Mild and selective mono-iodination of unprotected peptides as initial step for the synthesis of bioimaging probes

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Supporting Information

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Figure S1 – Iodination stock solution preparation

Iodination stock solution 50 mM in acetonitrile was prepared freshly. Selectfluor (15 mg, 42.3 µmol) was dissolved in 840 µL of acetonitrile by vigorous vortexing (solution **A**) followed by NaI (6.8 mg, 45.3 µmol). The addition of sodium iodide was easily visible by change in color: the transparent Selectfluor solution turned into a brown caramel mixture after 10 seconds (solution **B**).

Upon addition of the iodination stock solution to Tyr-peptides dissolved in DCM + 20% TFA (solution **C**), the reaction mixture turned light pink (solution **D**).

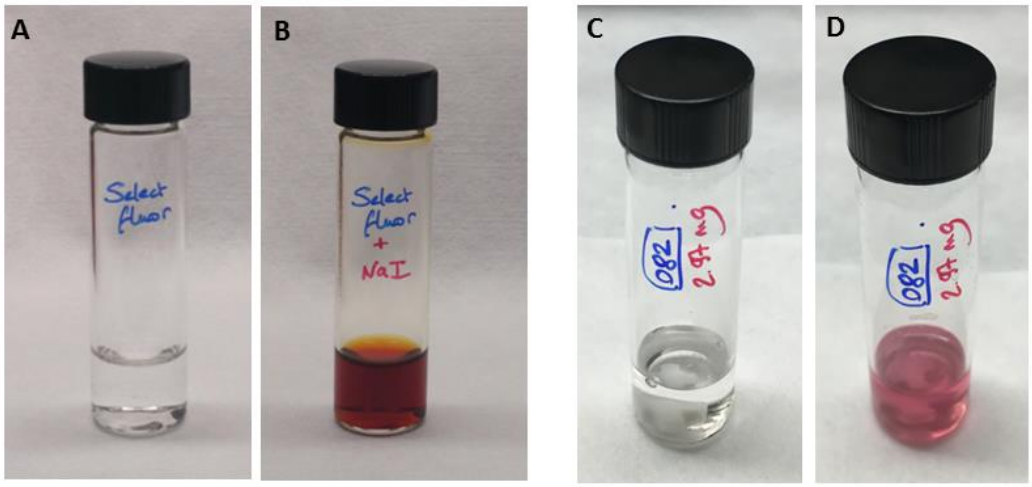


Figure S2 – Iodination reaction occurs quickly at room temperature

Ac-Tyr-NH-Me (2.0 mg, 8.5 µmol) was dissolved in 1 mL of DCM + 10% TFA. Then was added dropwise 1.1 eq of a 50 mM freshly prepared iodination stock solution. Reaction was allowed to stir at room temperature and monitored by LC-MS after 15 minutes and after 2 hours. No difference was observed. Analytical LC-MS chromatograms are shown below:

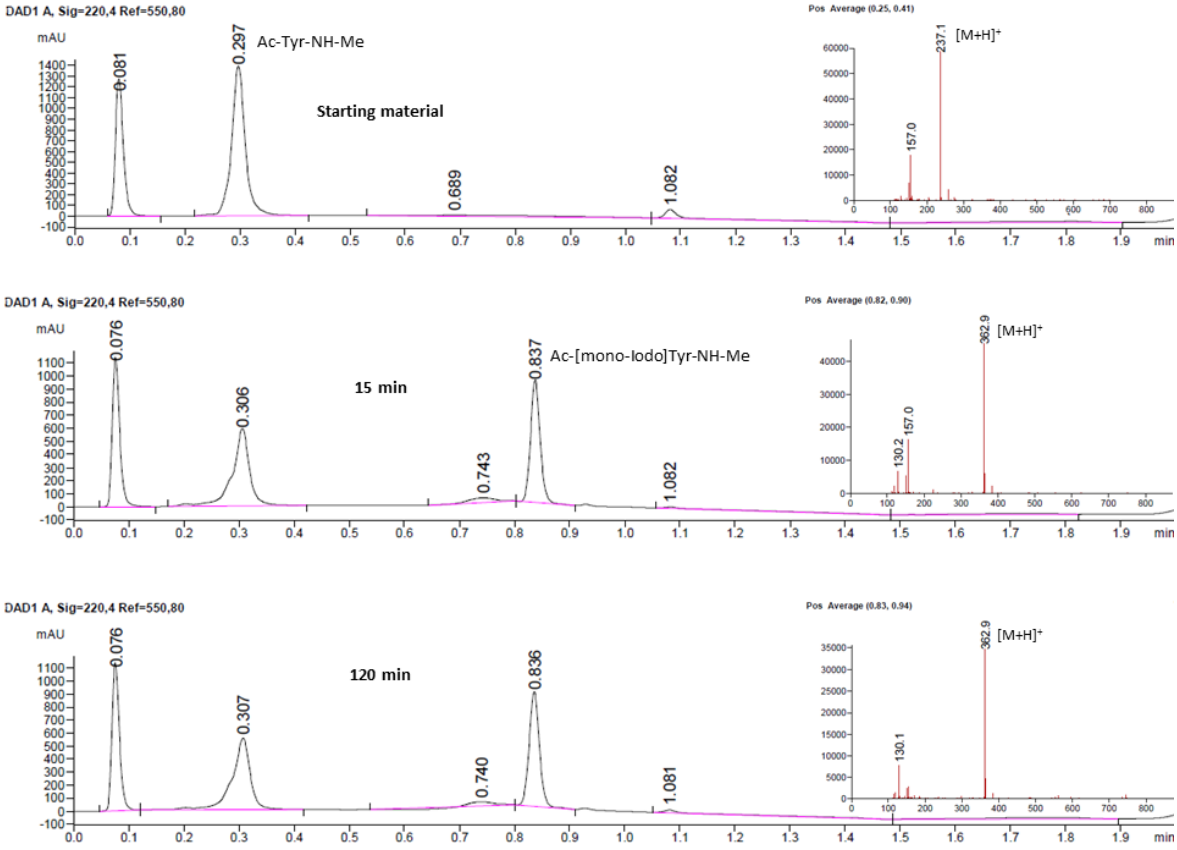
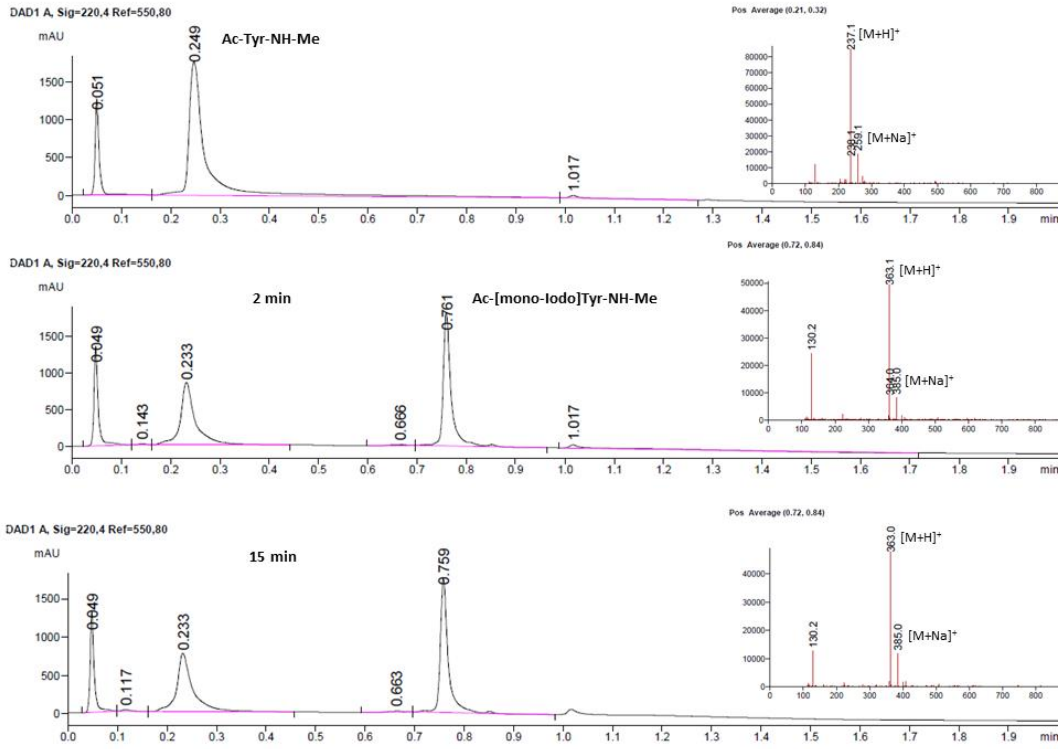
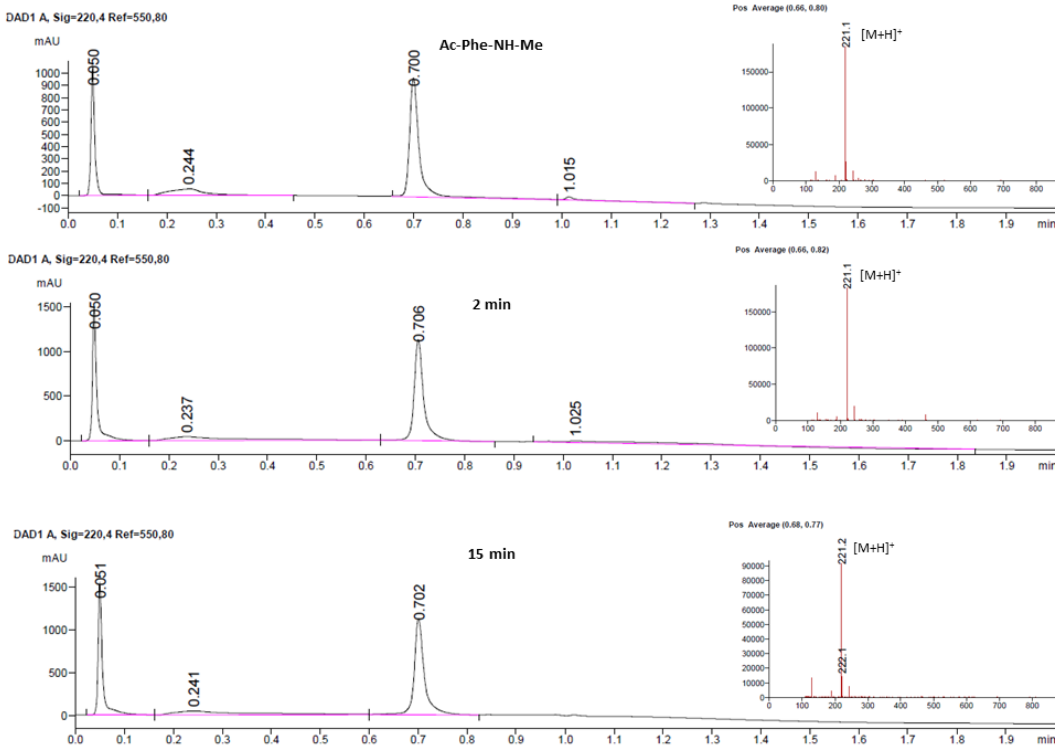
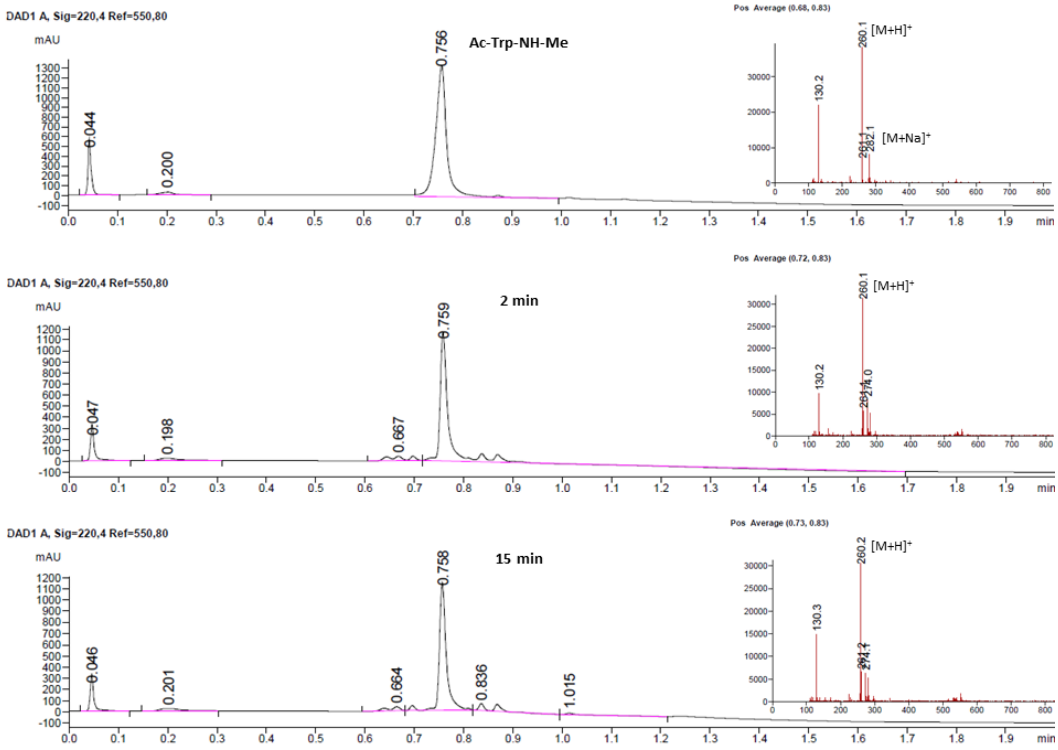


Figure S3 – Iodination is specific to Tyr residue

Ac-Tyr-NH-Me (2.0 mg, 8.5 µmol), Ac-Phe-Tyr-NH-Me, Ac-Phe-His-NH-Me and Ac-Phe-Trp-NH-Me were respectively dissolved in 1 mL of DCM + 10% TFA. Then was added dropwise 1.1 eq of a 50 mM freshly prepared iodination stock solution. Reaction was allowed to stir at room temperature and monitored by LC-MS after 2 minutes and after 15 minutes. Under these conditions, iodination proceeded only on Tyr residues. Analytical LC-MS chromatograms are shown below:




DAD1 A, Sig=220,4 Ref=550,80
mAU
Ac-Phe-NH-Me
0.050
0.244
0.700
1.015
Pos Average (0.66, 0.80)
221.1
[M+H]+
DAD1 A, Sig=220,4 Ref=550,80
mAU
2 min
0.050
0.237
0.706
1.025
Pos Average (0.66, 0.82)
221.1
[M+H]+
DAD1 A, Sig=220,4 Ref=550,80
mAU
15 min
0.051
0.241
0.702
Pos Average (0.68, 0.77)
221.2
222.1
[M+H]+
min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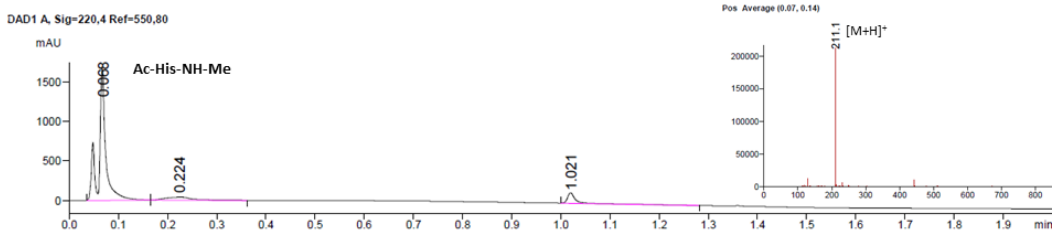
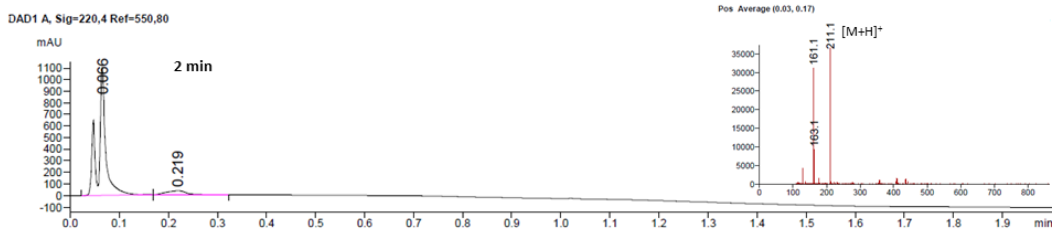
mAU
Ac-Trp-NH-Me
0.044
0.200
0.756
Pos Average (0.68, 0.83)
260.1
130.2
[M+H]+
[M+Na]+
DAD1 A, Sig=220,4 Ref=550,80
mAU
2 min
0.047
0.198
0.667
0.759
Pos Average (0.72, 0.83)
260.1
130.2
[M+H]+
DAD1 A, Sig=220,4 Ref=550,80
mAU
15 min
0.046
0.201
0.664
0.758
0.836
1.015
Pos Average (0.73, 0.83)
260.2
130.3
[M+H]+
min


