

## Supporting Information

### **Green Solvents Mixtures for Solid-Phase Peptide Synthesis (GM-SPPS). A DMF free high efficient synthesis of pharmaceutical grade peptides.**

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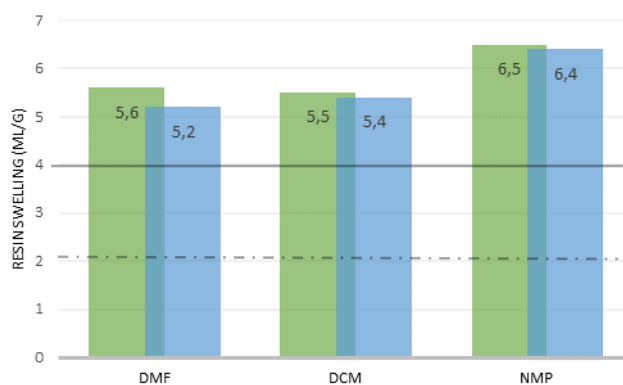
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Number of pages: 45

Number of figures: 35

Number of tables: 7



**Fig. S1** Swelling of PS-Wang-OH resin in three common solvents (green) and comparison with literature data (blue) (ref. Tetrahedron Lett., 1998, 39, 8951-8954) for the evaluation of procedure efficiency.

### Calculation of standard deviation for the swelling measurement of the reported resins.

**Tab. S1.** PS-Wang-OH resin.

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	7,7	7,7	7,7	7,9	0,1
Dimethyl carbonate	6,1	6,0	6,1	6,1	0,0
Diethyl carbonate	4,4	4,4	4,4	4,3	0,1
Anisole	6,8	6,9	6,8	6,8	0,0
Cyrene	1,6	1,5	1,7	1,7	0,1
Sulfolane	4,4	4,5	4,5	4,3	0,1
1a	1,6	1,6	1,6	1,5	0,1
2a	2,2	2,1	2,3	2,2	0,1
3a	2,0	2,0	2,0	2,1	0,0
4a	1,2	1,3	1,2	1,3	0,1
5a	1,2	1,3	1,2	1,1	0,1
6a	1,0	1,0	1,1	1,0	0,0
7a	1,0	1,0	1,0	1,0	0,0
1b	4,4	4,3	4,4	4,5	0,1
2b	5,0	5,0	5,1	5,0	0,0
3b	5,4	5,4	5,3	5,5	0,1
4b	5,4	5,4	5,4	5,5	0,1
5b	5,6	5,5	5,6	5,7	0,1
6b	5,0	5,1	4,9	4,9	0,1
7b	4,8	4,3	4,5	5,5	0,7
1c	6,8	6,7	6,8	6,9	0,1
2c	7,4	7,3	7,4	7,5	0,1
3c	10,0	9,8	10,1	10,0	0,1
4c	8,6	8,5	8,7	8,6	0,1
5c	8,4	8,3	8,5	8,5	0,1
6c	7,6	7,6	7,6	7,7	0,1
7c	7,2	7,2	7,3	7,1	0,1

**Tab. S2 PS-Rink Amide resin.**

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	7,7	7,7	7,7	7,9	0,1
Dimethyl carbonate	6,1	6,0	6,1	6,1	0,0
Diethyl carbonate	4,4	4,4	4,4	4,3	0,1
Anisole	6,8	6,9	6,8	6,8	0,0
Cyrene	1,6	1,5	1,7	1,7	0,1
Sulfolane	4,4	4,5	4,5	4,3	0,1
1a	1,6	1,6	1,6	1,5	0,1
2a	2,2	2,1	2,3	2,2	0,1
3a	2,0	2,0	2,0	2,1	0,0
4a	1,2	1,3	1,2	1,3	0,1
5a	1,2	1,3	1,2	1,1	0,1
6a	1,0	1,0	1,1	1,0	0,0
7a	1,0	1,0	1,0	1,0	0,0
1b	4,4	4,3	4,4	4,5	0,1
2b	5,0	5,0	5,1	5,0	0,0
3b	5,4	5,4	5,3	5,5	0,1
4b	5,4	5,4	5,4	5,5	0,1
5b	5,6	5,5	5,6	5,7	0,1
6b	5,0	5,1	4,9	4,9	0,1
7b	4,8	4,3	4,5	5,5	0,7
1c	6,8	6,7	6,8	6,9	0,1
2c	7,4	7,3	7,4	7,5	0,1
3c	10,0	9,8	10,1	10,0	0,1
4c	8,6	8,5	8,7	8,6	0,1
5c	8,4	8,3	8,5	8,5	0,1
6c	7,6	7,6	7,6	7,7	0,1
7c	7,2	7,2	7,3	7,1	0,1

**Tab. S3 TentaGel-Wang-OH resin.**

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	7,7	7,7	7,7	7,9	0,1
Dimethyl carbonate	6,1	6,0	6,1	6,1	0,0
Diethyl carbonate	4,4	4,4	4,4	4,3	0,1
Anisole	6,8	6,9	6,8	6,8	0,0
Cyrene	1,6	1,5	1,7	1,7	0,1
Sulfolane	4,4	4,5	4,5	4,3	0,1
1a	1,6	1,6	1,6	1,5	0,1
2a	2,2	2,1	2,3	2,2	0,1
3a	2,0	2,0	2,0	2,1	0,0
4a	1,2	1,3	1,2	1,3	0,1
5a	1,2	1,3	1,2	1,1	0,1

6a	1,0	1,0	1,1	1,0	0,0
7a	1,0	1,0	1,0	1,0	0,0
1b	4,4	4,3	4,4	4,5	0,1
2b	5,0	5,0	5,1	5,0	0,0
3b	5,4	5,4	5,3	5,5	0,1
4b	5,4	5,4	5,4	5,5	0,1
5b	5,6	5,5	5,6	5,7	0,1
6b	5,0	5,1	4,9	4,9	0,1
7b	4,8	4,3	4,5	5,5	0,7
1c	6,8	6,7	6,8	6,9	0,1
2c	7,4	7,3	7,4	7,5	0,1
3c	10,0	9,8	10,1	10,0	0,1
4c	8,6	8,5	8,7	8,6	0,1
5c	8,4	8,3	8,5	8,5	0,1
6c	7,6	7,6	7,6	7,7	0,1
7c	7,2	7,2	7,3	7,1	0,1

**Tab. S4 TentaGel-Rink Amide resin.**

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	6,2	6,2	6,2	6,1	0,1
Dimethyl carbonate	4,8	4,7	4,8	4,8	0,0
Diethyl carbonate	2,8	2,9	2,9	2,7	0,1
Anisole	5,6	5,6	5,6	5,6	0,0
Cyrene	1,6	1,5	1,6	1,6	0,1
Sulfolane	2,0	1,9	2,1	2,1	0,1
1a	4,4	4,4	4,5	4,4	0,0
2a	4,8	4,8	4,8	4,8	0,0
3a	3,2	3,2	3,3	3,2	0,1
4a	3,0	3,0	2,9	3,1	0,1
5a	4,0	4,1	4,0	3,9	0,1
6a	2,8	2,8	2,8	2,8	0,0
7a	2,6	2,5	2,6	2,7	0,1
1b	2,2	2,1	2,1	2,4	0,1
2b	2,2	2,3	2,2	2,1	0,1
3b	3,6	3,6	3,7	3,5	0,1
4b	2,8	2,9	2,9	2,8	0,1
5b	3,4	3,3	3,4	3,4	0,1
6b	3,6	3,5	3,7	3,7	0,1
7b	4,0	3,9	4,1	4,0	0,1
1c	5,2	5,1	5,3	5,1	0,1
2c	5,8	5,7	5,9	5,8	0,1
3c	5,6	5,6	5,6	5,7	0,1
4c	6,4	6,4	6,4	6,4	0,0
5c	5,8	5,7	5,8	5,9	0,1
6c	5,1	5,3	5,4	4,5	0,5
7c	4,4	4,3	4,4	4,5	0,1

**Tab. S5 ChemMatrix-Wang-OH resin.**

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	4,4	4,4	4,4	4,5	0,1
Dimethyl carbonate	5,4	5,4	5,5	5,4	0,1
Diethyl carbonate	3,2	3,2	3,2	3,1	0,1
Anisole	5,2	5,3	5,3	5,2	0,1
Cyrene	1,6	1,5	1,6	1,7	0,1
Sulfolane	3,6	3,5	3,6	3,7	0,1
1a	1,8	1,9	1,7	1,8	0,1
2a	1,8	1,8	1,8	1,9	0,0
3a	1,8	1,8	1,7	1,9	0,1
4a	1,2	1,1	1,3	1,3	0,1
5a	1,4	1,4	1,5	1,4	0,1
6a	1,2	1,1	1,2	1,2	0,1
7a	1,2	1,3	1,3	1,1	0,1
1b	6,8	6,7	6,8	6,8	0,0
2b	6,0	6,0	6,0	6,1	0,0
3b	4,0	4,1	4,0	4,0	0,1
4b	4,2	4,1	4,3	4,2	0,1
5b	4,8	4,8	4,8	4,8	0,0
6b	4,8	4,7	4,9	4,8	0,1
7b	4,4	4,3	4,5	4,5	0,1
1c	6,0	5,9	6,0	6,1	0,1
2c	6,0	6,0	6,0	6,0	0,0
3c	6,4	6,3	6,5	6,5	0,1
4c	5,6	5,6	5,6	5,7	0,0
5c	5,2	5,1	5,3	5,2	0,1
6c	5,6	5,6	5,6	5,7	0,1
7c	5,6	5,5	5,7	5,7	0,1

**Tab. S6 ChemMatrix-Rink Amide resin.**

solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	7,7	7,7	7,7	7,9	0,1
Dimethyl carbonate	6,1	6,0	6,1	6,1	0,0
Diethyl carbonate	4,4	4,4	4,4	4,3	0,1
Anisole	6,8	6,9	6,8	6,8	0,0
Cyrene	1,6	1,5	1,7	1,7	0,1
Sulfolane	4,4	4,5	4,5	4,3	0,1
1a	1,6	1,6	1,6	1,5	0,1
2a	2,2	2,1	2,3	2,2	0,1
3a	2,0	2,0	2,0	2,1	0,0
4a	1,2	1,3	1,2	1,3	0,1
5a	1,2	1,3	1,2	1,1	0,1
6a	1,0	1,0	1,1	1,0	0,0

7a	1,0	1,0	1,0	1,0	0,0
1b	4,4	4,3	4,4	4,5	0,1
2b	5,0	5,0	5,1	5,0	0,0
3b	5,4	5,4	5,3	5,5	0,1
4b	5,4	5,4	5,4	5,5	0,1
5b	5,6	5,5	5,6	5,7	0,1
6b	5,0	5,1	4,9	4,9	0,1
7b	4,8	4,3	4,5	5,5	0,7
1c	6,8	6,7	6,8	6,9	0,1
2c	7,4	7,3	7,4	7,5	0,1
3c	10,0	9,8	10,1	10,0	0,1
4c	8,6	8,5	8,7	8,6	0,1
5c	8,4	8,3	8,5	8,5	0,1
6c	7,6	7,6	7,6	7,7	0,1
7c	7,2	7,2	7,3	7,1	0,1

Representative examples of solubility of Fmoc-amino acids in Mixs B5 (Sulf/DEC=30:70) and C3 (An/DMC=70:30) in presence of OxymaPure/DIC.



**Fig. S2** Fmoc-Leu-OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (left) and Anisole:Dimethyl carbonate=70:30. (right).



**Fig. S3** Fmoc-Tyr(tBu)-OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (left) and Anisole:Dimethyl carbonate=70:30. (right).



**Fig. S4** Fmoc-Val OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (right) and Anisole:Dimethyl carbonate=70:30. (left).



**Fig. S5** Fmoc-Gln(Trt)-OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (right) and Anisole:Dimethyl carbonate=70:30. (left).

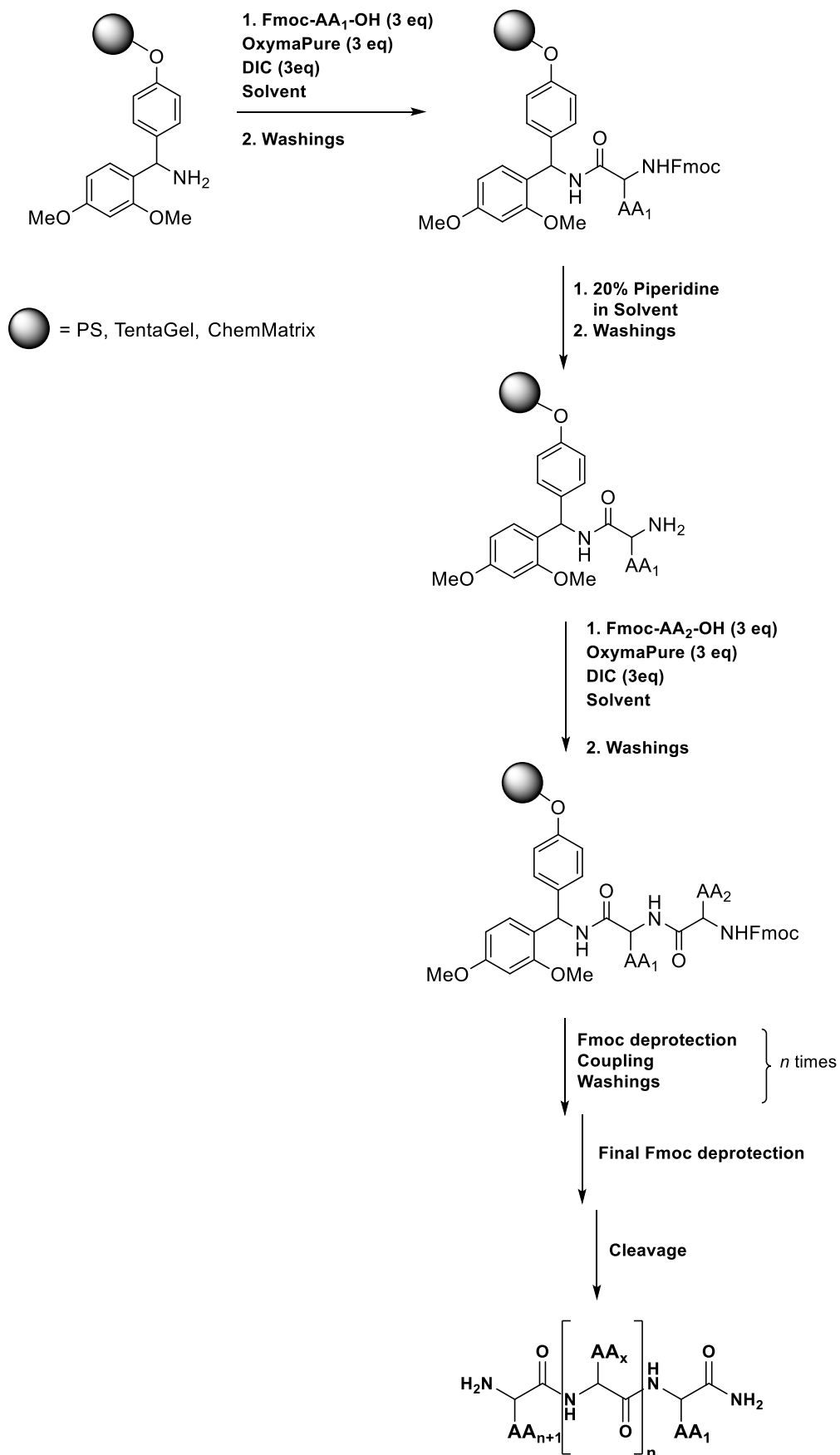


**Fig. S6** Fmoc-Gly-OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (right) and Anisole:Dimethyl carbonate=70:30. (left).

