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Supporting information

Efficient Synthesis of novel RGD Based Peptides and the Conjugation of Pyrazine Moiety to their *N*-Terminus

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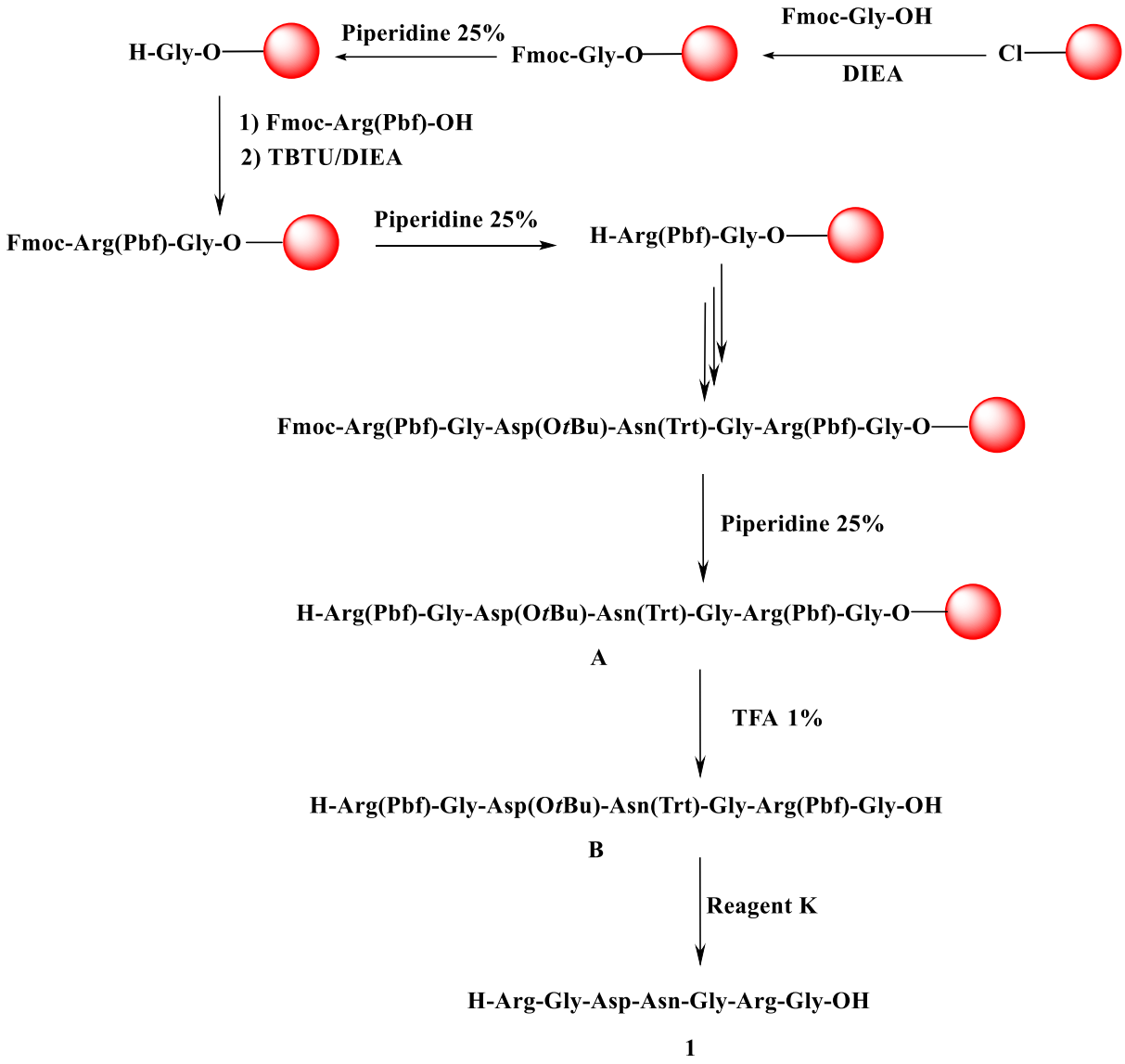
Table of Contents

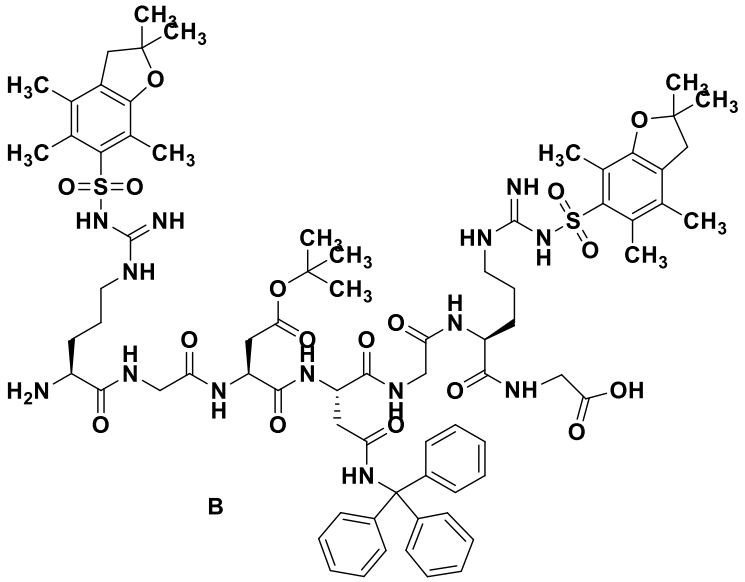
Peptide synthesis:

General Method 1: Synthesis of Heptapeptide RGDNGRG (1)



