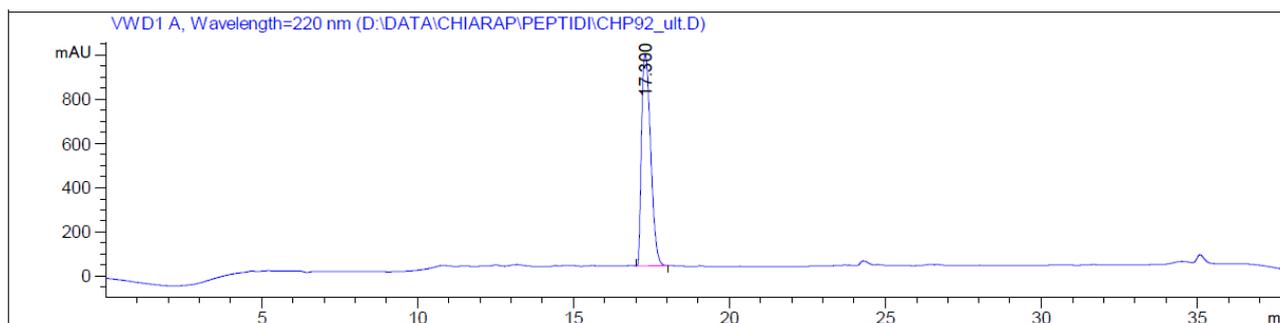
**Figure S37.** Chromatogram of a mixture of H-Phe-L-Cys-Gly-OH and H-Phe-D-Cys-Gly-OH



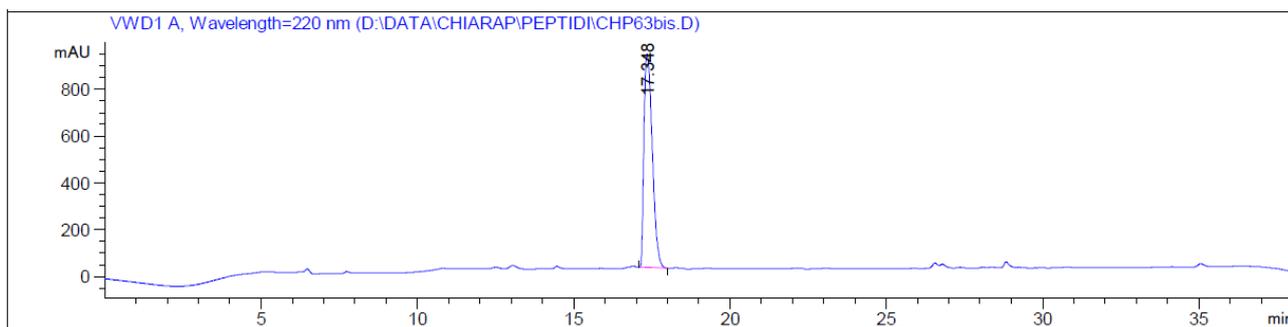
**Figure S38.** Overview of racemization tests with the tested bases in NOP during manual SPPS of H-Phe-L-Cys-Gly-OH



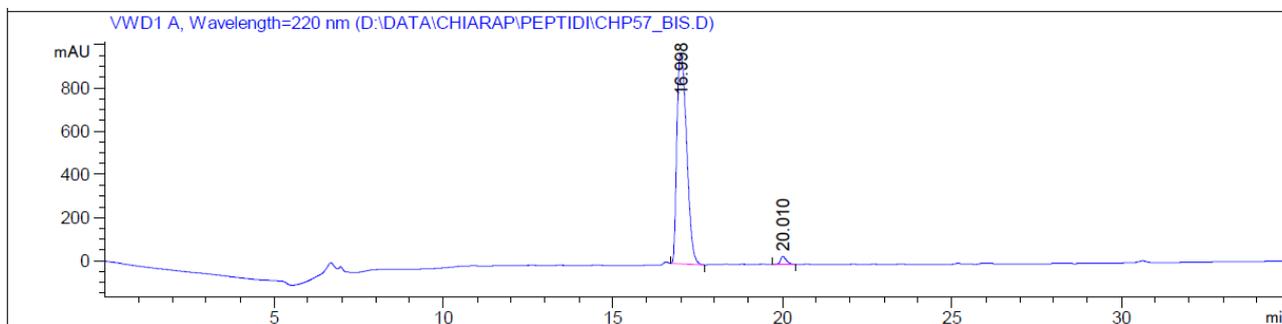
**Figure S39.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in NOP with 30% piperidine as deprotection solution (60 minutes deprotection time)



**Figure S40.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **30% TBA** as deprotection solution (60 minutes deprotection time)



**Figure S41.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **30% TMG** as deprotection solution (60 minutes deprotection time)



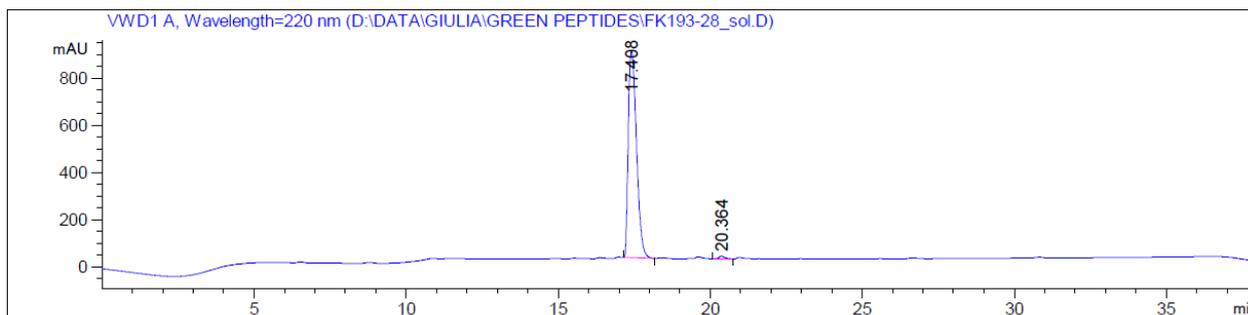
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.998	BB	0.3229	1.94418e4	973.92926	97.6394
2	20.010	BB	0.1805	470.04501	38.48532	2.3606

Totals : 1.99118e4 1012.41458

Product	Rt (min)	Area (%)
H-Phe-L-Cys-Gly-OH	16.998	97.6
H-Phe-D-Cys-Gly-OH	20.010	2.4

**Figure S42.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **20% TMG** as deprotection solution (60 minutes deprotection time)



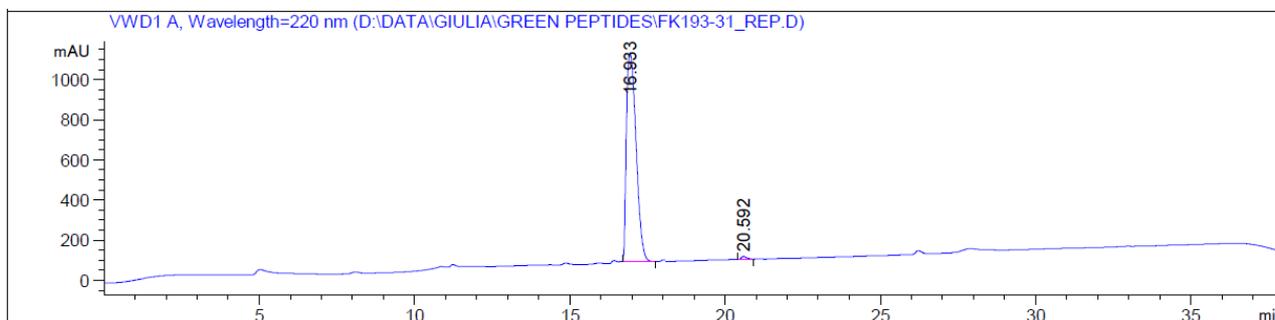
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.408	BB	0.3035	1.65004e4	876.89343	99.0386
2	20.364	BB	0.1971	160.17575	11.74310	0.9614

Totals : 1.66606e4 888.63653

Product	Rt (min)	Area (%)
H-Phe-L-Cys-Gly-OH	17.408	99.0
H-Phe-D-Cys-Gly-OH	20.364	1.0

**Figure S43.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **15% TMG** as deprotection solution (60 minutes deprotection time)



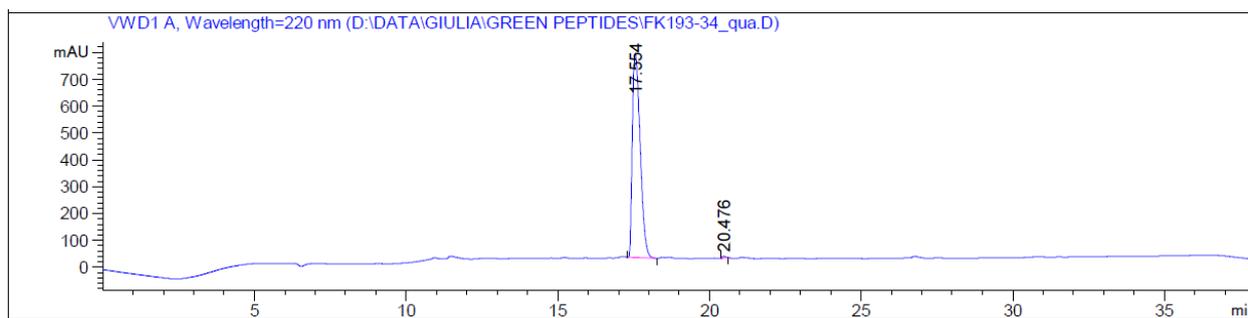
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.933	BB	0.3531	2.24273e4	1041.89746	99.2197
2	20.592	BB	0.1711	176.38608	15.22942	0.7803

Totals : 2.26037e4 1057.12688

Product	Rt (min)	Area (%)
H-Phe-L-Cys-Gly-OH	16.933	99.2
H-Phe-D-Cys-Gly-OH	20.592	0.8

**Figure S44.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **10% TMG** as deprotection solution (60 minutes deprotection time)



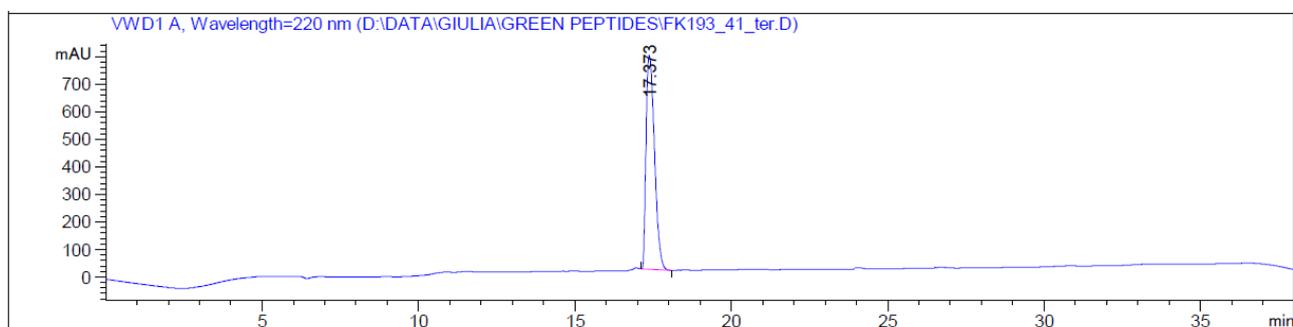
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.554	BB	0.2851	1.36381e4	760.82928	99.5663
2	20.476	MM	0.1449	59.40553	6.83167	0.4337

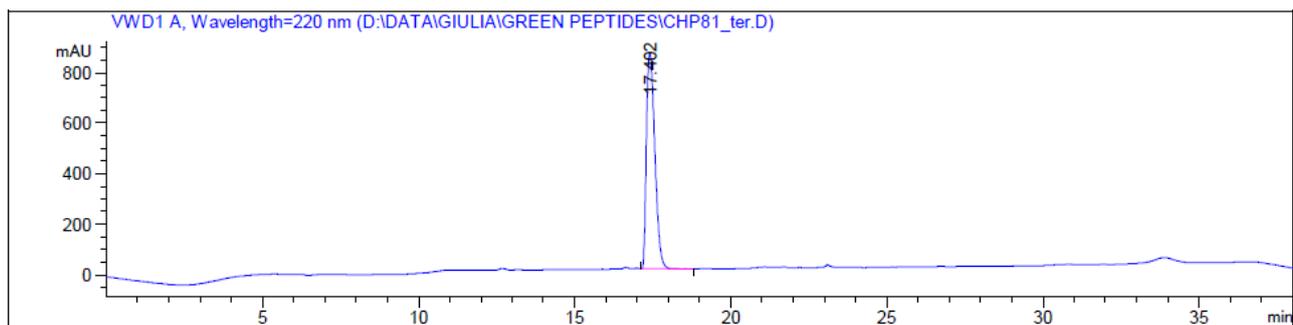
Totals :                            1.36975e4    767.66095

Product	Rt (min)	Area (%)
H-Phe-L-Cys-Gly-OH	17.554	99.6
H-Phe-D-Cys-Gly-OH	20.476	0.4

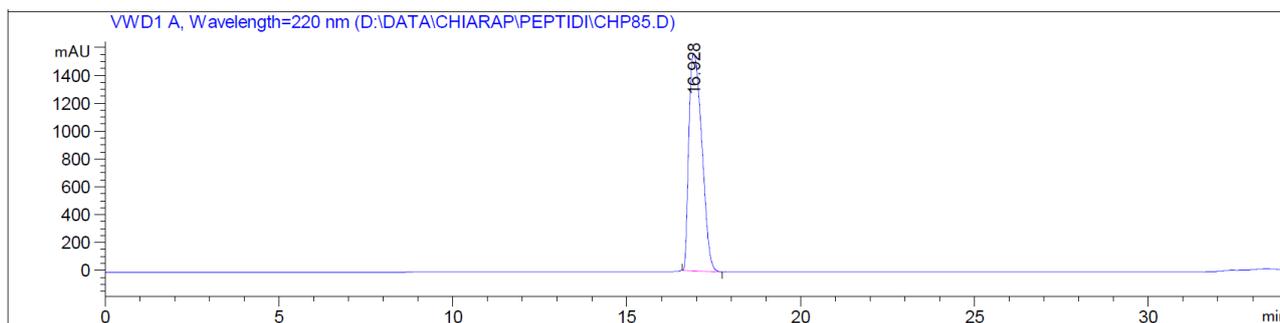
**Figure S45.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **5% TMG** as deprotection solution (60 minutes deprotection time)



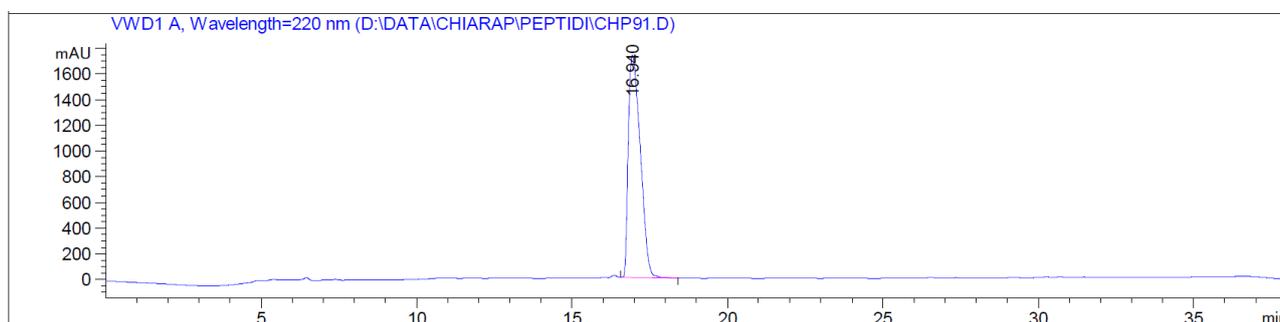
**Figure S46.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP** with **30% DEAPA** as deprotection solution (60 minutes deprotection time)



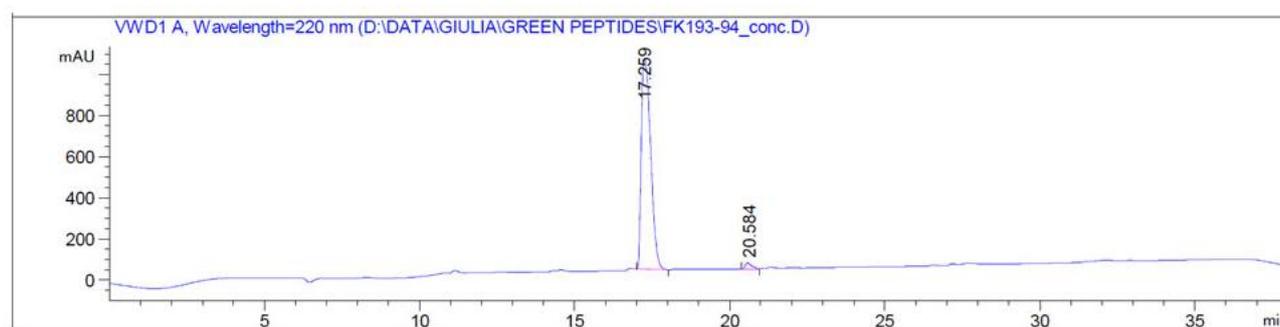
**Figure S47.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP/DMC 8/2** with **30% piperidine** as deprotection solution (60 minutes deprotection time)



**Figure S48.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP/DMC 8/2** with **30% TBA** as deprotection solution (60 minutes deprotection time)



**Figure S49.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP/DMC 8/2** with **30% TMG** as deprotection solution (60 minutes deprotection time)



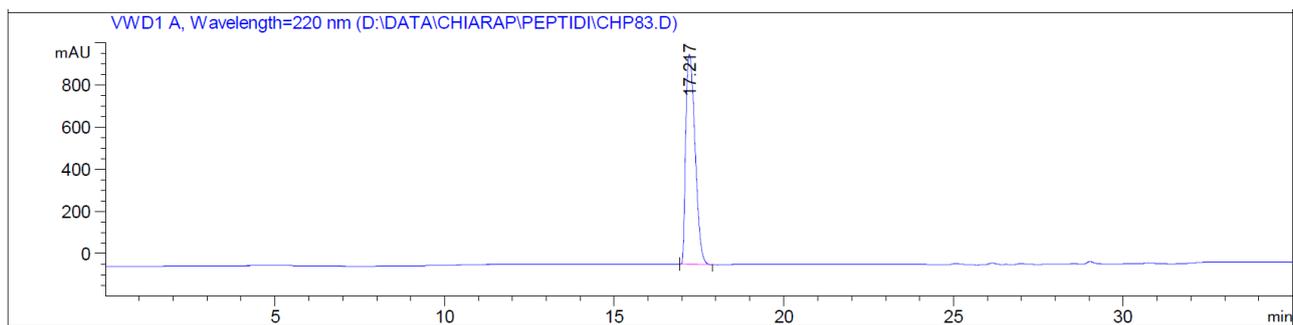
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.259	BB	0.3397	2.09280e4	1024.64478	97.7927
2	20.584	BB	0.1956	472.36931	43.08929	2.2073