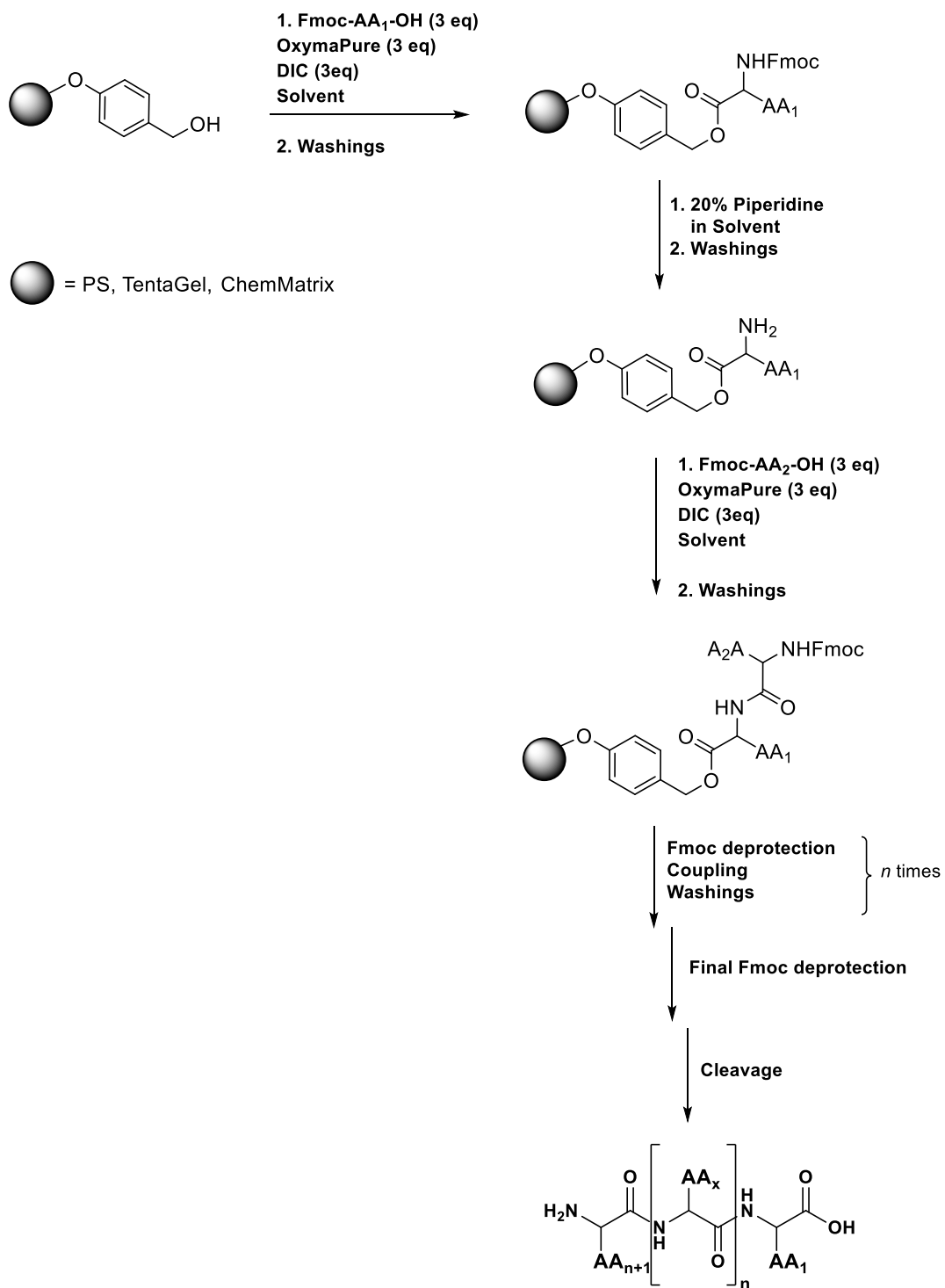


**Fig. S7** Fmoc-Phe-OH/DIC/Oxyma Pure=1/1/1 in Sulfolane: Diethyl carbonate=30:70 (left) and Anisole:Dimethyl carbonate=70:30. (right).





**Fig. S8** General procedure for peptide synthesis on RinkAmide functionalized resins.



**Fig. S9** General procedure for peptide synthesis on Wang functionalized resins.

**HPLC parameters for Aib-Enkephalin analysis:**

Instrument: Agilent 1260 InfinityLab coupled with Agilent InfinityLab LC/MSD.

Column: Luna Phenomenex®C18; Particle Size (µm): 5 µm; Pore Size (Å): 100 Å; Length (mm): 250; I.D. (mm):4.6.

Signal: 276 nm.

Polarity: Positive Scan.

Injection volume: 10 µL.

Flow: 1 mL/min.

Mobile Phase A: H<sub>2</sub>O+0,08% TFA

Mobile Phase B: ACN+0,08% TFA

Gradient:

	Mobile phase A	Mobile phase B
0 min	80	20
15 min	60	40
20 min	60	40
30 min	80	20

**HPLC parameters for Aib-ACP analysis:**

Instrument: Agilent 1260 InfinityLab coupled with Agilent InfinityLab LC/MSD.

Column: Luna Phenomenex®C18; Particle Size (µm): 5 µm; Pore Size (Å): 100 Å; Length (mm): 250; I.D. (mm):4.6.

Signal: 220 nm.

Polarity: Positive Scan.

Injection volume: 10 µL.

Flow: 1 mL/min.

Mobile Phase A: H<sub>2</sub>O+0,08% TFA

Mobile Phase B: ACN+0,08% TFA

Gradient:

	Mobile phase A	Mobile phase B
0 min	90	10
15 min	50	50
20 min	50	50
30 min	90	10

**HPLC parameters for Octreotide analysis:**

Instrument: Agilent 1260 InfinityLab coupled with Agilent InfinityLab LC/MSD.

Column: Luna Phenomenex®C18; Particle Size (µm): 5 µm; Pore Size (Å): 100 Å; Length (mm): 250; I.D. (mm):4.6.

Signal: 220 nm.

Polarity: Positive Scan.

Injection volume: 10 µL.

Flow: 1 mL/min.

Mobile Phase A: H<sub>2</sub>O+0,08% TFA

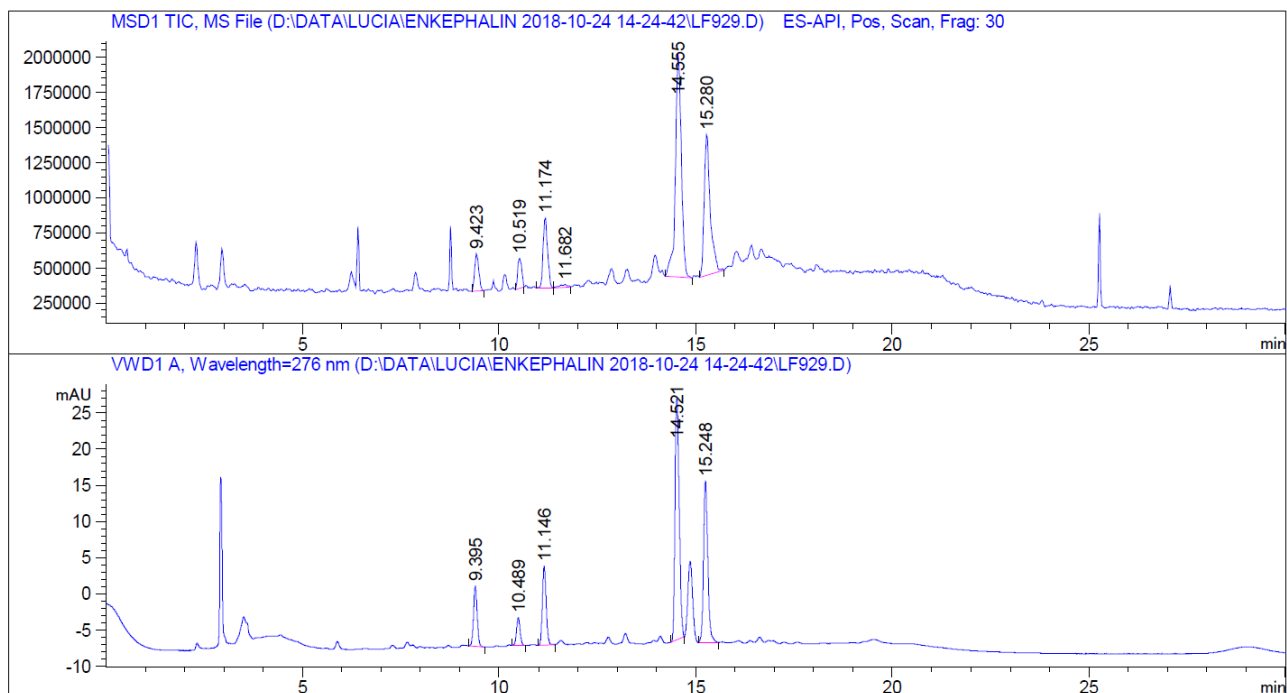
Mobile Phase B: ACN+0,08% TFA

Gradient:

	Mobile phase A	Mobile phase B
0 min	90	10
20 min	70	30
30 min	10	90
35 min	10	90
38	90	10
45	90	10

**Fig. S10** Chromatogram of pentapeptide, method 2 on PS-Wang-OH resin (entry 3, Table 3).

The not integrated peaks of the spectrum belong to impurities of the resin (not peptides).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 2: VWD1 A, Wavelength=276 nm

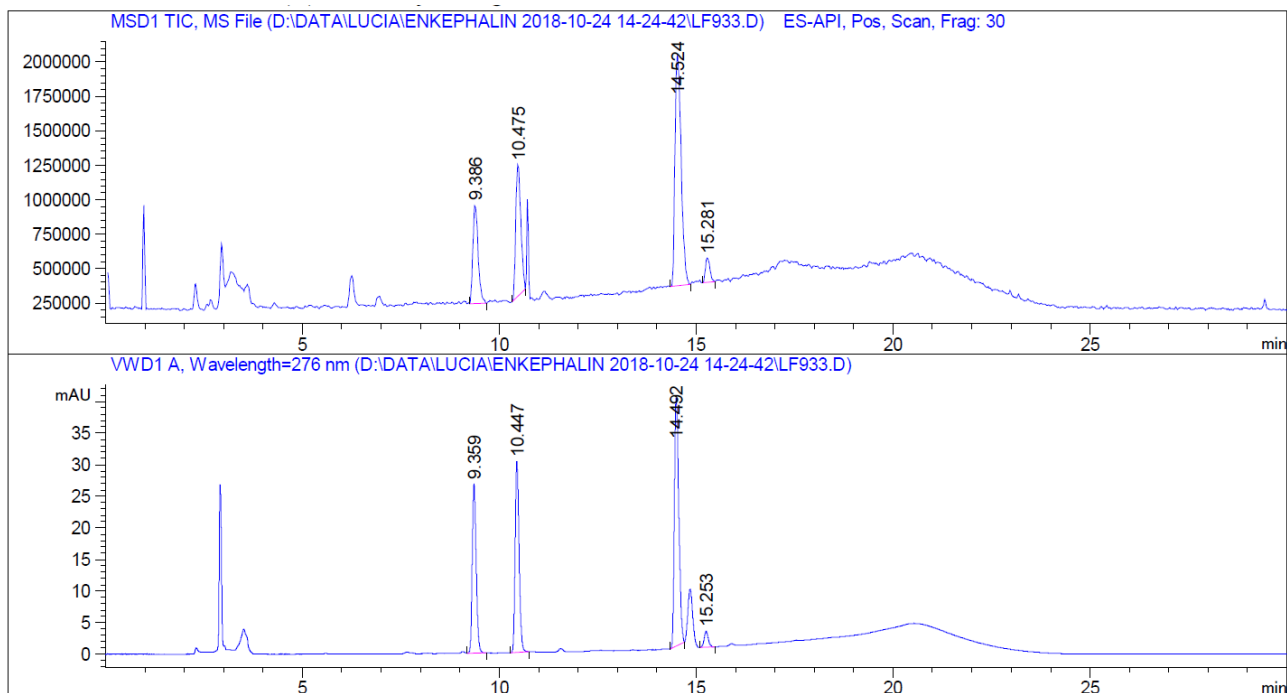
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.395	BB	0.0998	54.07289	8.32215	9.5208
2	10.489	BB	0.0979	24.63134	3.86501	4.3369
3	11.146	BB	0.1037	73.05728	10.89378	12.8634
4	14.521	BB	0.1157	247.38408	33.48820	43.5575
5	15.248	BB	0.1166	168.80214	22.35113	29.7214

Totals : 567.94773 78.92027

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 \*\*\* End of Report \*\*\*

**Fig. S11** Chromatogram of pentapeptide, method 3 on PS-Wang-OH resin (entry 4, Table 3).

The not integrated peaks of the spectrum belong to impurities of the resin (not peptides).