DAD1 A, Sig=220,4 Ref=550,80
mAU
Ac-His-NH-Me
0.068
0.224
1.021
Pos Average (0.07, 0.14)
211.1
[M+H]+
min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mAU
2 min
0.066
0.219
Pos Average (0.03, 0.17)
161.1
211.1
[M+H]+
min

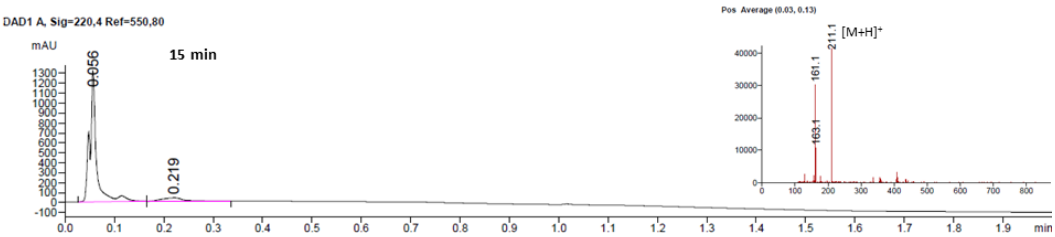
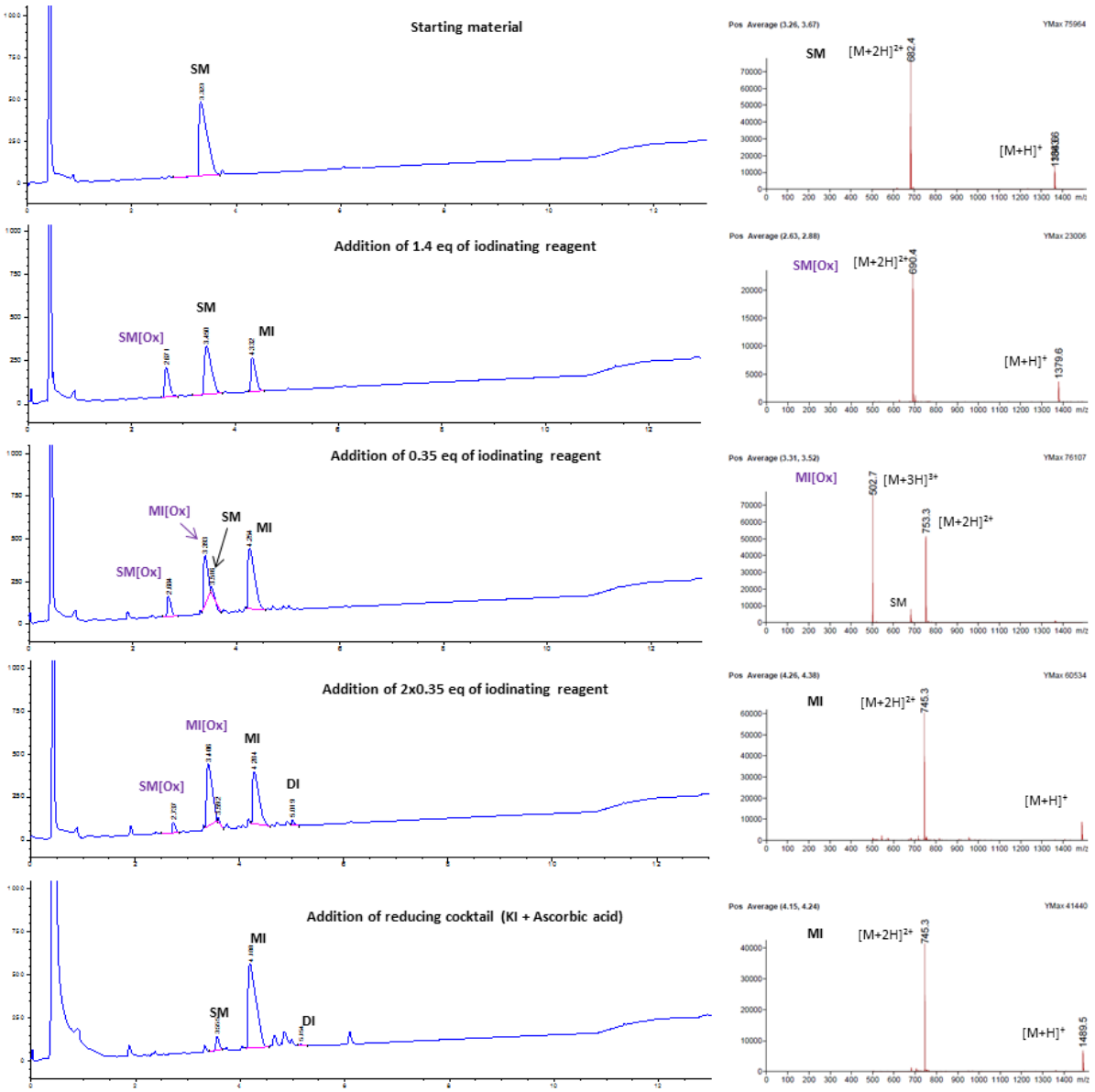
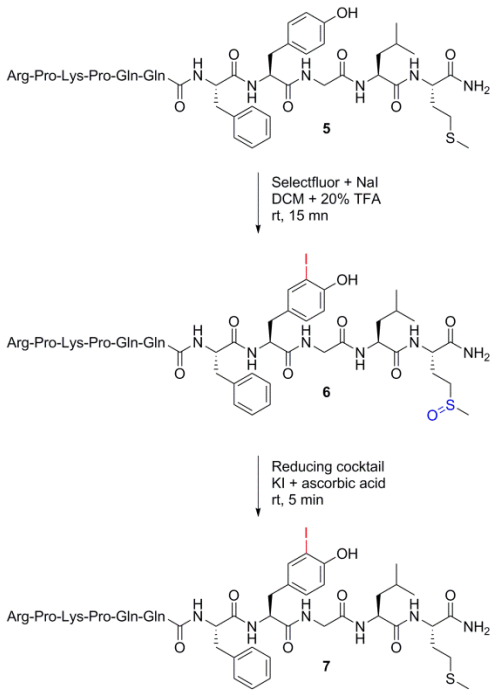

DAD1 A, Sig=220,4 Ref=550,80
mAU
15 min
0.056
0.219
Pos Average (0.03, 0.13)
161.1
211.1
[M+H]+
min

Figure S4 – Mono-iodination of a Met-containing peptide: [Tyr^{8}]-Substance P

[Tyr^{8}]-Substance P (0.81 mg, 0.59 µmol) was dissolved in 340 µL of DCM and 80 µL of TFA. Then were added dropwise 16 µL (1.4 eq.) of a 50 mM freshly prepared iodination stock solution. Reaction was allowed to stir at room temperature and monitored by LC-MS. A mixture containing the SM, the starting material with the oxidized methionine SM[Ox], and the MI. Addition of 4 µL (0.35 eq.) of a 50 mM iodination stock solution generated the oxidized methionine mono-iodinated compound MI[Ox]. Two other successive additions of 4 µL (3x 0.35 eq.) of a 50 mM iodination stock solution afforded MI and MI[Ox] as the major product. Finally, addition of 100 µL of the reducing cocktail (freshly prepared: potassium iodide KI (10 mg) and ascorbic acid (10 mg) were sonicated in 500 µL of TFA for 10 minutes) enabled reduction of MI[Ox] towards MI. Analytical LC-MS chromatograms are shown below:





[Tyr^{8}]-Substance P

SM: 5.9% / MI: 93.6% / DI: 0.5%

2.2 µmol - yield 72%

General methods

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers (Sigma Aldrich, ThermoFisher, Merck Millipore) and used without further purification.

Reactions were monitored by LC-MS. For small molecules: data were acquired using the Agilent 1100 MSD system with a Phenomenex Luna column (C-18, 100Å pore size, 3 µm particle size, 10x2.0 mm, flow: 1.1 mL/min). Gradient: 0 min 1% ACN (+0.05% TFA) / 99% H_2O (+0.05% TFA); 0.3 min % ACN (+0.05% TFA); 1.3 min 95% ACN (+0.05% TFA); 1.75 min 1% ACN (+0.05% TFA); 1.80 min 1% ACN (+0.05% TFA). Mass detection range: 110-1000MW. Temperature: 30 °C. For peptides: data were acquired using the Agilent 1100 MSD system with a Phenomenex Aeris Widepore column (XB-C18, 200Å pore size, 3.6 µm particle size, 100x2.1 mm, flow: 0.5 mL/min). Gradient: 0 min 5% ACN (+0.1% formic acid) / 95% H_2O (+0.1% formic acid) to 10 min - 50% ACN (+0.1% formic acid); 11 min 90% ACN (+0.1% formic acid) to 12.5 min; 12.5 min to 13.5 min 5% ACN (+0.1% formic acid). Mass detection range: 500-1500MW. Temperature: 38°C.

Purifications on reverse-phase preparative HPLC were performed on the HP-Agilent 1100 with either i) a column from Agilent (Zorbax Rx C18, 5 µm particle size, 250x9.4mm, flow: 4 mL/min). Gradient: 0 min to 5 min 10% ACN (+0.1% TFA) / 90% H_2O (+0.1% TFA); 5 min to 30 min 95% ACN (+0.1% TFA) / 5% H_2O (+0.1% TFA); 30 min to 32 min 95% ACN (+0.1% TFA) / 5% H_2O (+0.1% TFA); 32 min to 35 min 10% ACN (+0.1% TFA) / 90% H_2O (+0.1% TFA) or ii) a column from Waters (Xbridge Prep C18 OBD, 5 µm particle size, 250x19mm, flow: 16 mL/min). Gradient: 0 min to 5 min 10% ACN (+0.1% TFA) / 90% H_2O (+0.1% TFA); 5 min to 30 min 95% ACN (+0.1% TFA) / 5% H_2O (+0.1% TFA); 30 min to 32 min 95% ACN (+0.1% TFA) / 5% H_2O (+0.1% TFA); 32 min to 35 min 10% ACN (+0.1% TFA) / 90% H_2O (+0.1% TFA) or iii) a column from Waters (Acquity UPLC CSH C18, 130Å pore size, 1.7 µm particle size, 2.1x150mm, flow: 0.5 mL/min). Gradient: 0 min to 3 min 20% ACN (+0.05% TFA) / 80% H_2O (+0.05% TFA); 3 min to 23 min 75% ACN (+0.05% TFA) / 25% H_2O (+0.05% TFA); 23 min to 23.5 min 95% ACN (+0.05% TFA) / 5% H_2O (+0.05% TFA). 23.5 min to 25.5 min 95% ACN (+0.05% TFA) / 5% H_2O (+0.05% TFA). Temperature: 50 °C.

Purification on silica gel chromatography was performed on CombiFlash Rf-Isco Teledyne.

Final peptides were analyzed by UPLC-MS Waters Acquity (C-18 CSH column - 130Å pore size, 1.7 µm particle size, 150x2.1 mm, flow: 0.5 mL/min – Gradient 1: 0 min 10% ACN (+0.1% formic acid) / 90% H_2O (+0.1% formic acid) to 19.2 min - 90% ACN (+0.1% formic acid); 20 min 90% ACN (+0.1% formic acid). Gradient 2: 0 min 2% ACN (+0.1% formic acid) / 98% H_2O (+0.1% formic acid) to 9.12 min - 40% ACN (+0.1% formic acid); 12 min 40% ACN (+0.1% formic acid). Mass detection range: 500-2000MW. Temperature: 40 °C. High resolution mass HRMS were recorded on the Agilent 6200 Series Accurate-Mass Time-of-flight (TOF). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 or 600 systems in d_6-DMSO or $CDCl_3$. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet or unresolved, dd = doublets of doublet, br = broad. Coupling constants (*J* values) are given in Hertz (Hz). Absorption and Emission spectra were acquired with a Thermo Varioskan using the SkanIt 2.4.3 software.

Abbreviations

ACN = acetonitrile
AUC = area under the curve
Boc = *tert*-butyloxycarbonyl
DCM = dichloromethane
DI = di-iodinated product
DIEA = N,N-diisopropylethylamine
DMF = dimethylformamide
DODT = 3,6-Dioxa-1,8-octane-dithiol
ESI-TOF = electrospray ionization mass spectrometry – time of flight
EtOAc = ethyl acetate
Hept = heptane
HE-SPPS = high-efficiency solid phase peptide synthesis
HPLC = high performance liquid chromatography
HRMS = high resolution mass spectrometry
LC-MS = liquid chromatography - mass spectrometry
MI = mono-iodinated product
NMR = nuclear magnetic resonance
PEG = poly-ethylene glycol
SM = starting material
tBuOH = tert-butanol
TFA = trifluoroacetic acid
THPTA = tris(3-hydroxypropyltriazolylmethyl)amine
TIS = triisopropylsilane
UPLC-MS = ultra-performance liquid chromatography - mass spectrometry

Peptides

Tocinoic acid, Goserelin acetate and [Tyr^8]-Substance P were purchased from Sigma Aldrich. Cyclo(RGGyK) was purchased from Selleckchem. AcMeYVAD-CHO, [Tyr^0]-Bradykinin, and human GLP-1 (7-37) were purchased from Bachem.