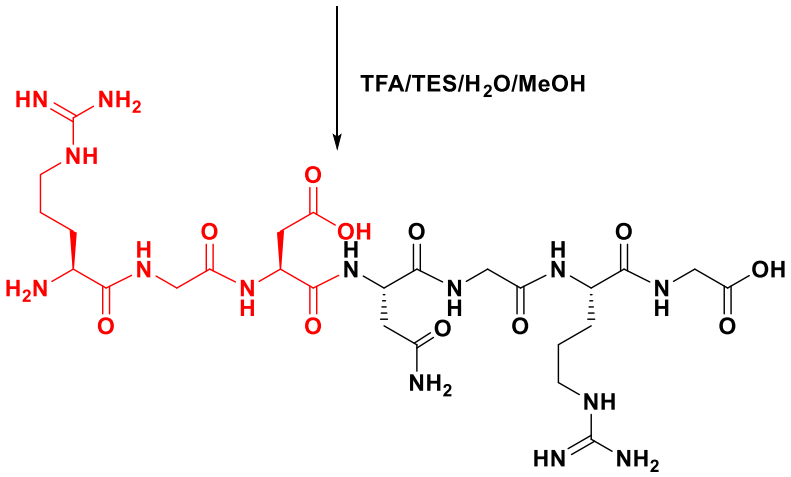
The Synthetic pathway for the synthesis of RGDNGRG peptide sequence

General Method 2: Final deprotection of A and formation of peptide 1



B

H-Arg (Pbf)-Gly-Asp (O*t*Bu)-Asn(Trt)-Gly-Arg(Pbf)-Gly-OH



H-RGDNGRG-OH

1 (67%)

(1.1g, 0.58 mmol) of the protected peptide (**B**) was slowly added to 7.5 ml of the reagent K TFA/TES/H_2O/MeOH (95%: 2%: 1.5%: 1.5%) and stirred at room temperature for 2 hours. This step removes all protected groups of the side chain within the peptide sequence. Then the solvent was evaporated and precipitated with Et_2O. The final peptide was dried under vacuum at 40 ºC (Isolated yield **67**%).

High Performance Liquid Chromatography:

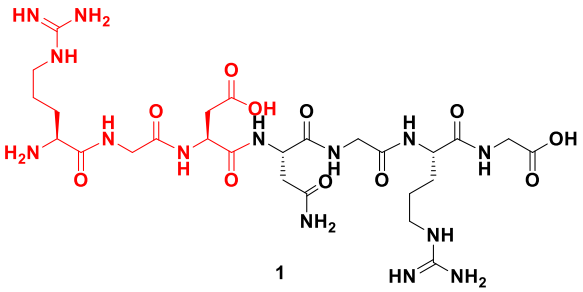
The samples were dissolved in solvent A for liquid chromatography. The mobile phase for all HPLC purifications consisted of solvent A (Acetonitrile/Water (70/30)) and solvent B (NaH_2PO_4/Water (10mM)) and separations were performed on an HPLC system (Knauer, Germany) equipped with a pump 1800 (Knauer, Germany), UV detector 2500 (Knauer, Germany). Peptide purification was performed at preparative scale using ODS-C_{18} column (120 mm × 20mm, 10 µm). The flow rate was set to 10 mL min^{-1} and the peptides were separated by linear gradients of solvent B between 100 and 50% at 10% min^{-1}. The elution profile was monitored via UV absorbance at 210 nm and peptides were collected manually according to their absorbance at 210 nm.

Entry	Flow (ml/min)	Time (min)	A%	B%
1	10	0	0	**100**
2	10	5	0	**100**
3	10	55	50	**50**
4	10	80	100	**0**
5	10	110	100	**0**

Analytical RP-HPLC separation was carried out using ODS-C_{18} column (250 mm × 4.6 mm, 3-5 µm) at a flow rate of 1 mL min^{-1}. The mobile phase for consisted of solvent A [Acetonitrile/Buffer B (80/20)] and solvent B Buffer B [TFA/Water (0.1%)] and separations were performed on an HPLC system (Knauer, Germany) equipped with a pump 1000 (Knauer, Germany), UV detector 2500 (Knauer, Germany). The employed elution program started at 95 % A and remained at this point for 5 min before changing to 55 % of solvent A over 45 min. at 1% min^{-1}.

Entry	Flow (ml/min)	Time (min)	A%	B%
1	1	0	95	**5**
2	1	5	95	**5**

3	1	45	55	**45**
4	1	65	0	**100**
5	1	80	0	**100**



1

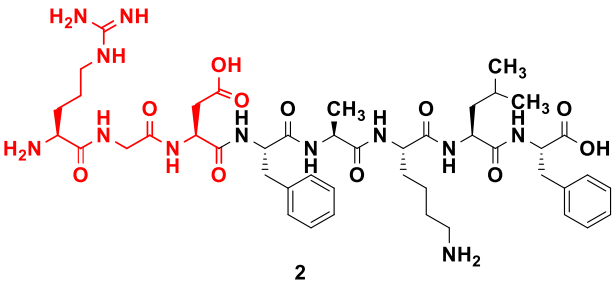
H-RGDNGRG-OH

HPLC analysis found that peptide **(1)** was obtained in 97 %< purity (t_R: 6. 25 min).

HR-Mass (ESI): $C_{26}H_{47}N_{14}O_{11}$ *m/z* = $[M+H]^+$ Found 731.3550, Calc. for 731.3543.

Synthesis of the octapeptide RGDFAKLF (2)

The same procedure was followed for the synthesis and purification of peptide **2** (Isolated yield **70**%).