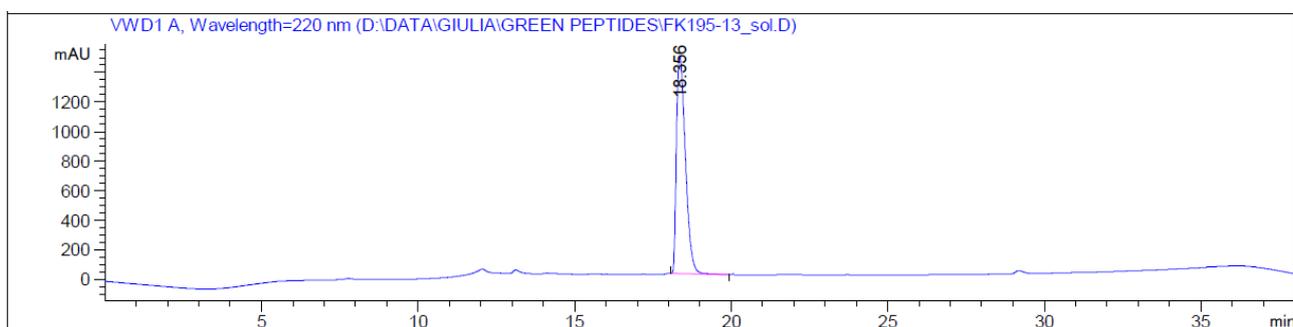
Totals : 2.14003e4 1067.73407

Product	Rt (min)	Area (%)
H-Phe-L-Cys-Gly-OH	17.259	97.8
H-Phe-D-Cys-Gly-OH	20.584	2.2

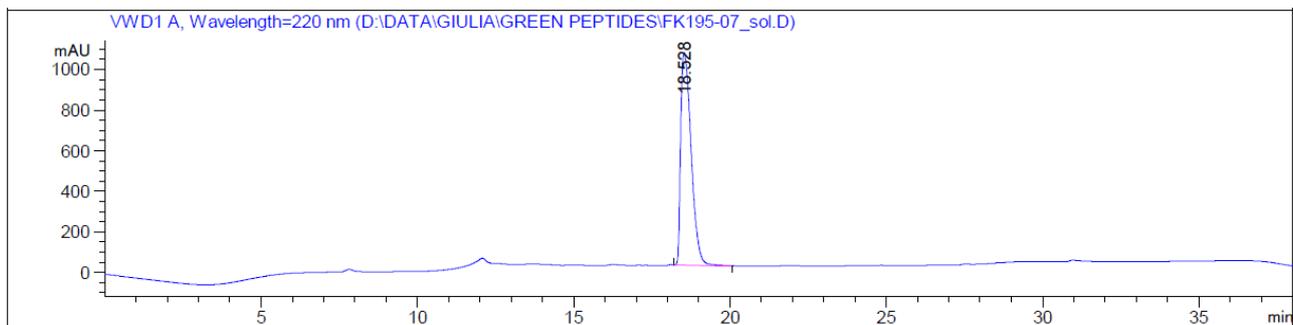
**Figure S50.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **NOP/DMC 8/2** with **30% DEAPA** as deprotection solution (60 minutes deprotection time)



**Figure S51.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **DMF** with **30% piperidine** as deprotection solution (60 minutes deprotection time)

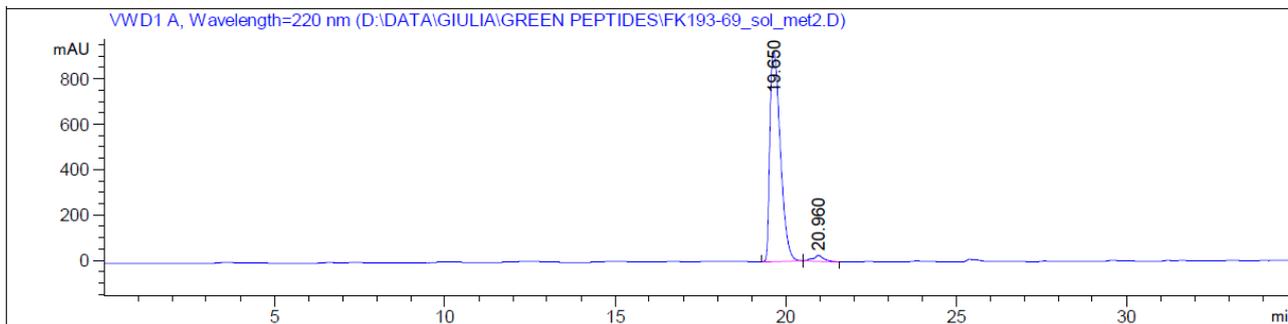


**Figure S52.** Chromatogram of H-Phe-Cys-Gly-OH, manual SPPS in **DMF** with **30% DEAPA** as deprotection solution (60 minutes deprotection time)



## Aspartimide formation

**Figure S53.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH, manual SPPS with standard conditions for reference compound



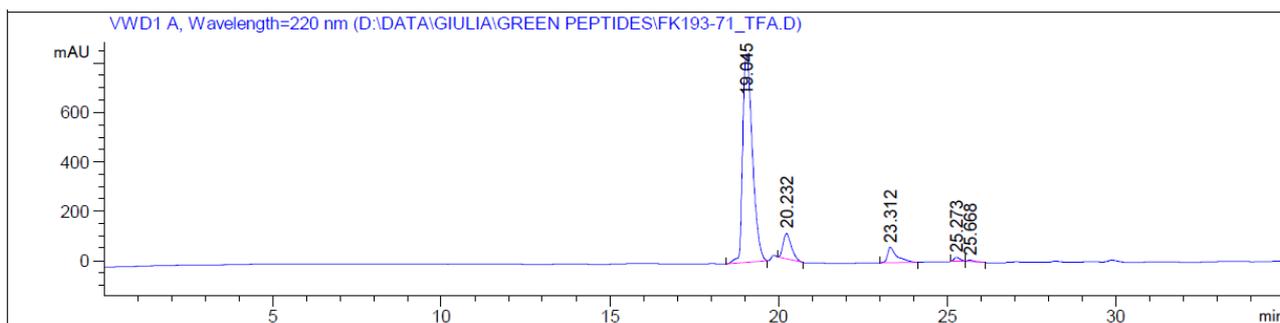
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.650	BB	0.3342	1.96926e4	925.58374	97.4196
2	20.960	BB	0.2812	521.60944	25.65905	2.5804

Totals : 2.02142e4 951.24279

Product	Rt (min)	Area (%)
Hexapeptide I	19.650	97.4
Aspartimide II	20.960	2.6

**Figure S54.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in DMF, 4 h at RT**



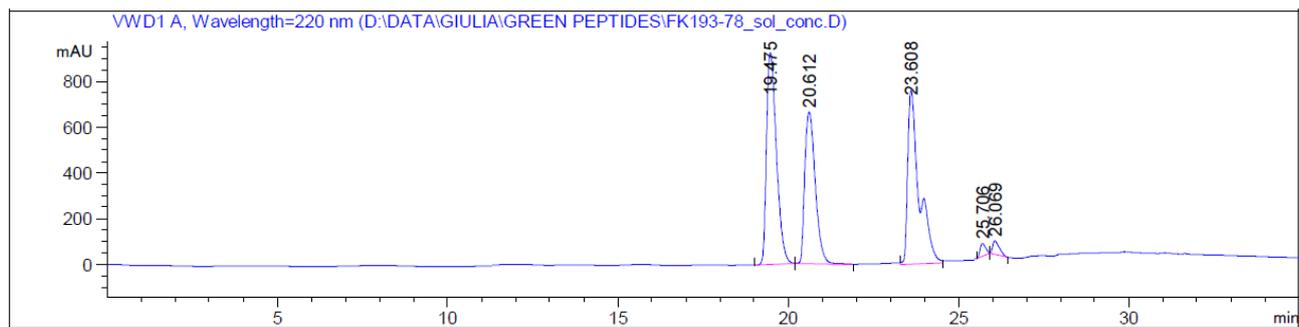
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.045	BB	0.3162	1.69608e4	844.82214	83.9320
2	20.232	BB	0.2624	1764.02014	102.71278	8.7294
3	23.312	BB	0.2735	1230.24805	62.52800	6.0880
4	25.273	BB	0.1836	185.95247	15.53874	0.9202
5	25.668	BB	0.1739	66.76701	5.73191	0.3304

Totals : 2.02078e4 1031.33357

Product	Rt (min)	Area (%)
Hexapeptide I	19.045	83.9
Aspartimide II	20.232	8.7
Piperidides III	23.312	6.1
	25.273	0.9
	25.668	0.3

**Figure S55.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in DMF, 4 h at 40°C**



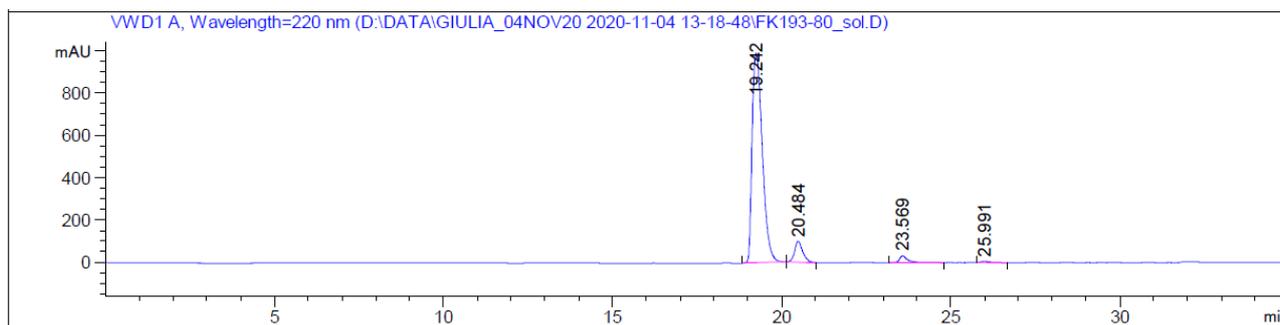
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.475	BB	0.3199	1.87052e4	925.16742	36.0138
2	20.612	BB	0.3418	1.41789e4	662.27148	27.2990
3	23.608	MM	0.3875	1.76323e4	758.41406	33.9480
4	25.706	BB	0.1763	626.37567	56.10244	1.2060
5	26.069	BB	0.2048	796.36420	58.49071	1.5333

Totals : 5.19391e4 2460.44612

Product	Rt (min)	Area (%)
Hexapeptide I	19.475	36.0
Aspartimide II	20.612	27.3
Piperidides III	23.608	34.0
	25.706	1.2
	26.069	1.5

**Figure S56.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in NBP, 4 h at RT**



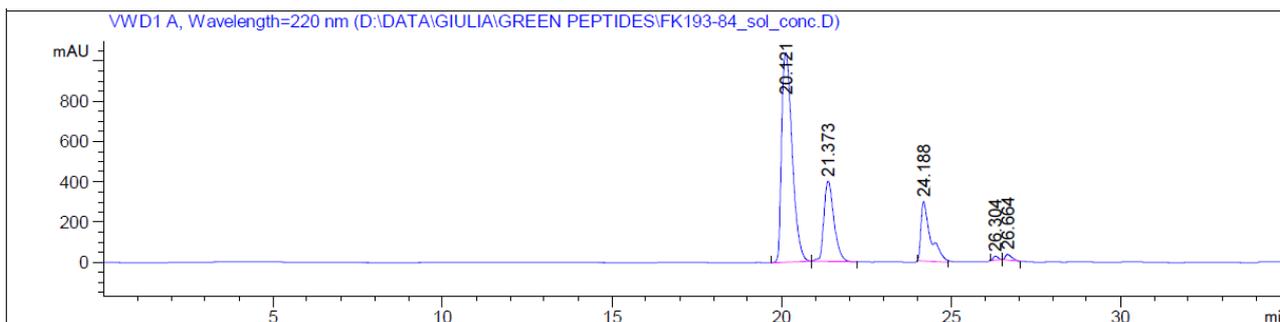
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.242	BB	0.3362	2.06937e4	988.57520	90.0042
2	20.484	BB	0.2451	1627.94458	98.36476	7.0805
3	23.569	BB	0.2536	565.35999	31.46964	2.4590
4	25.991	BB	0.2716	104.91269	5.78369	0.4563

Totals : 2.29919e4 1124.19329

Product	Rt (min)	Area (%)
Hexapeptide I	19.242	90.0
Aspartimide II	20.484	7.1
Piperidides III	23.569	2.4
	25.991	0.5

**Figure S57.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in NBP, 4 h at 40°C**



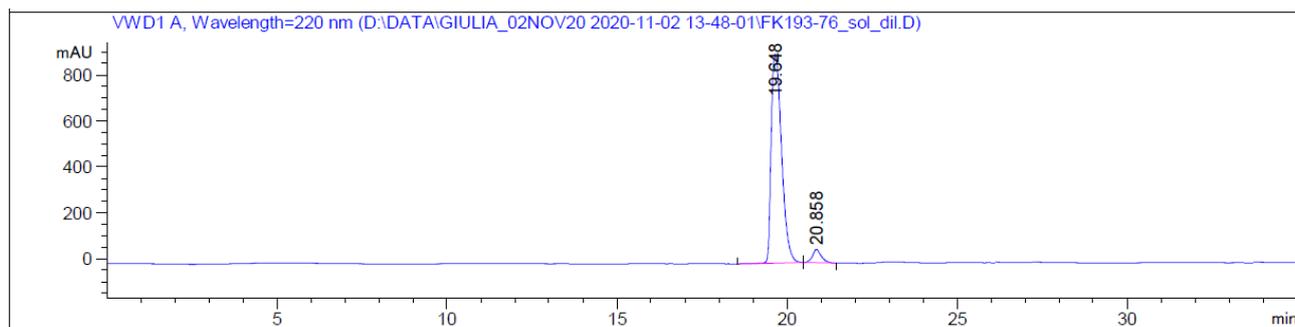
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.121	BB	0.3409	2.23503e4	1039.07520	60.4709
2	21.373	BB	0.3211	8260.61230	399.67758	22.3499
3	24.188	MM	0.3269	5760.25195	293.66702	15.5849
4	26.304	BB	0.1605	199.76794	19.66879	0.5405
5	26.664	BB	0.2015	389.51898	28.49531	1.0539

Totals : 3.69605e4 1780.58389

Product	Rt (min)	Area (%)
Hexapeptide I	20.121	60.5
Aspartimide II	21.373	22.3
Piperidides III	24.188	15.6
	26.304	0.5
	26.664	1.1

**Figure S58.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in NOP, 4 h at RT**



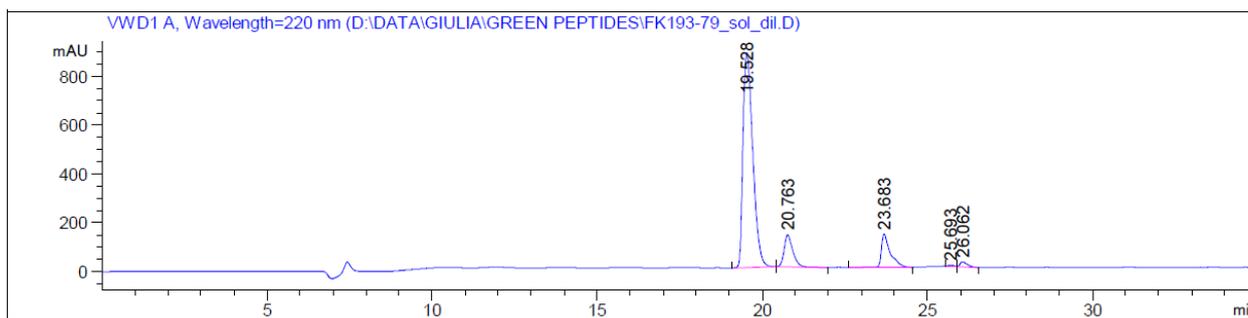
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.648	BB	0.3335	1.91386e4	909.46179	94.9092
2	20.858	BB	0.2556	1026.57214	57.68584	5.0908

Totals : 2.01652e4 967.14763

Product	Rt (min)	Area (%)
Hexapeptide I	19.648	94.9
Aspartimide II	20.858	5.1

**Figure S59.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% piperidine in NOP, 4 h at 40°C**



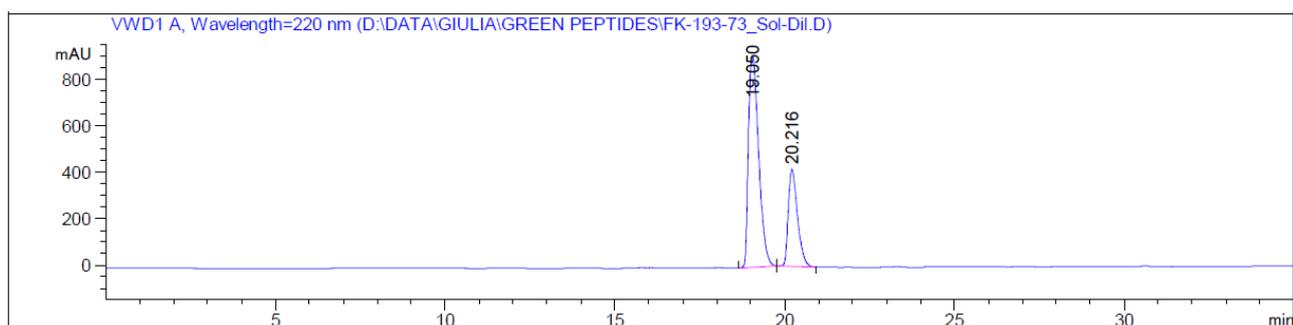
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.528	BB	0.3246	1.81352e4	879.33728	77.0344
2	20.763	BB	0.2690	2408.23291	131.89261	10.2296
3	23.683	BB	0.2714	2640.74023	137.92331	11.2173
4	25.693	BB	0.1527	63.87175	6.61015	0.2713
5	26.062	BB	0.2120	293.64609	20.39544	1.2473

Totals : 2.35417e4 1176.15879

Product	Rt (min)	Area (%)
Hexapeptide I	19.528	77.0
Aspartimide II	20.763	10.2
Piperidides III	23.683	11.2
	25.693	0.3
	26.062	1.3

**Figure S60.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% TBA in DMF, 4 h at RT**



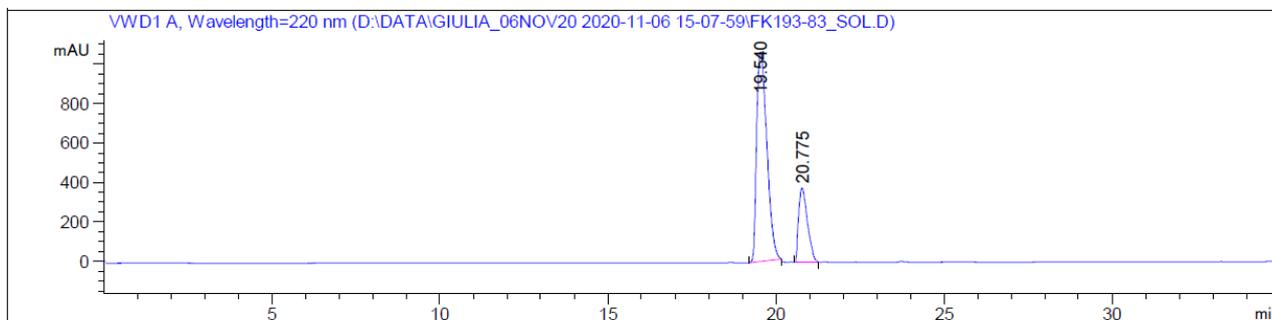
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.050	BB	0.3309	1.89397e4	909.70319	70.6713
2	20.216	BB	0.2920	7860.00977	416.85880	29.3287