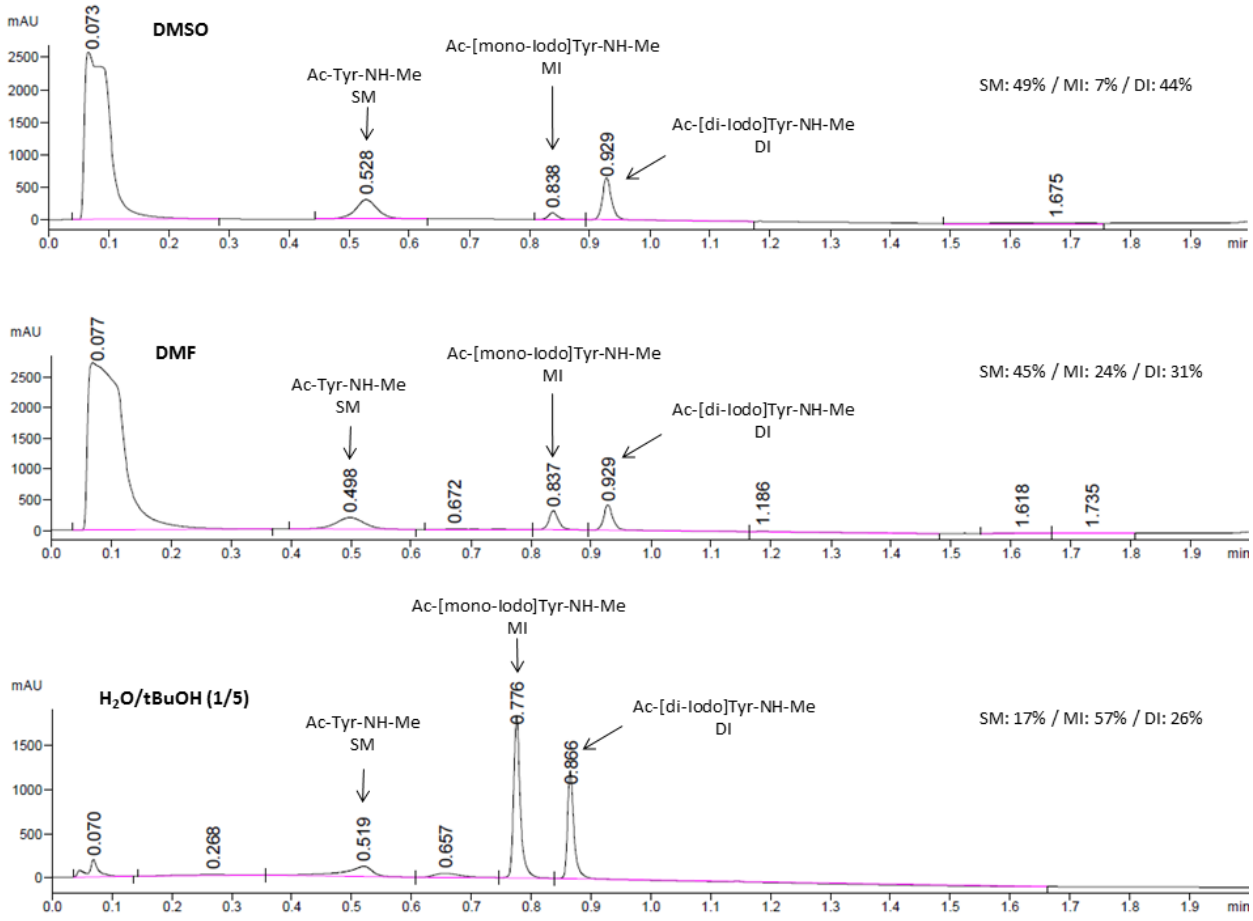
HE-SPPS

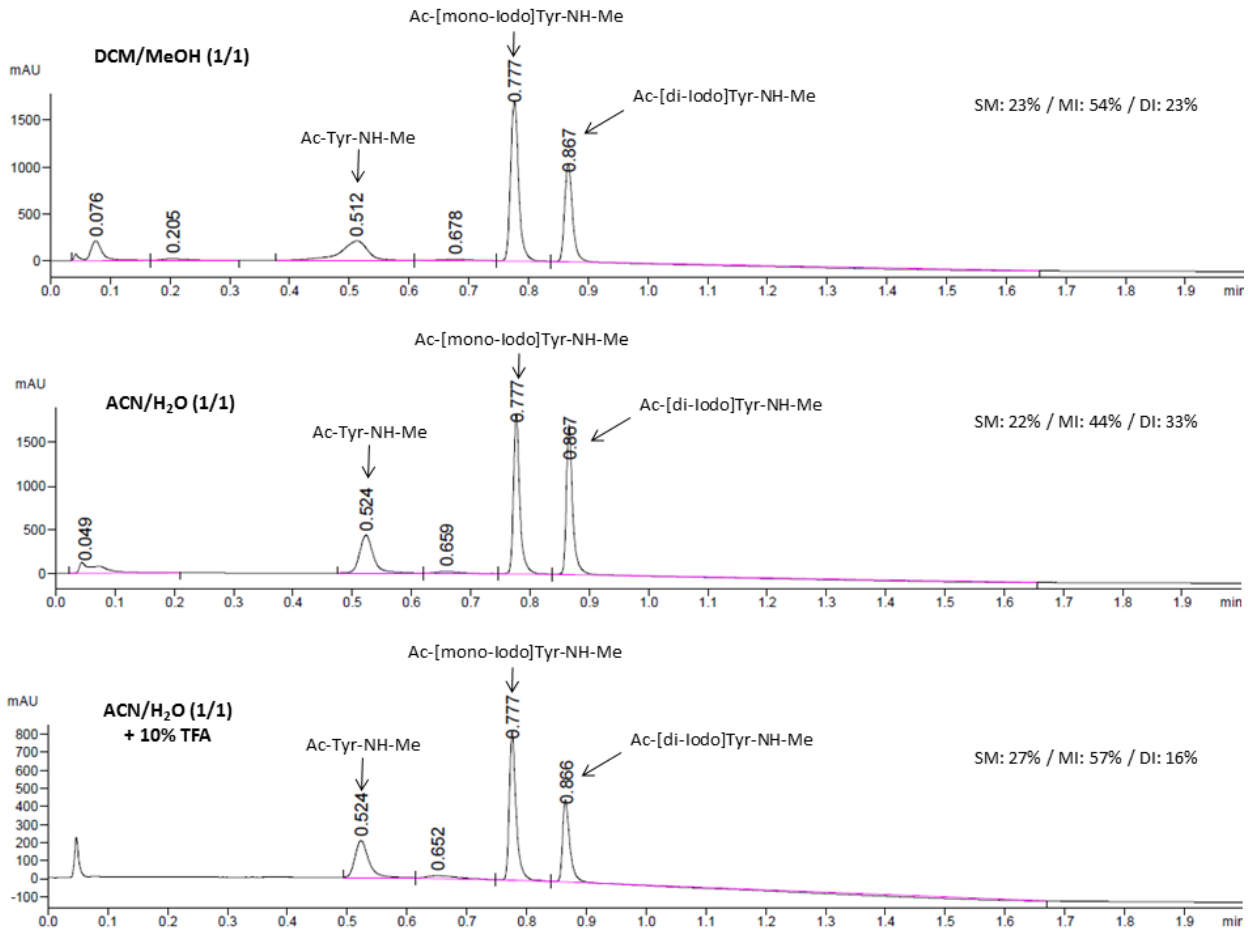
Leucin-Enkephalinamide, Angiotensin III and ACP fragment (65-74) were synthesized by High-Efficiency Solid Phase Peptide Synthesis[1] using a CEM Liberty Blue system on a 0.1 mmol scale with a Rink Amide AM resin low loading (0.29 mmol/g) 100-200 mesh from Novabiochem using 5-fold excess of reagents [0.2 M Fmoc amino acid solution (in DMF) with 0.5 M DIC (in DMF) and 1.0 M Oxyma (in DMF)] and 20% pipieridine in DMF for the Fmoc-deprotection cycles. Immediately after synthesis, the peptide resin was washed three times with 10 mL of DMF and then three times with 10 mL of DCM. Cleavage was then performed in all cases with 10 mL of a freshly prepared King's cocktail (TFA 82.5% / Phenol 5% / Thioanisol 5% / H_2O 5% / DODT 2.5%) for 3 hours before being precipitated in 70 mL of ice cold diisopropyl ether. Precipitate was centrifuged (4 min, 4000 rpm, 4 °C) and washed with ice cold diisopropyl ether three times. Finally, the resin was filtered off and the peptide precipitate was dissolved

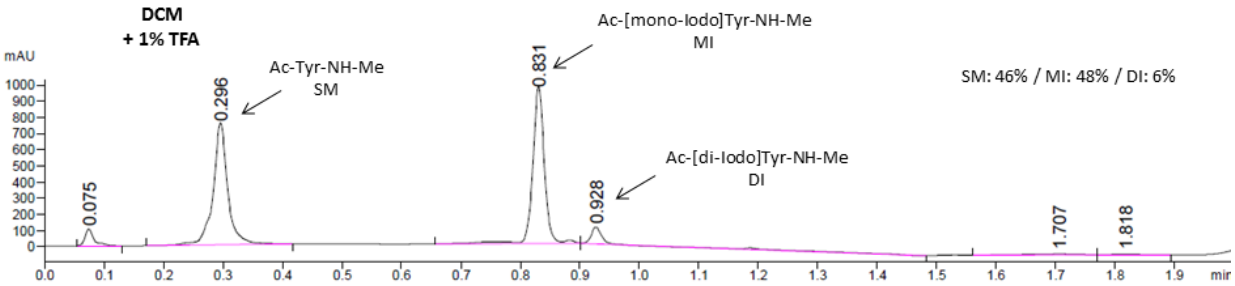
in H_2O + 25% ACN + 0.5% AcOH and lyophilized. Crude purity was > 95% for Leucin-Enkephalinamide. Angiotensin III and ACP fragment (65-74) were purified with reverse-phase preparative HPLC.

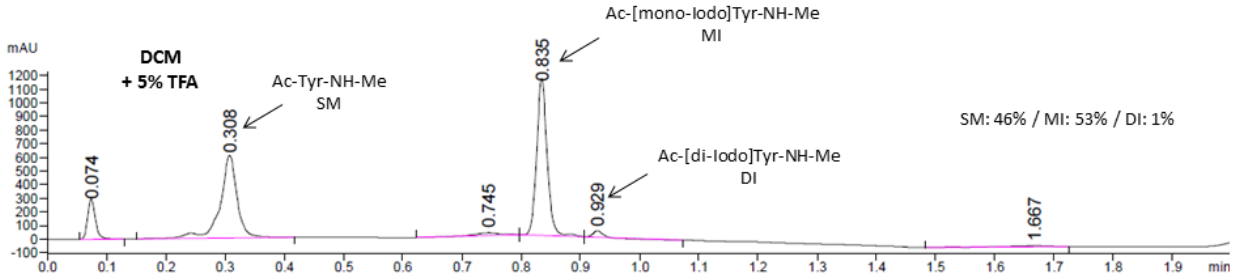
Solvent screening for Ac-Tyr-NH-Me mono-iodination

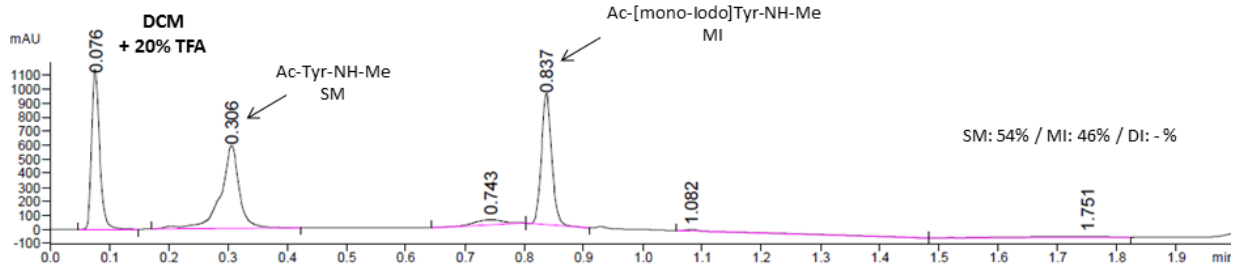
Ac-Tyr-NH-Me (2.0 mg, 8.5 µmol) was dissolved in 1 mL of different solvents (indicated in Table 1). Then was added dropwise 1.1 eq of a 50 mM freshly prepared iodination stock solution. Reaction was allowed to stir at room temperature and monitored by LC-MS instantly after addition of iodination solution, and after 15 minutes. Relative amounts of SM, MI, and DI shown in Table 1 were quantified using AUC integration (absorbance at 220 nm). Analytical LC-MS chromatograms are shown below:




DCM/MeOH (1/1)
mAU
Ac-[mono-Iodo]Tyr-NH-Me
Ac-[di-Iodo]Tyr-NH-Me
Ac-Tyr-NH-Me
0.076
0.205
0.512
0.678
0.777
0.867
SM: 23% / MI: 54% / DI: 23%
ACN/H2O (1/1)
0.049
0.524
0.659
0.777
0.867
SM: 22% / MI: 44% / DI: 33%
ACN/H2O (1/1)
+ 10% TFA
0.524
0.652
0.777
0.866
SM: 27% / MI: 57% / DI: 16%
min


DCM
+ 1% TFA
mAU
Ac-Tyr-NH-Me
SM
Ac-[mono-Iodo]Tyr-NH-Me
MI
Ac-[di-Iodo]Tyr-NH-Me
DI
SM: 46% / MI: 48% / DI: 6%
0.075
0.296
0.831
0.928
1.707
1.818
min

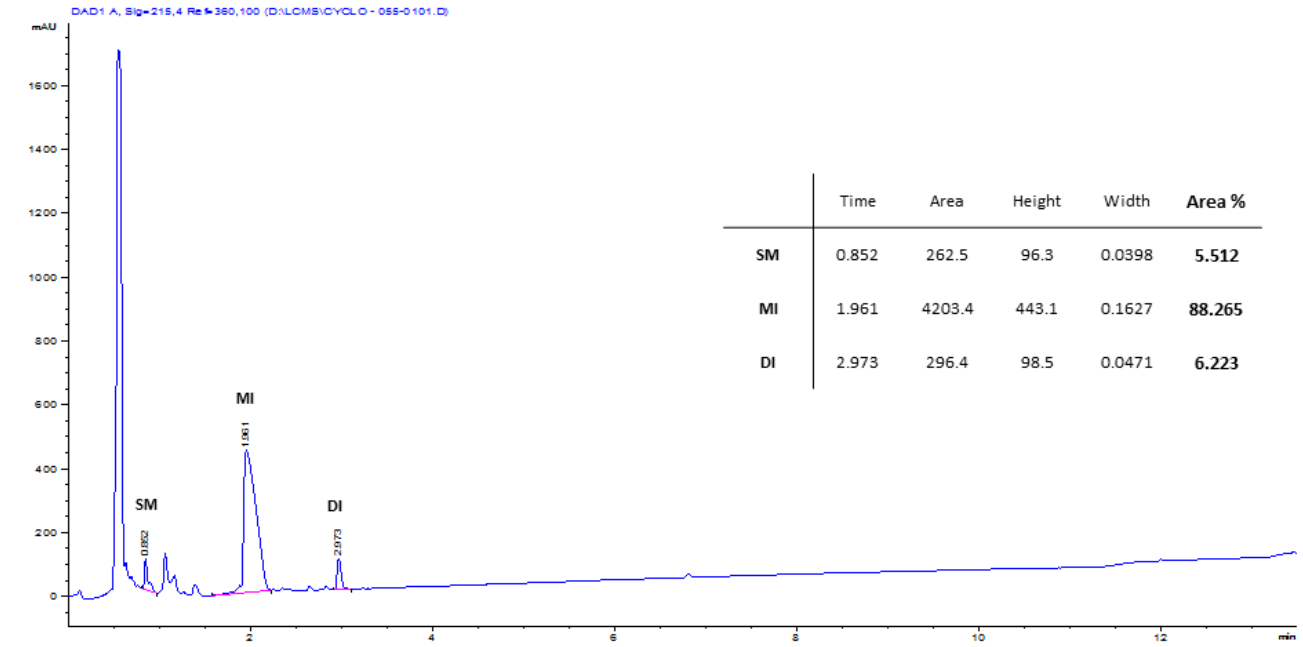

mAU
DCM
+ 5% TFA
Ac-Tyr-NH-Me
SM
Ac-[mono-Iodo]Tyr-NH-Me
MI
Ac-[di-Iodo]Tyr-NH-Me
DI
SM: 46% / MI: 53% / DI: 1%
0.074
0.308
0.745
0.835
0.929
1.667
min


mAU
DCM
+ 20% TFA
Ac-Tyr-NH-Me
SM
Ac-[mono-Iodo]Tyr-NH-Me
MI
SM: 54% / MI: 46% / DI: - %
0.076
0.306
0.743
0.837
1.082
1.751
min

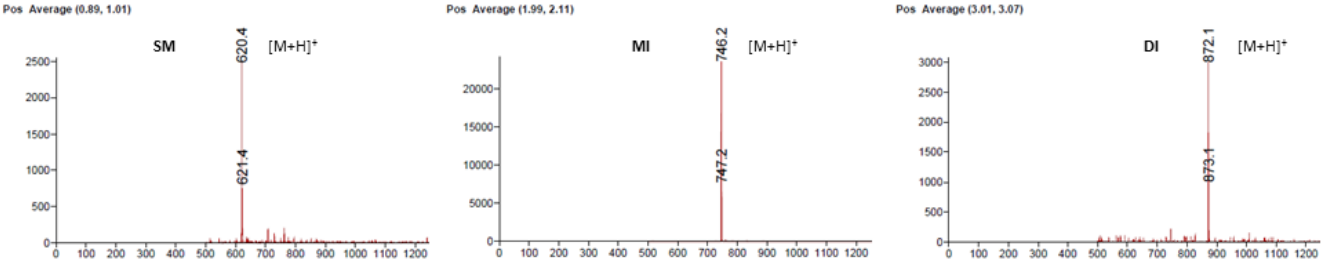
General Mono-iodination procedure

Tyr-containing peptide was dissolved in DCM+ 20% TFA at a concentration of 1-2 mM. Then were added dropwise 1.4 eq of a 50 mM freshly prepared iodination stock solution. Reaction was allowed to stir at room temperature for 15 minutes and was monitored by LC-MS as followed: 10 µL of the reaction mixture were quenched with 20 µL of distilled water which were submitted to analysis. Depending on the conversion, extra 0.25 eq of iodination stock solution were added sequentially to reach the described ratio of starting material (SM), mono-iodinated product (MI) and di-iodinated product (DI) in Scheme 2. Relative amounts of SM, MI and DI were quantified using AUC integration (absorbance at 215 nm) corresponding to their respective masses. Analytical LC-MS chromatograms are shown below:

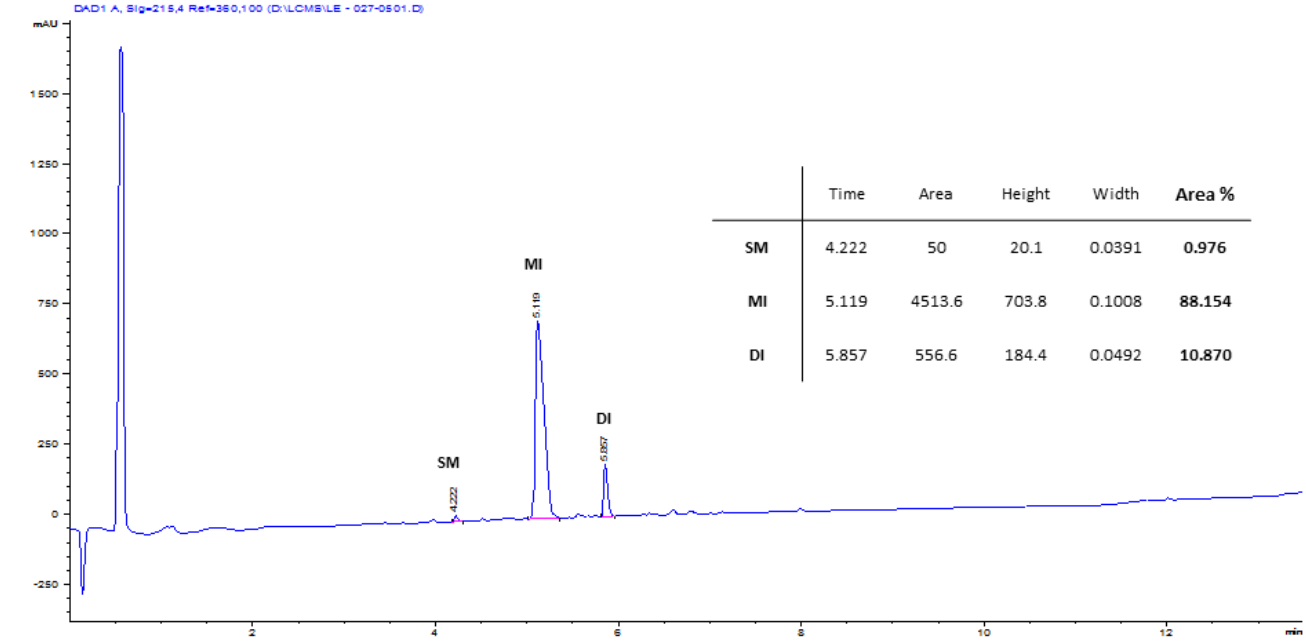
<u>Cyclo(RGD[mono-iodo]yK)</u>



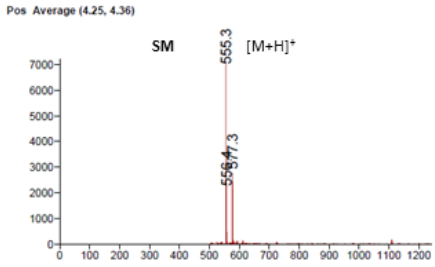
	Time	Area	Height	Width	Area %
SM	0.852	262.5	96.3	0.0398	**5.512**
MI	1.961	4203.4	443.1	0.1627	**88.265**
DI	2.973	296.4	98.5	0.0471	**6.223**

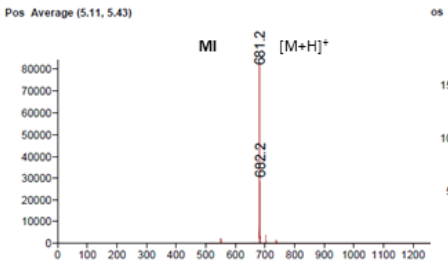


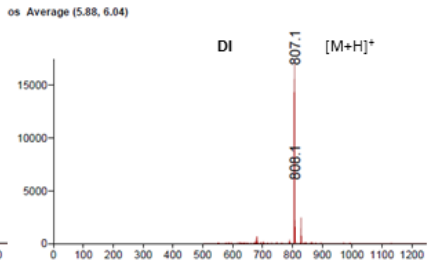
Leucin-Enkephalinamide



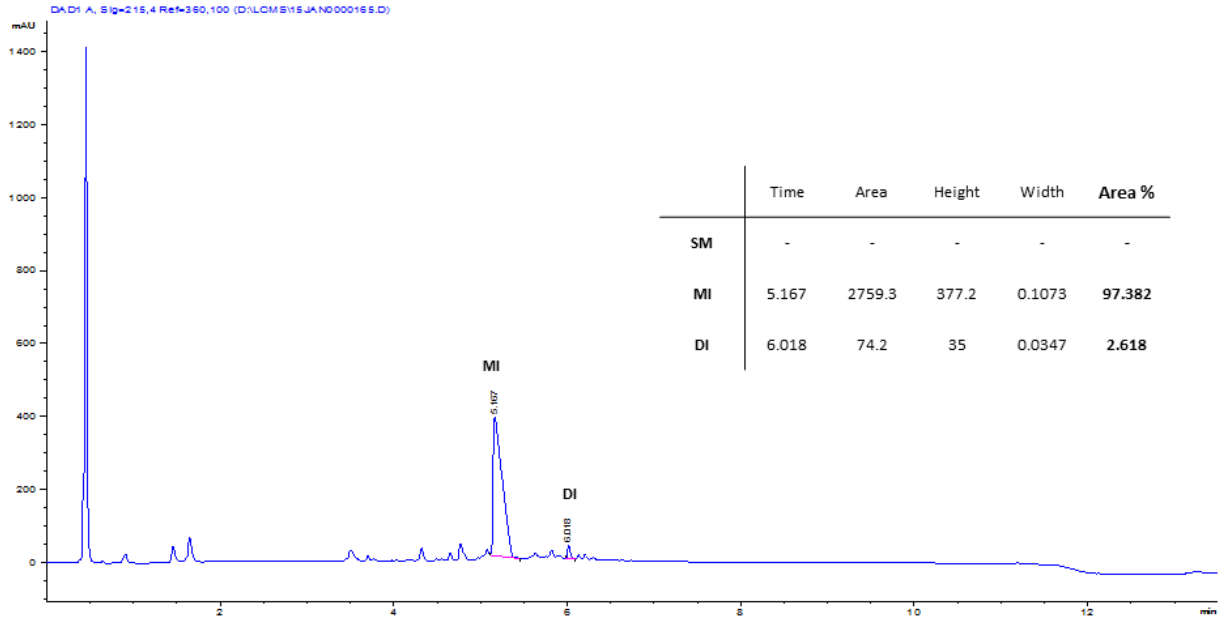
	Time	Area	Height	Width	Area %
SM	4.222	50	20.1	0.0391	**0.976**
MI	5.119	4513.6	703.8	0.1008	**88.154**
DI	5.857	556.6	184.4	0.0492	**10.870**





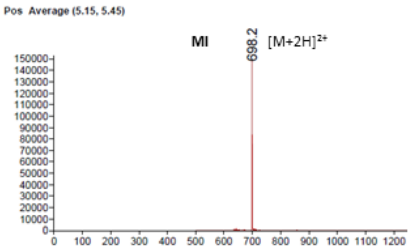


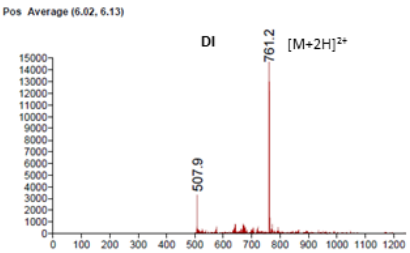
Goserelin



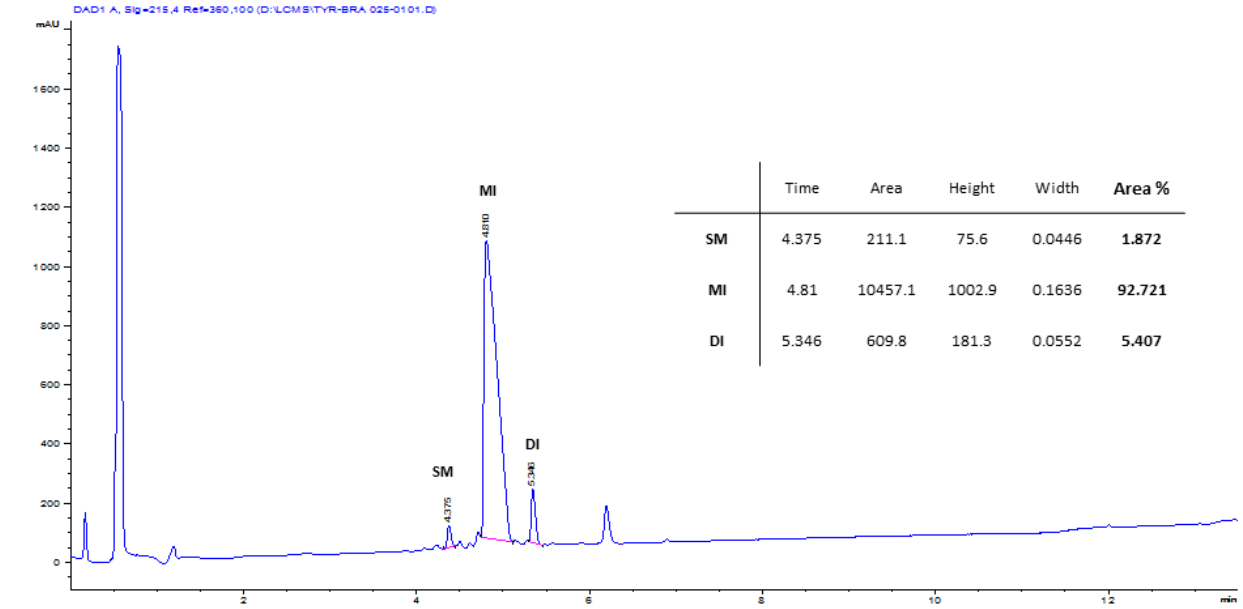
	Time	Area	Height	Width	Area %
SM	-	-	-	-	-
MI	5.167	2759.3	377.2	0.1073	**97.382**
DI	6.018	74.2	35	0.0347	**2.618**

Starting material was not detected

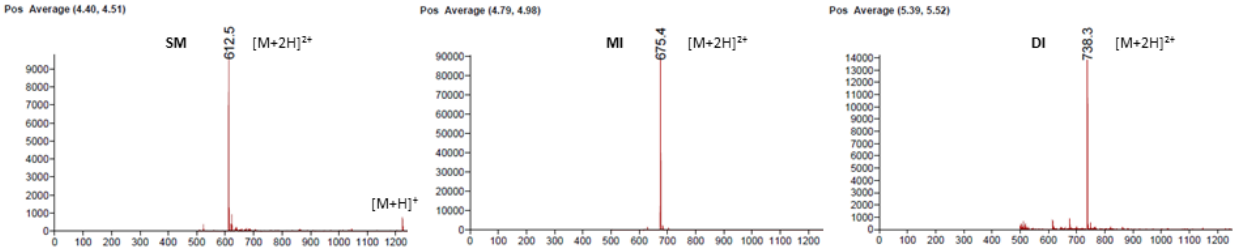




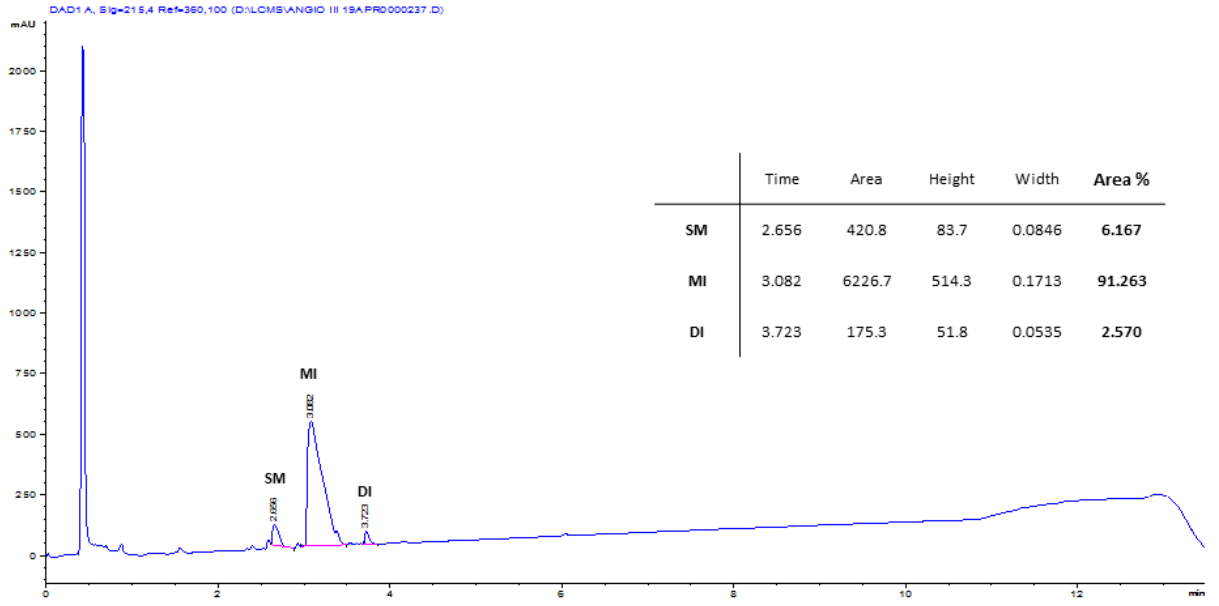
[Tyr^0]-Bradykinin



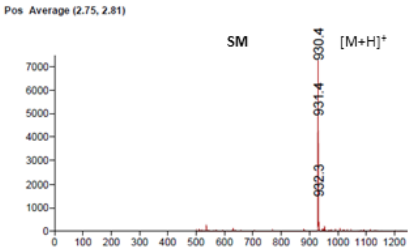
	Time	Area	Height	Width	Area %
SM	4.375	211.1	75.6	0.0446	**1.872**
MI	4.81	10457.1	1002.9	0.1636	**92.721**
DI	5.346	609.8	181.3	0.0552	**5.407**

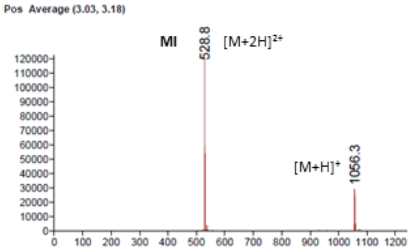


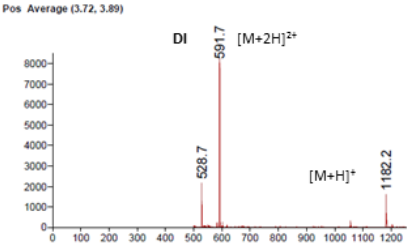
Angiotensin III



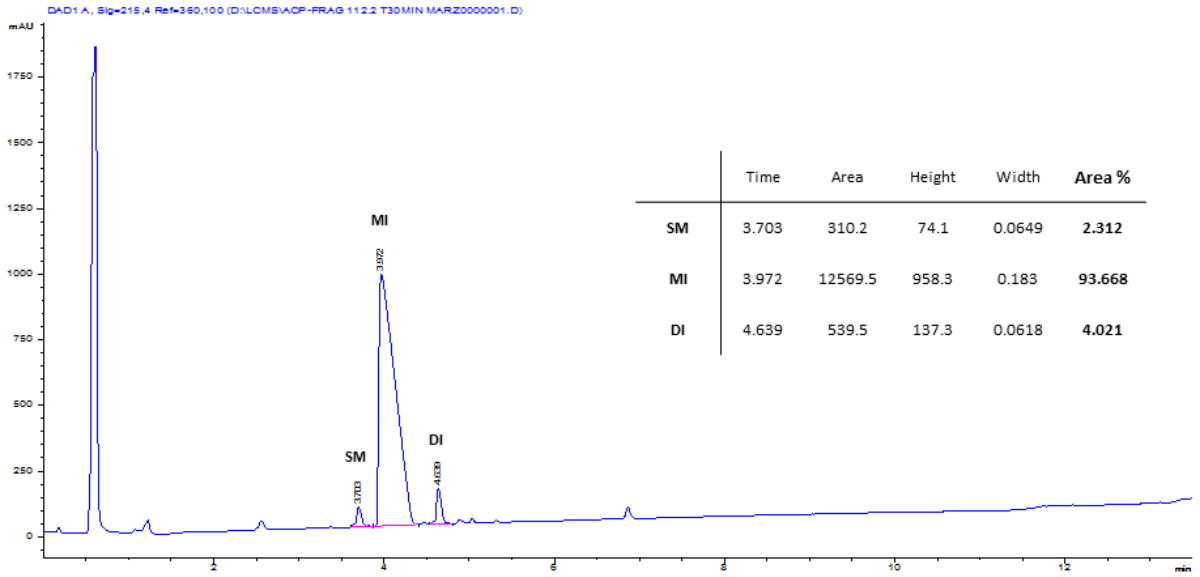
	Time	Area	Height	Width	Area %
SM	2.656	420.8	83.7	0.0846	**6.167**
MI	3.082	6226.7	514.3	0.1713	**91.263**
DI	3.723	175.3	51.8	0.0535	**2.570**



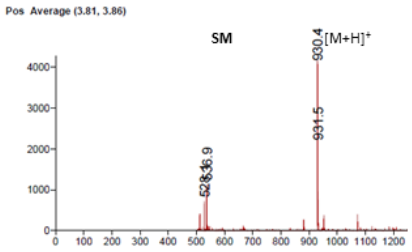


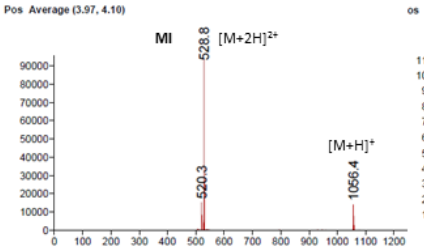


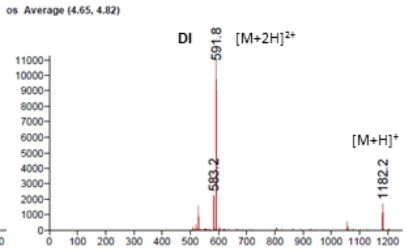
ACP fragment (65-74)