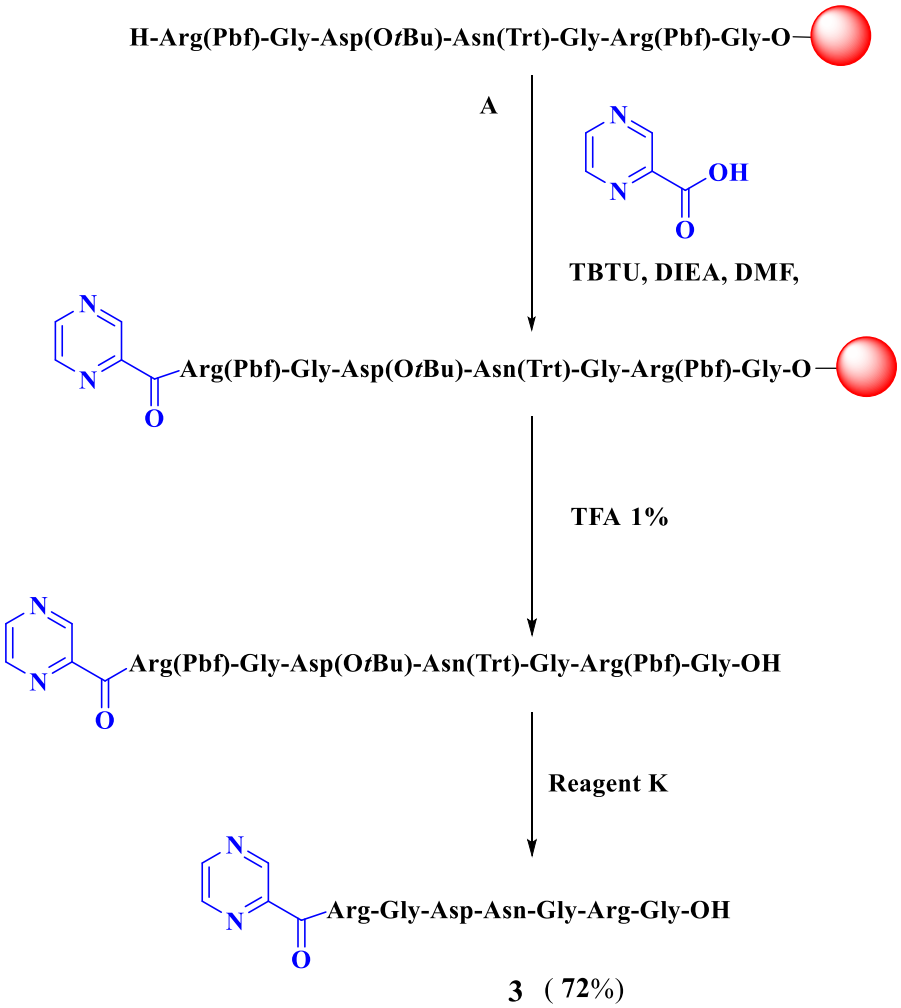
2

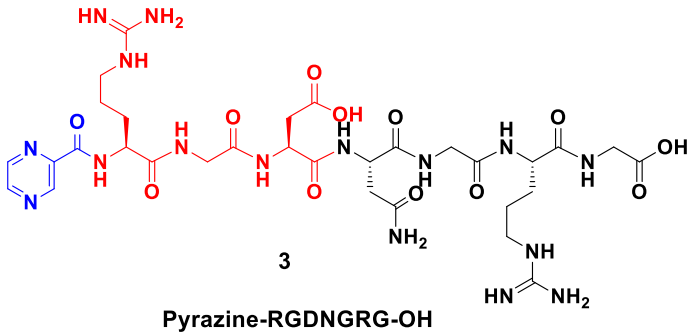
H-RGDFAKLF-OH

HPLC analysis found that peptide **(2)** was obtained in 96 %< purity (t_R: 42. 26 min).

HR-Mass (ESI): $C_{45}H_{69}N_{12}O_{11}$*m/z* =$[M+H]^+$ Found 953.5207, Calc. for 953.5203.

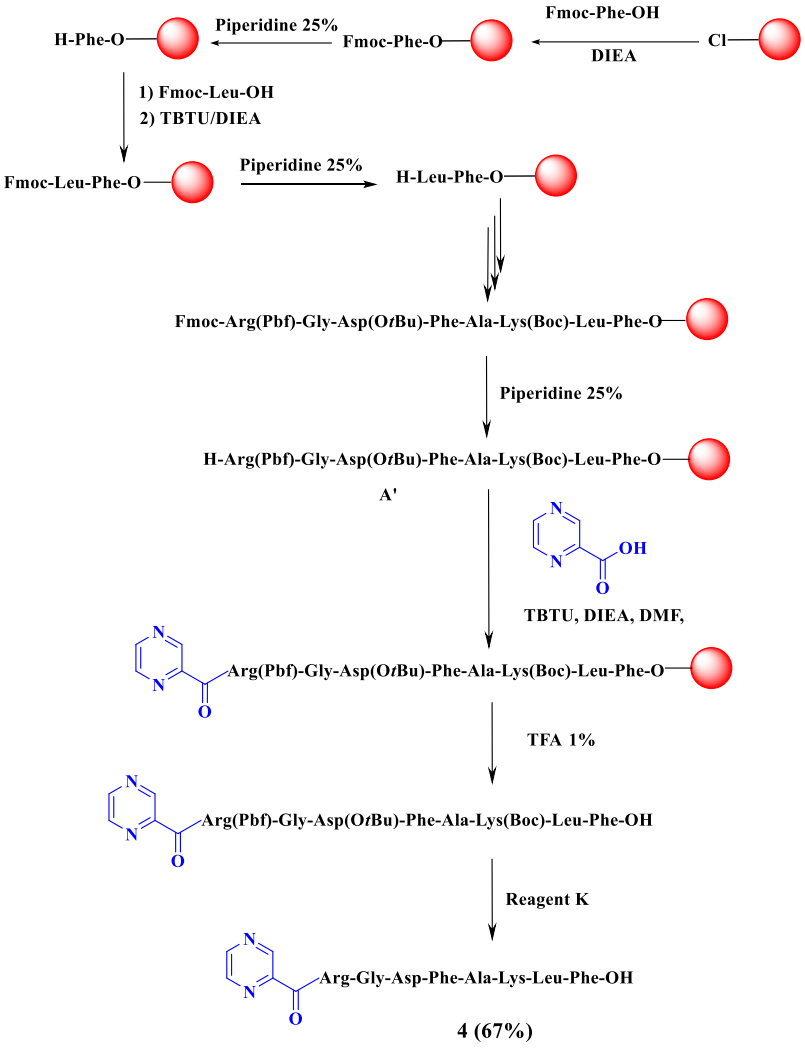
General Method for the synthesis of RGD based peptide (3, 4) by coupling pyrazine 2-carboxylic moiety to the *N*-terminus