Totals : 2.67998e4 1326.56198

Product	Rt (min)	Area (%)
Hexapeptide I	19.050	70.7
Aspartimide II	20.216	29.3

**Figure S61.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% TBA in NBP, 4 h at RT**



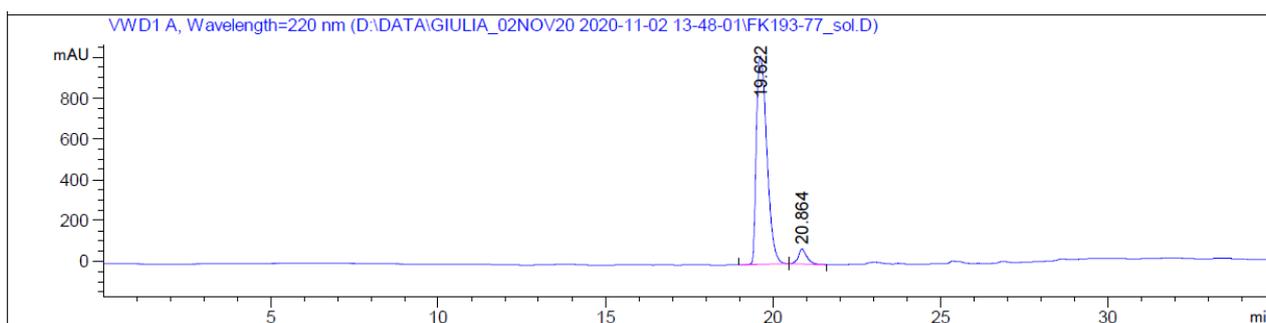
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.540	MM	0.3882	2.49102e4	1069.39648	79.4854
2	20.775	MM	0.2991	6429.13867	358.22852	20.5146

Totals : 3.13393e4 1427.62500

Product	Rt (min)	Area (%)
Hexapeptide I	19.540	79.5
Aspartimide II	20.775	20.5

**Figure S62.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **20% TBA in NOP, 4 h at RT**



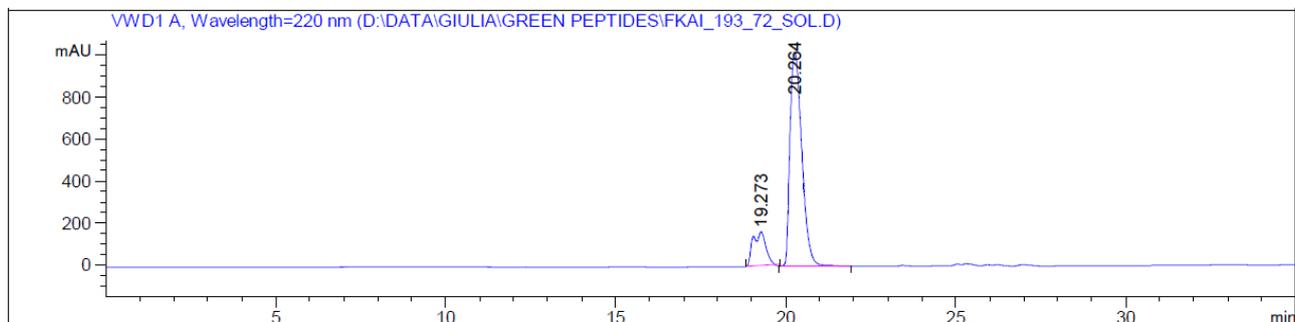
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.622	BB	0.3455	2.21684e4	1020.20471	94.5109
2	20.864	BB	0.2512	1287.50720	73.93987	5.4891

Totals : 2.34559e4 1094.14458

Product	Rt (min)	Area (%)
Hexapeptide I	19.622	94.5
Aspartimide II	20.864	5.5

**Figure S63.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with 5% TMG in DMF, 4 h at RT



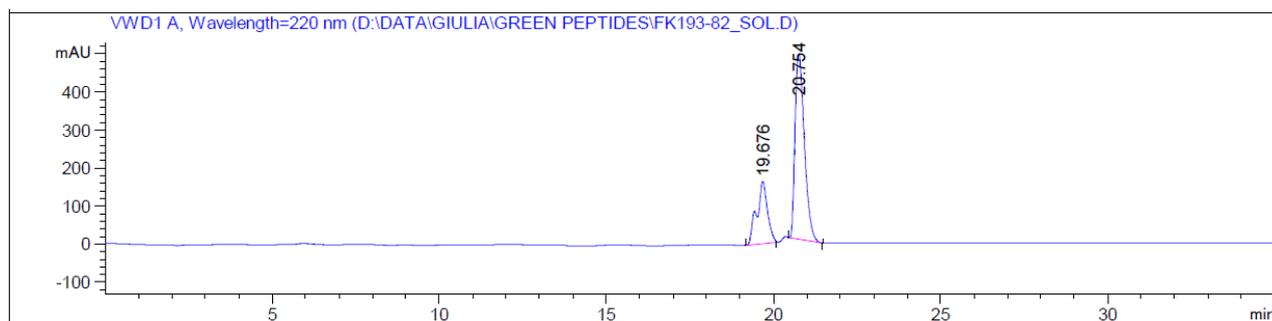
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.273	MM	0.4367	4177.47363	159.41856	14.4741
2	20.264	BB	0.3906	2.46842e4	1019.00739	85.5259

Totals : 2.88617e4 1178.42595

Product	Rt (min)	Area (%)
Hexapeptide I ( $\alpha$ and $\beta$ peptide)	19.273	14.5
Aspartimide II	20.264	85.5

**Figure S64.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with 5% TMG in NBP, 4 h at RT



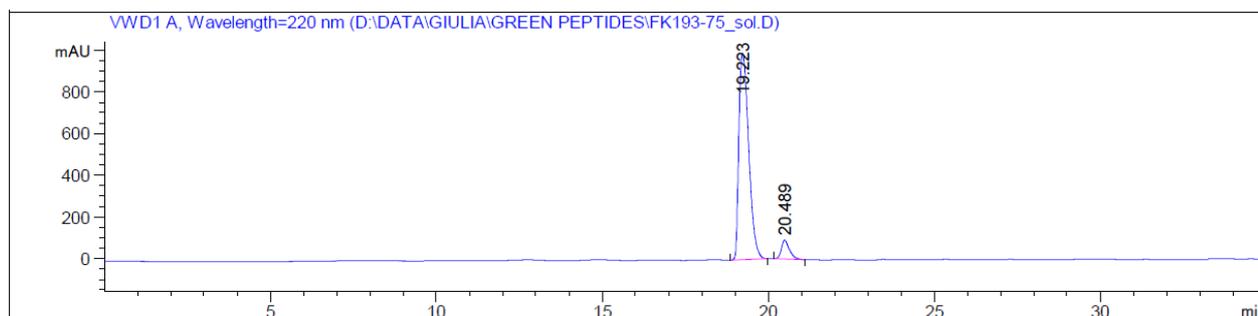
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.676	MM	0.3718	3682.07764	165.06819	27.8769
2	20.764	BB	0.3037	9526.28027	488.03809	72.1231

Totals : 1.32084e4 653.10628

Product	Rt (min)	Area (%)
Hexapeptide I ( $\alpha$ and $\beta$ peptide)	19.676	27.9
Aspartimide II	20.754	72.1

**Figure S65.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **5% TMG in NOP, 4 h at RT**



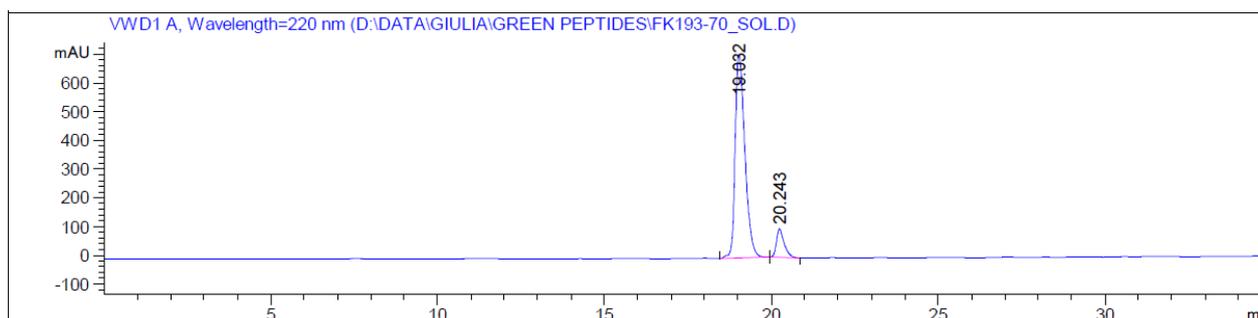
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.223	BB	0.3278	2.03609e4	990.78229	92.9980
2	20.489	BB	0.2487	1533.01807	90.93493	7.0020

Totals : 2.18939e4 1081.71722

Product	Rt (min)	Area (%)
Hexapeptide I	19.223	93.0
Aspartimide II	20.489	7.0

**Figure S66.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **10% DEAPA in DMF, 4 h at RT**



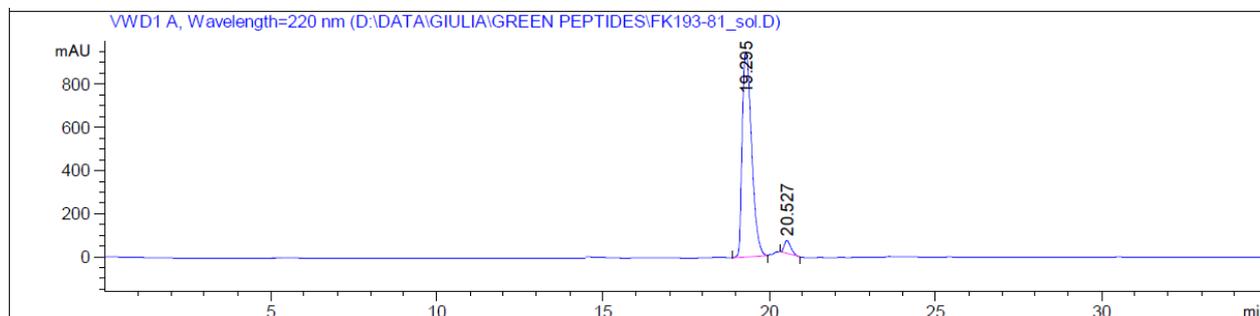
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.032	BB	0.3135	1.43151e4	709.01990	89.9476
2	20.243	BB	0.2394	1599.82861	98.56615	10.0524

Totals : 1.59149e4 807.58604

Product	Rt (min)	Area (%)
Hexapeptide I	19.032	89.9
Aspartimide II	20.243	10.1

**Figure S67.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **10% DEAPA in NBP, 4 h at RT**



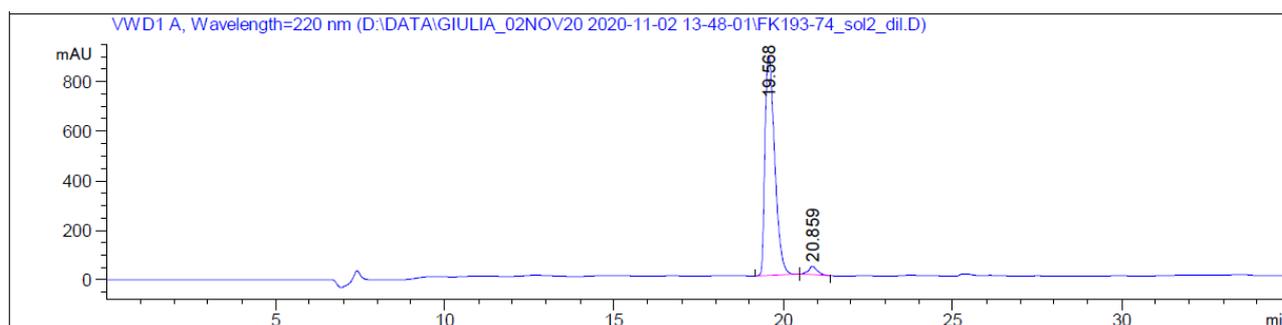
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.295	BB	0.3123	1.86919e4	946.90442	95.7341
2	20.527	BB	0.2062	832.91559	60.67286	4.2659

Totals : 1.95248e4 1007.57728

Product	Rt (min)	Area (%)
Hexapeptide I	19.295	95.7
Aspartimide II	20.527	4.3

**Figure S68.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **10% DEAPA in NOP, 4 h at RT**



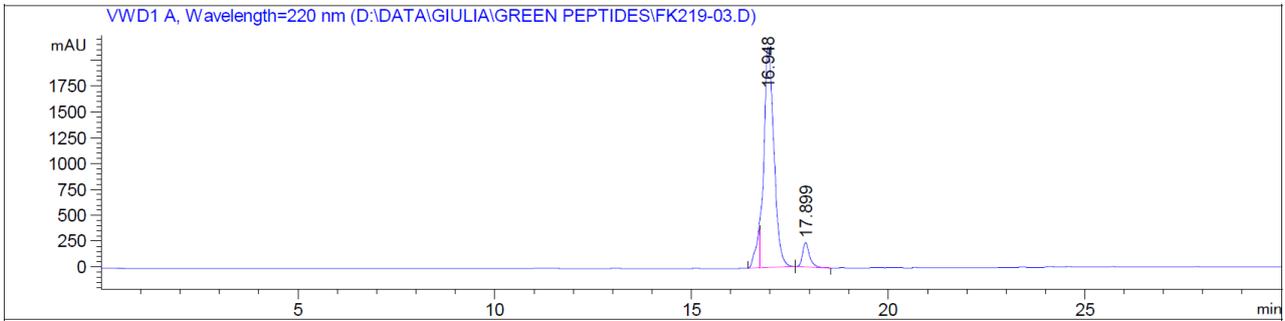
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.568	BB	0.3138	1.77667e4	886.47058	96.5934
2	20.859	BB	0.2590	626.59259	34.64211	3.4066

Totals : 1.83933e4 921.11269

Product	Rt (min)	Area (%)
Hexapeptide I	19.568	96.6
Aspartimide II	20.859	3.4

**Figure S69.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **10% DEAPA in NOP, 4 h at 40°C**



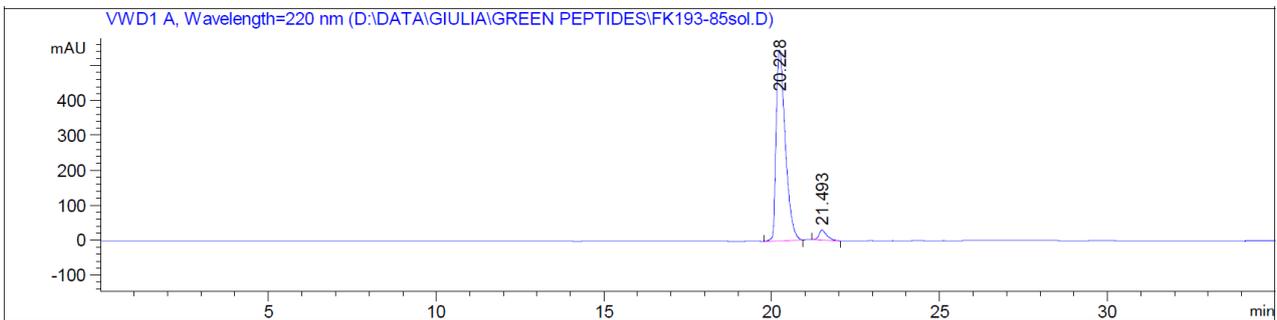
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.701	MM	0.1043	2232.41602	356.66306	4.9869
2	16.948	MM	0.3055	3.96522e4	2163.40869	88.5777
3	17.899	BB	0.1842	2880.84839	236.36681	6.4354

Totals : 4.47655e4 2756.43855

Product	Rt (min)	Area (%)
DEAPA adduct IV	16.701	5.0
Hexapeptide I	16.948	88.6
Aspartimide II	17.899	6.4

**Figure S70.** Chromatogram of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH after resin-bound peptide stress test with **10% DEAPA in NOP/DMC 8/2, 4 h at RT**



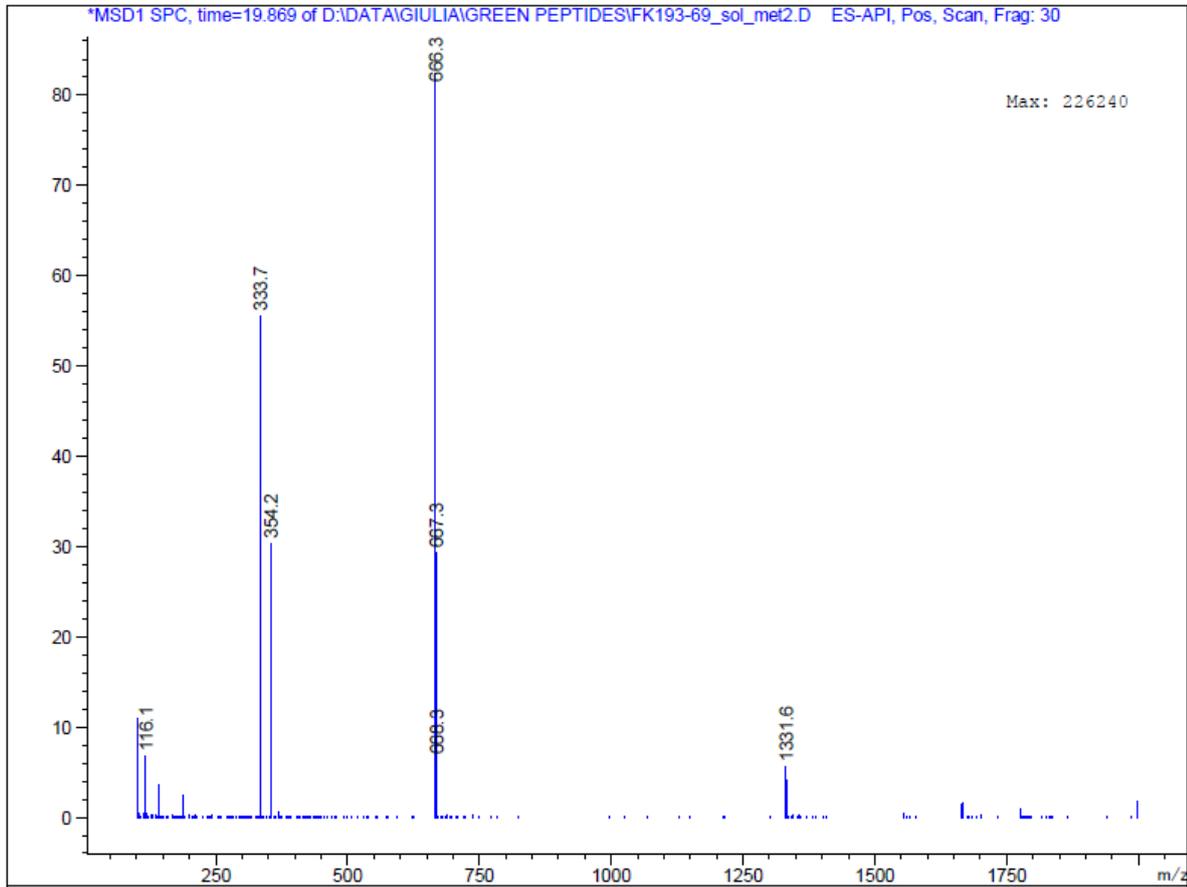
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.228	BB	0.2906	1.03250e4	546.04572	95.8007
2	21.493	BB	0.2327	452.58197	28.28045	4.1993