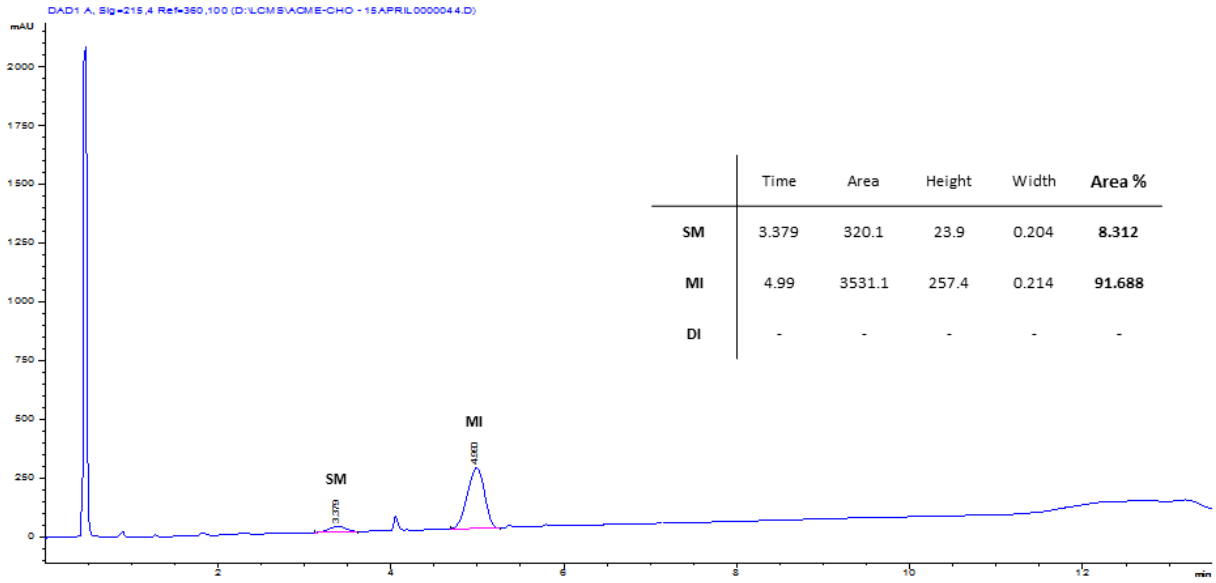
	Time	Area	Height	Width	Area %
SM	3.703	310.2	74.1	0.0649	**2.312**
MI	3.972	12569.5	958.3	0.183	**93.668**
DI	4.639	539.5	137.3	0.0618	**4.021**



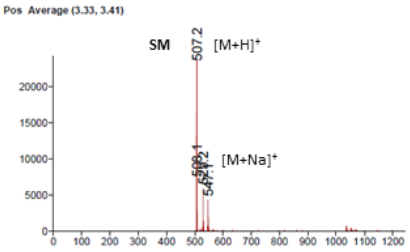


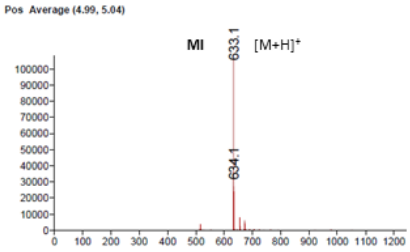


AcMeYVAD-CHO



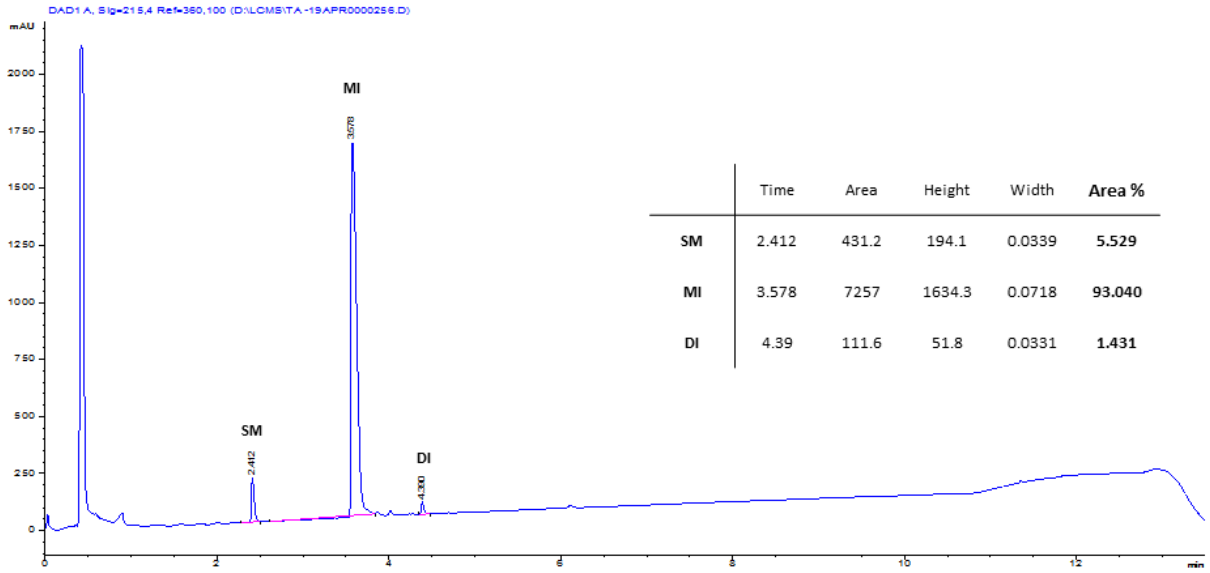
	Time	Area	Height	Width	Area %
SM	3.379	320.1	23.9	0.204	**8.312**
MI	4.99	3531.1	257.4	0.214	**91.688**
DI	-	-	-	-	-



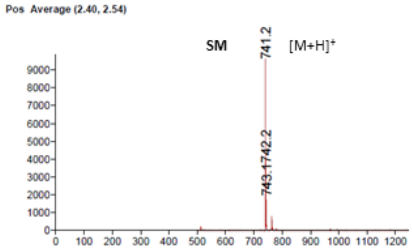


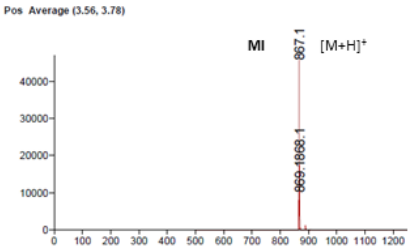
Di-iodinated product was not detected

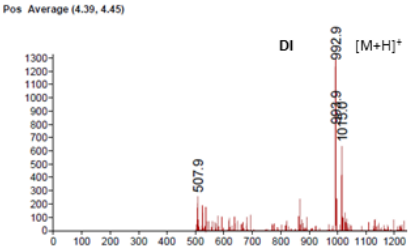
Tocinoic acid



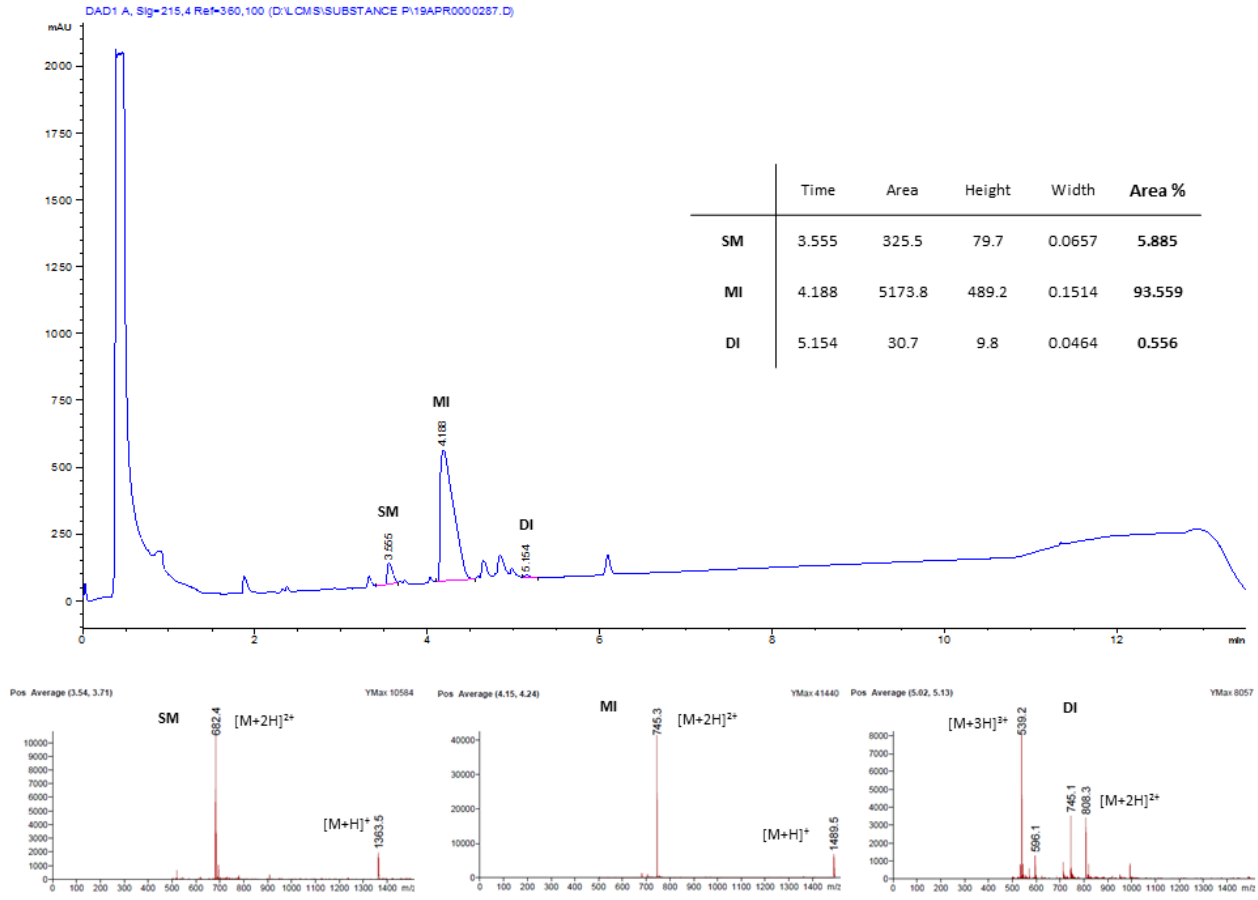
	Time	Area	Height	Width	Area %
SM	2.412	431.2	194.1	0.0339	**5.529**
MI	3.578	7257	1634.3	0.0718	**93.040**
DI	4.39	111.6	51.8	0.0331	**1.431**





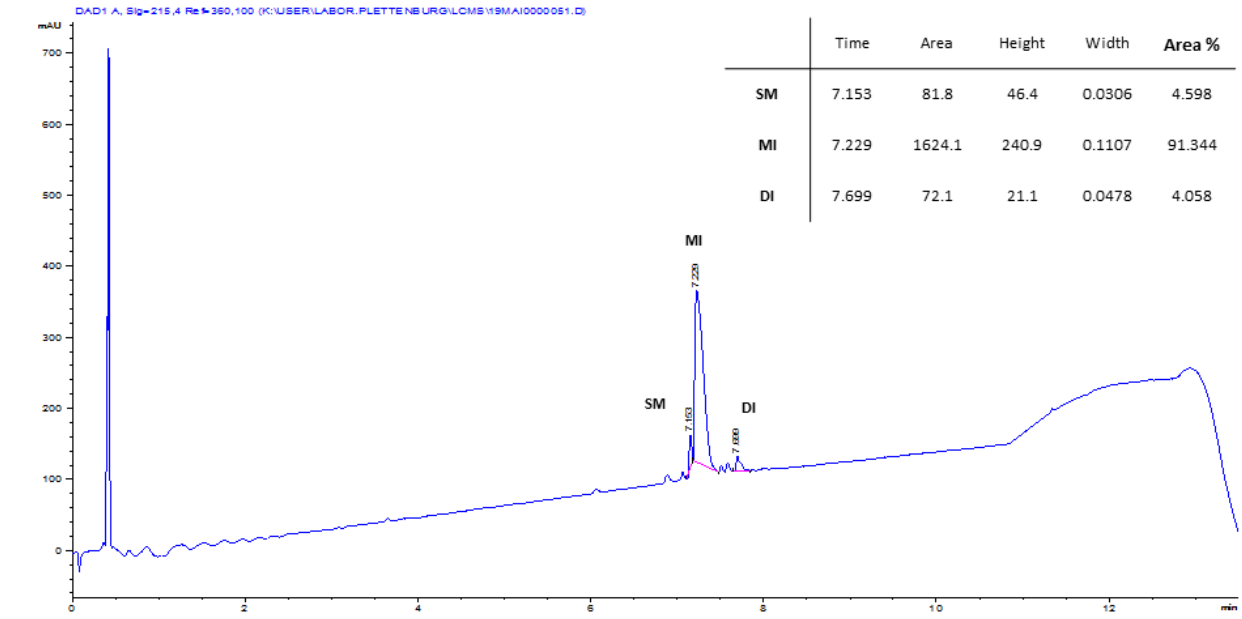


[Tyr^{8}]-Substance P

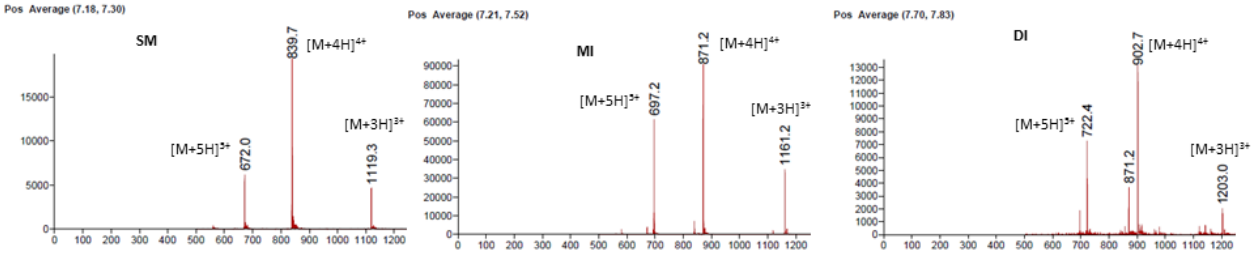


	Time	Area	Height	Width	Area %
SM	3.555	325.5	79.7	0.0657	**5.885**
MI	4.188	5173.8	489.2	0.1514	**93.559**
DI	5.154	30.7	9.8	0.0464	**0.556**

Human GLP-1 (7-37)



	Time	Area	Height	Width	Area %
SM	7.153	81.8	46.4	0.0306	4.598
MI	7.229	1624.1	240.9	0.1107	91.344
DI	7.699	72.1	21.1	0.0478	4.058



Mono-iodination of Cyclo(RGDyK)

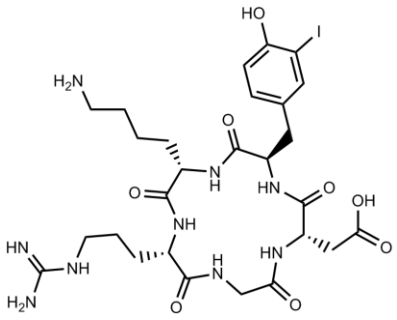
Cyclo(RGDyK) (1.02 mg, 1.18 µmol) was dissolved in 500 µL of DCM and 125 µL of TFA. Then were added dropwise 33 µL of a 50 mM iodination stock solution (1.65 µmol, 1.4 eq.) (stock solution freshly prepared: 29.1 mg of Selectfluor were dissolved in 1.6 mL of ACN before the addition of 12.5 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Two other additions of iodination solution (2 x 5 µL, 2 x 0.25 eq.) were needed to reach the following final ratio: SM: 5.5% - MI: 88.3% - DI: 6.2%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM) and redissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fraction was lyophilized to afford the desired product Cyclo(RGD[mono-iodo]yK) (m= 0.88 mg, 0.90 µmol, 77 % yield).