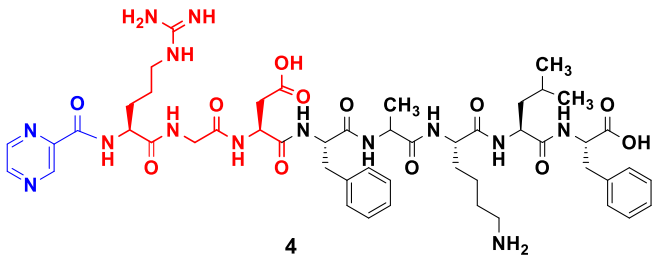




HPLC analysis found that peptide **(3)** was obtained in 97 %< purity (t_R: 18. 30 min).

HR-Mass (ESI): $C_{31}H_{49}N_{16}O_{12}$ *m/z* =$[M+H]^+$ Found 837.3717, Calc. for 837.3710.





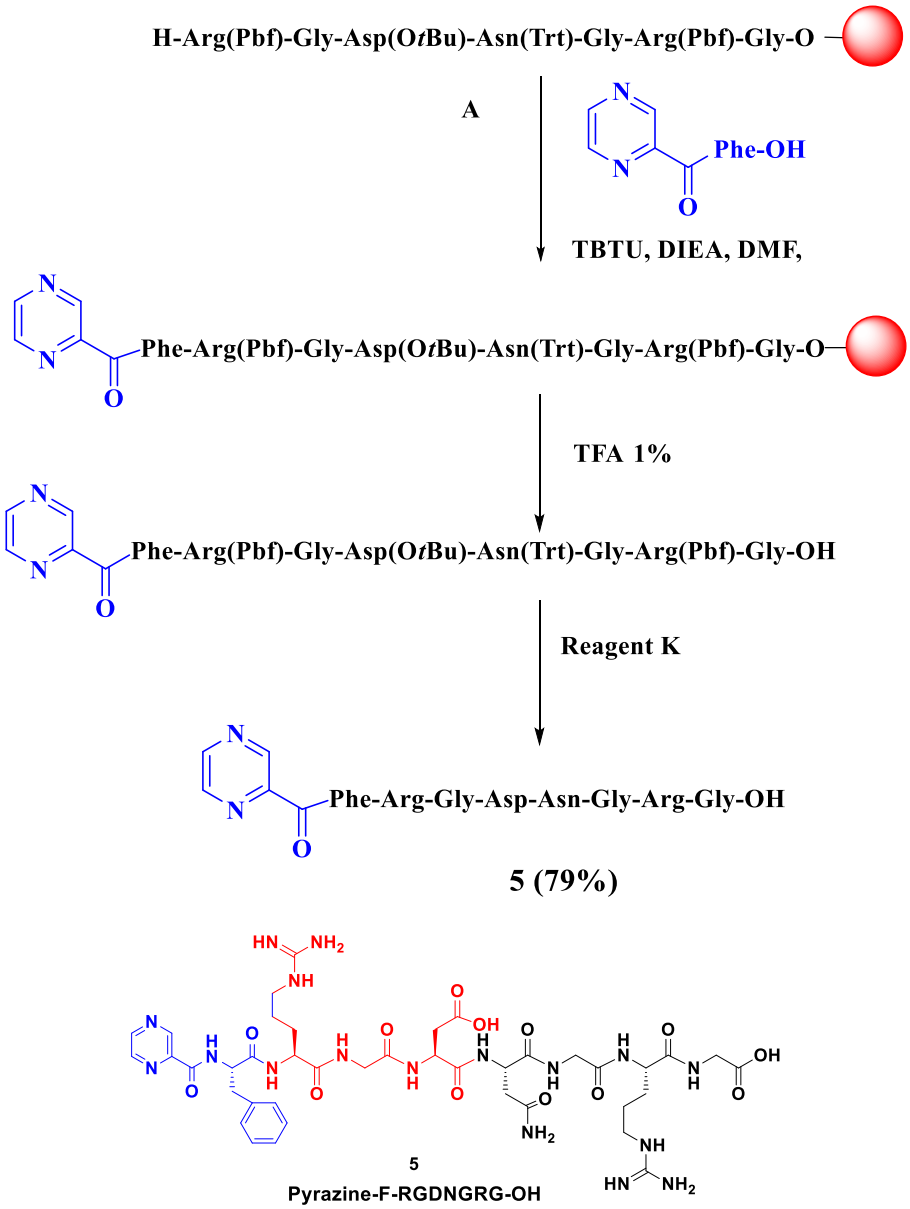
4

Pyrazine-RGDFAKLF-OH

HPLC analysis found that peptide **(4)** was obtained in 96 %< purity (t_R: 47. 29 min).