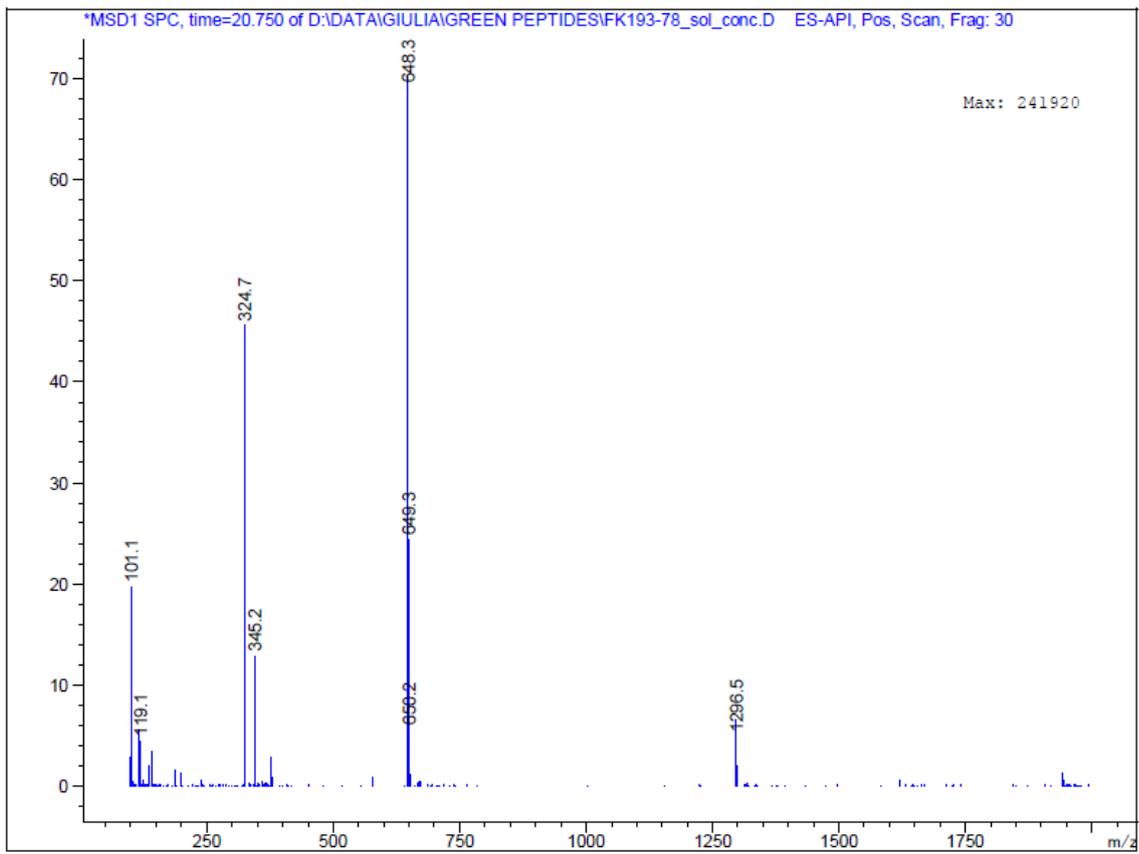
Totals : 1.07776e4 574.32617

Product	Rt (min)	Area (%)
Hexapeptide I	20.228	95.8
Aspartimide II	21.493	4.2

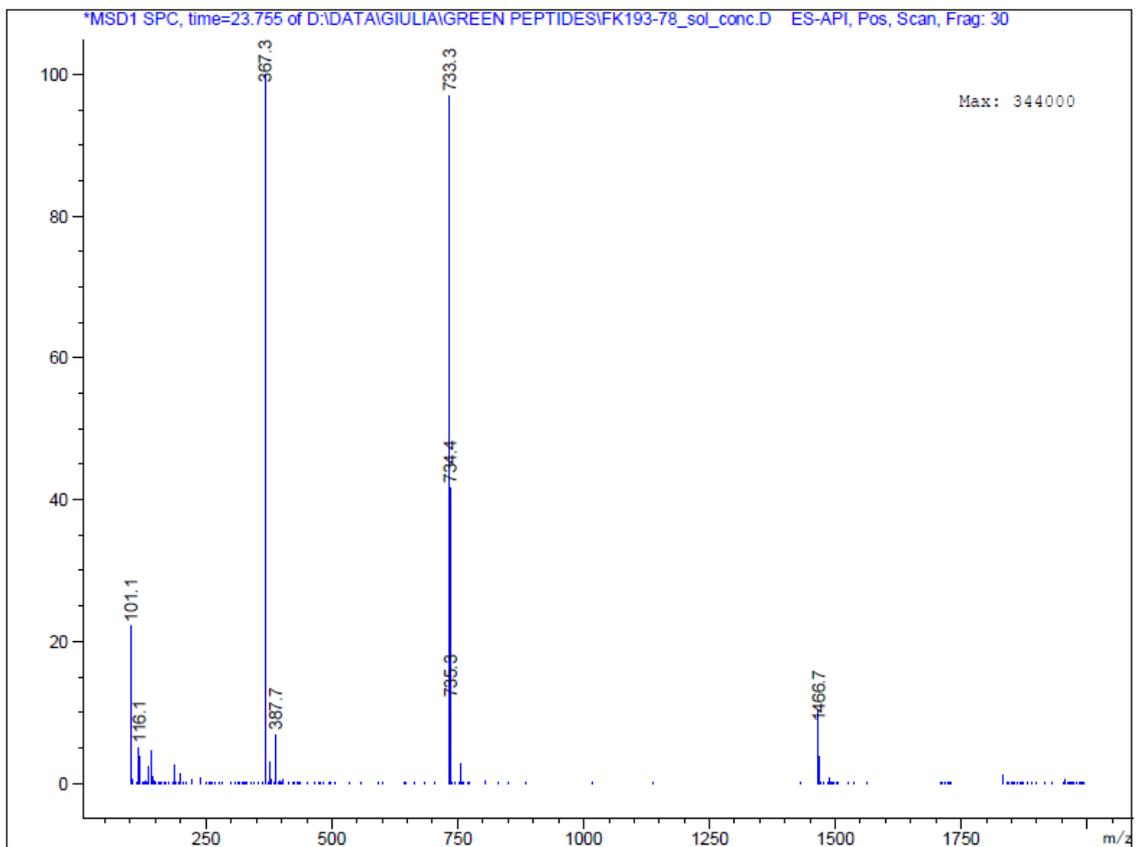
**Figure S71.** Mass spectrum of H-Ala-Lys-Asp-Gly-Tyr-Ile-OH (hexapeptide I)



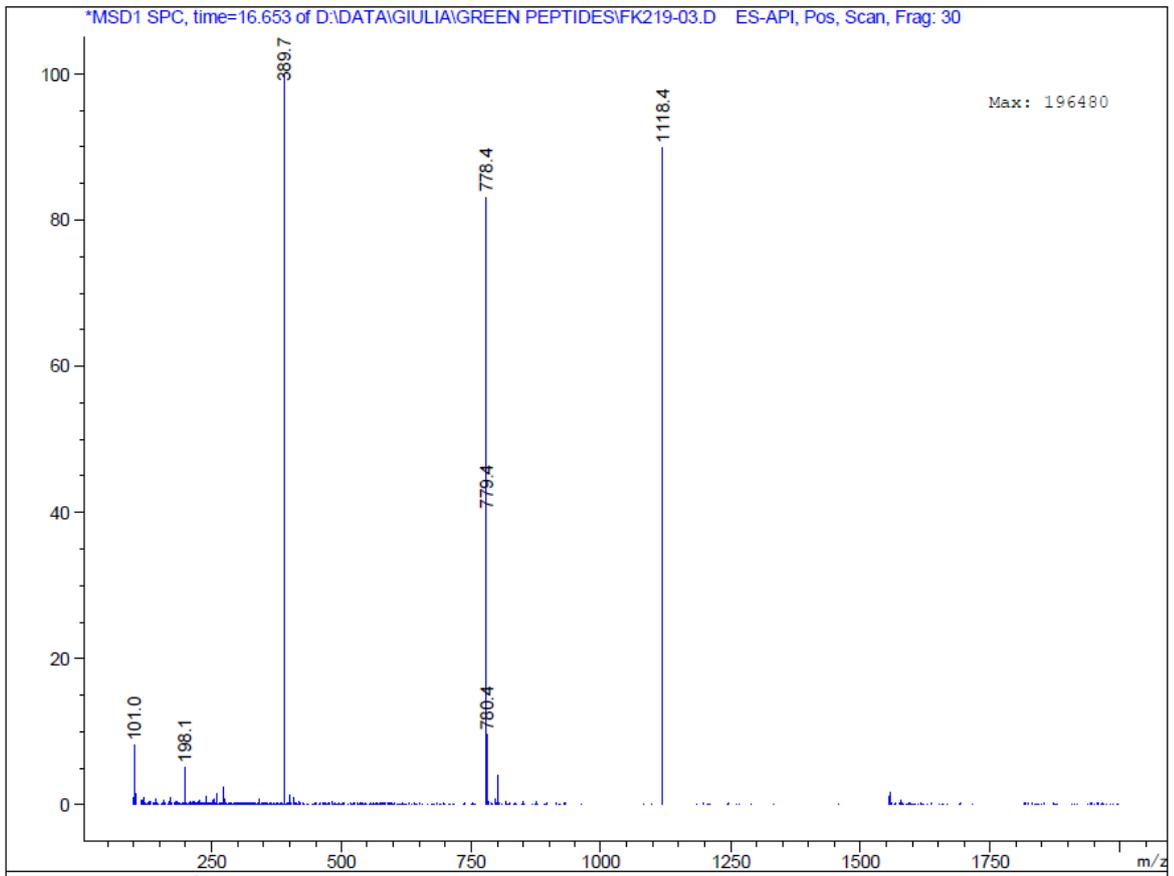
**Figure S72.** Mass spectrum of aspartimide II



**Figure S73.** Mass spectrum of piperidides III

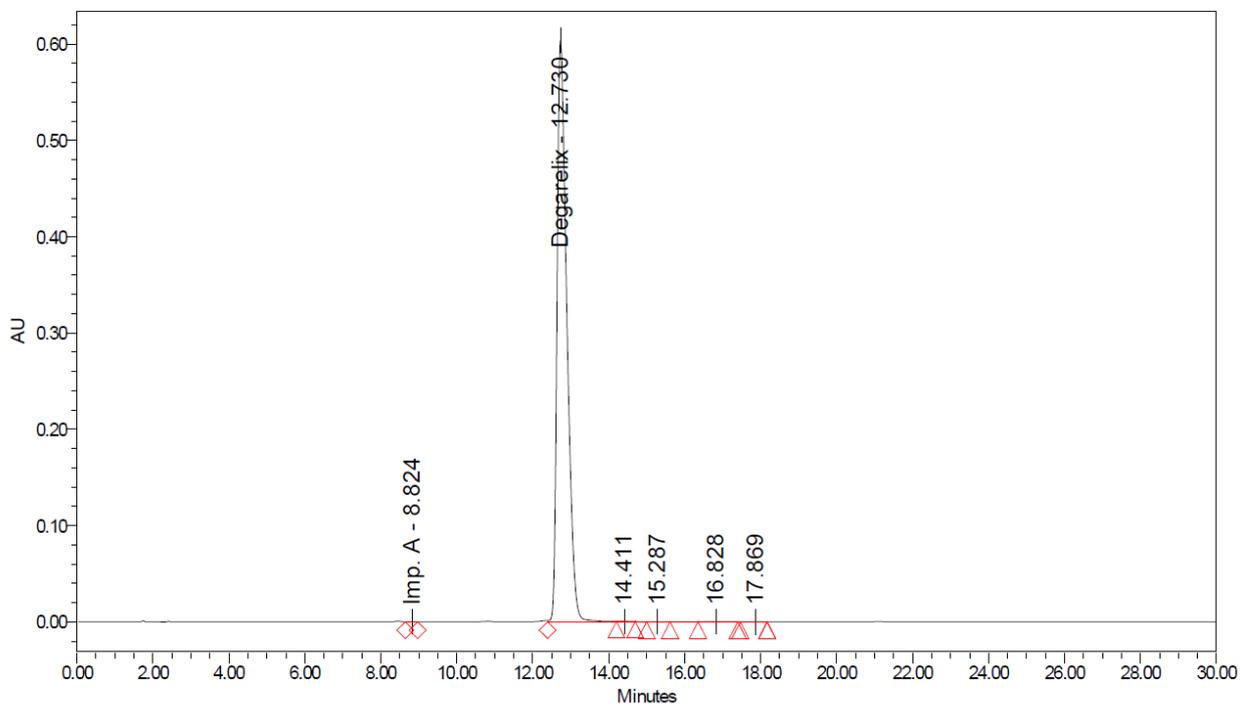


**Figure S74.** Mass spectrum of DEAPA adducts IV



# (L)-dihydroorotic fragment isomerization

**Figure S75.** Chromatogram of Degarelix at t=0

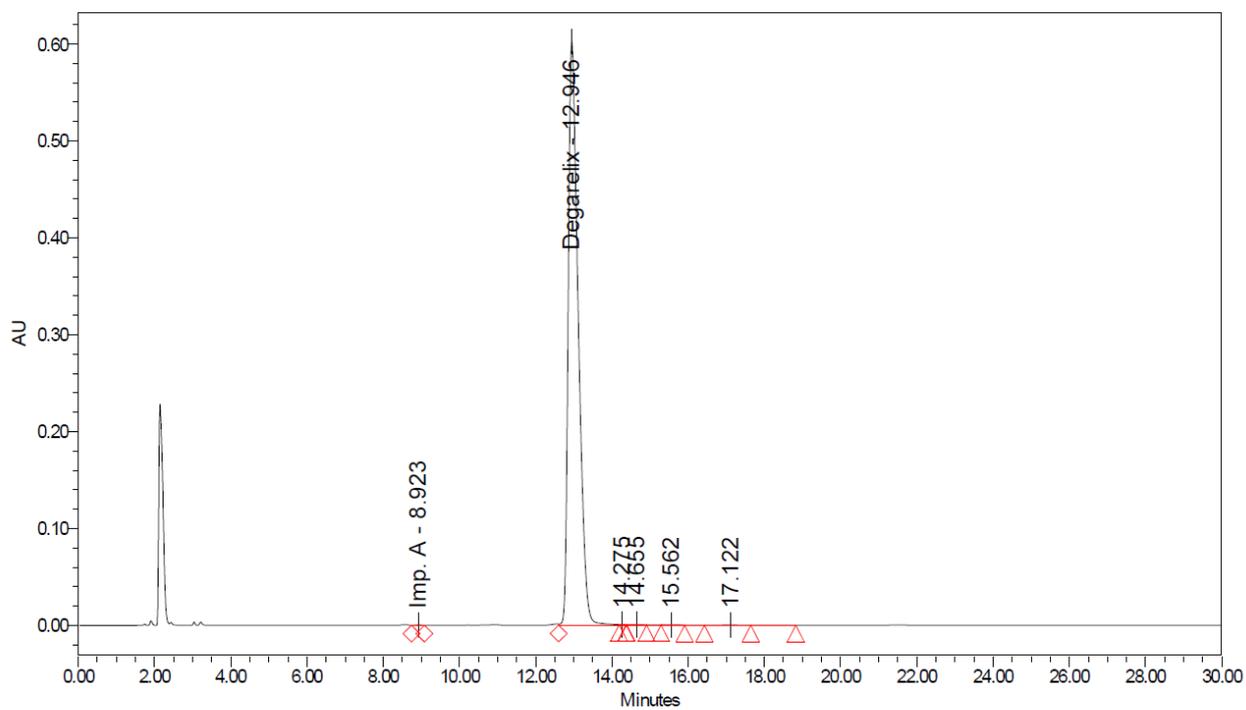


picchi > 0.025%

	Name	RT	Area	% Area	RRT
1	Imp. A	8.82	2938	0.03	0.693
2	Degarelix	12.73	10769657	99.91	1.000

Product	Rt (min)	Area (%)
Hydantoin VI	8.824	0.03
Degarelix V	12.730	99.97

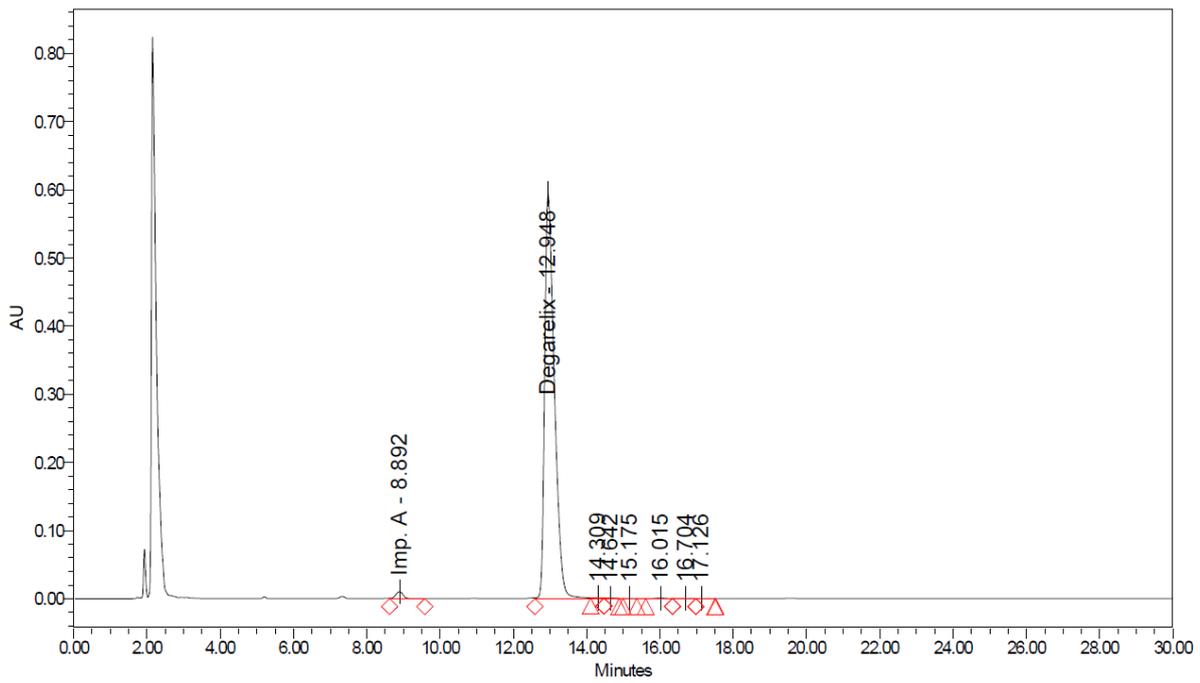
**Figure S76.** Chromatogram of Degarelix after stress test with **10% piperidine** in DMF, 24 h at RT