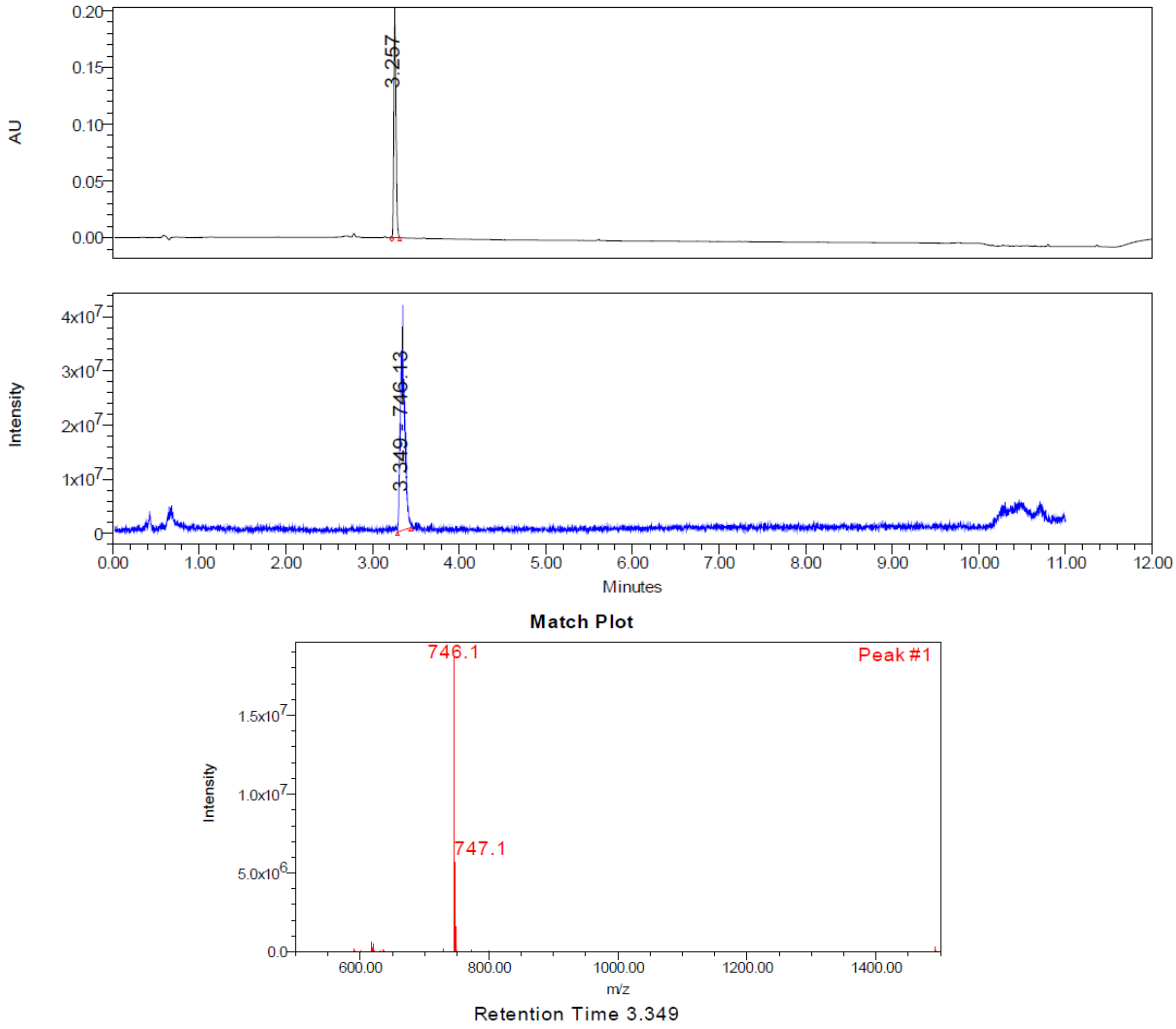
^{1}H NMR (700 MHz, d_6-DMSO, 308 K) δ: 1.10 (p, 2H), 1.36 (m, 2H), 1.45 (m, 4H), 1.57 (br, 1H), 1.72 (br, 1H), 2.39 (dd, 1 H), 2.70 (m, 4H), 2.78 (dd, 1H), 3.08 (q, 2H), 3.24 (dd, 1H), 3.94 (m, 1H), 4.04 (dd, 1H), 4.16 (q, 1H), 4.34 (q, 1H), 4.63 (q, 1H), 6.77 (d, 1H, J = 8.2 Hz), 6.87 (dd, 1H, J = 8.2, 2.0 Hz), 7.44 (d, 1H, J = 2.0 Hz), 7.52 (t, 1H), 7.61 (d, 1H), 7.68 (br, 3H), 8.00 (d, 1H), 8.09 (d, 1H), 8.11 (d, 1H), 8.44 (q, 1H), 10.10 (s, 1H), 12.20 (s, 1H).

^{13}C NMR (175 MHz, d_6-DMSO, 308 K) δ: 22.4, 25.1, 26.4, 28.5, 30.6, 35.0, 35.9, 38.6, 40.3, 43.2, 48.8, 51.8, 54.3, 54.5, 84.1, 114.6, 129.1, 130.2, 138.9, 155.0, 156.6, 169.4, 169.9, 170.5, 171.1, 171.5, 171.8.

HRMS (ESI-TOF) Calcd for $C_{27}H_{40}IN_9O_8$: 745.2054; Found: 745.2078.

UPLC-MS rt: 3.257 min (Gradient 2).




AU
0.20
0.15
0.10
0.05
0.00
3.257
Intensity
4x10^7
3x10^7
2x10^7
1x10^7
0
3.349 - 746.13
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
Minutes
Match Plot
746.1
Peak #1
1.5x10^7
1.0x10^7
5.0x10^6
0.0
747.1
600.00
800.00
1000.00
1200.00
1400.00
m/z
Retention Time 3.349

Mono-iodination of Leucin-Enkephalinamide (8)

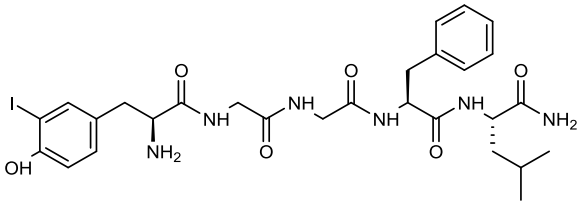
Leucin-Enkephalinamide (36.0 mg, 64.9 µmol) was dissolved in 26 mL of DCM and 6.5 mL of TFA. Then were added dropwise 1.8 mL of a 50 mM iodination stock solution (91 µmol, 1.4 eq.) (stock solution freshly prepared: 50.0 mg of Selectfluor were dissolved in 2.8 mL of ACN before the addition of 21.0 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Two other additions of iodination solution (2 x 320 µL, 2 x 0.25 eq.) were needed to reach the following final ratio: SM: 1.0% - MI: 88.2% - DI: 10.8%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM) and redissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Xbridge Prep C18, gradient: see general methods). The collected fraction was lyophilized to afford the desired product [mono-Iodo]-Leucin-Enkephalinamide (27.3 mg, 40.1 µmol, 62 % yield).

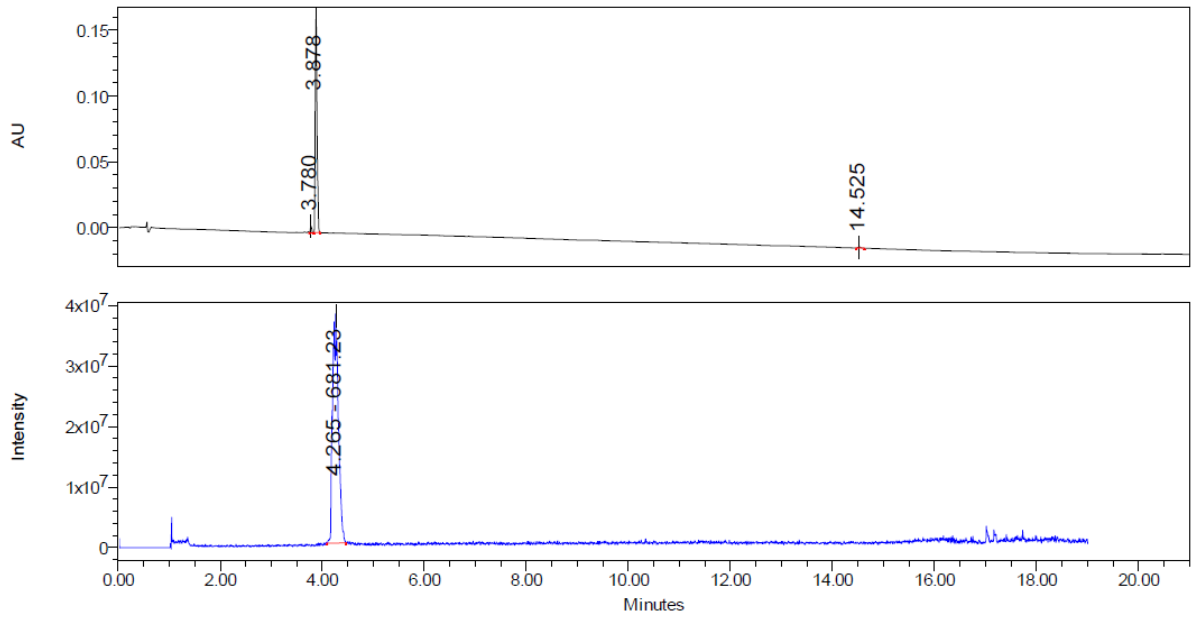
^{1}H NMR (500 MHz, d_6-DMSO, 300 K) δ: 0.84 (d, 3H), 0.88 (d, 3H), 1.47 (m, 2H), 1.57 (m, 1H), 2.53 (s, 1H), 2.80 (dd, 1H), 2.87 (dd, 1H), 3.02 (dd, 1H), 3.45 (q, 1H), 3.61 (dd, 1H), 3.70 (m, 3H), 4.18 (m, 1H), 4.49 (m, 1H), 6.77 (d, 1H, J = 8.2 Hz), 6.97 (s, 1H), 7.03 (dd, 1H, J = 8.2, 2.0 Hz), 7.17 (m, 1H), 7.25 (d, 4H, J = 4.4 Hz), 7.53 (d, 1H, J = 2.0 Hz), 7.96 (d, 1H, J = 8.2 Hz), 8.07 (d, 1H, J = 8.2 Hz), 8.11 (t, 1H, J = 5.6 Hz), 8.18 (s, 1H), 8.30 (s, 1H), 10.10 (br, 1H).

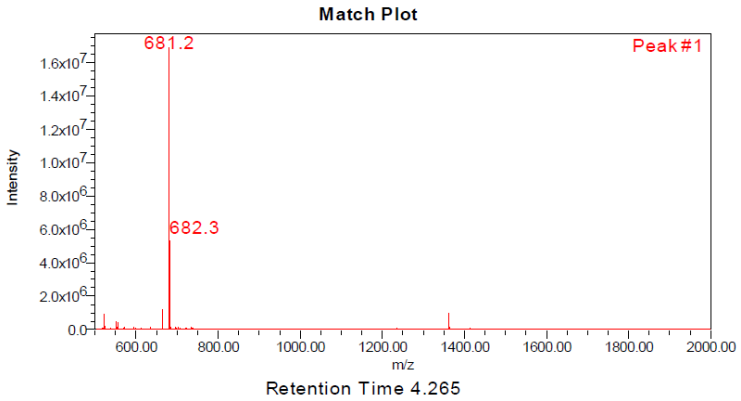
^{13}C NMR (125 MHz, d_6-DMSO, 300 K) δ: 21.56, 23.02, 24.19, 37.28, 38.53, 40.80, 41.90, 42.02, 50.99, 54.09, 55.77, 84.36, 114.64, 126.26, 128.04, 129.17, 130.40, 130.62, 137.78, 139.22, 155.08, 163.38, 168.75, 169.10, 170.69, 173.76, 173.88.

HRMS (ESI-TOF) Calcd for $C_{28}H_{38}IN_6O_6$: 681.1892; Found: 681.1891.

UPLC-MS rt: 3.878 min (Gradient 1).







Mono-iodination of [Tyr^0]-Bradykinin

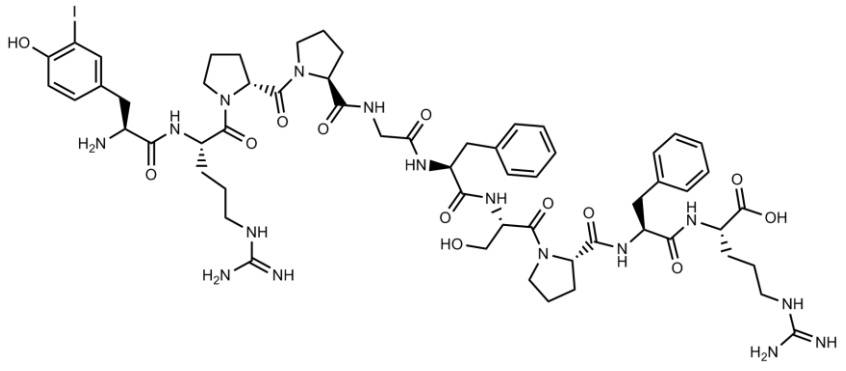
[Tyr^0]-Bradykinin (1.58 mg, 1.29 µmol) was dissolved in 740 µL of DCM and 180 µL of TFA. Then were added dropwise 36 µL of a 50 mM iodination stock solution (1.80 µmol, 1.4 eq.) (stock solution freshly prepared: 29.1 mg of Selectfluor were dissolved in 1.6 mL of ACN before the addition of 12.5 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Two other additions of iodination solution (2 x 6.5 µL, 2 x 0.25 eq.) were needed to reach the following final ratio: SM: 1.9% - MI: 92.7% - DI: 5.4%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM) and redissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fraction was lyophilized to afford the desired product [mono-Iodo-Tyr^0]-Bradykinin (1.46 mg, 0.93 µmol, 72 % yield).

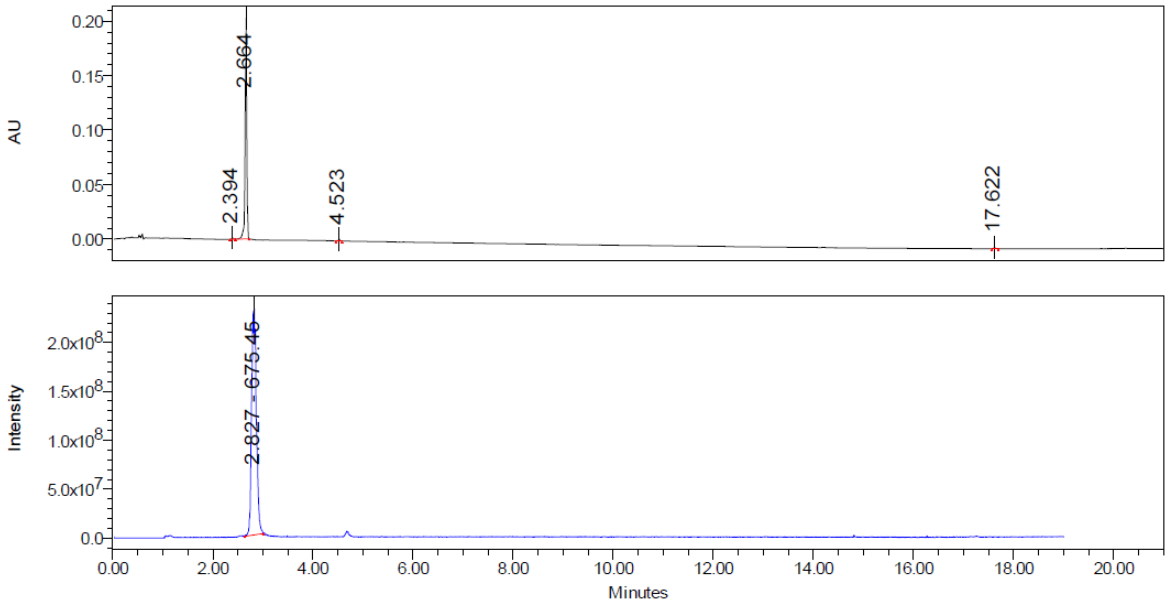
^{1}H NMR (500 MHz, d_6-DMSO, 300 K) δ: 1.43 (m, 1H), 1.51 (m, 5H), 1.61 (m, 2H), 1.70 (m, 2H), 1.79-2.05 (br, 9H), 2.18 (m, 1H), 2.72 (m, 2H), 2.79 (dd, 1H), 2.89 (dd, 1H), 2.96 (dd, 1H), 3.11 (m, 5H), 3.51 (m, 2H), 3.62 (m, 8H), 3.99 (m, 1H), 4.20 (m, 1H), 4.26 (dd, 1H), 4.30 (dd, 1H), 4.49 (m, 1H), 4.50-4.62 (m, 4H), 5.36 (s, 1H), 6.78 (d, 1H, J = 8.2 Hz), 7.00 (dd, 1H, J = 8.2, 2.0 Hz), 7.15-7.30 (m, 10H), 7.51 (d, 1H, J = 2.0 Hz), 7.56 (t, 1H), 7.61 (t, 1H), 7.70 (d, 1H), 7.94 (m, 2H), 8.08 (br, 2H), 8.13 (d, 1H), 8.31 (d, 1H), 8.66 (d, 1H), 10.31 (s, 1H), 12.72 (s, 1H).