HR-Mass (ESI): $C_{50}H_{71}N_{14}O_{12}$ *m/z* =$[M+H]^+$ Found 1059.5378, Calc. for 1059.5370.

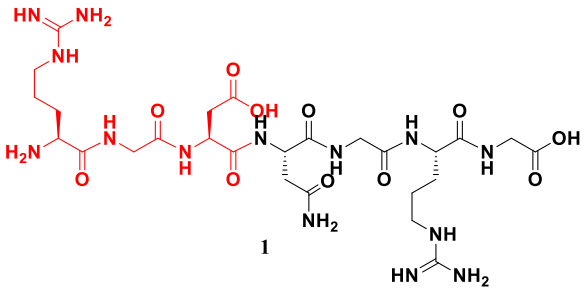
General Method for the synthesis of RGD based peptide (5) by coupling pyrazine 2-carboxylic moiety to the *N*-terminus



HPLC analysis found that peptide **(5)** was obtained in 99 %< purity (t_R: 32. 04 min).

HR-Mass (ESI): $C_{40}H_{57}N_{17}O_{13}$ *m/z* =$[M+H]^+$ Found 984.4403, Calc. for 984.4395.

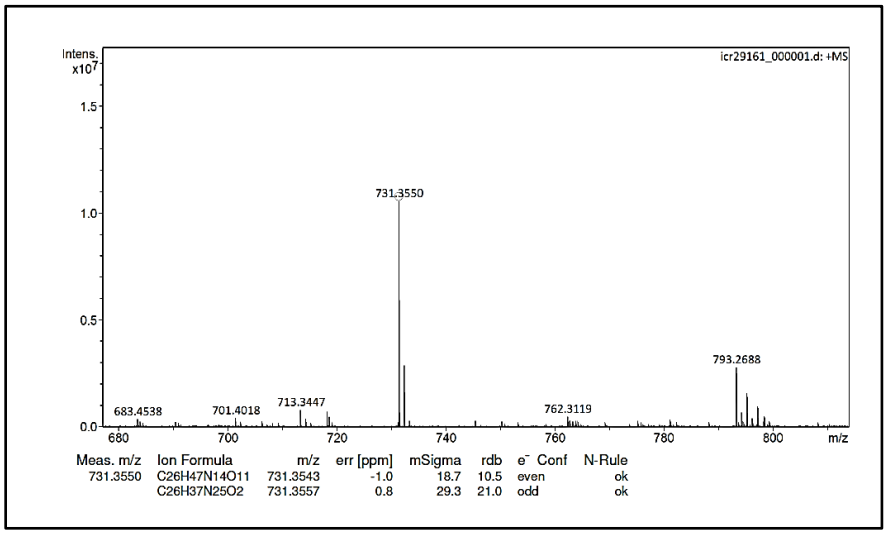
Peptide 1: H-Arg-Gly-Asp-Asn-Gly-Arg-Gly-OH (RGDNGRG)



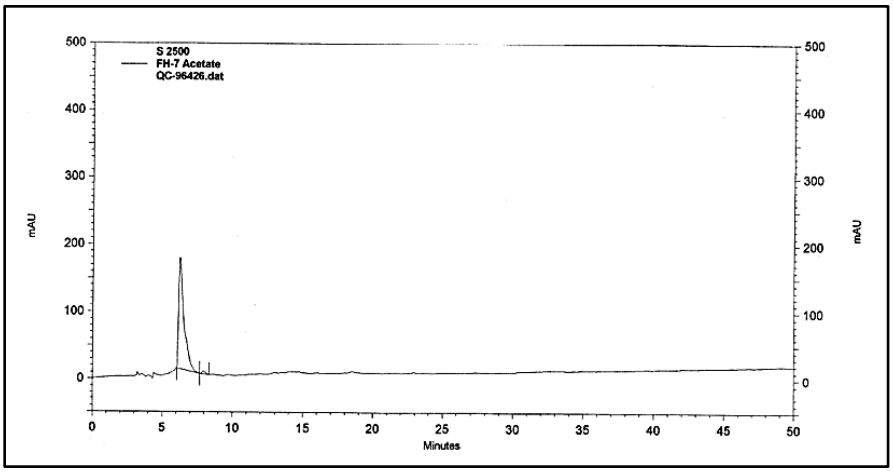
1

HPLC analysis found that peptide **(1)** was obtained in 97 %< purity (t_R: 6. 25 min).

HR-Mass (ESI): $C_{26}H_{47}N_{14}O_{11}$ *m/z* = $[M+H]^+$ Found 731.3550, Calc. for 731.3543.

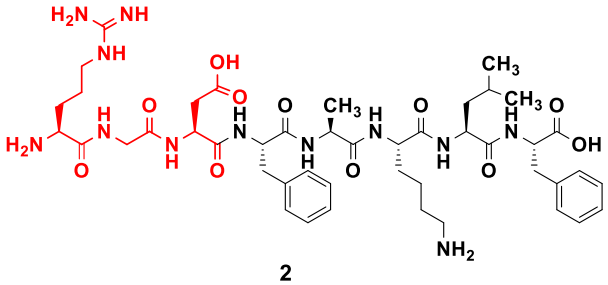


HR-MS (ESI) of peptide **1**.



Analytical HPLC chromatogram of peptide **1**.