	Name	RT	Area	% Area	RRT
1	Imp. A	8.92	3863	0.04	0.689
2	Degarelix	12.95	10966991	99.90	1.000
3		14.66	2978	0.03	1.132
4		17.12	3767	0.03	1.323

Product	Rt (min)	Area (%)
Hydantoin VI	8.923	0.04
Degarelix V	12.946	99.96

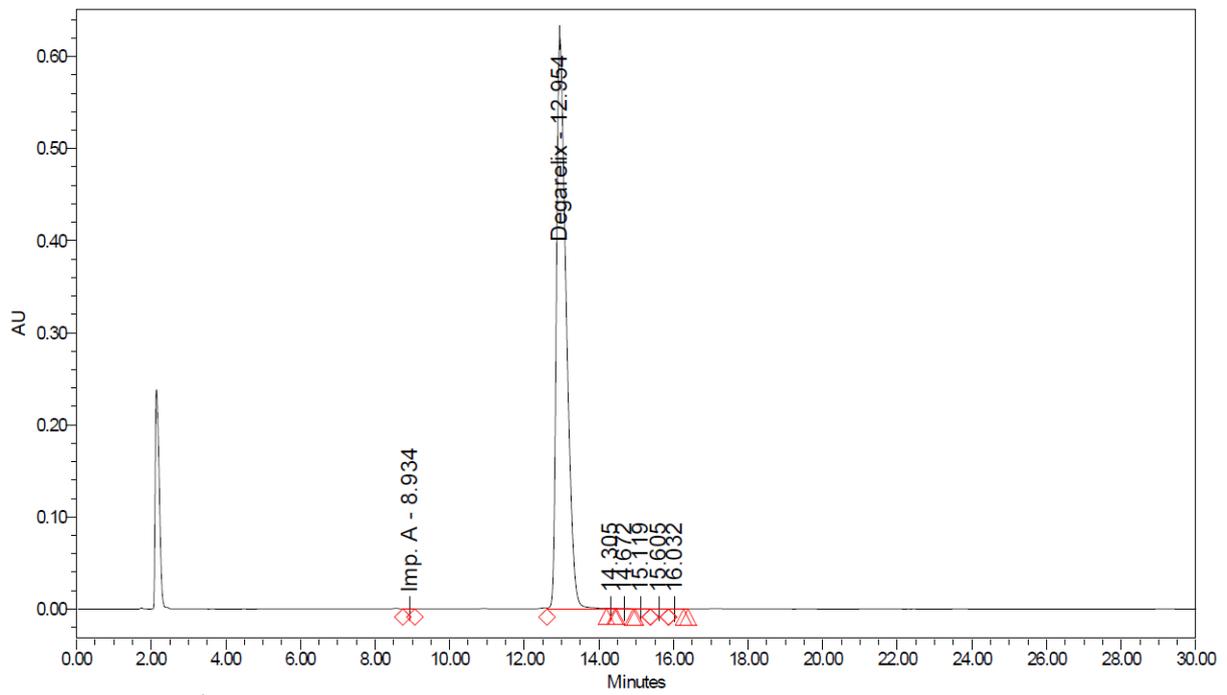
**Figure S77.** Chromatogram of Degarelix after stress test with **10% TMG** in DMF, 24 h at RT



	Name	RT	Area	% Area	RRT
1	Imp. A	8.89	142550	1.30	0.687
2	Degarelix	12.95	10840484	98.50	1.000
3		14.64	3262	0.03	1.131
4		16.01	10922	0.10	1.237
5		16.70	4136	0.04	1.290

Product	Rt (min)	Area (%)
Hydantoin VI	8.892	1.3
Degarelix V	12.948	98.7

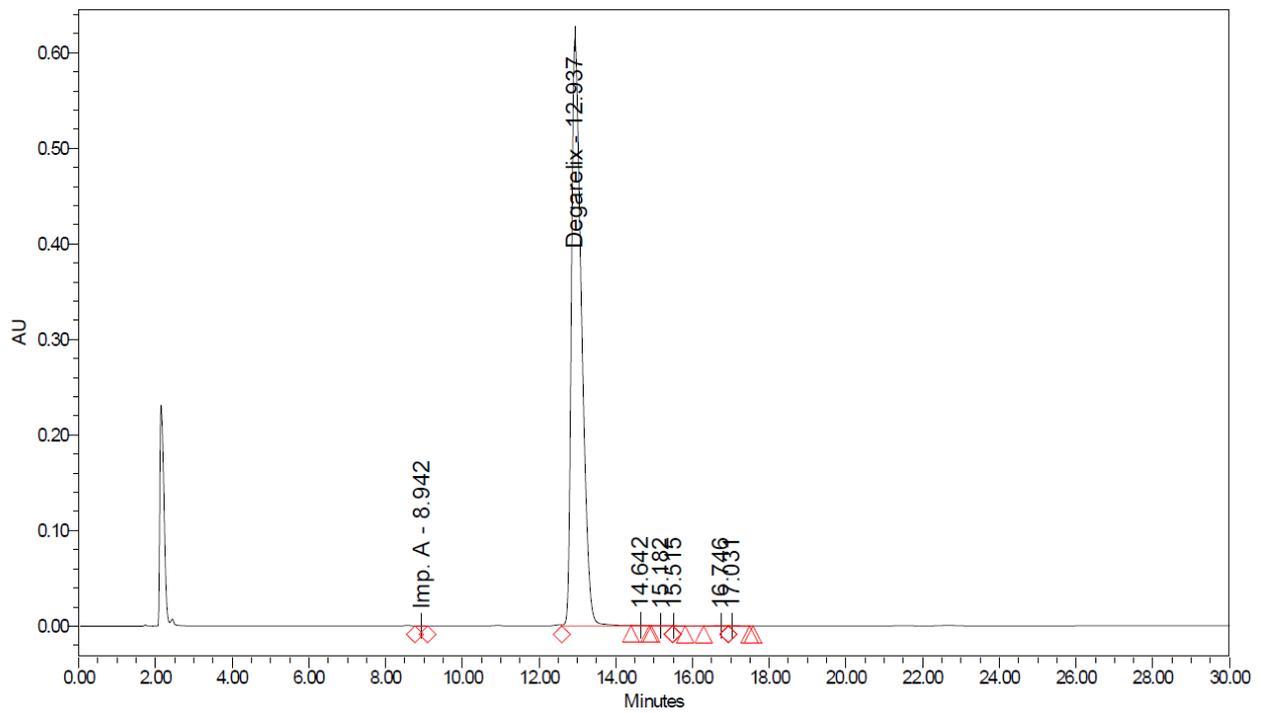
**Figure S78.** Chromatogram of Degarelix after stress test with **10% TBA** in DMF, 24 h at RT



	Name	RT	Area	% Area	RRT
1	Imp. A	8.93	4270	0.04	0.690
2	Degarelix	12.95	11313969	99.93	1.000

Product	Rt (min)	Area (%)
Hydantoin VI	8.934	0.04
Degarelix V	12.954	99.96

**Figure S79.** Chromatogram of Degarelix after stress test with **10% DEAPA** in DMF, 24 h at RT

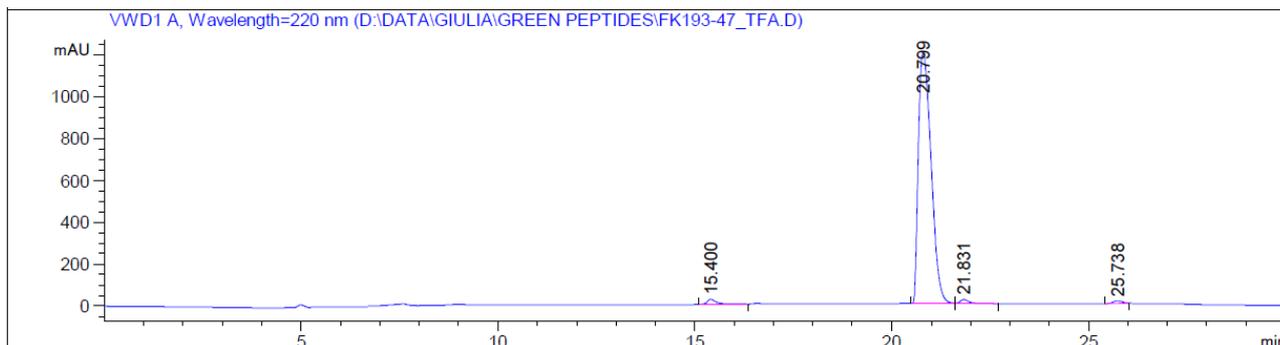


	Name	RT	Area	% Area	RRT
1	Imp. A	8.94	3599	0.03	0.691
2	Degarelix	12.94	11184556	99.86	1.000
3		14.64	2912	0.03	1.132
4		16.75	4097	0.04	1.294
5		17.03	3207	0.03	1.317

Product	Rt (min)	Area (%)
Hydantoin VI	8.942	0.03
Degarelix V	12.937	99.97

## SPPS of Aib-Enkephalin in NOP

**Figure S80.** Chromatogram of Aib-Enkephalin, manual SPPS in NOP with **20% TBA** as deprotection solution



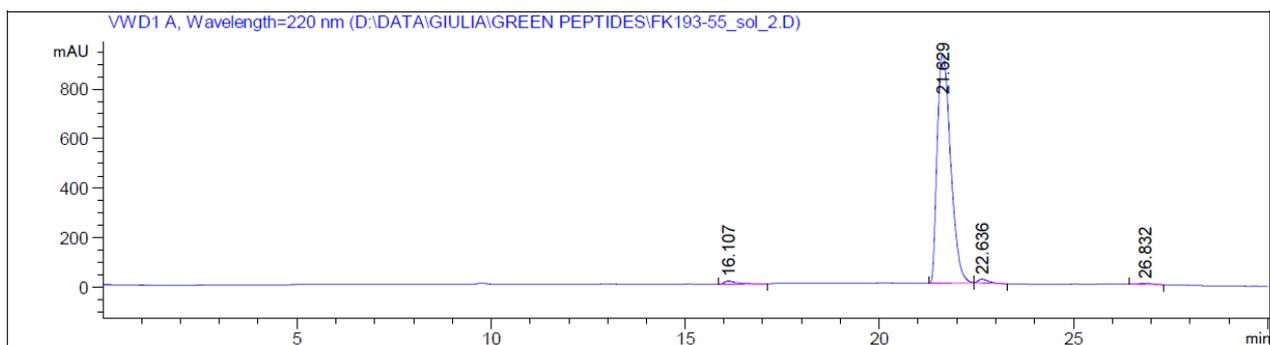
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.400	BB	0.2299	357.34210	22.67599	1.3046
2	20.799	BB	0.3597	2.66358e4	1196.59863	97.2463
3	21.831	BB	0.2096	229.77277	16.38626	0.8389
4	25.738	BB	0.2509	167.13870	10.54478	0.6102

Totals : 2.73901e4 1246.20566

Peptide	Rt (min)	Area (%)
Des-Phe	15.400	1.3
Aib-Enkephalin	20.799	97.3
Des-Aib	21.831	0.8
Aib-Enkephalin+TFA	25.738	0.6

**Figure S81.** Chromatogram of Aib-Enkephalin, manual SPPS in NOP with **5% TMG** as deprotection solution



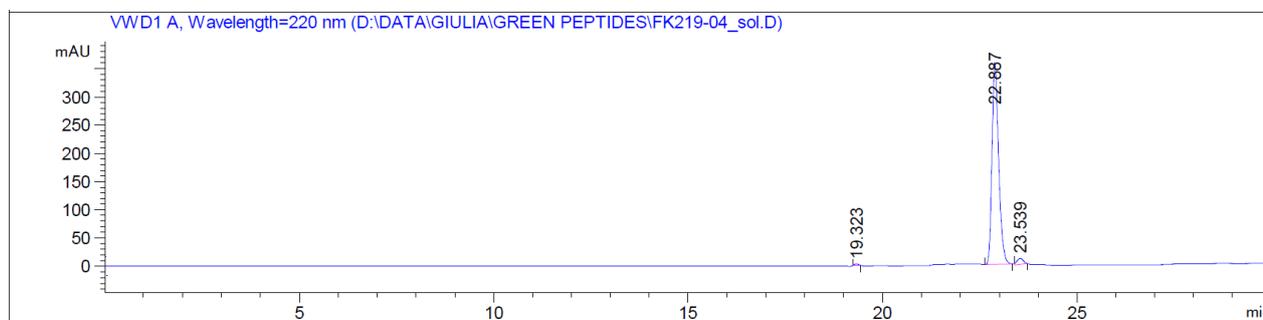
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.107	BB	0.3246	258.79755	11.49601	1.1419
2	21.629	BB	0.3805	2.20396e4	923.18396	97.2419
3	22.636	BB	0.2685	256.51566	14.77845	1.1318
4	26.832	BB	0.3401	109.81307	5.00234	0.4845

Totals : 2.26647e4 954.46076

Peptide	Rt (min)	Area (%)
Des-Phe	16.107	1.1
Aib-Enkephalin	21.629	97.3
Des-Aib	22.636	1.1
Aib-Enkephalin+TFA	26.832	0.5

**Figure S82.** Chromatogram of Aib-Enkephalin, manual SPPS in NOP with **10% DEAPA** as deprotection solution



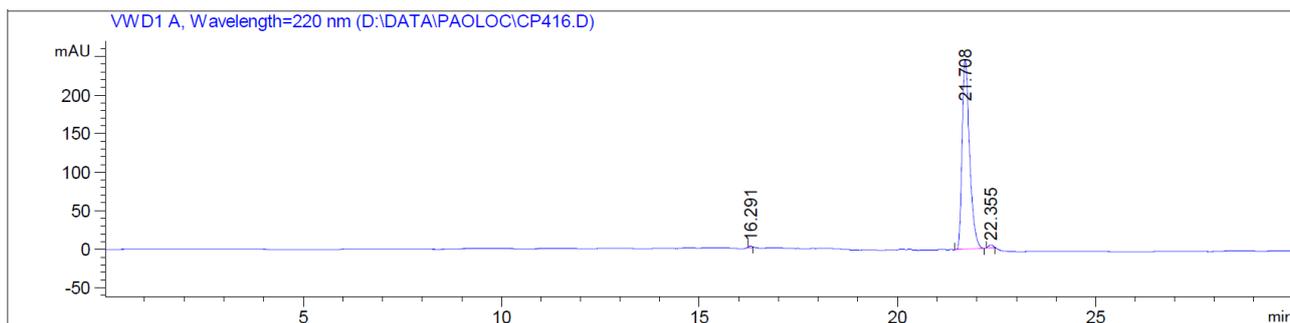
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.323	MM	0.1034	14.80165	2.38595	0.3369
2	22.887	BB	0.1835	4266.15869	356.70010	97.0994
3	23.539	MM	0.1795	112.63808	10.45851	2.5637

Totals :                      4393.59843   369.54456

Peptide	Rt (min)	Area (%)
Des-Tyr	19.323	0.3
Aib-Enkephalin	22.887	97.1
Des-Aib	23.539	2.6

**Figure S83.** Chromatogram of Aib-Enkephalin, manual SPPS in NOP with **10% DEAPA** as deprotection solution and deprotection steps pre Fmoc-Aib-OH insertion at 40°C



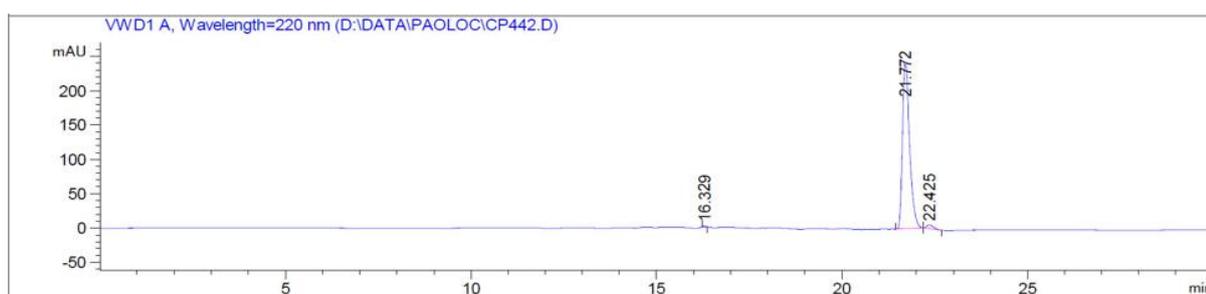
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.291	MM	0.0987	13.42192	2.26539	0.4094
2	21.708	BB	0.1991	3230.95776	246.19342	98.5618
3	22.355	MM	0.1381	33.72318	4.06926	1.0287

Totals : 3278.10286 252.52807

Peptide	Rt (min)	Area (%)
Des-Phe	16.291	0.4
Aib-Enkephalin	21.708	98.6
Des-Aib	22.355	1.0

**Figure S84.** Chromatogram of Aib-Enkephalin, manual SPPS in NOP/DMC 8/2 with **10% DEAPA** as deprotection solution



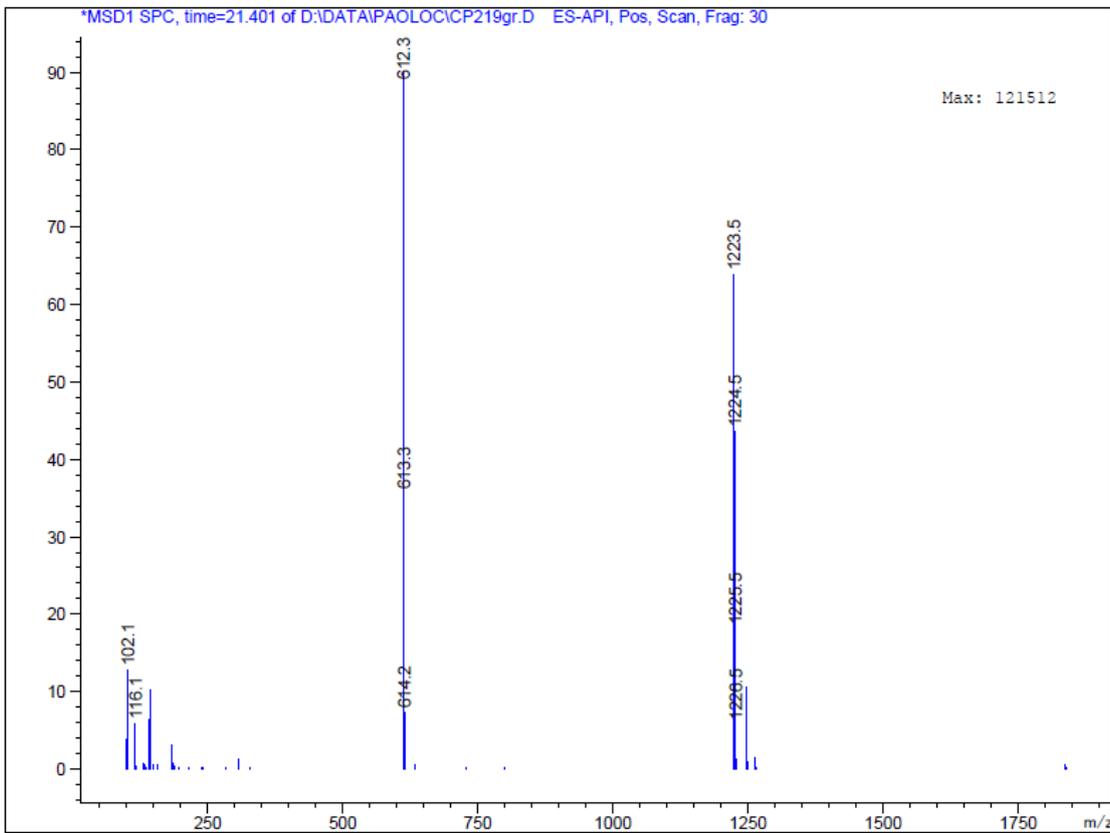
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.329	MM	0.1097	14.90592	2.26539	0.4497
2	21.772	BB	0.1991	3230.95776	246.19342	97.4781
3	22.425	BB	0.1875	68.68263	5.66201	2.0722