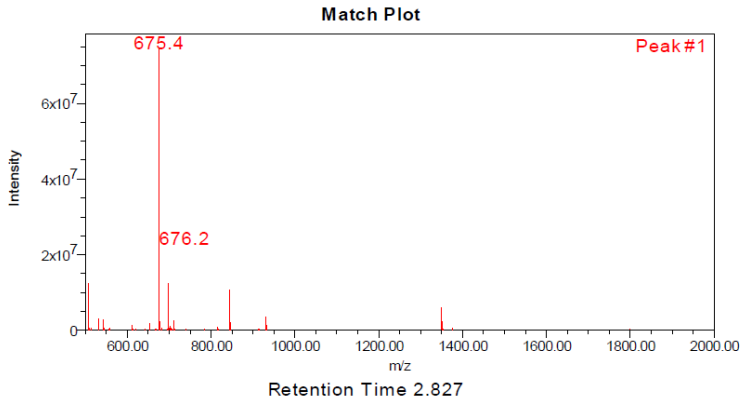
^{13}C NMR (125 MHz, d_6-DMSO, 300 K) δ: 23.72, 24.43, 24.52, 25.09, 27.90, 28.12, 28.58, 28.81, 29.08, 35.47, 37.26, 37.72, 40.27, 40.47, 41.67, 46.82, 46.90, 50.01, 51.49, 52.60, 53.12, 53.38, 53.45, 57.63, 59.44, 59.60, 61.73, 84.78, 114.66, 126.26, 126.29, 126.90, 127.97, 128.02, 129.10, 129.13, 130.71, 137.57, 137.68, 139.39, 155.85, 156.66, 156.74, 167.52, 168.48, 168.50, 169.63, 170.04, 170.85, 170.95, 170.96, 171.77, 173.08.

HRMS (ESI-TOF) Calcd for $C_{59}H_{81}IN_{16}O_{13}$: 1348.5214; Found: 1348.5255.

UPLC-MS rt: 2.664 min (Gradient 1).




AU
2.664
2.394
4.523
17.622
Intensity
675.46
2.827
Minutes


Match Plot
675.4
Peak#1
676.2
Intensity
m/z
Retention Time 2.827

Mono-iodination of Angiotensin III

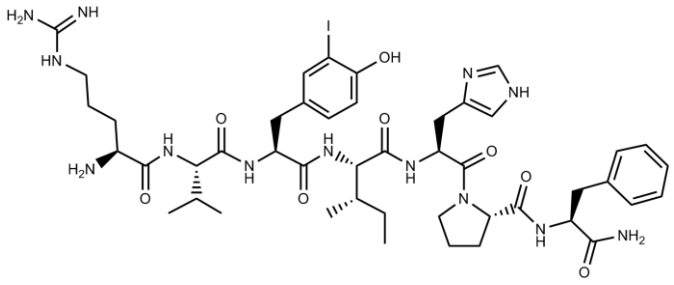
Angiotensin III (20.0 mg, 15.7 µmol) was dissolved in 8.5 mL of DCM and 2.0 mL of TFA. Then were added dropwise 440 µL of a 50 mM iodination stock solution (22.0 µmol, 1.4 eq.) (stock solution freshly prepared: 14.8 mg of Selectfluor were dissolved in 830 µL of ACN before the addition of 6.9 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Three other additions of iodination solution (3 x 80 µL, 3 x 0.25 eq.) were needed to reach the following final ratio: SM: 6.2% - MI: 91.3% - DI: 2.5%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM) and redissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Xbridge Prep C18, gradient: see general methods). The collected fraction was lyophilized to afford the desired product [mono-Iodo]-Angiotensin III (14.9 mg, 11.6 µmol, 74 % yield).

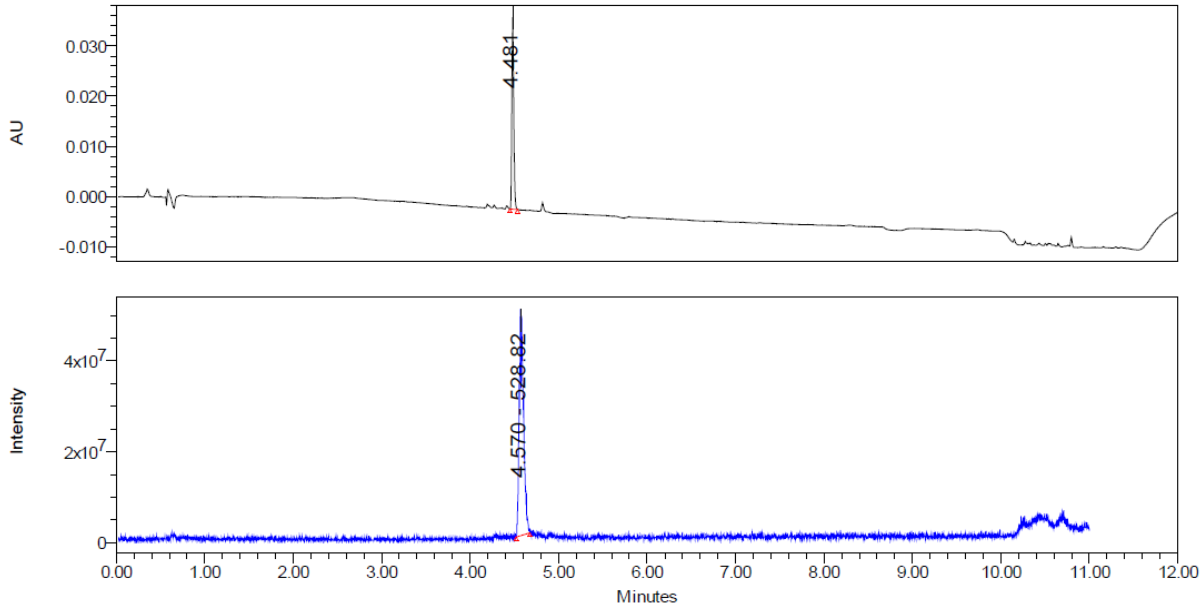
^{1}H NMR (500 MHz, d_6-DMSO, 300 K) δ: 0.75 (d, 3H), 0.77 (t, 3H), 0.79 (d, 3H), 0.84 (d, 3H), 1.05 (p, 1H), 1.36 (m, 1H), 1.46 (m, 2H), 1.64 (m, 4H), 1.77 (br, 2H), 1.99 (m, 2H), 2.59 (dd, 1H), 2.77 (dd, 1H), 2.85 (dd, 1H), 2.93 (dd, 1H), 3.07 (m, 4H), 3.46 (br, 1H), 3.61 (m, 1H), 3.87 (br, 1H), 4.15 (t, 1H), 4.27 (m, 2H), 4.38 (dd, 1H), 4.49 (m, 1H), 4.79 (br, 1H), 6.75 (d, J = 8.4 Hz, 1H), 7.08 (m, 2H), 7.17 (m, 1H), 7.23 (m, 5H), 7.38 (br, 1H), 7.60 (m, 2H), 7.99 (s, 1H), 8.01 (s, 1H), 8.10 (br, 4H), 8.35 (d, 2H), 8.41 (d, 2H), 8.93 (br, 1H), 10.17 (s, 1H), 14.19 (br, 1H).

^{13}C NMR (125 MHz, d_6-DMSO, 300 K) δ: 10.85, 15.19, 17.57, 19.19, 24.10, 24.25, 24.28, 26.30, 28.62, 29.13, 31.11, 35.77, 36.62, 37.01, 40.12, 46.99, 47.7, 51.72, 53.83, 53.93, 56.65, 57.12, 59.87, 84.27, 114.41, 117.1, 126.2, 128.00, 129.17, 130.29, 130.36, 133.8, 137.88, 138.97, 155.02, 156.71, 168.22, 170.48, 170.85, 170.97, 171.43, 172.55.

HRMS (ESI-TOF) Calcd for $C_{46}H_{66}IN_{13}O_8$: 1055.4202; Found: 1055.4242.

UPLC-MS rt: 4.481 min (Gradient 2).

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0.010
0.000
-0.010
4.481
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2x10^7
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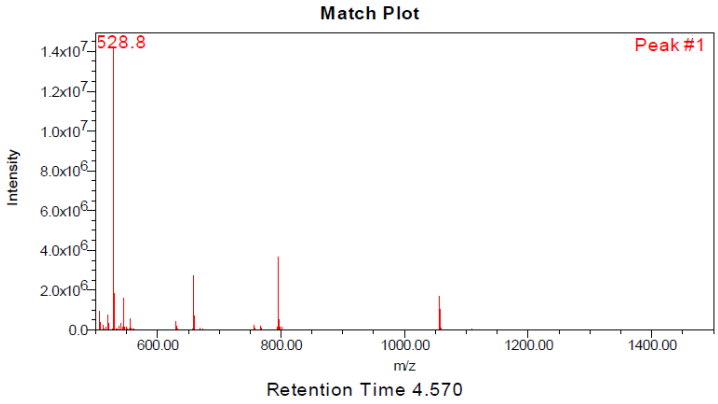
4.570
528.82
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
Minutes


Match Plot
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8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
600.00
800.00
1000.00
1200.00
1400.00
m/z
Retention Time 4.570

Mono-iodination of ACP-fragment 65-74 (11)

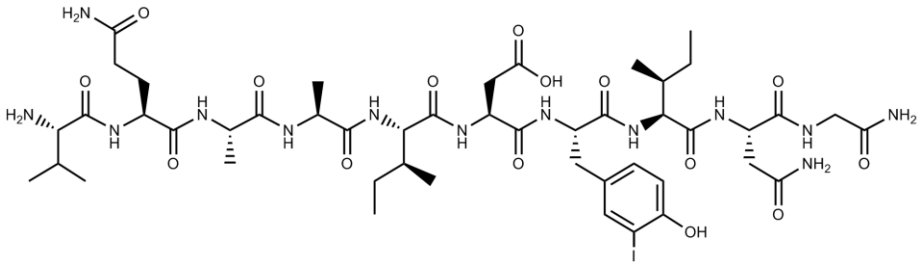
ACP fragment 65-74 (18.0 mg, 15.3 µmol) was dissolved in 6.0 mL of DCM and 1.5 mL of TFA. Then were added dropwise 428 µL of a 50 mM iodination stock solution (21.4 µmol, 1.4 eq.) (stock solution freshly prepared: 15.3 mg of Selectfluor were dissolved in 860 µL of ACN before the addition of 7.0 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Two other additions of iodination solution (2 x 45 µL, 2 x 0.1 eq.) were needed to reach the following final ratio: SM: 2.3% - MI: 93.7% - DI: 4.0%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM) and redissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Xbridge Prep C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product [mono-Iodo]-ACP fragment 65-74 (11.5 mg, 9.7 µmol, 63 % yield).

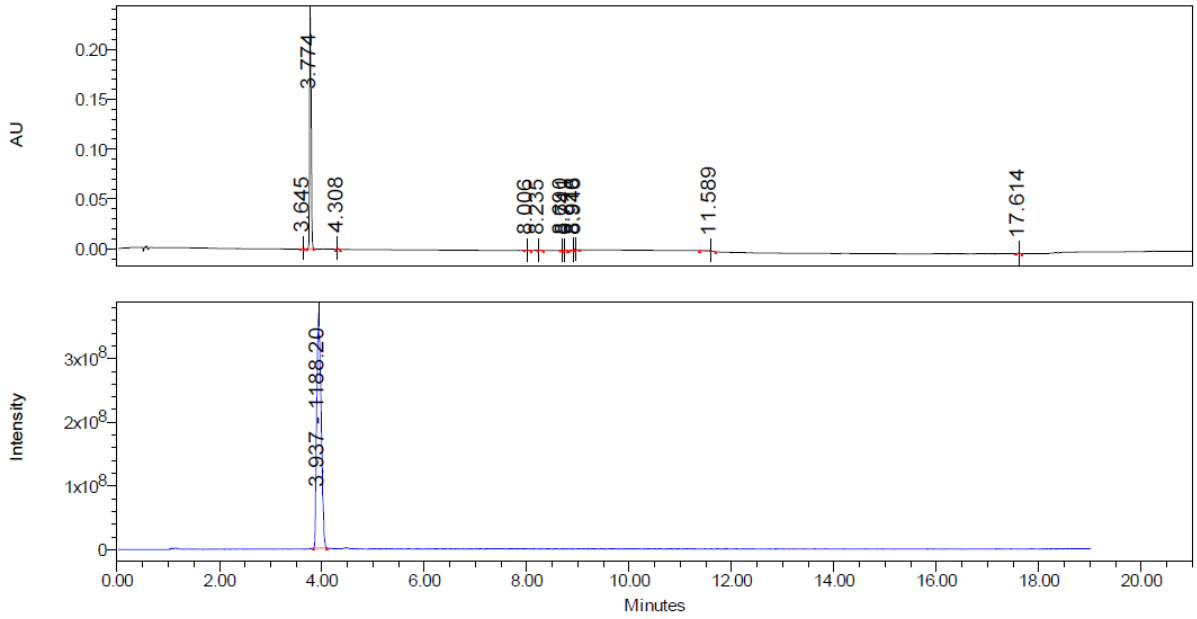
^{1}H NMR (500 MHz, d_6-DMSO, 295 K) δ: 0.73 (d, 3H), 0.76 (t, 3H), 0.80 (m, 6H), 0.91 (d, 3H), 0.92 (d, 3H), 1.00 (m, 1H), 1.06 (m, 1H), 1.17 (d, 3H), 1.18 (d, 3H), 1.35 (m, 1H), 1.43 (m, 1H), 1.66 (m, 2H), 1.76 (m, 1H), 1.87 (m, 1H), 2.03 (m, 1H), 2.13 (m, 2H), 2.43 (m, 1H), 2.51 (m, 1H), 2.62 (m, 3H), 2.84 (dd, 1H), 3.58 (m, 3H), 4.09 (t, 1H), 4.12 (t, 1H), 4.28 (m, 1H), 4.32 (m, 2H), 4.44 (m, 2H), 4.51 (m, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.84 (s, 1H), 6.99 (br, 1H), 7.02 (dd, J = 8.2, 1.8 Hz, 1H), 7.14 (s, 1H), 7.19 (s, 1H), 7.28 (s, 1H), 7.46 (s, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.67 (d, 1H), 7.77 (d, 1H), 8.04 (m, 5H), 8.10 (d, 1H), 8.17 (m, 2H), 8.28 (d, 1H), 8.52 (d, 1H), 10.07 (s, 1H), 12.35 (s, 1H).

^{13}C NMR (125 MHz, d_6-DMSO, 295 K) δ: 11.08, 11.11, 15.15, 15.27, 17.07, 17.79, 18.22, 18.33, 24.01, 24.45, 28.02, 29.89, 31.29, 35.96, 36.27, 36.43, 36.57, 36.99, 42.41, 47.97, 48.02, 49.54, 49.92, 52.07, 53.94, 56.6, 57.18, 57.25, 84.22, 114.49, 130.19, 130.34, 139.1, 155.01, 167.63, 170.29, 170.47, 170.6, 170.83, 171.01, 171.07, 171.11, 171.65, 171.82, 171.86, 171.91, 173.73.

HRMS (ESI-TOF) Calcd for $C_{47}H_{74}IN_{13}O_{15}$: 1187.4472; Found: 1187.4526.

UPLC-MS rt: 3.774 min (Gradient 1).

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2x10^8
1x10^8
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3.937
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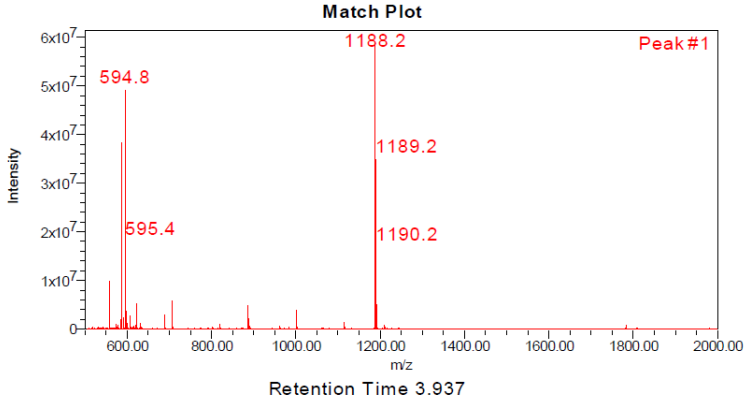
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes


Match Plot
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4x10^7
3x10^7
2x10^7
1x10^7
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Peak#1
594.8
1189.2
595.4
1190.2
600.00
800.00
1000.00
1200.00
1400.00
1600.00
1800.00
2000.00
m/z
Retention Time 3.937

Mono-iodination of [Tyr^8]-Substance P (7)

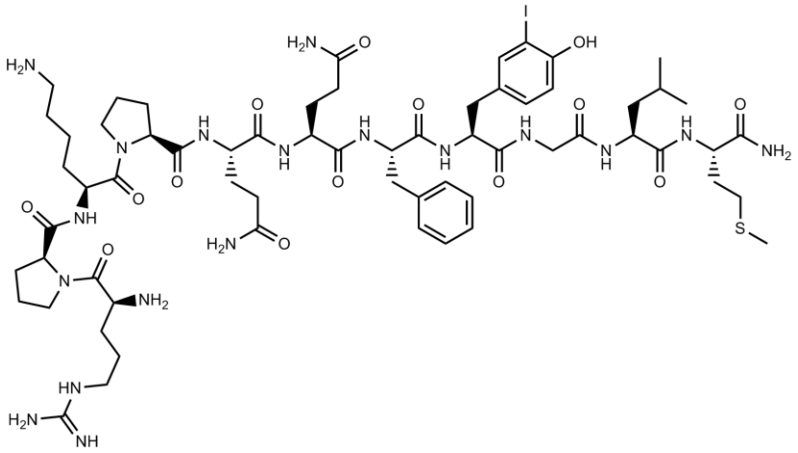
[Tyr^8]-Substance P (3.0 mg, 2.2 µmol) was dissolved in 1.4 mL of DCM and 300 µL of TFA. Then were added dropwise 60 µL of a 50 mM iodination stock solution (3.0 µmol, 1.4 eq.) (stock solution freshly prepared: 15.0 mg of Selectfluor were dissolved in 840 µL of ACN before the addition of 7.2 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Three other additions of iodination solution (3 x 15 µL, 3 x 0.35 eq.) were needed to reach the following final ratio: SM: 5.9% - MI: 93.6% - DI: 0.5%. The reaction mixture was then quenched with 400 µL of reducing cocktail (freshly prepared: KI (10 mg) and ascorbic acid (10 mg) were sonicated in 500 µL of TFA for 10 minutes) and evaporated under reduced pressure (TFA was co-evaporated three times with DCM). The crude was dissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product [mono-Iodo-Tyr^8]-Substance P (2.9 mg, 1.6 µmol, 72 % yield).