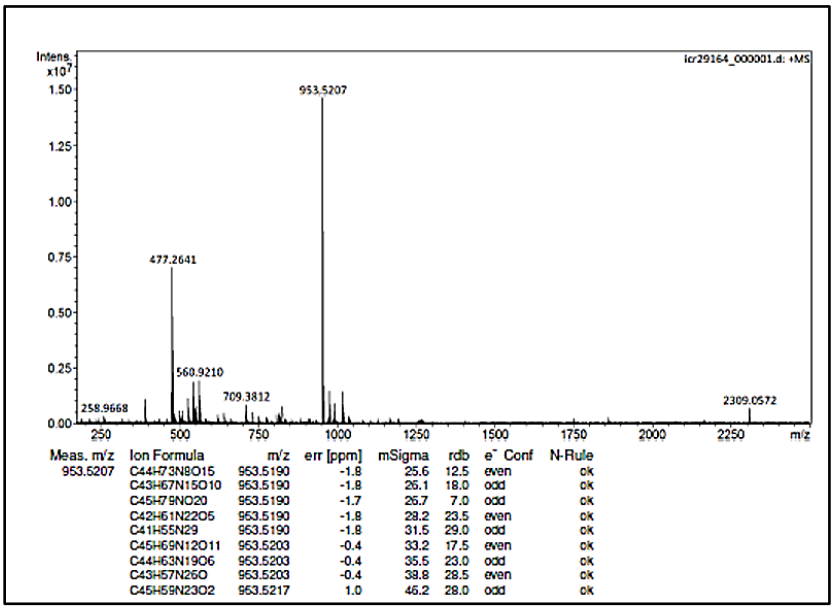
Peptide 2: H-Arg-Gly-Asp-Phe-Ala-Lys-Leu-Phe-OH (RGDFAKLF)



2

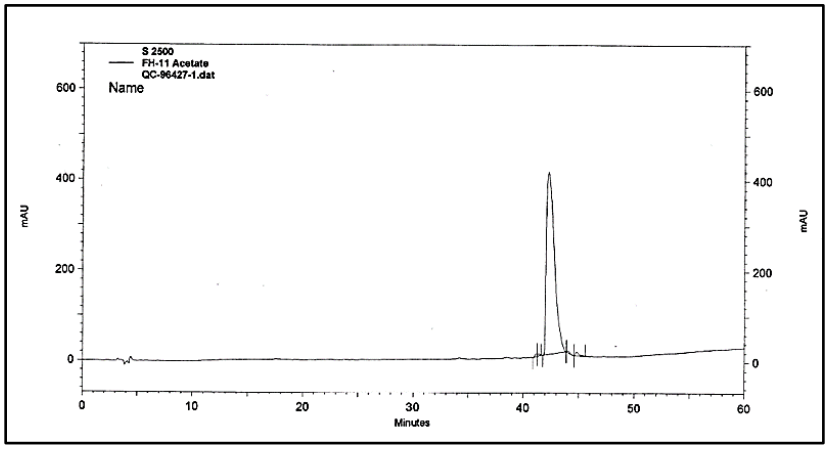
HPLC analysis found that peptide **(2)** was obtained in 96 %< purity (t_R: 42. 26 min).

HR-Mass (ESI): $C_{45}H_{69}N_{12}O_{11}$*m/z* =$[M+H]^+$ Found 953.5207, Calc. for 953.5203.



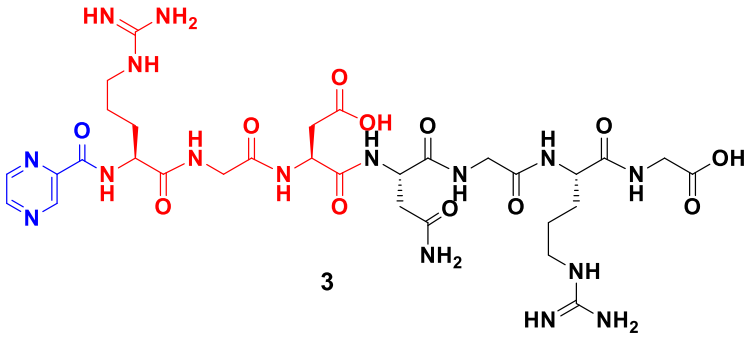
Meas. m/z	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
953.5207	C44H73N8O15	953.5190	-1.8	25.6	12.5	even	ok
	C43H67N15O10	953.5190	-1.8	26.1	18.0	odd	ok
	C45H79NO20	953.5190	-1.7	26.7	7.0	odd	ok
	C42H61N22O5	953.5190	-1.8	28.2	23.5	even	ok
	C41H55N29	953.5190	-1.8	31.5	29.0	odd	ok
	C45H69N12O11	953.5203	-0.4	33.2	17.5	even	ok
	C44H63N19O6	953.5203	-0.4	35.5	23.0	odd	ok
	C43H57N26O	953.5203	-0.4	38.8	28.5	even	ok
	C45H59N23O2	953.5217	1.0	46.2	28.0	odd	ok

HR-MS (ESI) of peptide **2**.



Analytical HPLC chromatogram of peptide **2**.