Totals : 3314.54631 254.12082

Peptide	Rt (min)	Area (%)
Des-Phe	16.329	0.4
Aib-Enkephalin	21.772	97.5
Des-Aib	22.425	2.1

**Figure S85.** Mass spectrum of H-Tyr-Aib-Aib-Phe-Leu-OH (Aib-Enkephalin)



**Figure S86.** Mass spectrum of H-Tyr-Aib-Aib-Phe-Leu-OH + TFA (Aib-Enkephalin+TFA)

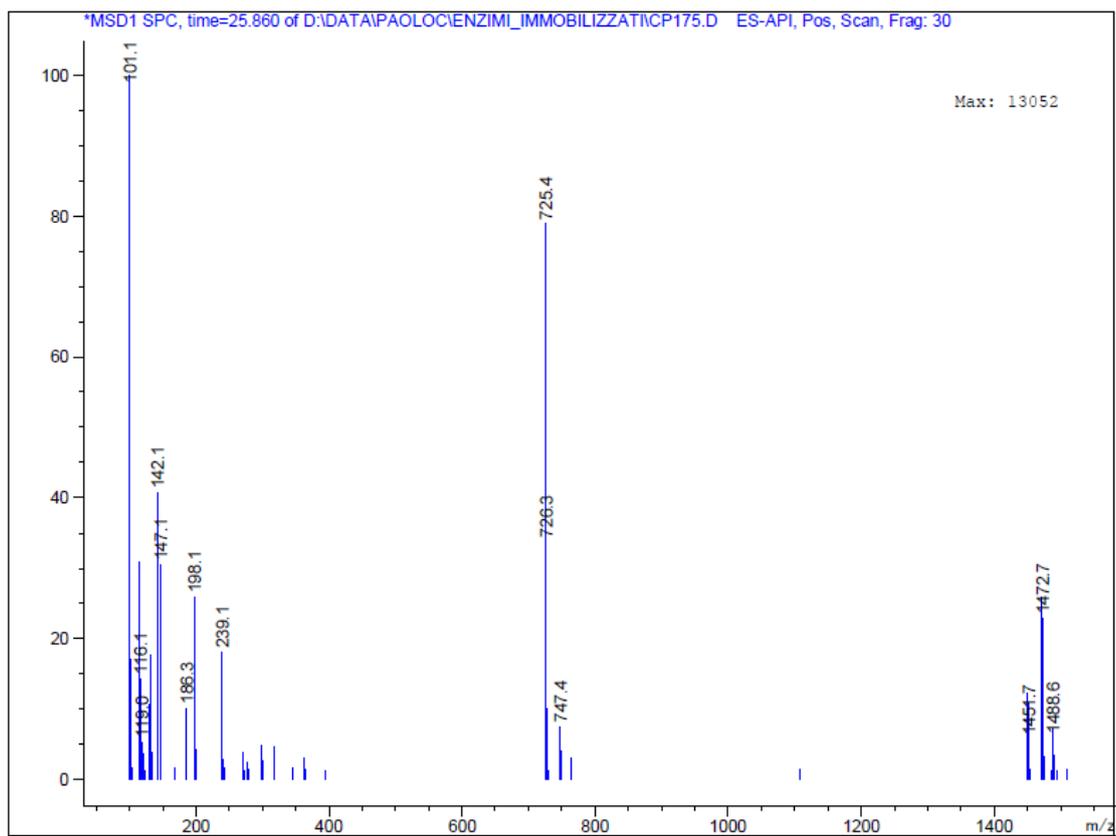


Figure S87. Mass spectrum of H-Tyr-Aib-Phe-Leu-OH (des-Aib)

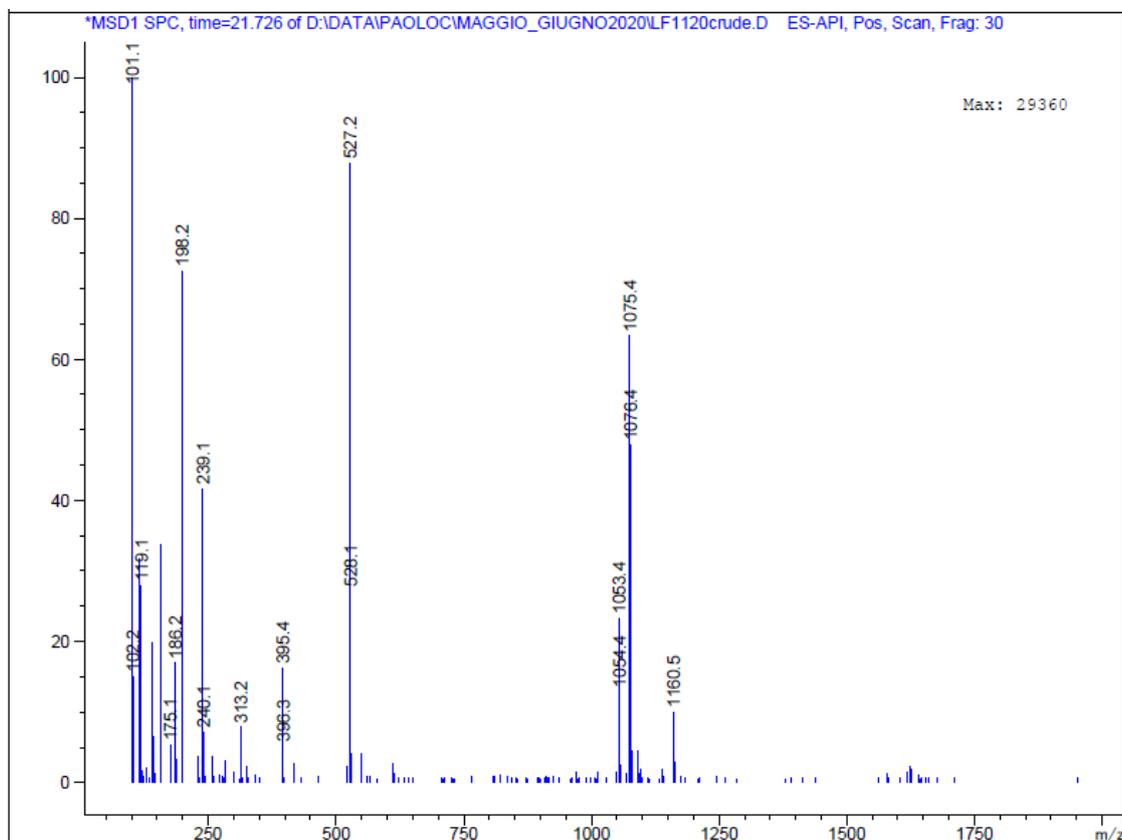


Figure S88. Mass spectrum of H-Tyr-Aib-Aib-Leu-OH (des-Phe)

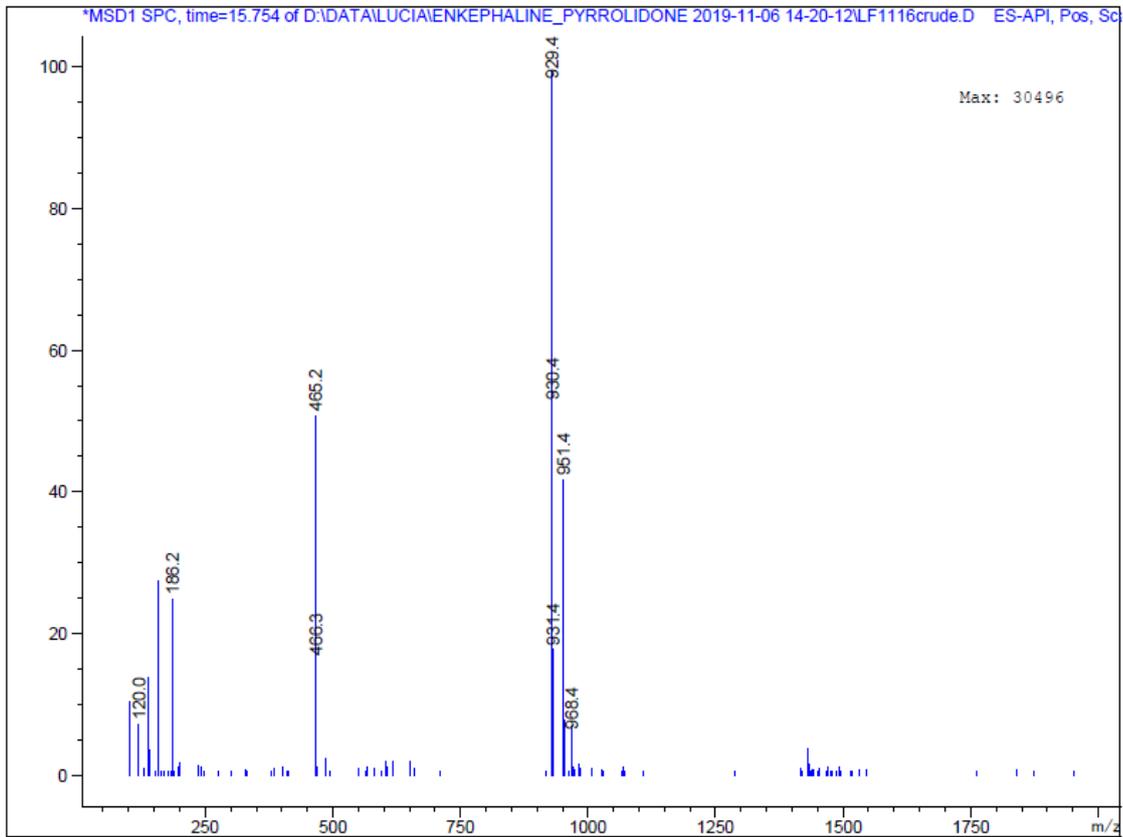
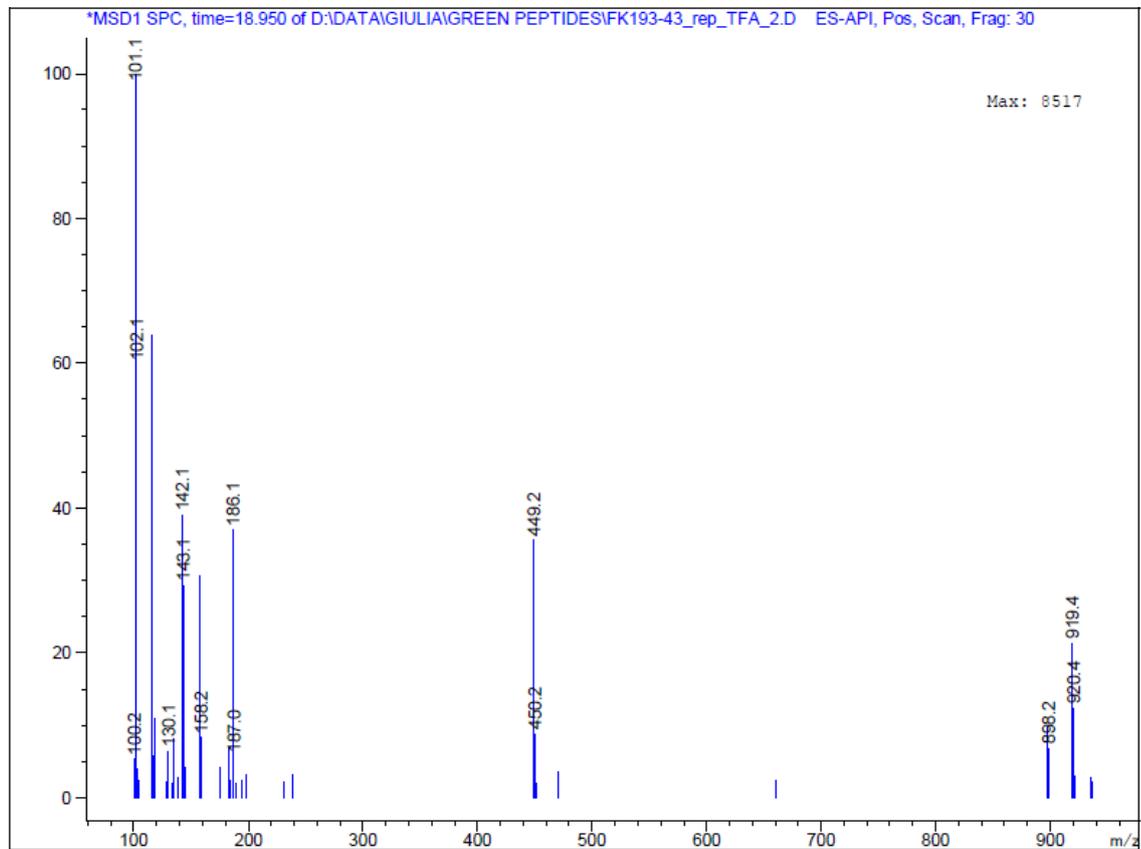
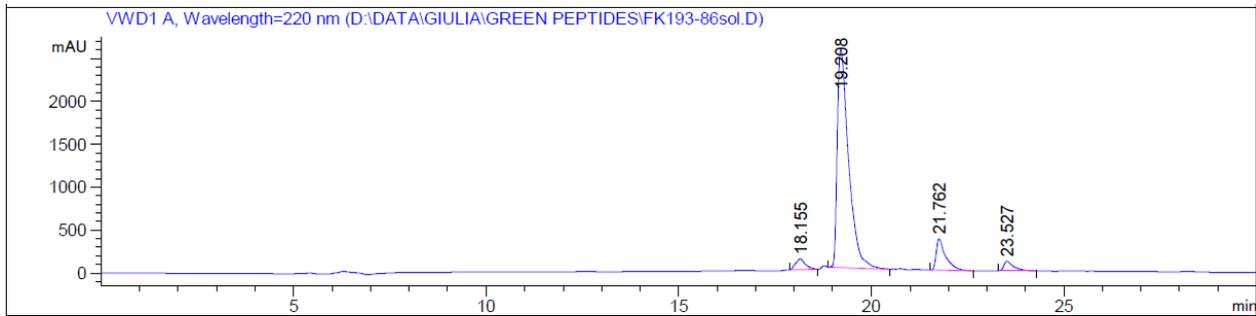


Figure S89. Mass spectrum of H-Aib-Aib-Phe-Leu-OH (des-Tyr)



## SPPS of linear Octreotide

**Figure S90.** Chromatogram of linear Octreotide, manual SPPS in DMF with 10% DEAPA as deprotection solution



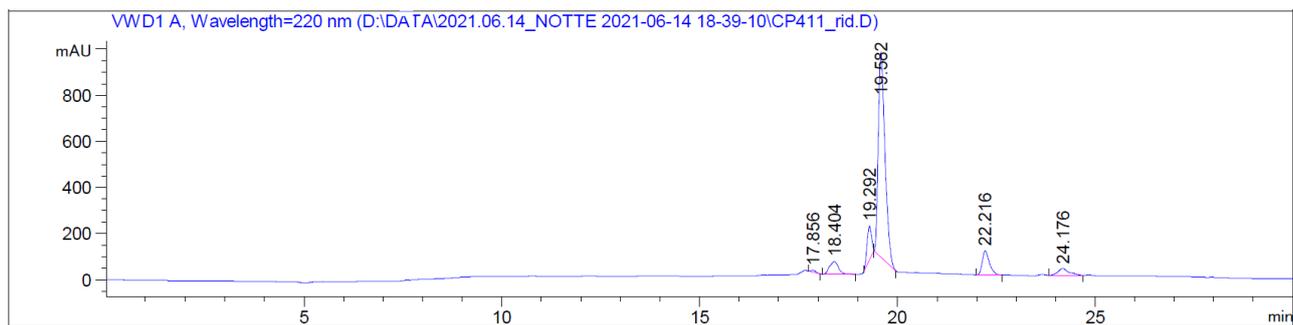
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.155	BB	0.2431	2267.19751	128.98524	3.6195
2	19.208	BB	0.3053	5.23553e4	2552.87720	83.5828
3	21.762	BB	0.2335	6061.45654	365.42905	9.6768
4	23.527	BB	0.2534	1954.90027	109.96827	3.1209

Totals : 6.26388e4 3157.25976

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 2	18.155	3.6
Linear Octreotide	19.208	83.6
Linear Octreotide+tBu	21.762	9.7
Linear Octreotide+tBu2	23.527	3.1

**Figure S91.** Chromatogram of linear Octreotide, manual SPPS in NOP with 10% DEAPA as deprotection solution



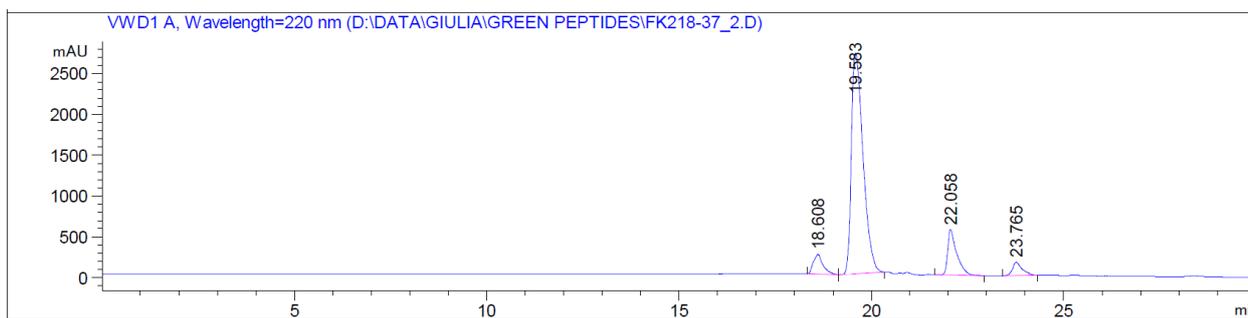
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.856	BB	0.1324	68.70734	8.31694	0.4721
2	18.404	BB	0.2212	858.56342	55.26545	5.8990
3	19.292	BB	0.1331	1210.08337	148.49017	8.3143
4	19.582	BB	0.1824	1.05137e4	886.10333	72.2380
5	22.216	BB	0.1878	1316.99585	105.35062	9.0488
6	24.176	BB	0.2722	586.20697	30.50037	4.0277