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 Area Percent Report  
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Sorted By : Signal  
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 Use Multiplier & Dilution Factor with ISTDs

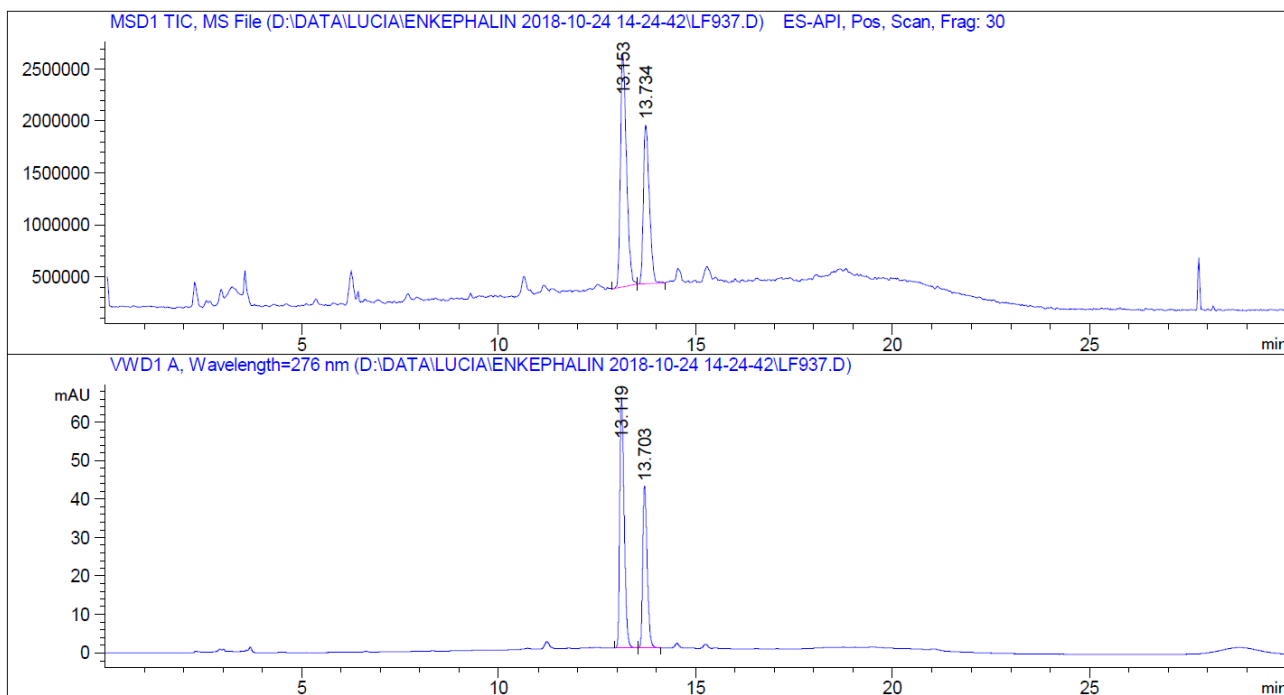
Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.359	BB	0.1072	185.49585	26.81006	25.7585
2	10.447	BB	0.1083	210.43890	30.20205	29.2221
3	14.492	BB	0.1217	305.85422	39.32333	42.4718
4	15.253	BB	0.1036	18.34597	2.51432	2.5476

Totals : 720.13494 98.84976

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 \*\*\* End of Report \*\*\*

**Fig. S12** Chromatogram of pentapeptide, method 3 on ChemMatrix-RinkAmide (entry 5, Table 3).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

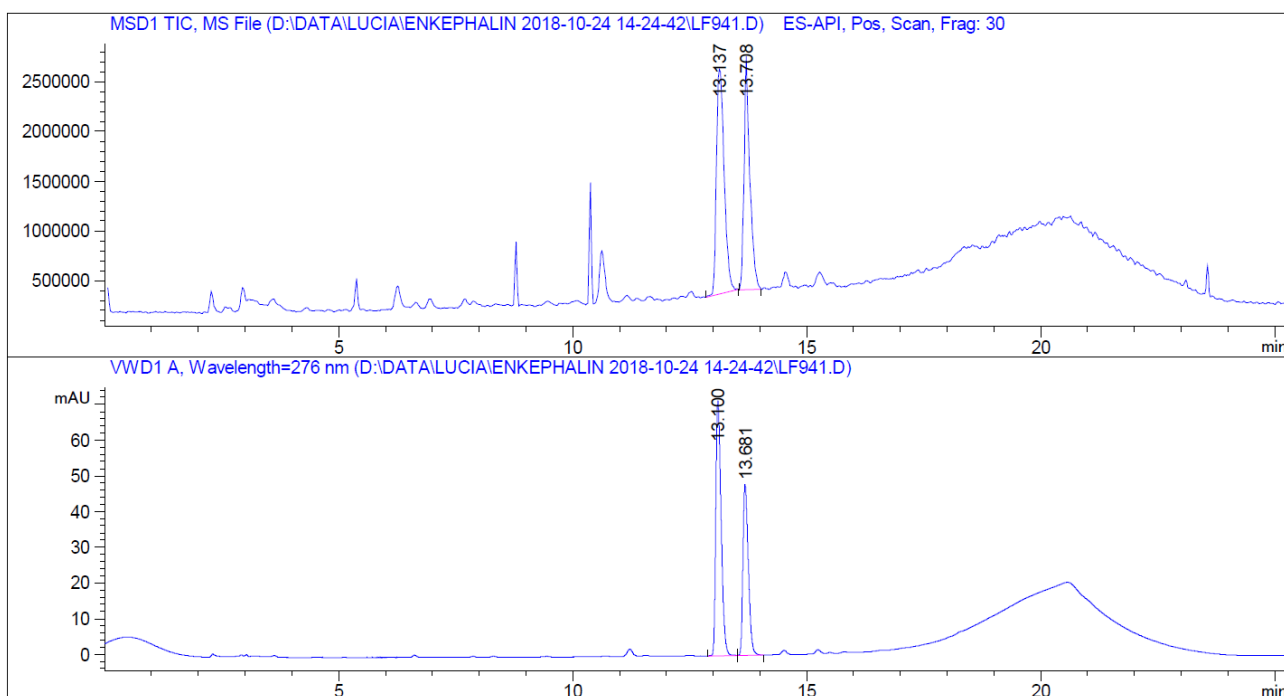
Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.119	BB	0.1222	507.93332	65.00964	60.6194
2	13.703	BB	0.1224	329.97208	42.11088	39.3806

Totals : 837.90540 107.12053

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 \*\*\* End of Report \*\*\*

**Fig. S13** Chromatogram of pentapeptide, method 4 on ChemMatrix-RinkAmide (entry 6, Table 3).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.100	BB	0.1231	567.35846	71.46362	59.8120
2	13.681	BB	0.1224	381.21094	47.85302	40.1880

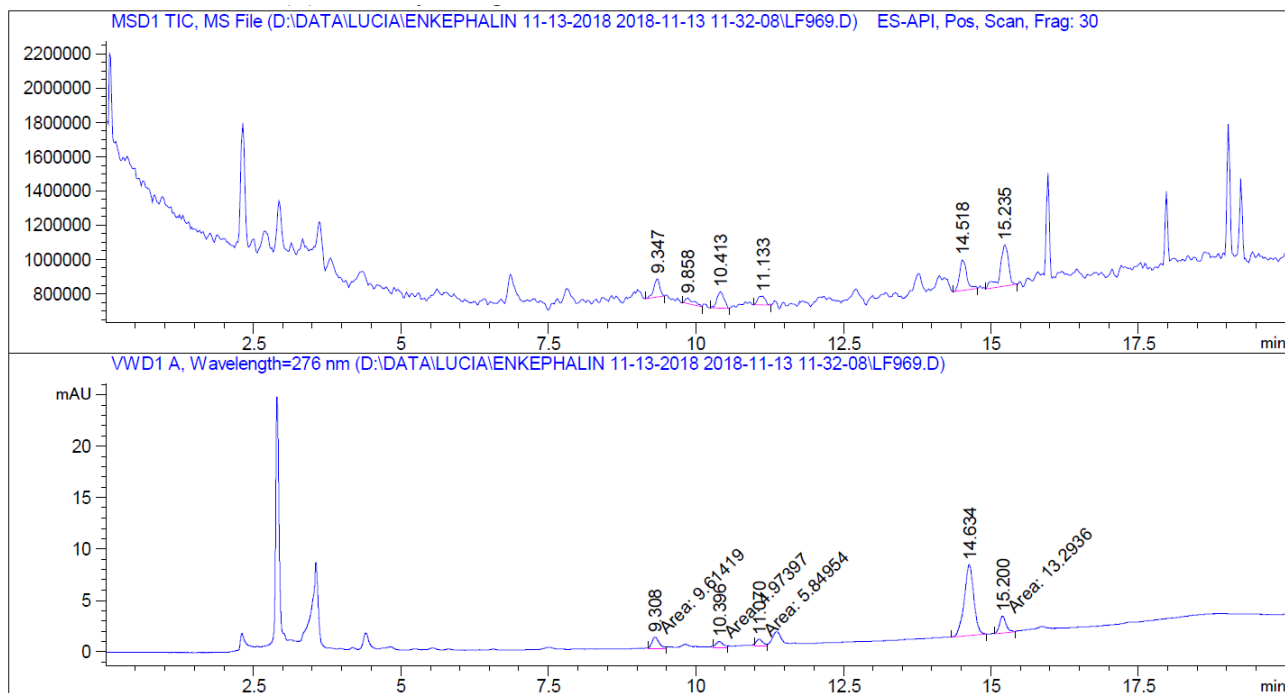
Totals : 948.56940 119.31664

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 \*\*\* End of Report \*\*\*



**Fig. S14** Chromatogram of pentapeptide, method 5 on PS-Wang-OH (entry 11, Table 3).

The not integrated peaks of the spectrum belong to impurities of the resin (not peptides).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 2: VWD1 A, Wavelength=276 nm

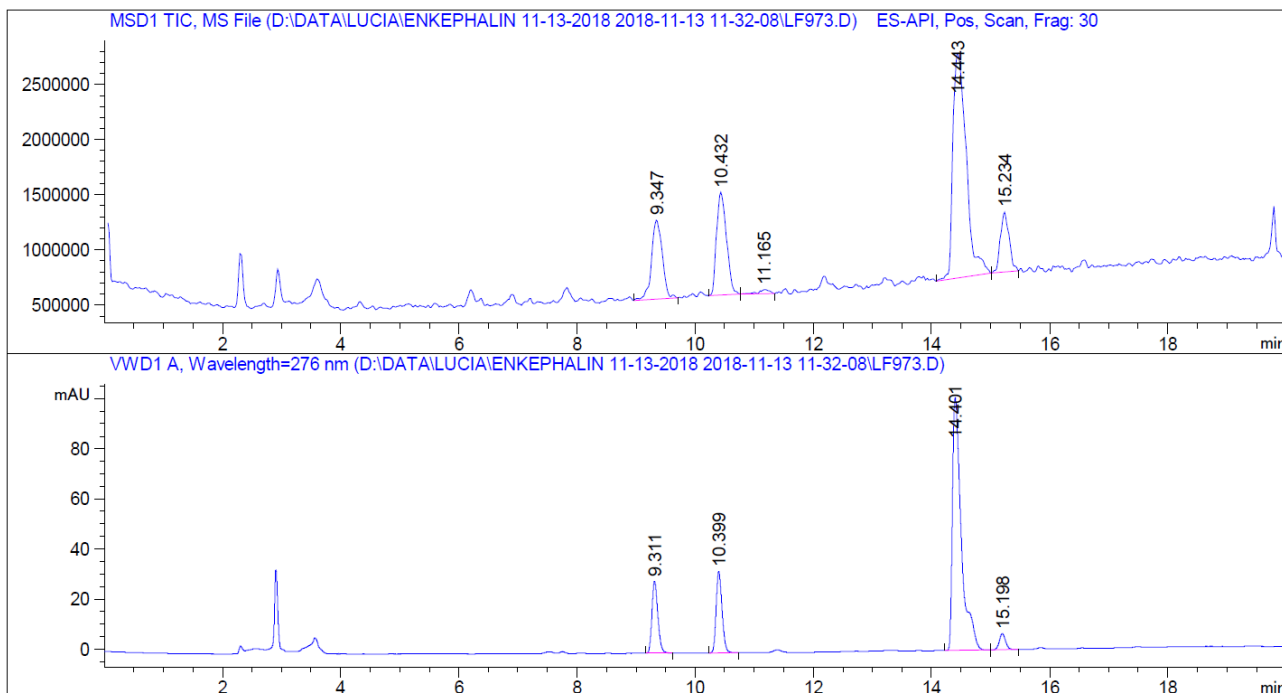
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.308	MM T	0.1437	9.61419	1.11494	8.6879
2	10.396	MM T	0.1423	4.97397	5.82673e-1	4.4947
3	11.070	MM T	0.1374	5.84954	7.09487e-1	5.2860
4	14.634	BB	0.1567	76.93060	6.86468	69.5186
5	15.200	MM	0.1330	13.29361	1.66563	12.0128

Totals : 110.66192 10.93740

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 \*\*\* End of Report \*\*\*

**Fig. S15** Chromatogram of pentapeptide, method 6 on PS-Wang-OH (entry 12, Table 3).

The not integrated peaks of the spectrum belong to impurities of the resin (not peptides).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

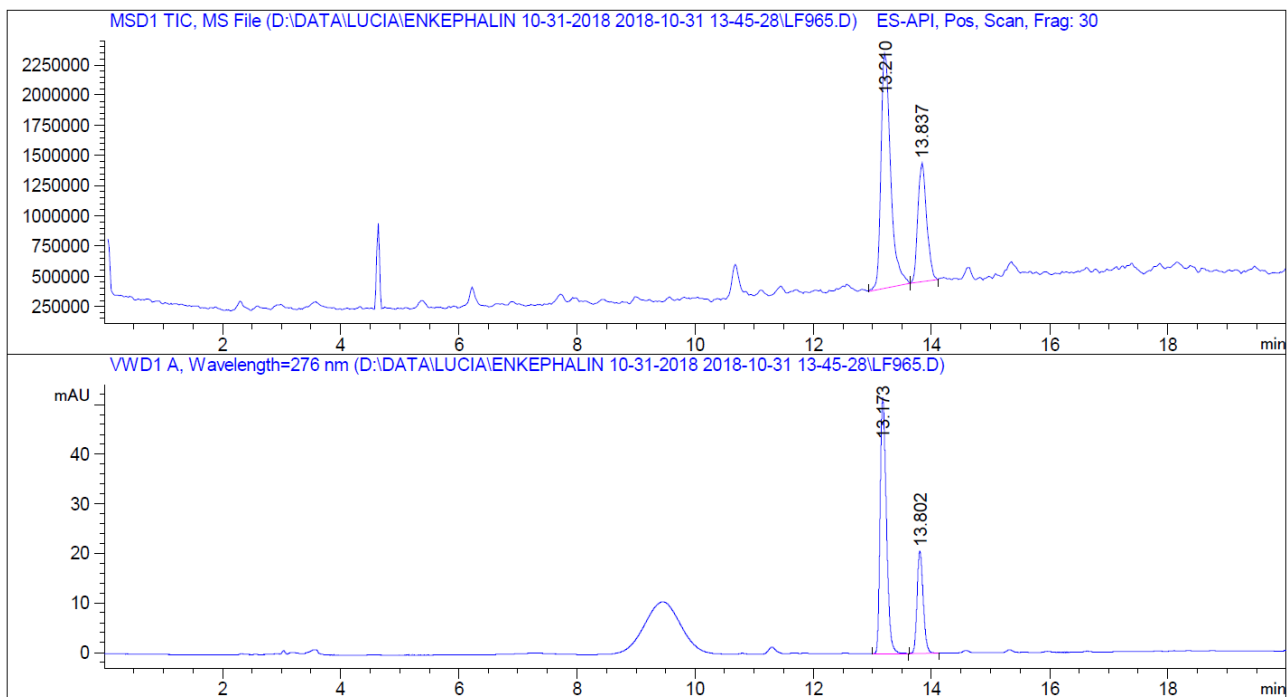
Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.311	BB	0.1077	199.67712	28.69429	13.0282
2	10.399	BB	0.1070	226.08812	32.54913	14.7515
3	14.401	BB	0.1568	1058.64038	101.01575	69.0726
4	15.198	BB	0.1135	48.24219	6.46934	3.1476

Totals : 1532.64781 168.72850

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 \*\*\* End of Report \*\*\*

**Fig. S16** Chromatogram of pentapeptide, method 6 on ChemMatrix-RinkAmide (entry 9, Table 3).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