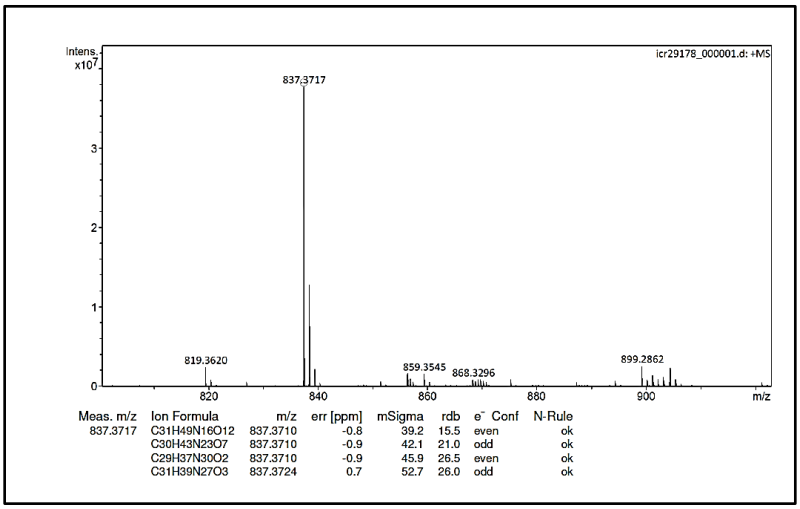
Peptide 3: Pyrazine-Arg-Gly-Asp-Asn-Gly-Arg-Gly-OH (Pyrazine-RGDNGRG)



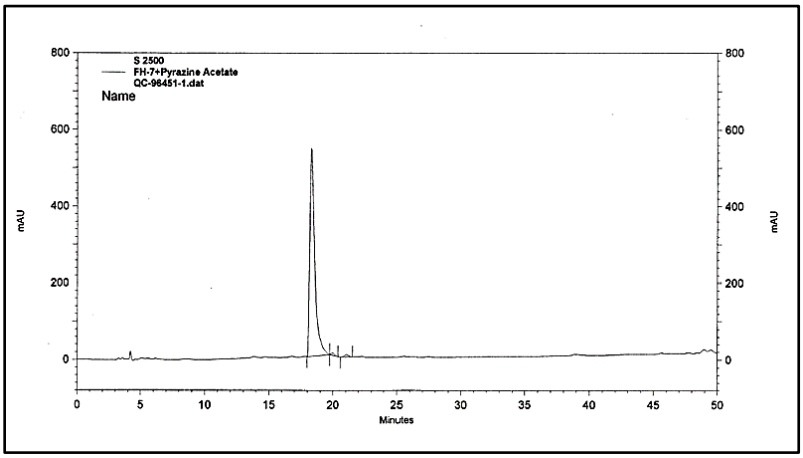
3

HPLC analysis found that peptide **(3)** was obtained in 97 %< purity (t_R: 18. 30 min).

HR-Mass (ESI): $C_{31}H_{49}N_{16}O_{12}$ *m/z* =$[M+H]^+$ Found 837.3717, Calc. for 837.3710.

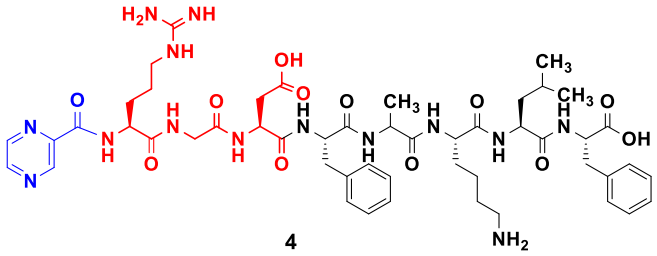


HR-MS (ESI) of peptide **3**.



Analytical HPLC chromatogram of peptide **3**.

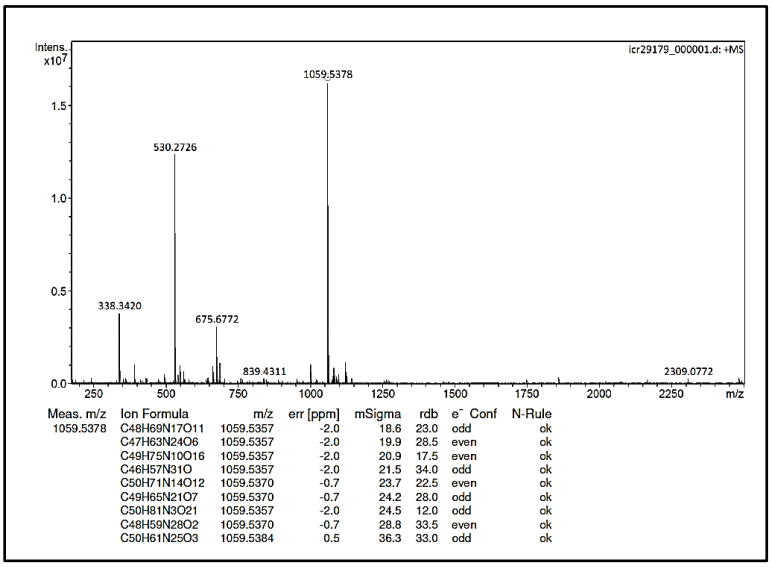
Peptide 4: Pyrazine-Arg-Gly-Asp-Phe-Ala-Lys-Leu-Phe-OH (Pyrazine-RGDFAKLF)



4

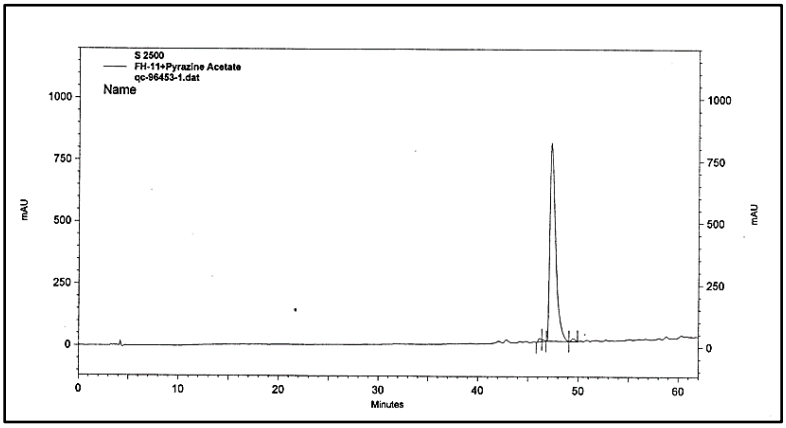
HPLC analysis found that peptide **(4)** was obtained in 96 %< purity (t_R: 47. 29 min).