Totals : 1.45543e4 1234.02690

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 1	17.856	0.5
Linear Octreotide N,O-shift 2	18.404	5.9
Des-Cys <sup>7</sup>	19.292	8.3
Linear Octreotide	19.582	72.2
Linear Octreotide+tBu	22.216	9.0
Linear Octreotide+tBu2	24.176	4.0

**Figure S92.** Chromatogram of linear Octreotide, manual SPPS in NOP with 10% DEAPA as deprotection solution and 4h initial swelling times



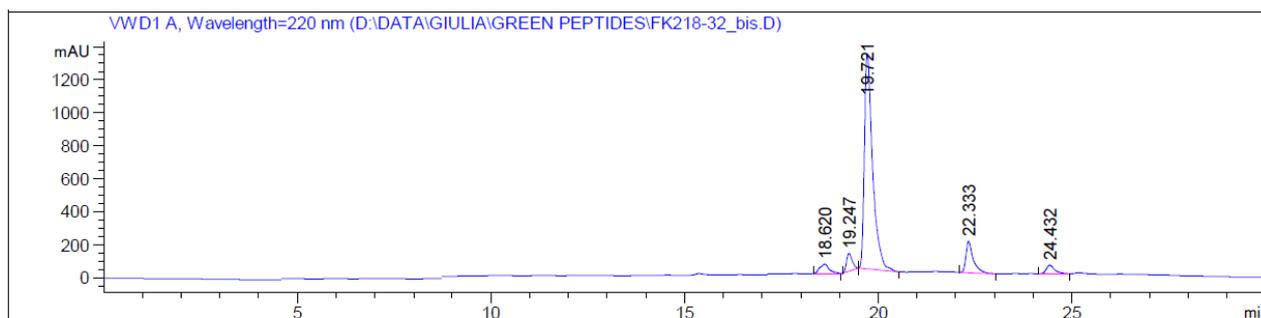
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.608	BB	0.2379	4155.06592	240.08929	5.5821
2	19.583	BB	0.3289	5.81646e4	2705.12622	78.1415
3	22.058	BB	0.2324	9134.56543	559.85425	12.2719
4	23.765	BB	0.2571	2980.73828	164.73466	4.0045

Totals : 7.44349e4 3669.80443

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 2	18.620	5.6
Linear Octreotide	19.721	78.1
Linear Octreotide+tBu	22.333	12.3
Linear Octreotide+tBu2	24.432	4.0

**Figure S93.** Chromatogram of linear Octreotide, manual SPPS at 40°C in NOP with 10% DEAPA as deprotection solution



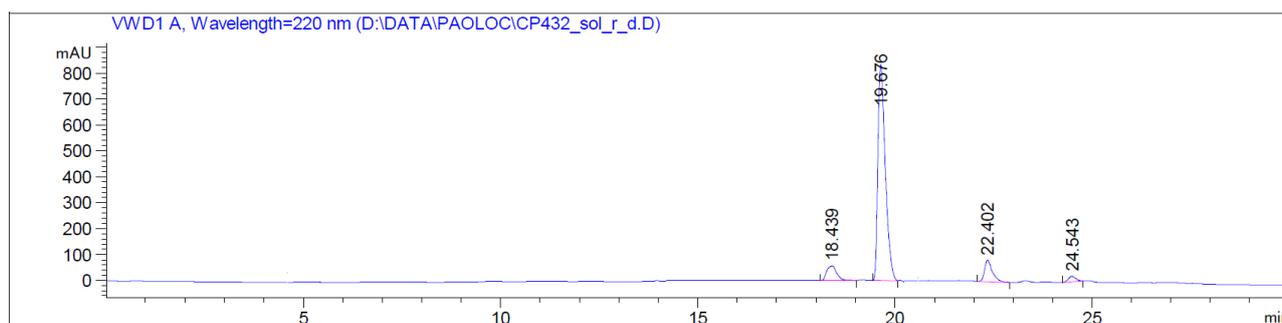
Signal 3: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.620	BB	0.2310	956.72913	56.67263	3.8480
2	19.247	BB	0.1570	1105.90088	108.45225	4.4480
3	19.721	BB	0.2217	1.94325e4	1305.05444	78.1583
4	22.333	BB	0.1933	2539.91138	190.86345	10.2156
5	24.432	BB	0.2317	827.98010	53.17313	3.3302

Totals : 2.48630e4 1714.21590

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 2	18.620	3.8
Linear Octreotide + CO <sub>2</sub>	19.247	4.4
Linear Octreotide	19.721	78.2
Linear Octreotide+tBu	22.333	10.2
Linear Octreotide+tBu2	24.432	3.4

**Figure S94.** Chromatogram of linear Octreotide, manual SPPS in **NOP/DMC 8/2 with 10% DEAPA** as deprotection solution (swelling and first Fmoc removal at 40°C)



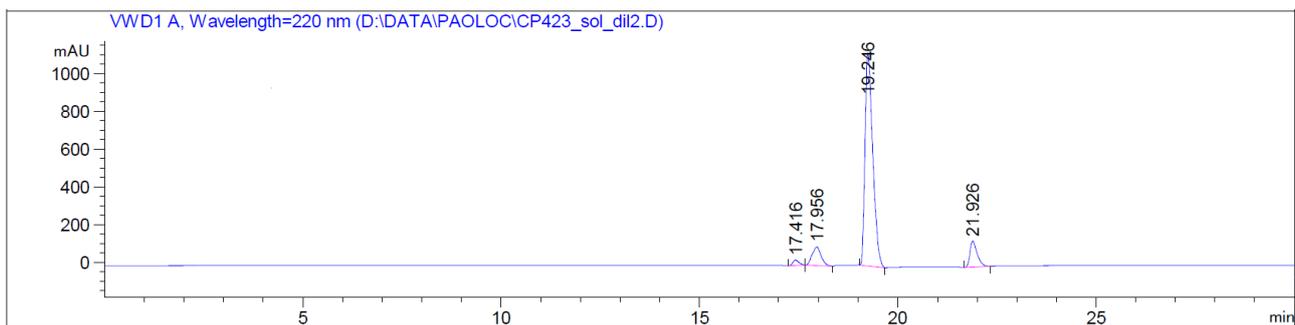
Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.439	BB	0.2761	988.99408	56.52509	7.4996
2	19.676	BB	0.1952	1.07978e4	833.08105	81.8802
3	22.402	BB	0.2005	1120.30371	82.48135	8.4953
4	24.543	BB	0.2035	280.22736	21.30368	2.1250

Totals : 1.31873e4 993.39118

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 2	18.439	7.5
Linear Octreotide	19.676	81.9
Linear Octreotide+tBu	22.402	8.5
Linear Octreotide+tBu2	24.543	2.1

**Figure S95.** Chromatogram of linear Octreotide, manual SPPS at 40°C in NOP/DMC 80:20 with 10% DEAPA as deprotection solution



Signal 2: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.416	BB	0.1721	316.09729	28.35434	1.6695
2	17.956	BB	0.2228	1543.02795	98.46199	8.1498
3	19.246	BB	0.2065	1.53523e4	1130.12085	81.0866
4	21.926	BB	0.1883	1721.79199	137.30876	9.0940

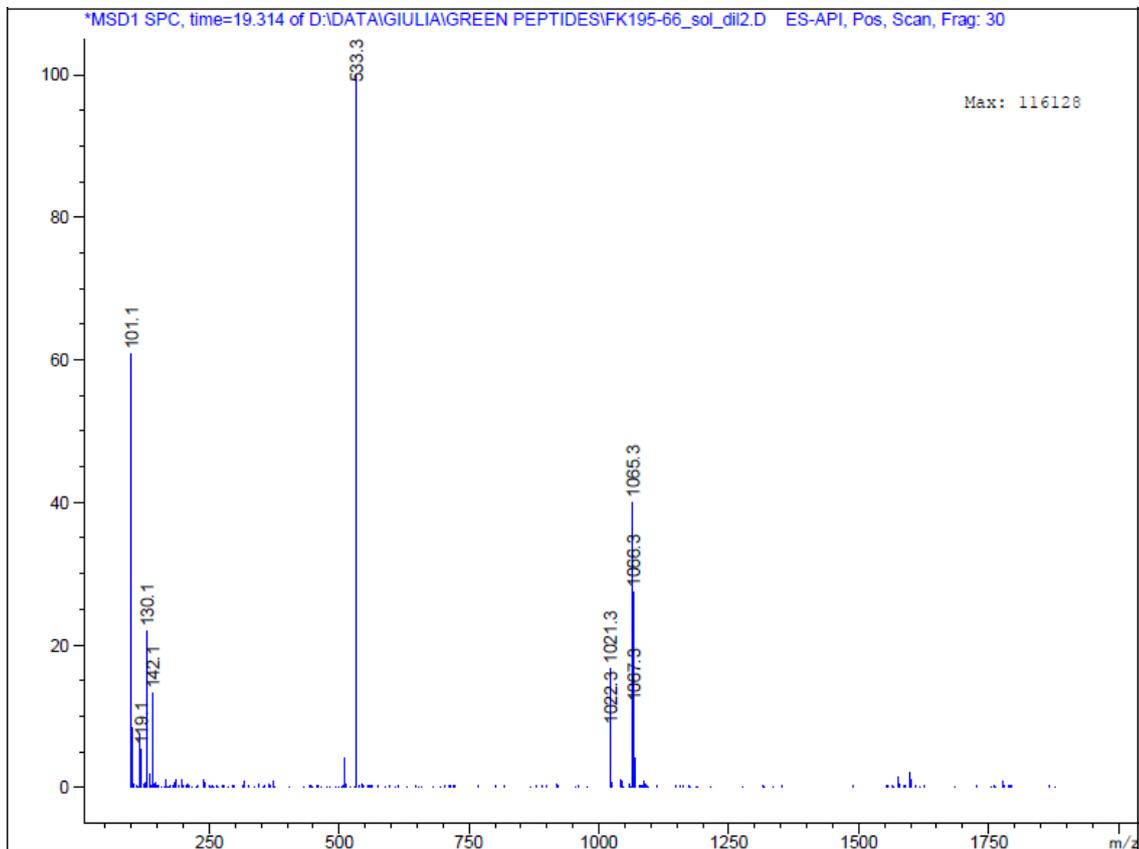
Totals : 1.89333e4 1394.24594

Peptide	Rt (min)	Area (%)
Linear Octreotide N,O-shift 1	17.416	1.7
Linear Octreotide N,O-shift 2	17.956	8.1
Linear Octreotide	19.246	81.1
Linear Octreotide+tBu	21.926	9.1

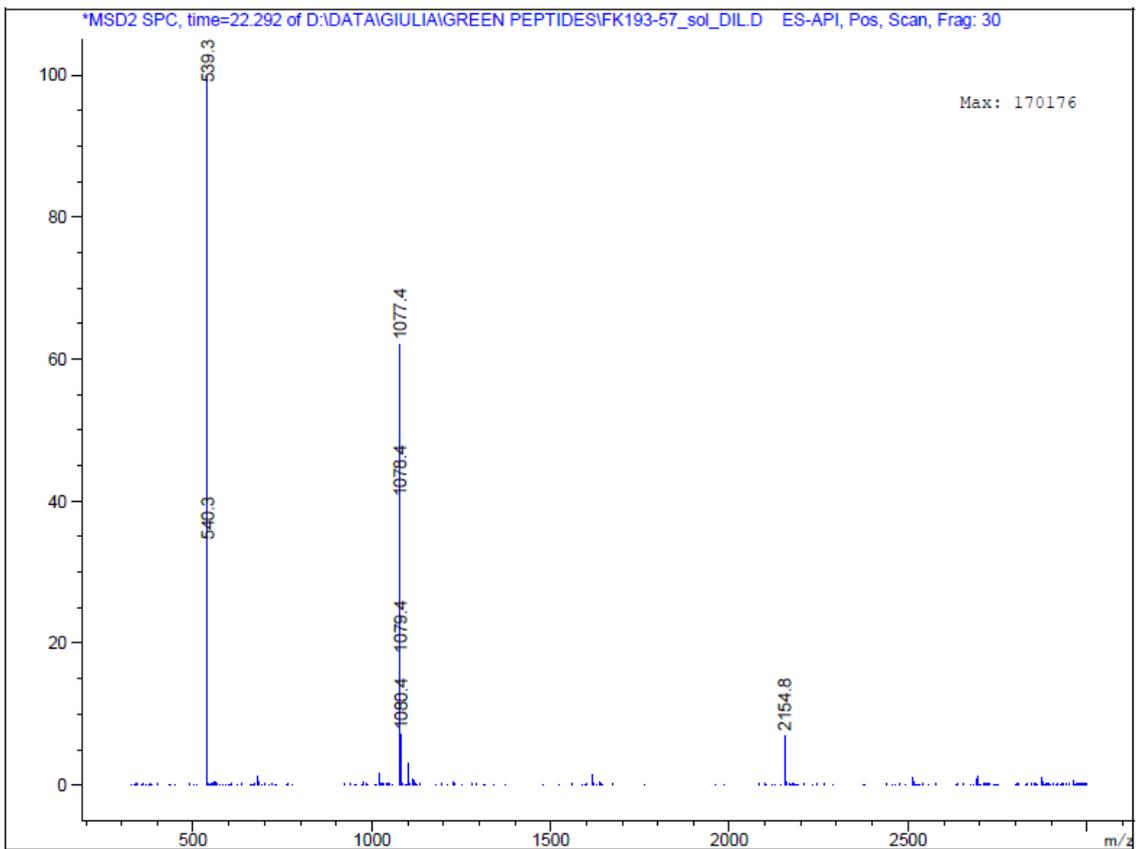
**Figure S96.** Mass spectrum of linear Octreotide



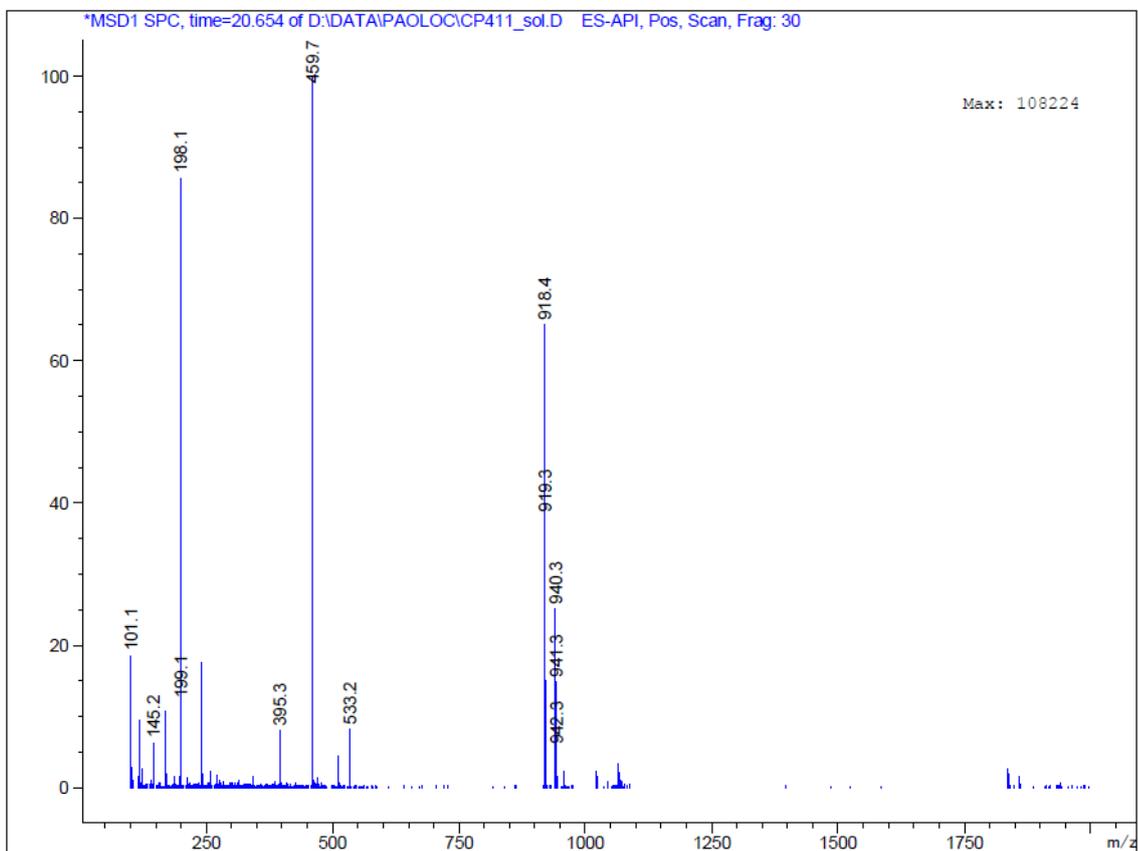
**Figure S97.** Mass spectrum of linear Octreotide+CO<sub>2</sub>



**Figure S98.** Mass spectrum of linear Octreotide+tBu



**Figure S99.** Mass spectrum of H<sub>2</sub>N-D-Phe-Cys<sup>2</sup>-Phe-D-Trp-Lys-Thr-Thr-ol (des-Cys<sup>7</sup>)



## Solvent and base recovery from waste stream of linear Octreotide SPPS

### *General considerations*

Solid phase peptide syntheses of linear Octreotide (see Table 8) were performed using the same procedure in all cases (both for manually and automated processes), except for the employed solvent, according to the protocol reported in the Experimental Section. The scale of the syntheses was 0.22 mmol in all cases, and the amount of the yielded crude linear Octreotide was comparable for all processes. Therefore, crude mass of linear Octreotide was considered to be the same in all cases (0.204 g) for PMI calculations. The total amount of solvent consumption derived from the different SPPS was the same in all cases; when the mixture NOP/DMC 8/2 was used, the ratio between the two solvents was maintained along all the synthetic steps.

Methods of linear Octreotide SPPS reported in Table 8 are compared to calculate the PMI of the processes: i) conventional synthesis in DMF; ii) synthesis in NOP at RT with 4 hours swelling time; iii) synthesis in NOP at 40°C; iv) synthesis in NOP/DMC 8/2 at RT (swelling and first deprotection at 40°C); v) synthesis in NOP/DMC 8/2 at 40°C. In cases from ii) to v), coupling and deprotection stream wastes were distilled separately to obtain recovery of solvent or of solvent and base, respectively, as reported in the Experimental Section.

### *PMI calculations for linear Octreotide SPPS*

Process Mass Intensity (PMI) is defined as the ratio between the total mass of materials and the mass of the isolated product as reported below:

$$PMI = \frac{\sum \text{mass of materials}}{\text{mass of isolated product}}$$

In our case, the total mass of materials includes:

- Starting materials: Fmoc-AA-OH, DIC, OxymaPure®, resin, TFA + scavengers (TIPS, 1-dodecanthiol)
- Solvents: DMF or NOP or NOP/DMC 8/2, DCM (for washings of the resin pre-cleavage) and Et<sub>2</sub>O (for crude peptide precipitation)
- Base (DEAPA)

The total mass of starting materials, base, DCM and Et<sub>2</sub>O does not change along all SPPS independently from the solvent employed for the synthesis; an overview of these “fixed” reactants with their relative masses is reported in Table S2; when the syntheses were performed at 40°C, the amount of employed Fmoc-AA-OH, DIC and OxymaPure® was halved; accordingly, the PMI slightly decreases in these cases (Table S3)

The total mass of the solvent employed for the synthesis (DMF or NOP or NOP/DMC 8/2), even if does not change in volume, slightly changes according to solvents’ different densities; an overview of the employed solvents with their relative masses is reported in Table S4.