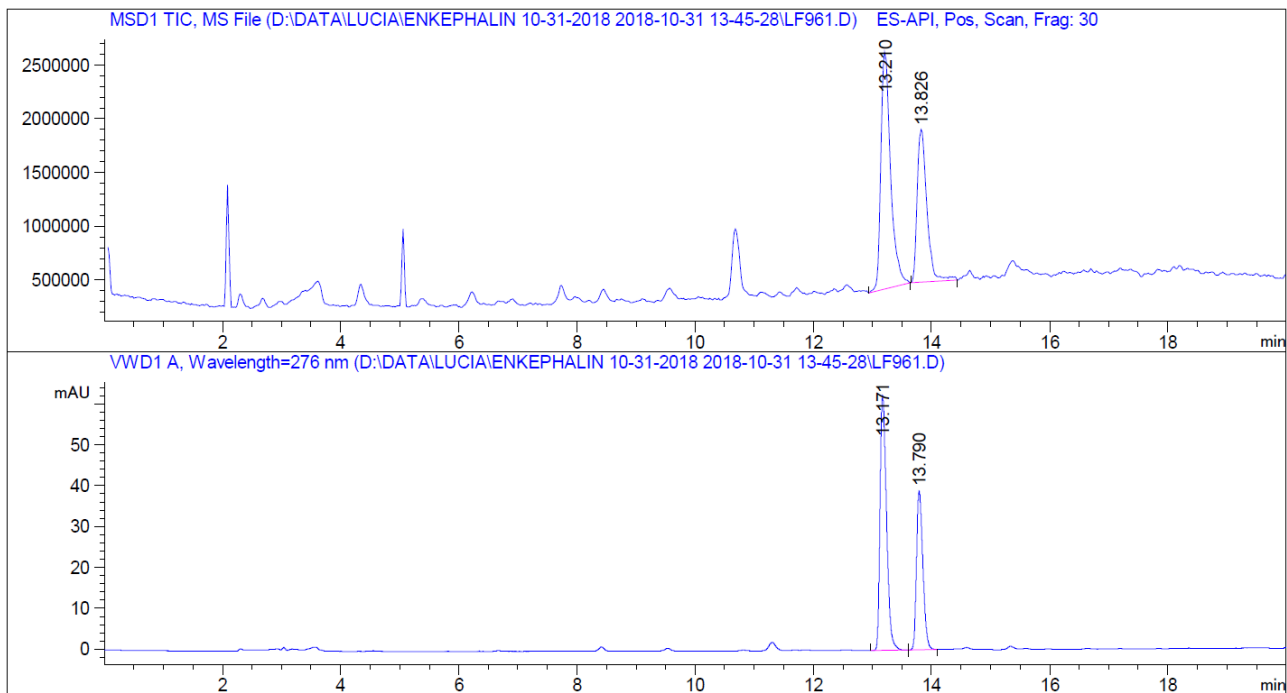
Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.173	BB	0.1148	381.45523	51.56120	72.0614
2	13.802	BB	0.1100	147.89229	20.66583	27.9386

Totals : 529.34752 72.22704

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 \*\*\* End of Report \*\*\*

**Fig. S17** Chromatogram of pentapeptide, method 7 on ChemMatrix-RinkAmide (entry 10, Table 3).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

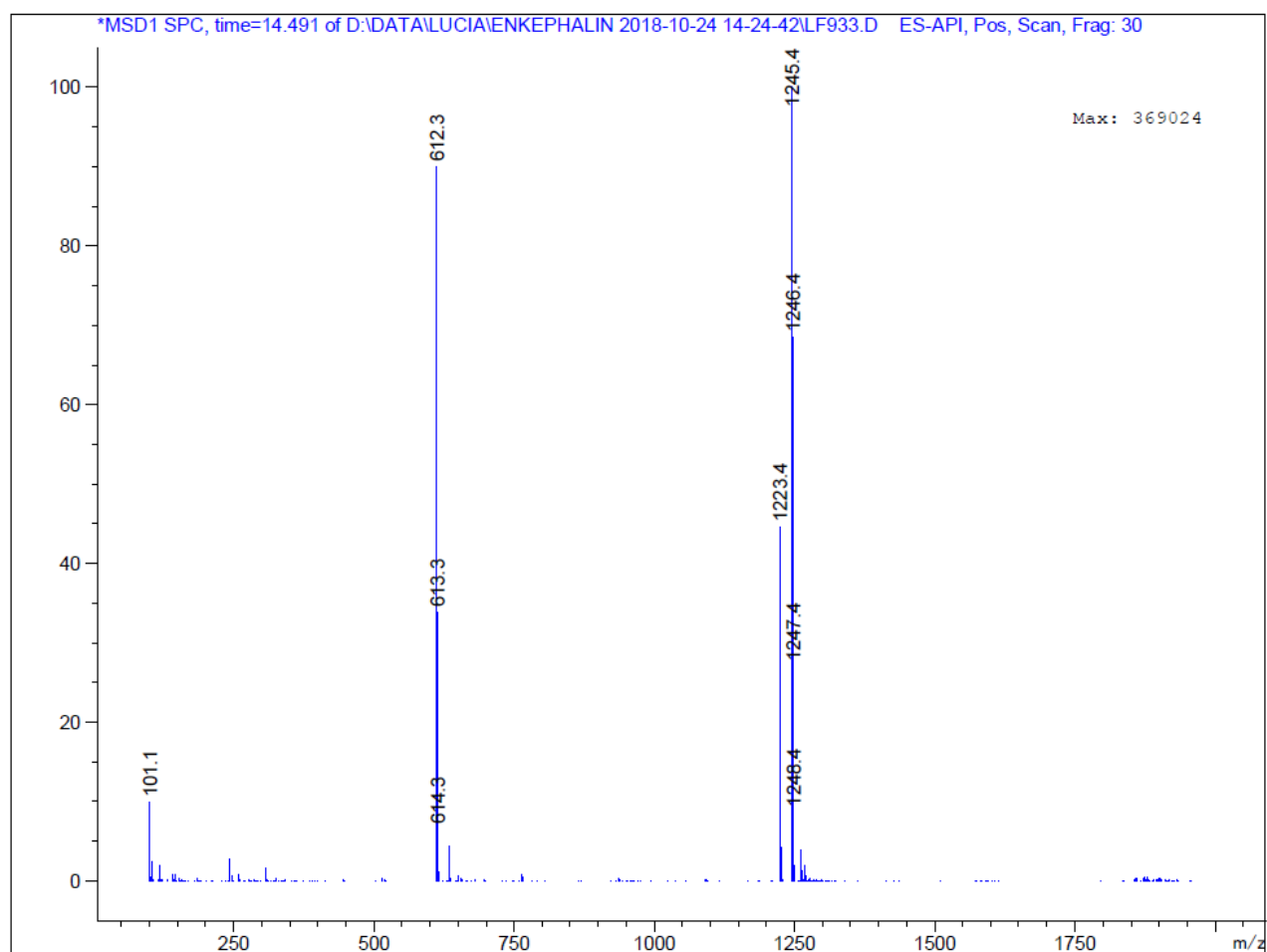
Signal 2: VWD1 A, Wavelength=276 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.171	BB	0.1187	477.19122	62.40409	62.1102
2	13.790	BB	0.1168	291.10593	38.88131	37.8898

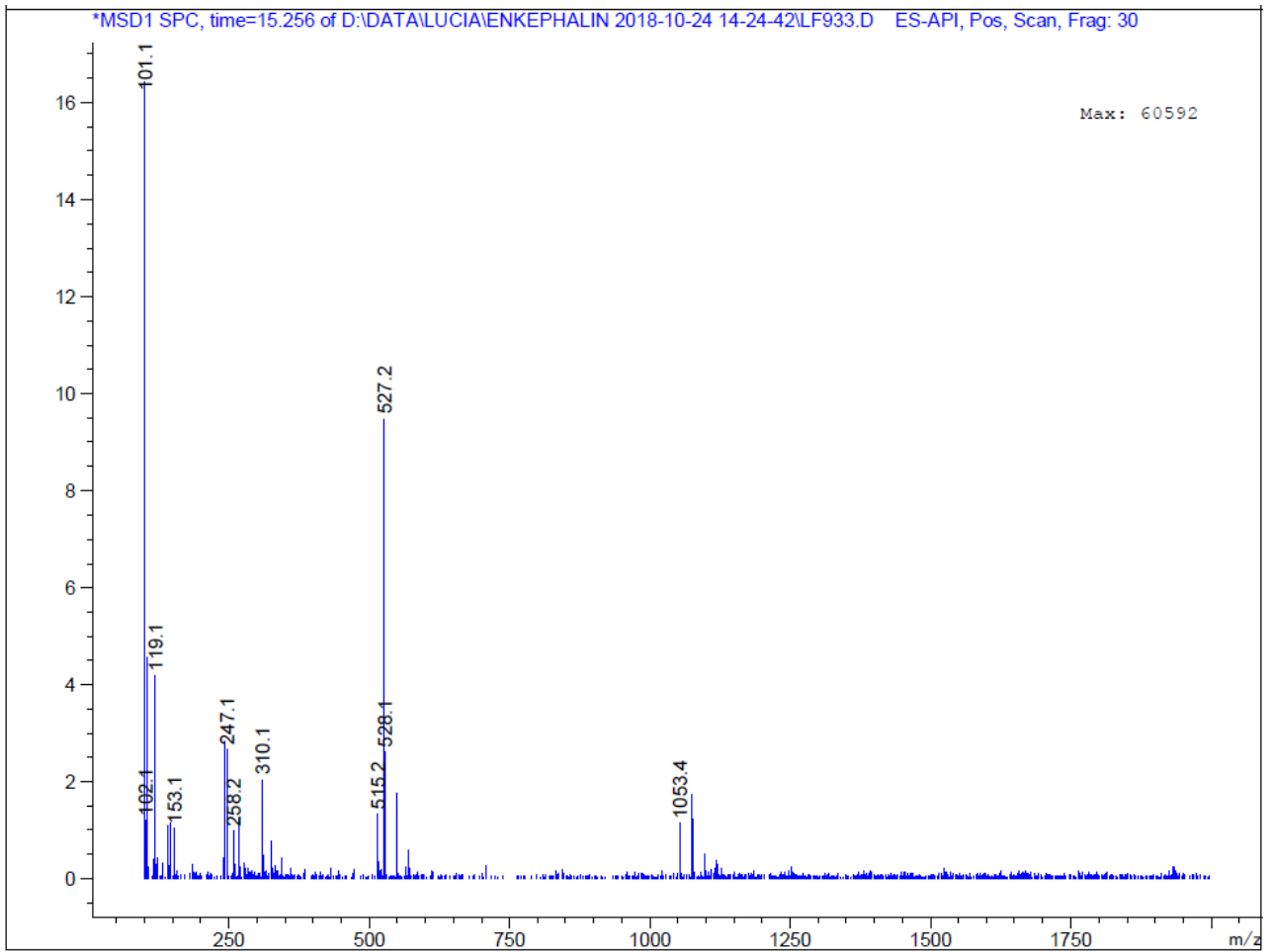
Totals : 768.29715 101.28540

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 \*\*\* End of Report \*\*\*

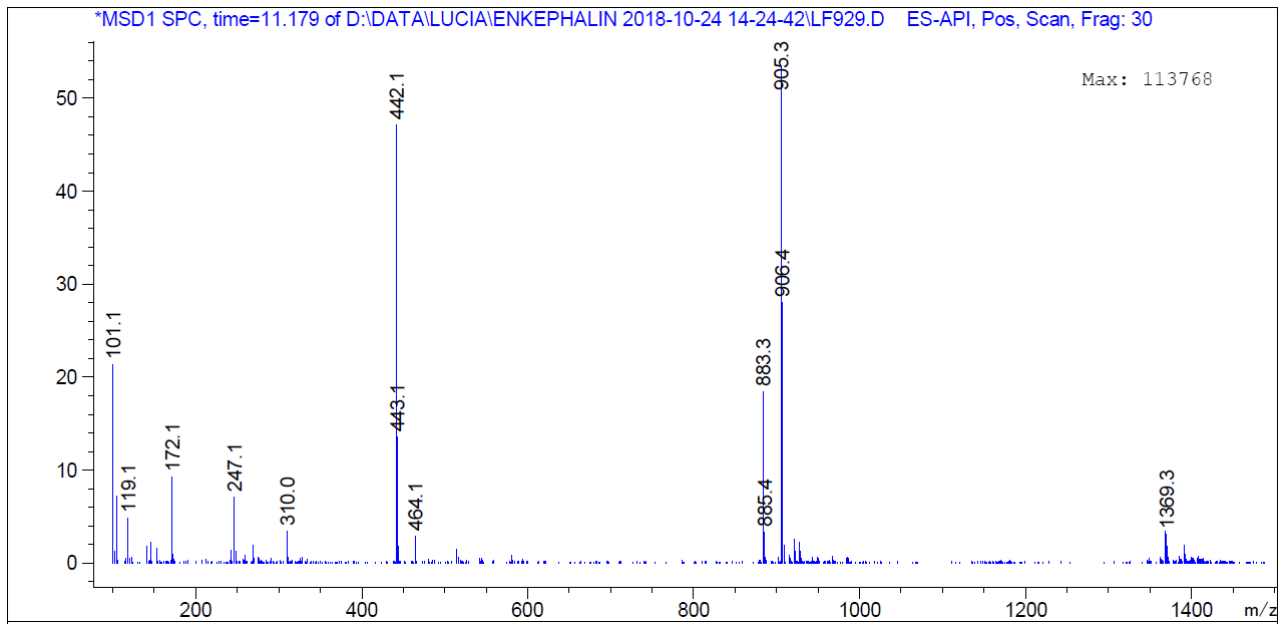
**Fig. S18** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Aib-Phe-Leu-COOH.