HR-Mass (ESI): $C_{50}H_{71}N_{14}O_{12}$*m/z* =$[M+H]^+$ Found 1059.5378, Calc. for 1059.5370.



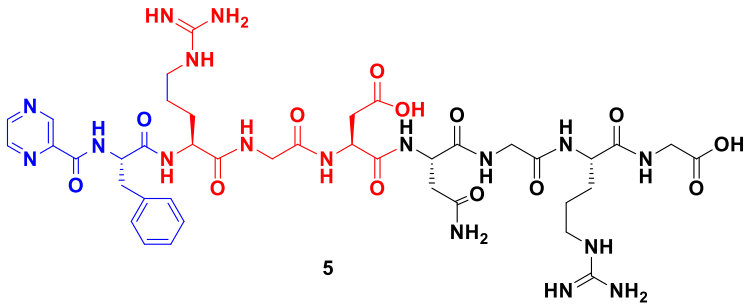
Meas. m/z	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1059.5378	C48H69N17O11	1059.5357	-2.0	18.6	23.0	odd	ok
	C47H63N24O6	1059.5357	-2.0	19.9	28.5	even	ok
	C49H75N10O16	1059.5357	-2.0	20.9	17.5	even	ok
	C46H57N31O	1059.5357	-2.0	21.5	34.0	odd	ok
	C50H71N14O12	1059.5370	-0.7	23.7	22.5	even	ok
	C49H65N21O7	1059.5370	-0.7	24.2	29.0	odd	ok
	C50H81N8O21	1059.5357	-2.0	24.5	12.0	odd	ok
	C48H59N28O2	1059.5370	-0.7	28.8	33.5	even	ok
	C50H61N25O3	1059.5384	0.5	36.3	33.0	odd	ok

HR-MS (ESI) of peptide **4**.



Analytical HPLC chromatogram of peptide **4**.

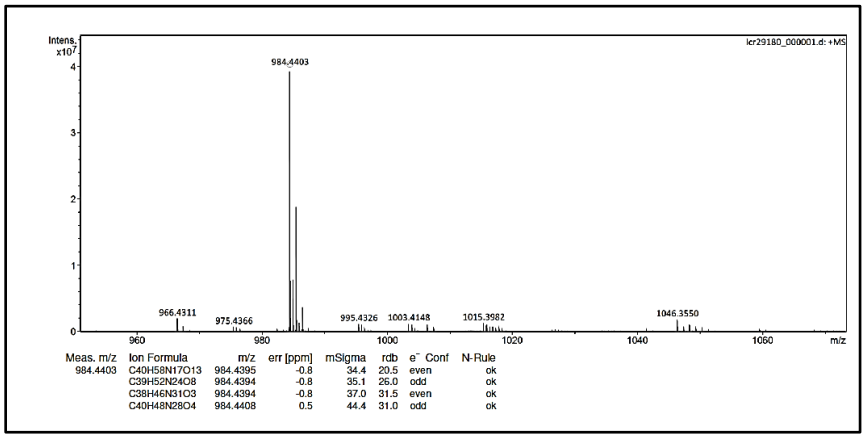
Peptide 5: Pyrazine-Phe-Arg-Gly-Asp-Asn-Gly-Arg-Gly-OH (Pyrazine-F-RGDNGRG)



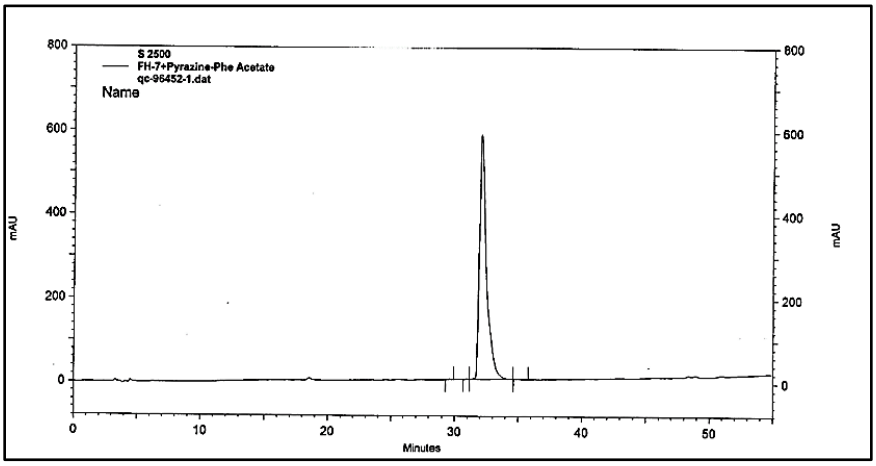
5

HPLC analysis found that peptide **(5)** was obtained in 99 %< purity (t_R: 32. 04 min).

HR-Mass (ESI): $C_{40}H_{57}N_{17}O_{13}$*m/z* =$[M+H]^+$ Found 984.4403, Calc. for 984.4395.



HR-MS (ESI) of peptide **5**.



Analytical HPLC chromatogram of peptide **5**.