An overview of the total mass of materials employed in the syntheses at RT and at 40°C with no solvent and base recovery is reported in Table S5 and Table S6, respectively.

**Table S2.** Overview of starting materials, base, DCM and Et<sub>2</sub>O employed in **SPPS of linear Octreotide at RT** and their relative masses

	MW (g/mol)	d (g/mL)	eq	mmol	Volume (mL)	Mass (g)	repetitions	Total mass (g)
Fmoc-Thr(tBu)-ol-Trt- PS resin			1	0,22		0,2	1	0,2
<b><i>Coupling</i></b>								
Fmoc-Cys(Trt)-OH	585,71		3	0,66		0,39	3	1,16
Fmoc-Thr(tBu)-OH	397,43		3	0,66		0,26	1	0,26
Fmoc-Lys(Boc)-OH	468,54		3	0,66		0,31	1	0,31
Fmoc-D-Trp(Boc)-OH	526,28		3	0,66		0,35	1	0,35
Fmoc-Phe-OH	387,43		3	0,66		0,26	1	0,26
Fmoc-D-Phe-OH	387,43		3	0,66		0,26	1	0,26
OxymaPure	142,11		3	0,66		0,09	8	0,75
DIC	126,2	0,815	3	0,66		0,08	8	0,67
<b><i>Deprotection</i></b>								
DEAPA	130,23	0,826			0,2	0,165	16	2,64
<b><i>Cleavage and precipitation</i></b>								
TFA	114,02	1,489			4,50	6,70	1	6,70
TIPS	158,36	0,773			0,25	0,19	1	0,19
1-dodecanthiol	18,02	0,997			0,25	0,21	1	0,21
DCM	84,93	1,325			2	2,65	3	7,95
Et <sub>2</sub> O	102,17	0,725			25	18,13	1	18,13
							<b>Total</b>	<b>40,03</b>

**Table S3.** Overview of starting materials, base, DCM and Et<sub>2</sub>O employed in **SPPS of linear Octreotide at 40°C** and their relative masses

	MW (g/mol)	d (g/mL)	eq	mmol	Volume (mL)	Mass (g)	repetitions	Total mass (g)
Fmoc-Thr(tBu)-ol-Trt- PS resin			1	0,22		0,2	1	0,2
<b><i>Coupling</i></b>								
Fmoc-Cys(Trt)-OH	585,71		1,5	0,33		0,19	3	0,58
Fmoc-Thr(tBu)-OH	397,43		1,5	0,33		0,13	1	0,13
Fmoc-Lys(Boc)-OH	468,54		1,5	0,33		0,15	1	0,15
Fmoc-D-Trp(Boc)-OH	526,28		1,5	0,33		0,17	1	0,17
Fmoc-Phe-OH	387,43		1,5	0,33		0,13	1	0,13
Fmoc-D-Phe-OH	387,43		1,5	0,33		0,13	1	0,13
OxymaPure	142,11		1,5	0,33		0,05	8	0,38
DIC	126,2	0,815	1,5	0,33		0,04	8	0,33
<b><i>Deprotection</i></b>								
DEAPA	130,23	0,826			0,2	0,165	16	2,64
<b><i>Cleavage and precipitation</i></b>								
TFA	114,02	1,489			4,50	6,70	1	6,70
TIPS	158,36	0,773			0,25	0,19	1	0,19
1-dodecanthiol	18,02	0,997			0,25	0,21	1	0,21
DCM	84,93	1,325			2	2,65	3	7,95
Et <sub>2</sub> O	102,17	0,725			25	18,13	1	18,13
							<b>Total</b>	<b>38,02</b>

**Table S4.** Overview of the solvents (DMF, NOP or NOP/DMC 8/2) employed in SPPS of linear Octreotide at RT and 40°C and their relative masses

	MW (g/mol)	d (g/mL)	Volume (mL)	Mass (g)	repetitions	Total mass (g)
<b>DMF</b>						
DMF swelling	73,09	0,944	2	1,9	1	1,89
DMF couplings	73,09	0,944	2,5	2,36	8	18,88
DMF washings after couplings	73,09	0,944	4,5	4,25	7	29,74
DMF deprotection	73,09	0,944	1,8	1,70	16	27,19
DMF washings after deprotection	73,09	0,944	4,5	4,25	8	33,98
					<b>Total</b>	<b>111,68</b>
<b>NOP</b>						
NOP swelling	197,32	0,92	2	1,80	1	1,84
NOP couplings	197,32	0,92	2,5	2,30	8	18,40
NOP washings after couplings	197,32	0,92	4,5	4,14	7	28,98
NOP deprotection	197,32	0,92	1,8	1,66	16	26,50
NOP washings after deprotection	197,32	0,92	4,5	4,14	8	33,12
					<b>Total</b>	<b>108,84</b>
<b>NOP/DMC 8/2</b>						
NOP swelling	197,32	0,92	1,6	1,5	1	1,47
NOP couplings	197,32	0,92	2	1,84	8	14,72
NOP washings after couplings	197,32	0,92	3,6	3,31	7	23,18
DMC swelling	90,08	1,07	0,4	0,43	7	3,00
DMC couplings	90,08	1,07	0,5	0,54	7	3,75
DMC washings after couplings	90,08	1,07	0,9	0,96	7	6,74
NOP deprotection	197,32	0,92	1,44	1,32	16	21,20
NOP washings after deprotection	197,32	0,92	3,6	3,312	8	26,50
DMC deprotection	90,08	1,07	0,36	0,39	16	6,16
DMC washings after deprotection	90,08	1,07	0,9	0,963	8	7,70
					<b>Total</b>	<b>114,42</b>

**Table S5.** Overview of total mass of materials employed for SPPS of linear Octreotide at RT without recycling

	SPPS solvent		
	DMF	NOP	NOP/DMC 8/2
$\Sigma$ starting materials <sup>a</sup> (g)	11,3	11,3	11,3
$\Sigma$ solvents <sup>b</sup> (g)	137,8	134,9	140,5
$\Sigma$ base (g)	2,6	2,6	2,6
<b>Total (g)</b>	<b>151,7</b>	<b>148,8</b>	<b>154,4</b>

<sup>a</sup>Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure<sup>®</sup>, resin, cleavage cocktail (TFA+scavengers)

<sup>b</sup>Solvents include DCM and Et<sub>2</sub>O

**Table S6.** Overview of total mass of materials employed for SPPS of linear Octreotide at 40°C without recycling

	SPPS solvent	
	NOP	NOP/DMC 8/2
$\Sigma$ starting materials <sup>a</sup> (g)	9,3	9,3
$\Sigma$ solvents <sup>b</sup> (g)	134,9	140,5
$\Sigma$ base (g)	2,6	2,6
<b>Total (g)</b>	<b>146,8</b>	<b>152,4</b>

<sup>a</sup>Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure<sup>®</sup>, resin, cleavage cocktail (TFA+scavengers)

<sup>b</sup>Solvents include DCM and Et<sub>2</sub>O

#### *PMI calculations for linear Octreotide SPPS after solvents and base recovery*

When solvents (NOP or NOP/DMC 8/2) and base (DEAPA) were recovered, the value of the final PMI was calculated by subtracting the mass of recovery materials from the mass of used materials, according to the following equation:

$$PMI \text{ (for SPPS with recycling)} = \frac{\Sigma \text{ mass of materials} - \Sigma \text{ mass of recovered materials}}{\text{mass of isolated product}}$$

NOP was recovered with 85% yield, DMC with 95% yield, DEAPA with 92% yield. The residual 8% of DEAPA includes DEAPA involved in the formation of DBF-DEAPA adduct that is not recoverable. The amount of DBF-DEAPA adduct was estimated in 3% of the total DEAPA mass, considering a 38% conversion of DBF in DEAPA-DBF adduct at the end of a model SPPS (HPLC monitoring of deprotection stream waste at the end of a synthesis).

The amount of the total recovered mass of NOP or NOP/DMC 8/2 and DEAPA is reported in Table S7. Final PMI values for all considered SPPS are reported in Table 8 (main body text) and in Table S8 and S9.

**Table S7.** Total mass of solvents (NOP or NOP/DMC 8/2) and DEAPA employed for SPPS and their recovered mass

	SPPS solvent	
	NOP	NOP/DMC 8/2
$\Sigma$ NOP (g)	108,8	87,1
$\Sigma$ NOP recovered (g)	92,5	74,0
$\Sigma$ DMC (g)	-	27,4
$\Sigma$ DMC recovered (g)	-	26,0
$\Sigma$ DEAPA (g)	2,6	2,6
$\Sigma$ base recovered (g)	2,4	2,4
<b>Total solvents + base recycled (g)</b>	<b>94,9</b>	<b>102,4</b>

**Table S8.** PMI values for linear Octreotide SPPS processes at RT

	SPPS solvent				
	DMF	NOP (25)	NOP (25) + recovery	NOP/DMC 8/2 (25)	NOP/DMC 8/2 (25) + recovery
$\Sigma$ starting materials <sup>a</sup> (g)	11,3	11,3	11,3	11,3	11,3
$\Sigma$ solvents <sup>b</sup> (g)	137,8	134,9	42,4	140,5	40,5
$\Sigma$ base (g)	2,6	2,6	0,2	2,6	0,2
<b>PMI<sup>c</sup></b>	<b>743,7</b>	<b>729,7</b>	<b>264,3</b>	<b>757,1</b>	<b>254,6</b>

<sup>a</sup>Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure®, resin, cleavage cocktail (TFA + scavengers)

<sup>b</sup>Solvents include DCM and Et<sub>2</sub>O

<sup>c</sup>PMI =  $\Sigma m$  (starting materials) +  $\Sigma m$  (solvents) +  $\Sigma m$  (base) /  $m$  (crude linear Octreotide product)

**Table S9.** PMI values for linear Octreotide SPPS processes at 40°C

	SPPS solvent			
	NOP (40)	NOP (40) + recovery	NOP/DMC 8/2 (40)	NOP/DMC 8/2 (40) + recovery
$\Sigma$ starting materials <sup>a</sup> (g)	9,3	9,3	9,3	9,3
$\Sigma$ solvents <sup>b</sup> (g)	134,9	42,4	140,5	40,5
$\Sigma$ base (g)	2,6	0,2	2,6	0,2
<b>PMI<sup>c</sup></b>	<b>719,9</b>	<b>254,5</b>	<b>747,3</b>	<b>245,0</b>

<sup>a</sup>Starting materials include Fmoc-AA-OH, DIC, Oxyma Pure®, resin, cleavage cocktail (TFA + scavengers)

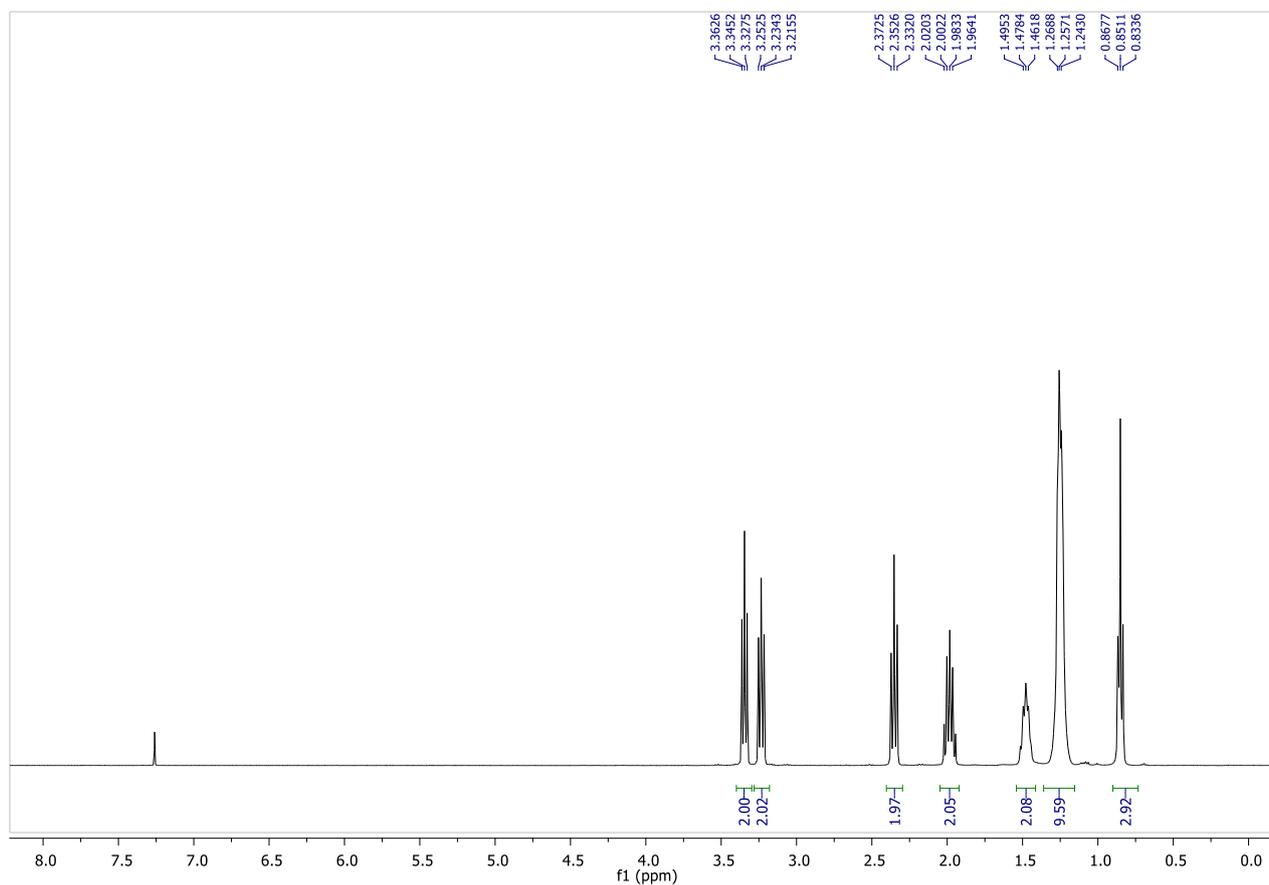
<sup>b</sup>Solvents include DCM and Et<sub>2</sub>O

<sup>c</sup>PMI =  $\Sigma m$  (starting materials) +  $\Sigma m$  (solvents) +  $\Sigma m$  (base) /  $m$  (crude linear Octreotide product)

Recovered NOP, DMC and DEAPA were assessed as having purities > 95% from  $^1\text{H}$  NMR (Figures S95-S97) and were re-used as such.

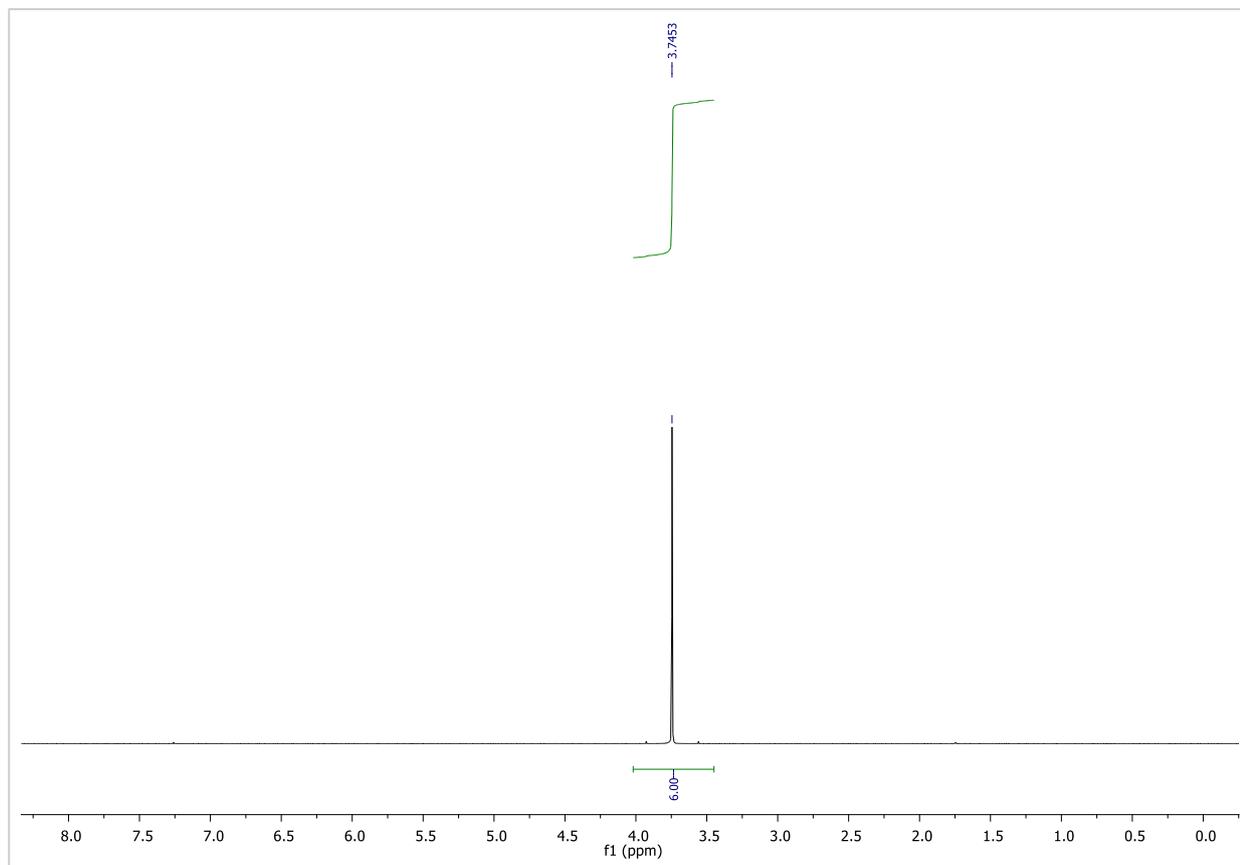
**Figure S100.**  $^1\text{H}$  NMR spectrum of NOP recovered from distillation

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 3.35 (t,  $J = 7.0$  Hz, 2H), 3.24 (t,  $J = 7.4$  Hz, 2H), 2.36 (t,  $J = 8.1$  Hz, 2H), 2.04 – 1.93 (m, 2H), 1.54 – 1.43 (m, 2H), 1.18 – 1.30 (m, 10H), 0.86 (t,  $J = 6.8$  Hz, 3H).



**Figure S101.**  $^1\text{H}$  NMR spectrum of DMC recovered from distillation

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 3.75 (s, 6H)



**Figure S102.**  $^1\text{H}$  NMR spectrum of DEAPA recovered from distillation

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 2.84 (bs, 2H), 2.79 (t,  $J = 6.6$  Hz, 2H), 2.52 (dt,  $J = 12.4, 7.2$  Hz, 6H), 1.68 – 1.58 (m, 2H), 1.02 (t,  $J = 7.2$  Hz, 6H).