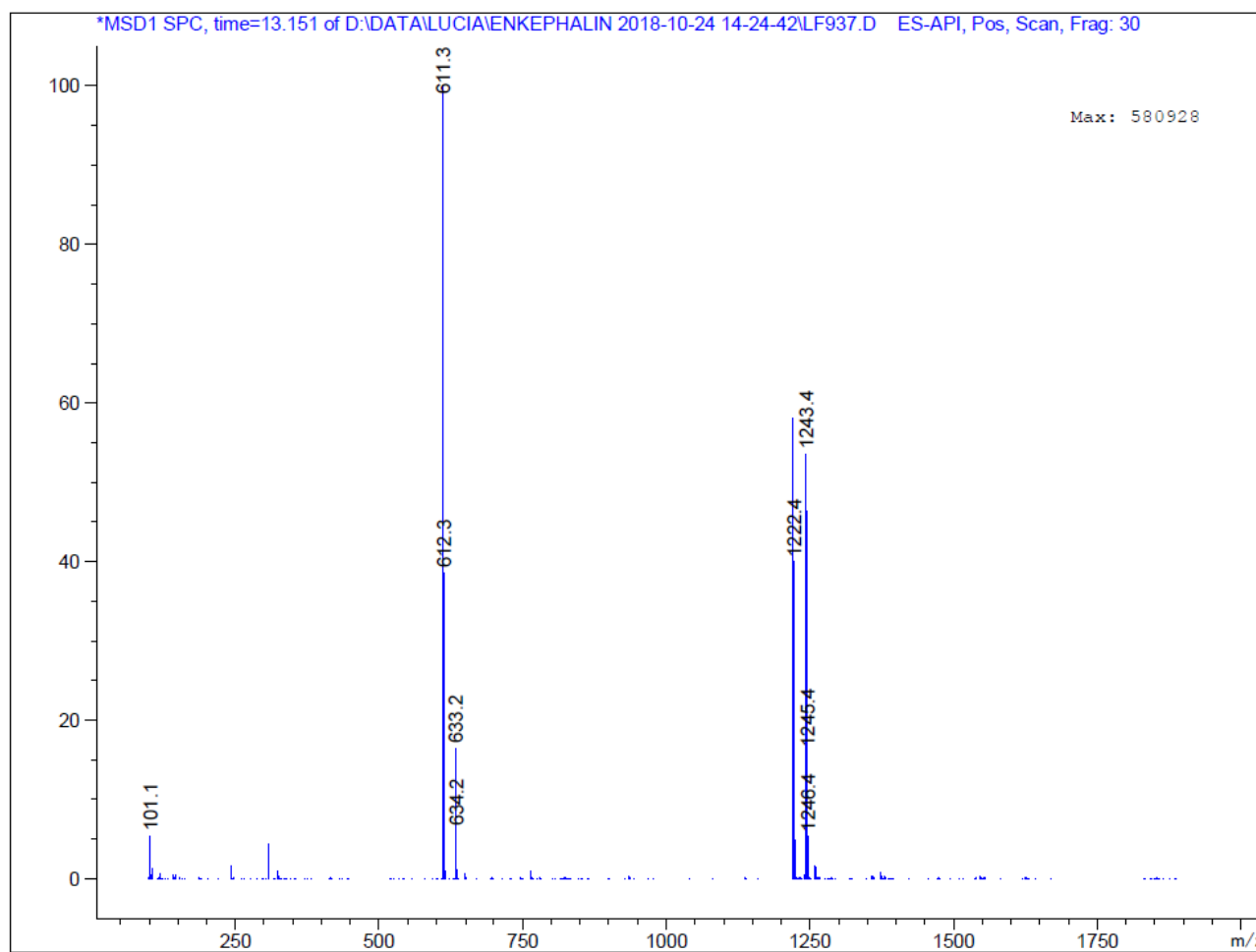
**Fig. S19** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Phe-Leu-COOH.



**Fig. S20** Mass spectrum of H<sub>2</sub>N-Tyr-Phe-Leu-COOH.

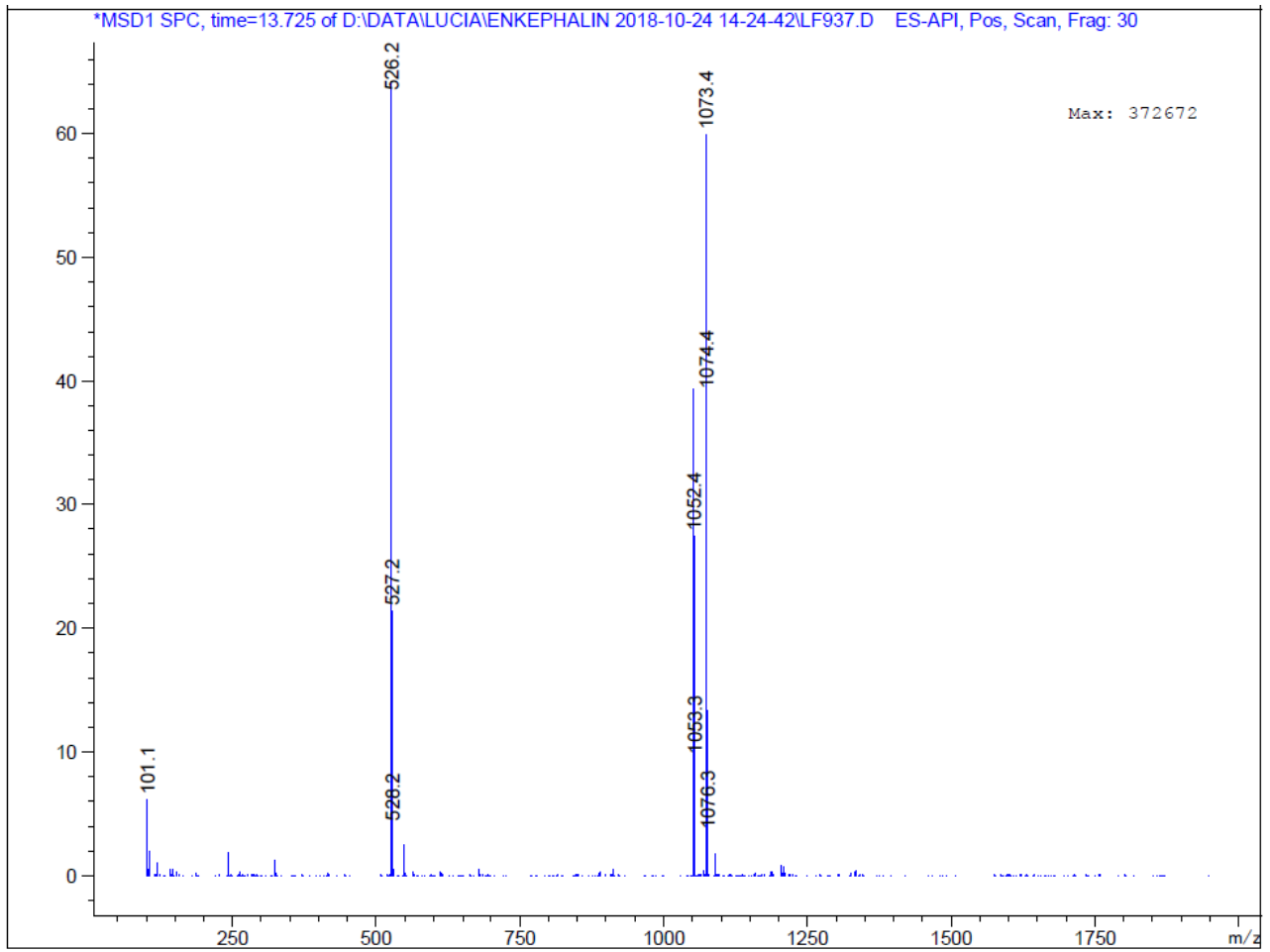


**Fig. S21** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Aib-Phe-Leu-CONH<sub>2</sub>.

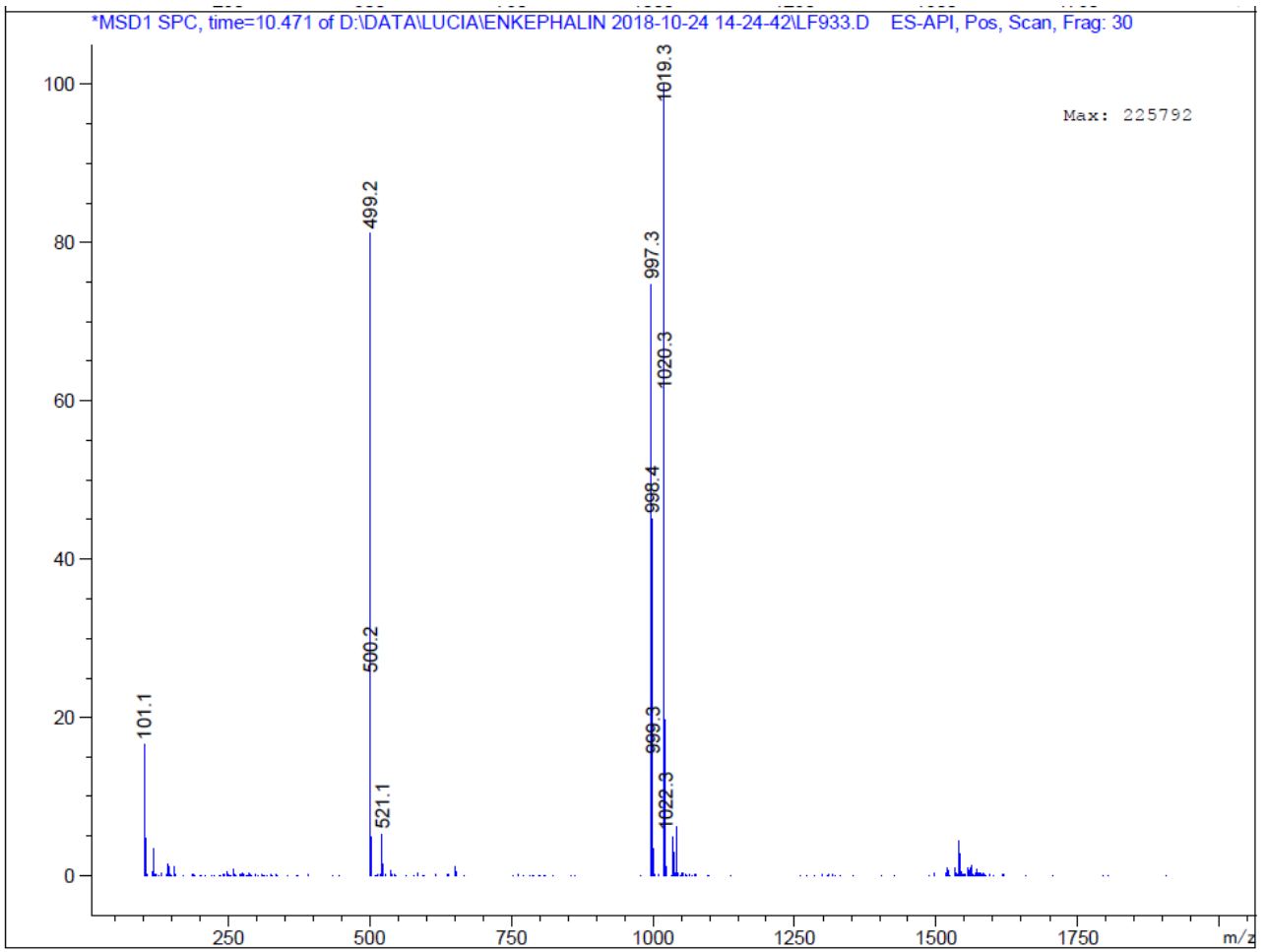




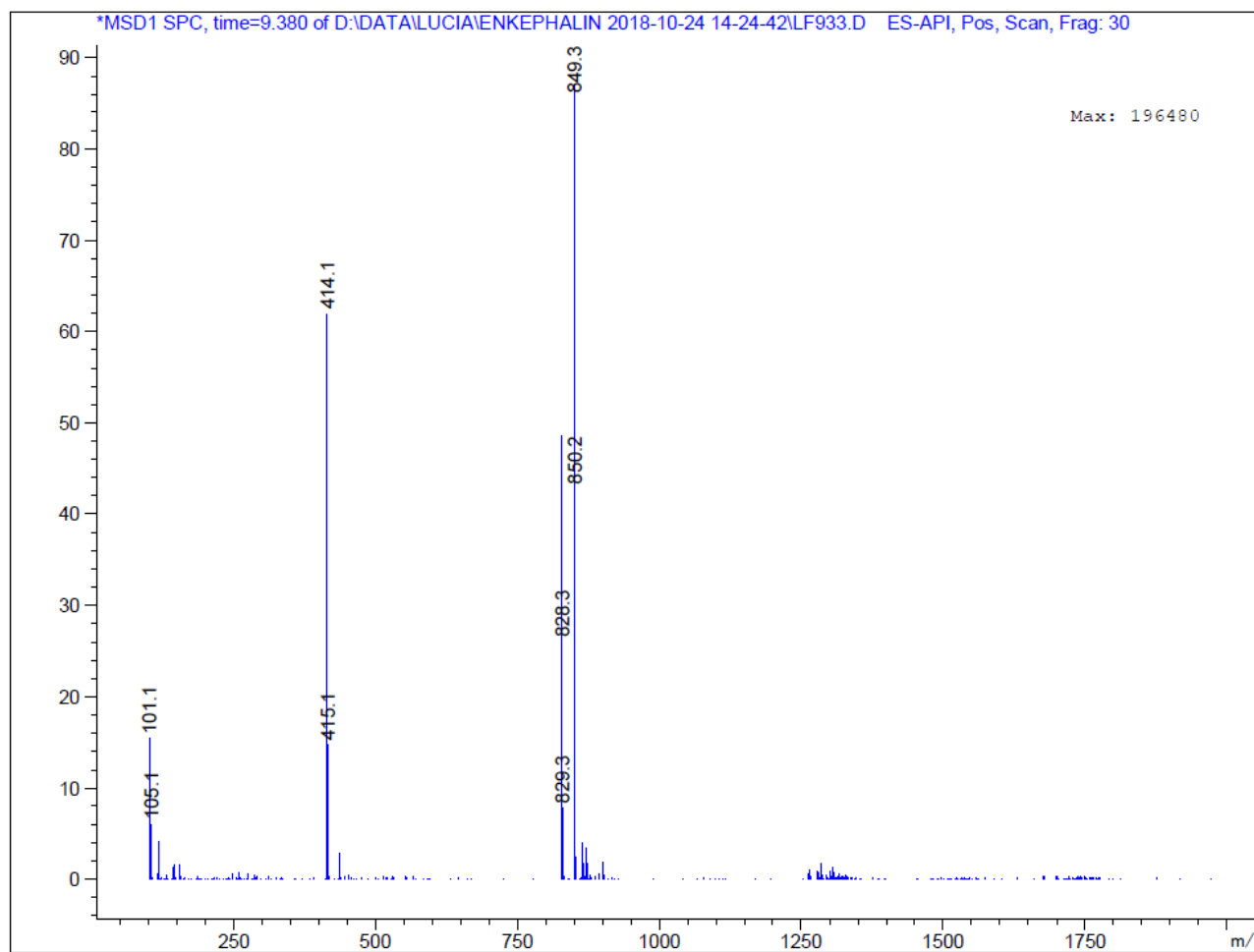
**Fig. S22** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Phe-Leu-CONH<sub>2</sub>.



**Fig. S23** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Aib-Phe-COOH.

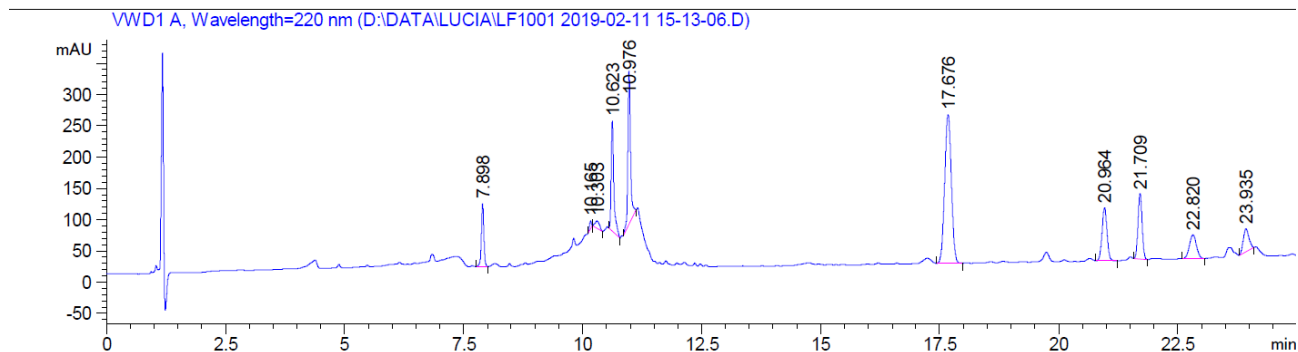


**Fig. S24** Mass spectrum of H<sub>2</sub>N-Tyr-Aib-Phe-COOH.



Supporting Information

**Fig. S25** Chromatogram of Aib-ACP decapeptide, method 6 on ChemMatrix-RinkAmide (entry 2, Table 4).



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 Area Percent Report  
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Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

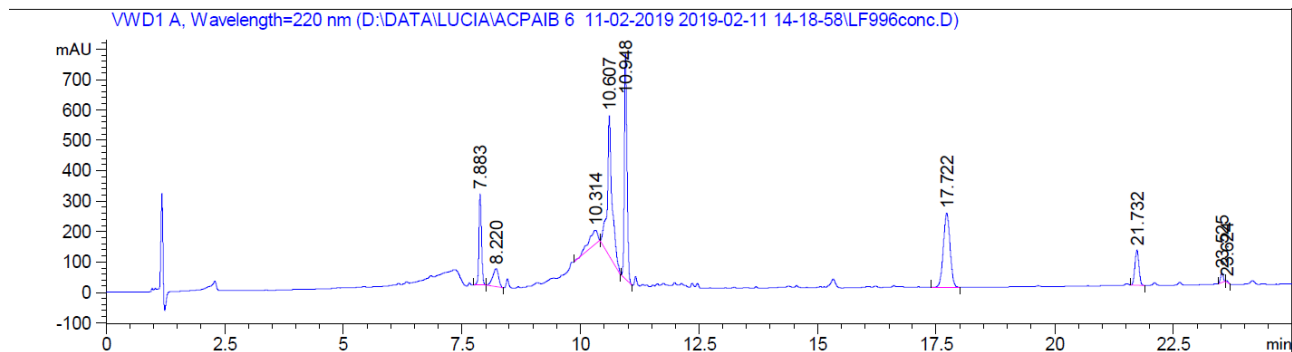
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.898	BB	0.0546	359.25238	100.63807	5.6658
2	10.165	BB	0.0422	33.62323	12.58262	0.5303
3	10.303	BB	0.1044	77.07565	11.77406	1.2156
4	10.623	BB	0.0571	682.52112	176.42404	10.7641
5	10.976	BB	0.0574	931.96509	244.97026	14.6980
6	17.676	BB	0.1520	2324.04102	237.84325	36.6525
7	20.964	BB	0.1139	614.98016	84.46383	9.6989
8	21.709	BB	0.0936	625.25092	104.74144	9.8608
9	22.820	BB	0.1608	390.24460	37.99164	6.1546
10	23.935	BB	0.1256	301.78726	36.81104	4.7595

Totals : 6340.74142 1048.24024

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 \*\*\* End of Report \*\*\*

**Fig. S26** Chromatogram of Aib-ACP decapeptide, method 7 on ChemMatrix-RinkAmide (entry 3, Table 4).



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 Area Percent Report  
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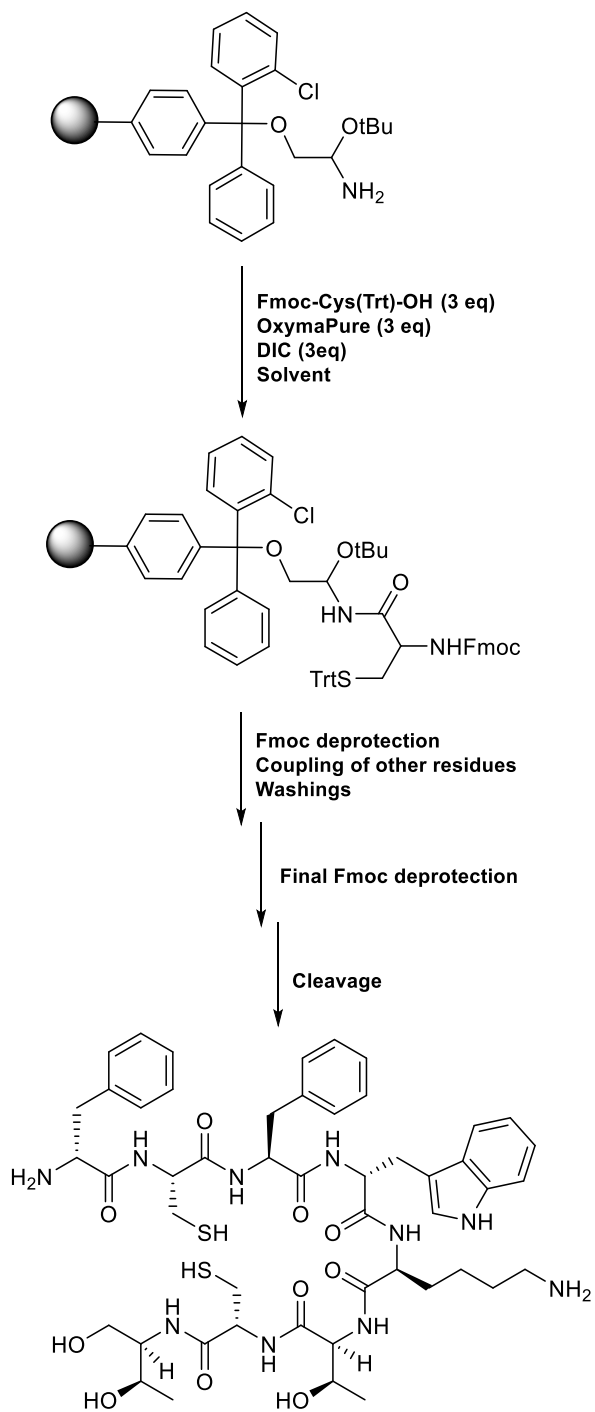
Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

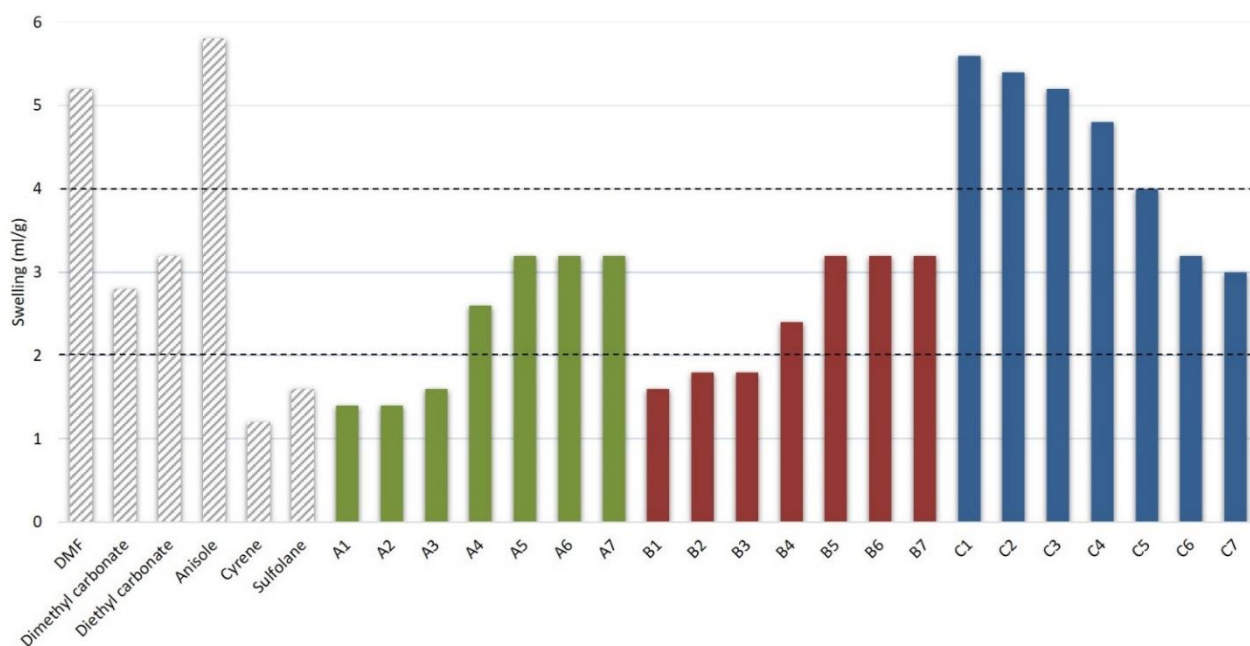
Signal 1: MSD1 TIC, MS File

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.883	BB	0.0575	1119.12122	300.41644	9.5759
2	8.220	BB	0.1286	517.20032	59.96790	4.4255
3	10.314	BB	0.1965	665.54077	46.07932	5.6948
4	10.607	BB	0.0954	3256.67822	461.05157	27.8663
5	10.948	BB	0.0598	2917.69360	743.74475	24.9657
6	17.722	BB	0.1536	2388.45508	243.23308	20.4372
7	21.732	BB	0.0917	681.79248	115.70737	5.8339
8	23.525	BB	0.0644	123.09662	30.97960	1.0533
9	23.624	BB	0.0517	17.21664	5.47248	0.1473

Totals : 1.16868e4 2006.65252

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 \*\*\* End of Report \*\*\*

**Fig. S27** General procedure for synthesis of linear Octreotide on H-Thr(tBu)-ol-2CT-PS resin.

**Fig. S28** Swelling of H-Thr(tBu)-ol-2CT resin.**Tab. S7** H-Thr(tBu)-ol-2CT resin.

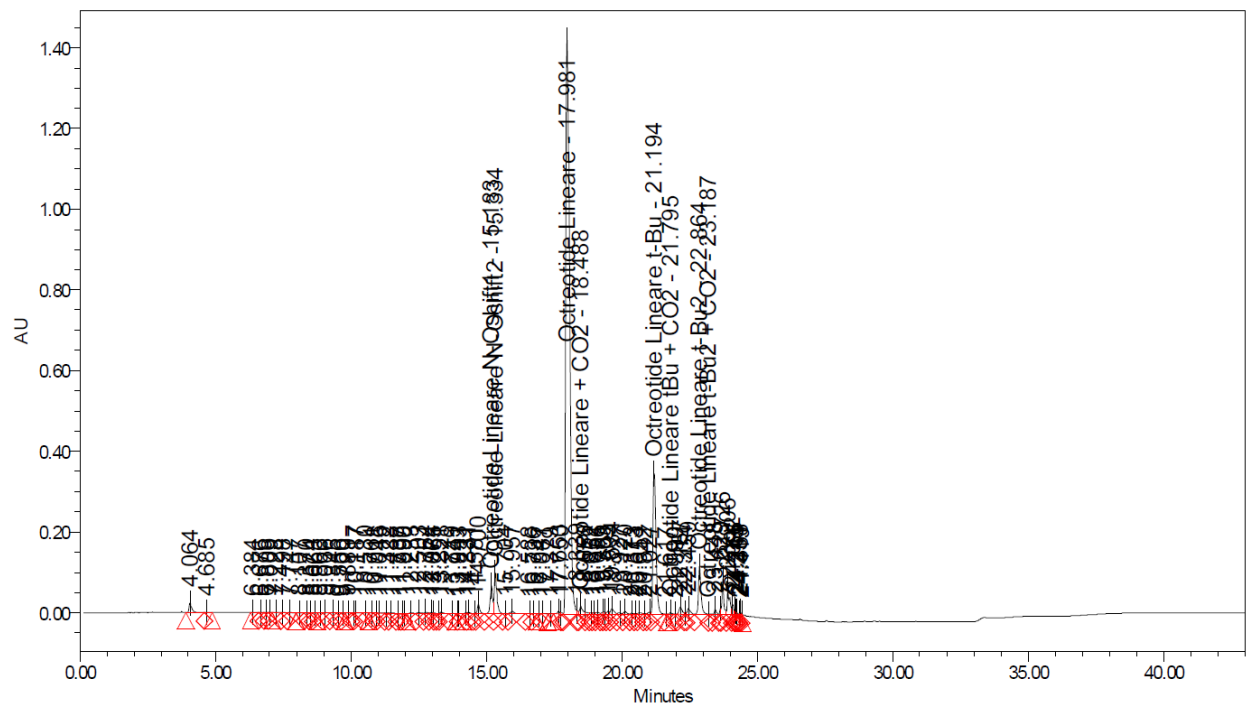
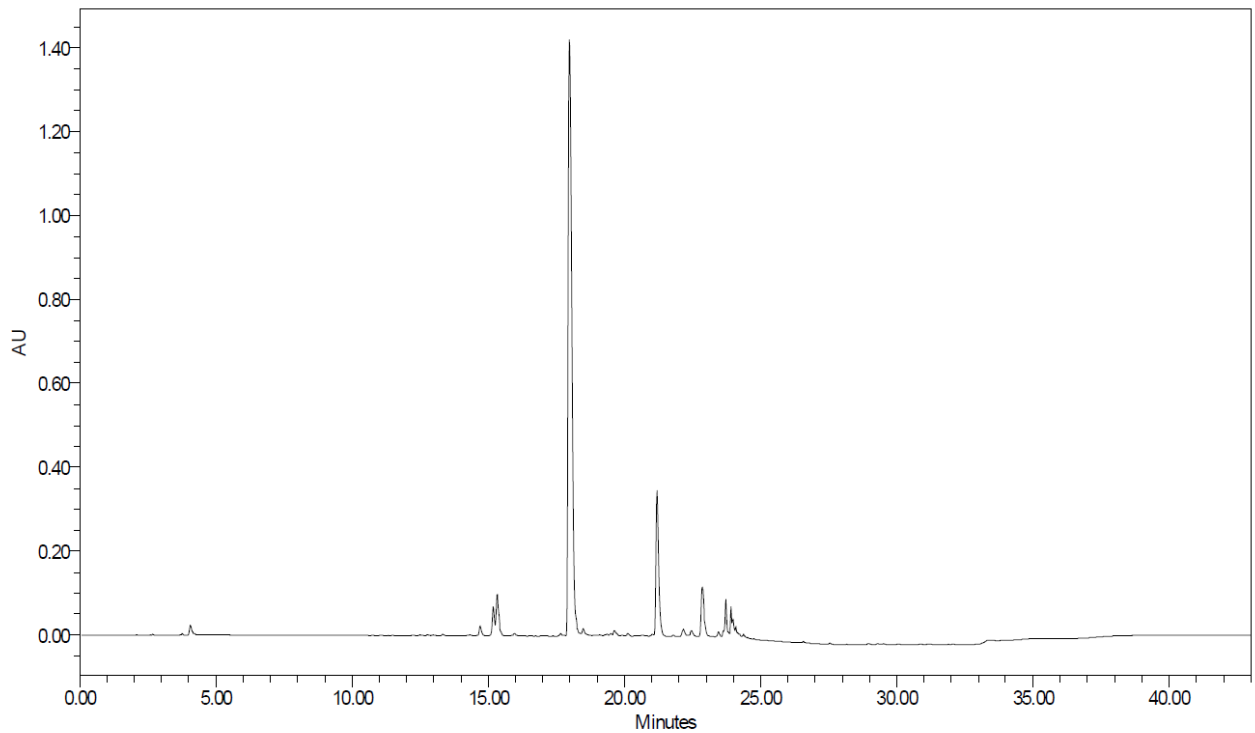
solvent	volume (medium)	t1	t2	t3	DEV.ST
DMF	5,2	5,2	5,2	5,2	0,0
Dimethyl carbonate	2,8	2,8	2,8	2,8	0,0
Diethyl carbonate	3,2	3,1	3,2	3,3	0,1
Anisole	5,8	5,8	5,8	5,9	0,1
Cyrene	1,2	1,2	1,2	1,2	0,0
Sulfolane	1,6	1,6	1,7	1,6	0,0
1a	1,4	1,5	1,4	1,5	0,0
2a	1,4	1,4	1,5	1,4	0,0
3a	1,6	1,6	1,6	1,7	0,1
4a	2,6	2,6	2,7	2,7	0,1
5a	3,3	3,3	3,2	3,3	0,0
6a	3,2	3,2	3,3	3,2	0,1
7a	3,2	3,3	3,3	3,2	0,1
1b	1,6	1,5	1,6	1,7	0,1
2b	1,8	1,8	1,8	1,9	0,1
3b	1,8	1,7	1,8	1,9	0,1
4b	2,4	2,5	2,5	2,3	0,1
5b	3,2	3,2	3,2	3,3	0,1
6b	3,2	3,2	3,3	3,2	0,0
7b	3,2	3,2	3,1	3,4	0,1
1c	5,6	5,5	5,6	5,6	0,1
2c	5,4	5,3	5,4	5,5	0,1
3c	5,2	5,3	5,2	5,3	0,0

## Supporting Information

4c	5,1	4,8	4,9	5,8	0,6
5c	4,0	3,9	4,2	4,0	0,2
6c	3,2	3,2	3,2	3,3	0,1
7c	3,0	3,0	3,1	3,0	0,1



**Fig. S29** Chromatogram of linear Octreotide, method 1 (DMF): the same chromatogram is reported without integrations and with integrations.



## Supporting Information

Picchi >= 0.05

	Tempo min	Picco	Area	% Area	RRT
1	4.064		163904	0.78	0.226
2	12.503		20175	0.10	0.695
3	12.764		19089	0.09	0.710
4	12.974		15422	0.07	0.722
5	13.328		25624	0.12	0.741
6	14.331		21190	0.10	0.797
7	14.700		152368	0.72	0.818
8	15.183	Octreotide Lineare N-Oshift1	409431	1.94	0.844
9	15.334	Octreotide Lineare N-Oshift2	663391	3.14	0.853
10	15.957		56554	0.27	0.887
11	17.663		36596	0.17	0.982
12	17.981	Octreotide Lineare	13966116	66.15	1.000
13	18.339		28524	0.14	1.020

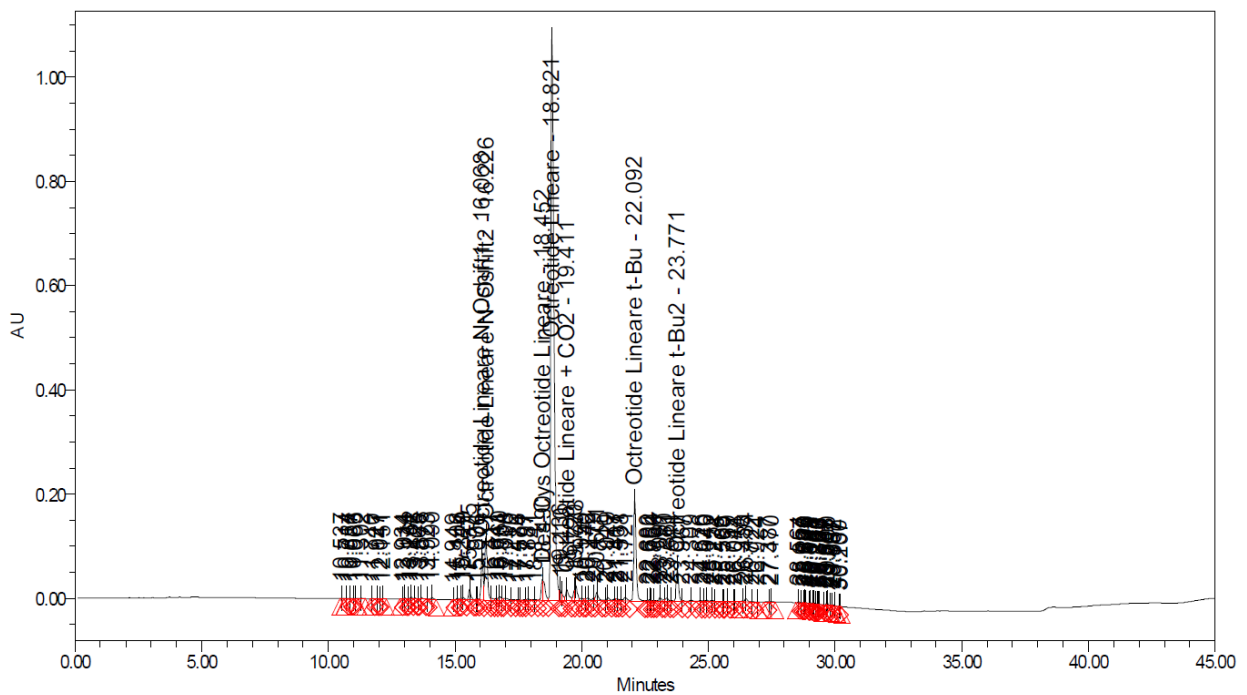
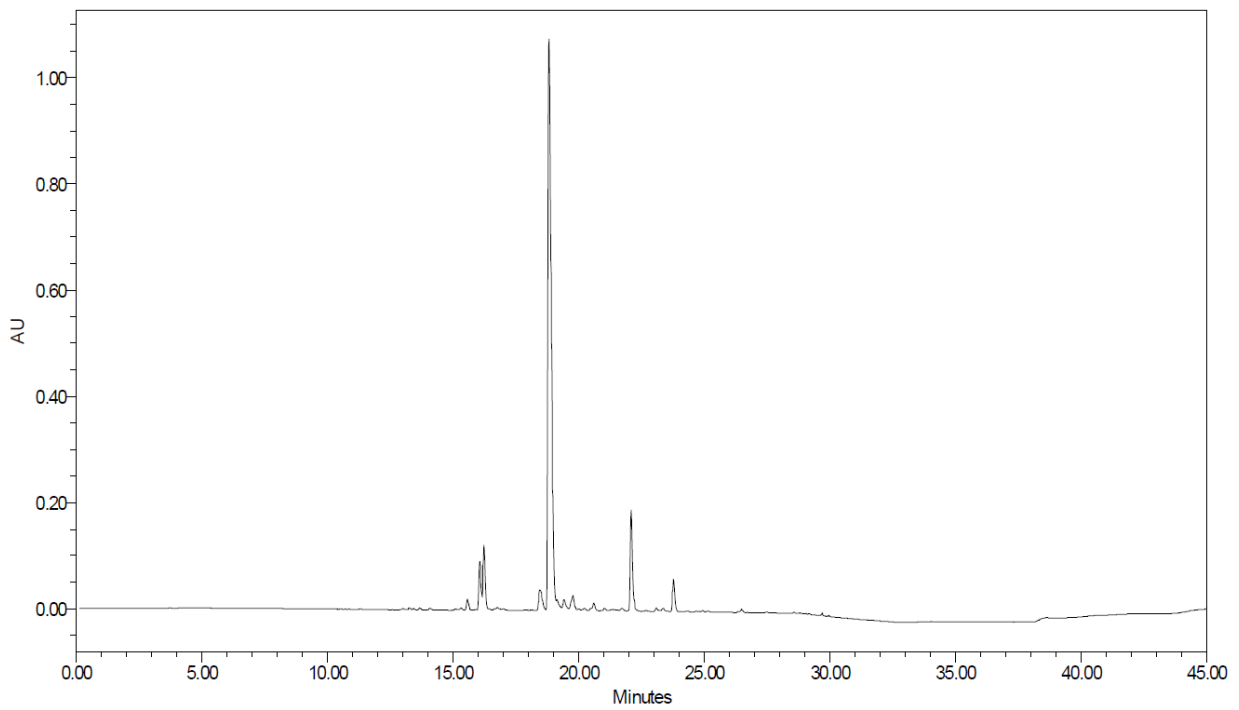
	Tempo min	Picco	Area	% Area	RRT
14	18.488	Octreotide Lineare + CO2	142393	0.67	1.028
15	18.650		24892	0.12	1.037
16	18.872		12339	0.06	1.050
17	18.951		12723	0.06	1.054
18	19.096		31361	0.15	1.062
19	19.309		18232	0.09	1.074
20	19.351		28271	0.13	1.076
21	19.505		45140	0.21	1.085
22	19.634		100913	0.48	1.092
23	19.927		22591	0.11	1.108
24	20.120		41549	0.20	1.119
25	20.373		12304	0.06	1.133
26	20.651		26893	0.13	1.148

	Tempo min	Picco	Area	% Area	RRT
27	21.027		32492	0.15	1.169
28	21.194	Octreotide Lineare t-Bu	2531609	11.99	1.179
29	21.795	Octreotide Lineare tBu + CO2	22078	0.10	1.212
30	22.167		139180	0.66	1.233
31	22.334		17248	0.08	1.242
32	22.459		103682	0.49	1.249
33	22.864	Octreotide Lineare t-Bu2	841211	3.98	1.272
34	23.448		77158	0.37	1.304
35	23.629		39077	0.19	1.314
36	23.726		429880	2.04	1.319
37	23.906		439957	2.08	1.329
38	24.092		91513	0.43	1.340
39	24.166		30782	0.15	1.344
40	24.215		20720	0.10	1.347
41	24.241		15600	0.07	1.348
42	24.368		20783	0.10	1.355

**Tab. S1** HPLC purity for linear Octreotide assembled on H-Thr(tBu)-ol-2CT-PS applying MixC3 at 40°C for deprotections and couplings.

Description	Mix C3 at 40°C for deprotections and couplings
Target product N,O-shifted 1	3.4
Target product N,O-shifted 2	5.0
des-Cys <sup>7</sup>	1.4
Target product	64.4
Target product + CO <sub>2</sub>	1.3
Target product + tBu	8.2
Target product + tBu <sub>2</sub>	2.6
Sum of unknown	15.2
Product purity <sup>[a]</sup>	84.9

**Fig. S31** Chromatogram of linear Octreotide when MixC3 at 40°C for couplings and deprotections: the same chromatogram is reported without integrations and with integrations.



## Supporting Information

Picchi >= 0.05

	Tempo min	Picco	Area	% Area	RRT
1	13.014		14947	0.10	0.691
2	13.262		25214	0.16	0.705
3	13.425		14052	0.09	0.713
4	13.676		21576	0.14	0.727
5	14.090		23781	0.16	0.749
6	15.106		16084	0.11	0.803
7	15.320		31105	0.20	0.814
8	15.575		123734	0.81	0.828
9	15.906		10840	0.07	0.845
10	16.068	Octreotide Lineare N-Oshift1	515103	3.37	0.854
11	16.226	Octreotide Lineare N-Oshift2	769757	5.04	0.862
12	16.417		18970	0.12	0.872
13	16.641		17694	0.12	0.884

	Tempo min	Picco	Area	% Area	RRT
14	16.760		41718	0.27	0.890
15	16.834		15777	0.10	0.894
16	17.008		35556	0.23	0.904
17	17.486		7843	0.05	0.929
18	17.881		19752	0.13	0.950
19	18.141		20432	0.13	0.964
20	18.452	Des-Cys Octreotide Lineare	208315	1.36	0.980
21	18.490		187043	1.22	0.982
22	18.821	Octreotide Lineare	9834390	64.34	1.000
23	19.136		125853	0.82	1.017
24	19.210		61726	0.40	1.021
25	19.411	Octreotide Lineare + CO2	193335	1.26	1.031
26	19.720		82174	0.54	1.048

	Tempo min	Picco	Area	% Area	RRT
27	19.778		202350	1.32	1.051
28	19.999		38622	0.25	1.063
29	20.170		15535	0.10	1.072
30	20.242		45038	0.29	1.076
31	20.479		30610	0.20	1.088
32	20.601		125289	0.82	1.095
33	20.940		10196	0.07	1.113
34	21.029		52064	0.34	1.117
35	21.317		29209	0.19	1.133
36	21.401		23687	0.15	1.137
37	21.538		23401	0.15	1.144
38	21.721		63648	0.42	1.154
39	22.092	Octreotide Lineare t-Bu	1258506	8.23	1.174
40	22.682		17586	0.12	1.205
41	23.097		51671	0.34	1.227
42	23.280		14686	0.10	1.237
43	23.361		53967	0.35	1.241
44	23.541		10799	0.07	1.251
45	23.771	Octreotide Lineare t-Bu2	401126	2.62	1.263
46	23.967		10572	0.07	1.273
47	24.309		23818	0.16	1.292
48	24.676		16266	0.11	1.311
49	24.820		9967	0.07	1.319
50	24.940		20192	0.13	1.325
51	25.152		19936	0.13	1.336
52	26.356		10756	0.07	1.400
53	26.484		42568	0.28	1.407
54	27.470		10388	0.07	1.460

	Tempo min	Picco	Area	% Area	RRT
55	28.564		12174	0.08	1.518
56	28.846		11671	0.08	1.533
57	29.048		9241	0.06	1.543
58	29.181		8803	0.06	1.550
59	29.700		19023	0.12	1.578



## Supporting Information

Picchi >= 0.05

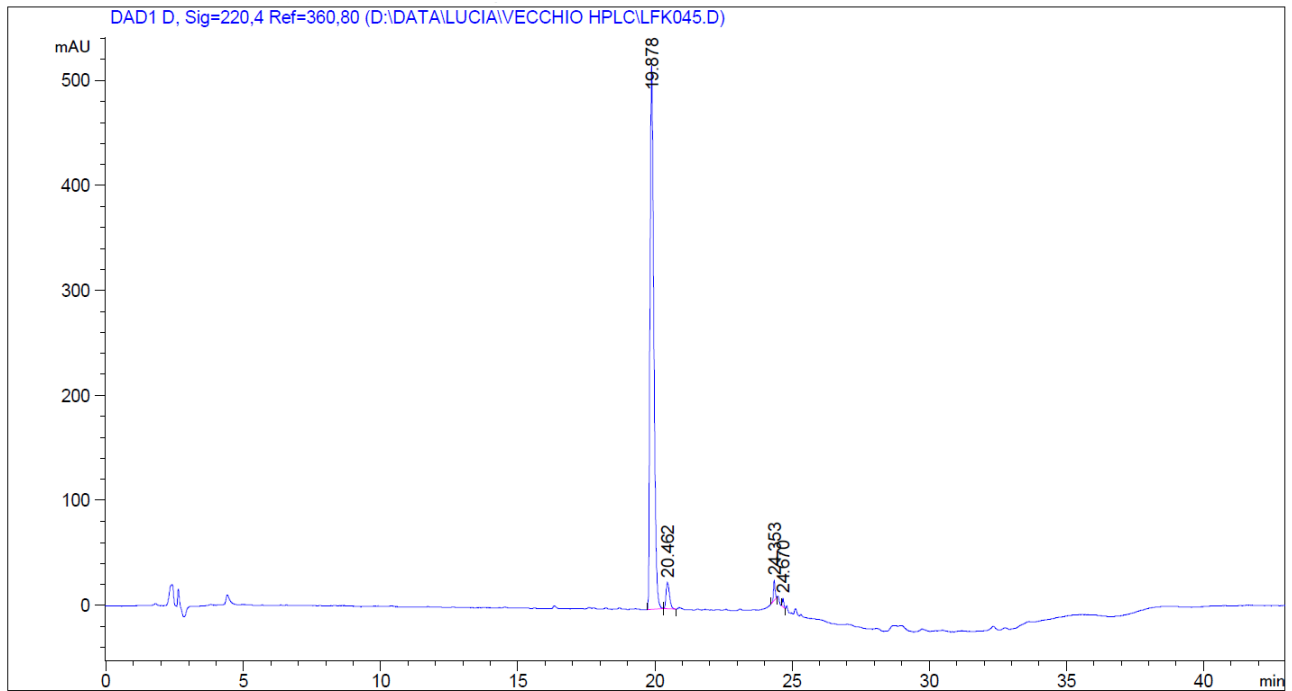
	Tempo min	Picco	Area	% Area	RRT
1	11.060		5577	0.05	0.587
2	11.286		13822	0.12	0.599
3	11.742		17324	0.16	0.623
4	11.926		23381	0.21	0.633
5	12.143		5757	0.05	0.645
6	12.599		14028	0.13	0.669
7	12.762		20926	0.19	0.677
8	13.253		50332	0.45	0.703
9	13.424		21467	0.19	0.712
10	13.678		25416	0.23	0.726
11	14.088		214785	1.94	0.748
12	15.118		144519	1.30	0.802
13	15.316		7789	0.07	0.813

	Tempo min	Picco	Area	% Area	RRT
14	15.582		56550	0.51	0.827
15	16.074	Octreotide Lineare N-Oshift1	356325	3.21	0.853
16	16.231	Octreotide Lineare N-Oshift2	453604	4.09	0.861
17	16.510		11556	0.10	0.876
18	16.765		31711	0.29	0.890
19	16.999		12650	0.11	0.902
20	17.497		17339	0.16	0.929
21	17.876		39210	0.35	0.949
22	18.109		8639	0.08	0.961
23	18.457	Des-Cys Octreotide Lineare	94892	0.86	0.980
24	18.577		13397	0.12	0.986
25	18.841	Octreotide Lineare	6952528	62.70	1.000
26	19.136		84689	0.76	1.016

	Tempo min	Picco	Area	% Area	RRT
27	19.237		112133	1.01	1.021
28	19.406	Octreotide Lineare + CO2	126580	1.14	1.030
29	19.779		138939	1.25	1.050
30	20.003		23492	0.21	1.062
31	20.132		14746	0.13	1.068
32	20.214		12722	0.11	1.073
33	20.469		39460	0.36	1.086
34	20.604		95722	0.86	1.094
35	21.020		29827	0.27	1.116
36	21.087		7424	0.07	1.119
37	21.268		16428	0.15	1.129
38	21.412		9315	0.08	1.136
39	21.547		15800	0.14	1.144
40	21.741		17849	0.16	1.154
41	22.097	Octreotide Lineare t-Bu	986504	8.90	1.173
42	22.681		13939	0.13	1.204
43	22.889		7835	0.07	1.215
44	23.103		24154	0.22	1.226
45	23.290		9495	0.09	1.236
46	23.358		28479	0.26	1.240
47	23.780	Octreotide Lineare t-Bu2	252093	2.27	1.262
48	24.174		8667	0.08	1.283
49	24.359		7556	0.07	1.293
50	24.685		11000	0.10	1.310
51	24.827		9440	0.09	1.318
52	24.947		17815	0.16	1.324
53	25.157		13196	0.12	1.335
54	25.766		70727	0.64	1.368

	Tempo min	Picco	Area	% Area	RRT
55	26.493		30340	0.27	1.406
56	27.479		7328	0.07	1.458
57	28.997		8140	0.07	1.539
58	29.046		6496	0.06	1.542
59	29.212		8363	0.08	1.550
60	29.698		8126	0.07	1.576
61	29.969		22912	0.21	1.591
62	31.462		6706	0.06	1.670
63	36.033		20872	0.19	1.912

**Fig. S33** Chromatogram of Octreotide obtained with cyclization of linear peptide synthesized with method 1.



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 Area Percent Report  
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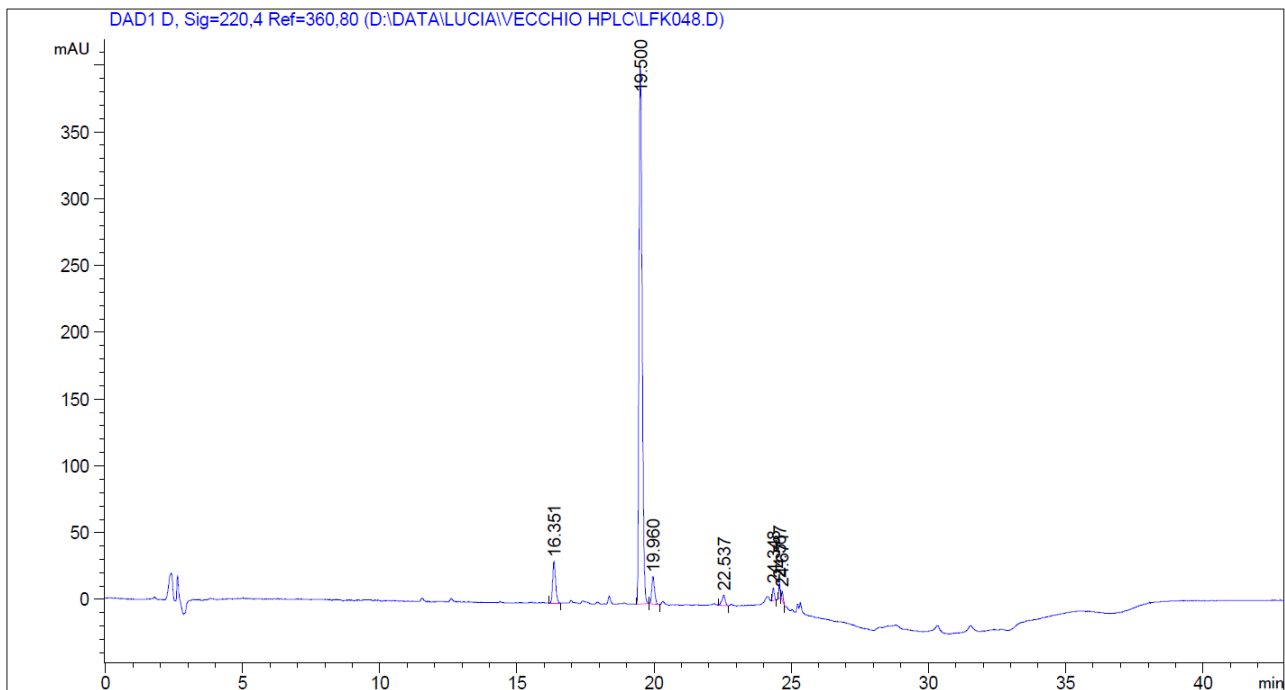
Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 D, Sig=220,4 Ref=360,80

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.878	BB	0.1494	4951.01221	518.33740	93.9297
2	20.462	BB	0.1299	214.05878	25.00788	4.0611
3	24.353	BB	0.0618	73.68929	18.76126	1.3980
4	24.670	BB	0.0602	32.21850	8.49851	0.6112



**Fig. S34** Chromatogram of Octreotide obtained with cyclization of linear peptide synthesized with method 11.



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 Area Percent Report  
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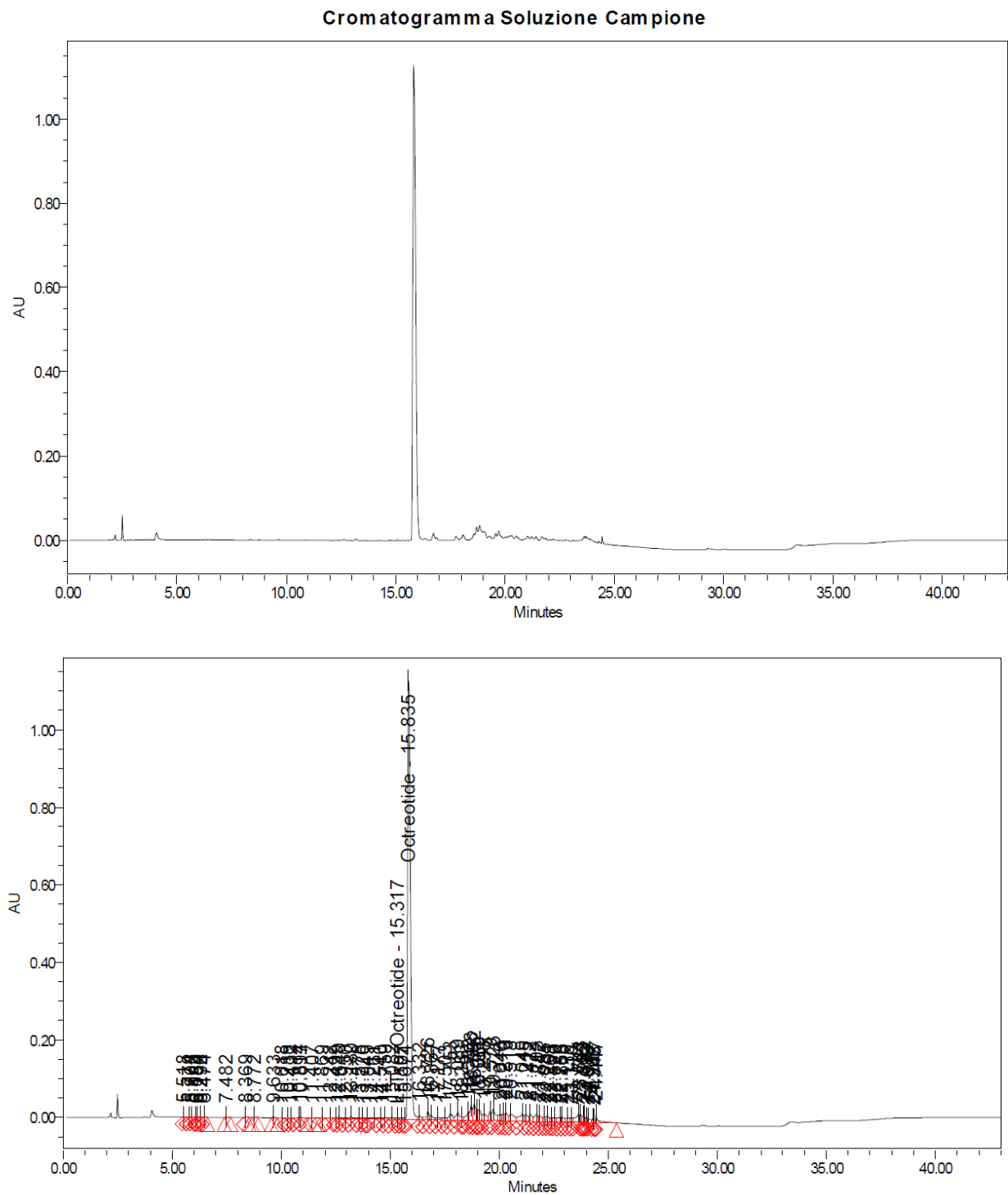
Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 D, Sig=220,4 Ref=360,80

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.351	BB	0.1167	235.35461	30.97034	6.4482
2	19.500	BB	0.1218	3092.68970	401.66891	84.7324
3	19.960	BB	0.1105	147.27272	20.33549	4.0349
4	22.537	BB	0.1233	67.89483	7.97610	1.8602
5	24.348	BB	0.0714	42.08096	9.19674	1.1529
6	24.567	BB	0.0568	39.80103	11.38967	1.0905
7	24.671	BB	0.0633	24.85385	6.40481	0.6809

Totals : 3649.94769 487.94207

**Fig. S35** Chromatogram of Octreotide by injection with USP method, according to American Pharmacopeia: the same chromatogram is reported without integrations and with integrations.



## Supporting Information

### Picchi > 0.05

	Tempo min	Picco	Area	% Area	RRT
1	9.633		11894	0.07	0.608
2	12.242		12128	0.07	0.773
3	12.499		11814	0.07	0.789
4	12.640		29069	0.17	0.798
5	12.950		17289	0.10	0.818
6	13.180		50033	0.29	0.832
7	13.578		19061	0.11	0.857
8	13.720		20266	0.12	0.866
9	13.949		10020	0.06	0.881
10	14.261		52999	0.31	0.901
11	14.540		39680	0.23	0.918
12	14.759		47239	0.27	0.932
13	15.082		61256	0.35	0.952

	Tempo min	Picco	Area	% Area	RRT
14	15.317	L-Trp-Octreotide	38352	0.22	0.967
15	15.507		38714	0.22	0.979
16	15.674		28679	0.17	0.990
17	15.835	Octreotide	10043274	58.10	1.000
18	16.332		112326	0.65	1.031
19	16.726		204254	1.18	1.056
20	16.867		118390	0.68	1.065
21	17.177		101273	0.59	1.085
22	17.503		79495	0.46	1.105
23	17.753		200598	1.16	1.121
24	18.089		238377	1.38	1.142
25	18.283		53641	0.31	1.155
26	18.582		219965	1.27	1.173

Supporting Information

	Tempo min	Picco	Area	% Area	RRT		Tempo min	Picco	Area	% Area	RRT
27	18.708		265072	1.53	1.181	55	23.992		77230	0.45	1.515
28	18.852		350760	2.03	1.190	56	24.079		76055	0.44	1.521
29	18.975		130133	0.75	1.198	57	24.282		55607	0.32	1.533
30	19.069		246277	1.42	1.204	58	24.348		21348	0.12	1.538
31	19.298		224126	1.30	1.219	59	24.447		162505	0.94	1.544
32	19.579		200969	1.16	1.236						
33	19.726		350095	2.03	1.246						
34	20.046		160485	0.93	1.266						
35	20.210		150012	0.87	1.276						
36	20.313		168419	0.97	1.283						
37	20.518		297555	1.72	1.296						
38	21.049		286202	1.66	1.329						
39	21.215		181493	1.05	1.340						
40	21.422		196015	1.13	1.353						
41	21.703		192068	1.11	1.371						
42	21.842		152504	0.88	1.379						
43	22.085		100831	0.58	1.395						
44	22.201		135886	0.79	1.402						
45	22.368		78672	0.46	1.413						
46	22.520		118750	0.69	1.422						
47	22.786		118103	0.68	1.439						
48	22.853		115504	0.67	1.443						
49	23.139		133898	0.77	1.461						
50	23.316		86819	0.50	1.472						
51	23.641		270681	1.57	1.493						
52	23.723		133389	0.77	1.498						
53	23.835		52216	0.30	1.505						
54	23.893		68412	0.40	1.509						