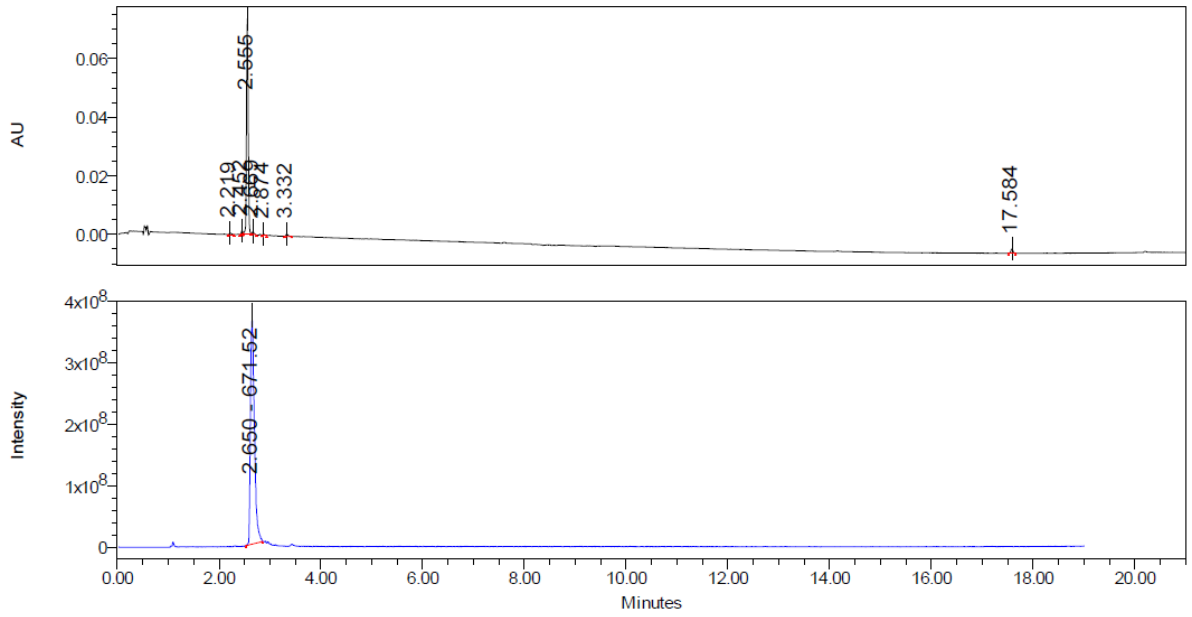
^{1}H NMR (700 MHz, d_6-DMSO, 295 K) δ: 0.84 (d, 3H), 0.88 (d, 3H), 1.39 (m, 2H), 1.47 (m, 2H), 1.51 (m, 1H), 1.54 (m, 2H), 1.61 (m, 3H), 1.67-1.77 (m, 6H), 1.78-1.88 (m, 6H), 1.91 (m, 3H), 2.03 (m, 4H), 2.11 (m, 4H), 2.38 (m, 1H), 2.45 (m, 1H), 2.67 (dd, 1H), 2.75 (m, 3H), 2.92 (m, 2H), 3.13 (m, 2H), 3.60 (m, 2H), 3.72 (m, 3H), 4.15 (m, 3H), 4.23 (m, 1H), 4.30 (m, 2H), 4.43 (m, 4H), 6.77 (d, 1H, J = 8.2 Hz), 6.87 (s, 2H), 7.08 (m, 2H), 7.15 (m, 3H), 7.19 (m, 3H), 7.32 (s, 2H), 7.58 (d, 1H, J = 1.8 Hz), 7.69 (t, 1H), 7.77 (br, 3H), 7.84 (d, 1H), 7.97 (t, 2H), 8.01 (t, 2H), 8.18 (br, 3H), 8.23 (t, 1H), 8.27 (d, 1H), 8.31 (d, 1H), 10.10 (s, 1H).

^{13}C NMR (175 MHz, d_6-DMSO, 295 K) δ: 14.65, 21.59, 21.84, 23.13, 23.62, 24.12, 24.67, 24.68, 26.56, 27.11, 27.39, 27.90, 29.03, 29.22, 29.70, 30.12, 31.48, 31.57, 31.59, 36.18, 37.50, 38.58, 40.22, 40.70, 41.99, 49.88, 47.04, 50.43, 50.48, 51.21, 51.76, 52.30, 52.80, 54.13, 54.29, 59.24, 59.83, 84.28, 114.63, 126.20, 128.04, 129.11, 130.22, 130.33, 137.59, 139.21, 155.10, 156.79, 166.84, 168.64, 170.38, 170.87, 170.92, 170.98, 171.30, 171.93, 172.04, 173.08, 174.19, 174.24.

HRMS (ESI-TOF) Calcd for $C_{63}H_{97}IN_{18}O_{14}S$: 1488.6197; Found: 1488.6244.

UPLC-MS rt: 2.555 min (Gradient 1).

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17.584
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2x10^8
1x10^8
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2.650 - 671.52
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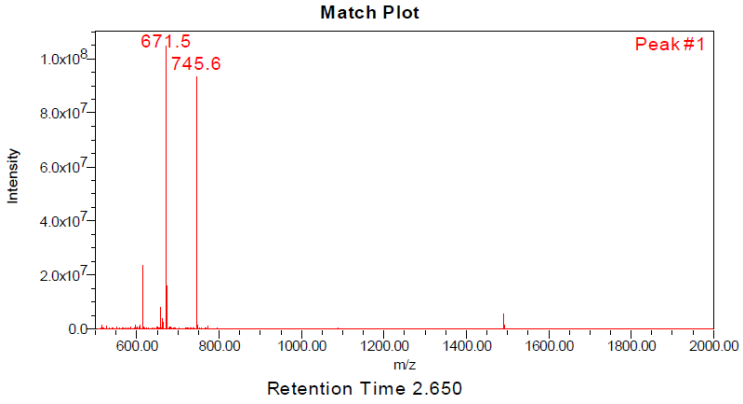
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes


Match Plot
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Peak #1
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4.0x10^7
2.0x10^7
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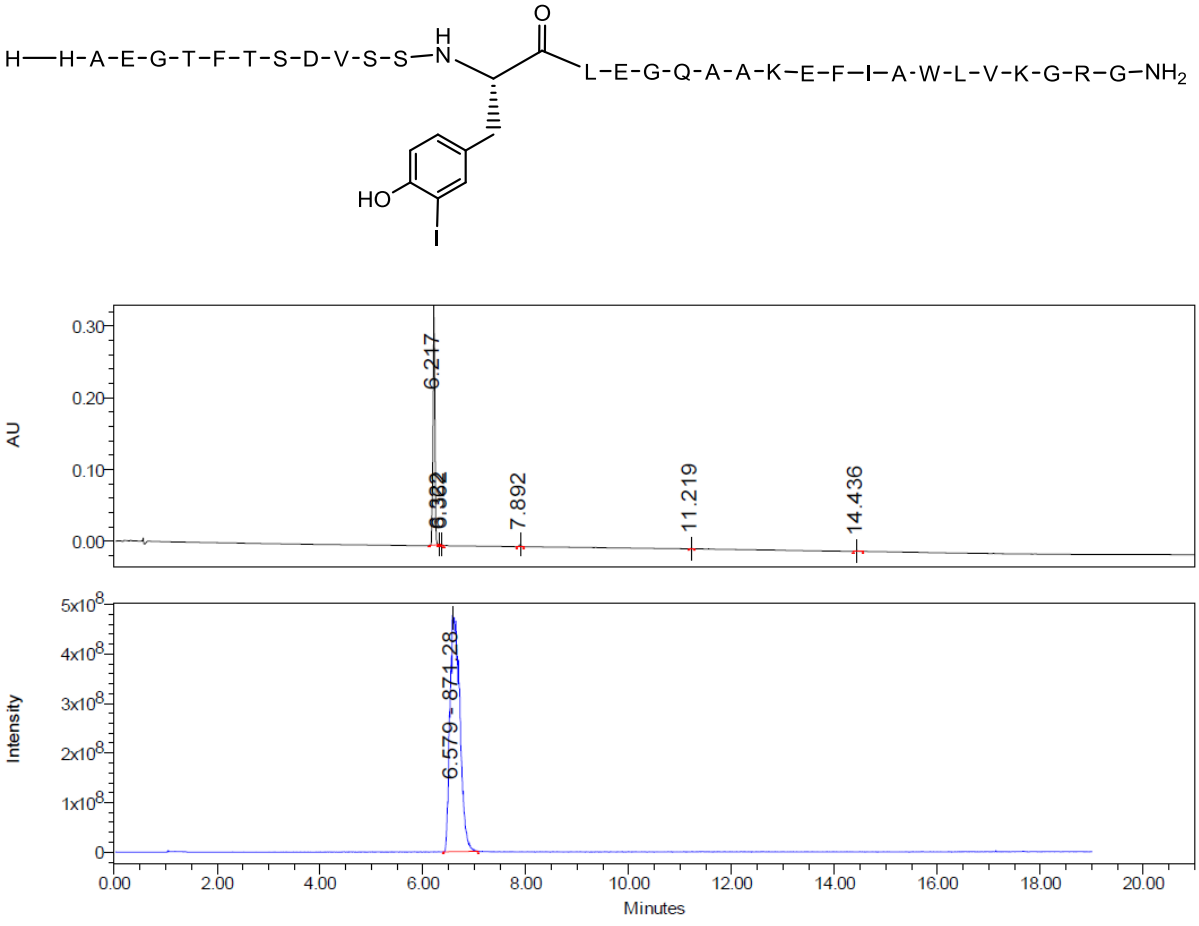
800.00
1000.00
1200.00
1400.00
1600.00
1800.00
2000.00
m/z
Retention Time 2.650

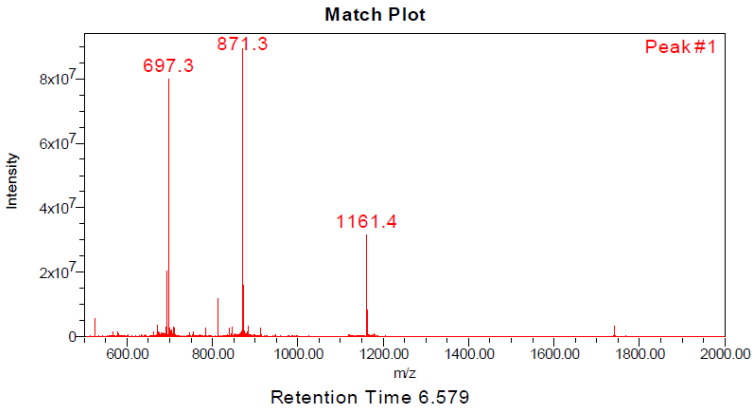
Mono-iodination of GLP-1(7-37)

GLP-1(7-37) (40.0 mg, 11.9 µmol) was dissolved in 5.6 mL of DCM and 1.4 mL of TFA. Then were added dropwise 330 µL of a 50 mM iodination stock solution (3.0 µmol, 1.4 eq.) (stock solution freshly prepared: 15.0 mg of Selectfluor were dissolved in 840 µL of ACN before the addition of 7.2 mg of NaI). The reaction mixture was allowed to stir at room temperature for 15 minutes. Two other additions of iodination solution (2 x 55 µL, 3 x 0.25 eq.) were needed to reach the following final ratio: SM: 4.6% - MI: 91.3% - DI: 4.1%. The reaction mixture was then evaporated under reduced pressure (TFA was co-evaporated three times with DCM). The crude was dissolved in water/acetonitrile (1/1) before being submitted to HPLC (purification performed on Waters Acquity CSH C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product [mono-Iodo]-GLP-1(7-37) (21.5 mg, 6.2 µmol, 52 % yield).

HRMS (ESI-TOF) Calcd for $C_{151}H_{227}IN_{40}O_{47}$ $[M+3H]^{3+}$ 1741.2900; Found: 1741.2999.

UPLC-MS rt: 6.217 min (Gradient 1).





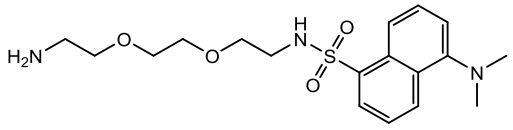
Synthesis of Amino-PEG$_2$-Dansyl

Dansyl chloride (100 mg, 370 µmol) was dissolved in 1.0 mL of DCM before the addition dropwise of tert-butyl(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (105 µL, 445 µmol) previously dissolved in 1 mL of DCM. The mixture was allowed to stir at room temperature for 2 hours. Reaction was monitored by LC-MS. The mixture was then diluted with 4 mL of brine, washed with water three times before being dried over magnesium sulfate and filtered. Subsequently 450 µL of TFA were added to the mixture (~10% v/v DCM) to induce Boc deprotection. After 15 min, LC-MS showed completion of reaction. The mixture was evaporated under reduced pressure and redissolved in acetonitrile and water before lyophilization to afford the desired product as pale yellow oil (201 mg, 346 µmol, 93 % yield).

^{1}H NMR (400 MHz, d_6-DMSO, 295 K) δ: 1.28 (s, 1H), 2.07 (s, 1H), 2.84 (s, 6H), 2.96 (m, 5H), 3.56 (m, 4 H), 4.60 (s, 1H), 5.37 (br s, 3H), 5.75 (br s, 1H), 7.27 (d, J=7.46 Hz, 1H), 7.61 (m, 2H), 7.72 (br s, 3H), 7.99 (t, J=5.81, 5.81 Hz, 1H), 8.11 (dd, J=7.34, 0.98 Hz, 1H), 8.30 (d, J=8.68 Hz, 1H), 8.47 (d, J=8.44 Hz, 1H).

^{13}C NMR (150 MHz, d_6-DMSO, 300 K) δ: 38.54, 42.16, 45.06 (2C), 66.58, 69.01, 69.30, 69.43, 115.13, 119.23, 123.56, 127.75, 128.00, 128.99, 129.03, 129.27, 136.21, 151.17.

HRMS (ESI-TOF) Calcd for $C_{18}H_{27}N_3O_4S$: 382.1795; Found: 382.1795.



Synthesis of Boronic pinacol ester-PEG_2-Dansyl (9)

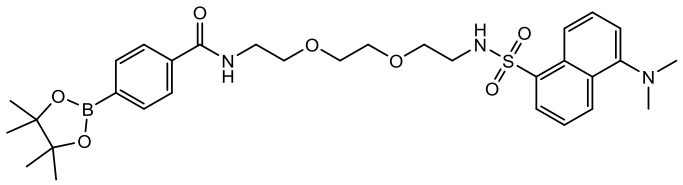
4-Carboxylphenylboronic acid pinacol ester (31 mg, 125 µmol), DIEA (45 µL, 258 µmol) and propylphosphonic anhydride T3P (75 µL, 125 µmol - 50% wt. solution in ethyl acetate) were dissolved in 1 mL of DCM and stirred at room temperature for 5 minutes before the dropwise addition of amino-PEG_2-Dansyl (60 mg, 104 µmol) and DIEA (45 µL, 258 µmol) previously dissolved in 750 µL of DCM. The reaction mixture was allowed to stir at room temperature. After 30 min, LC-MS showed completion of reaction. The crude was evaporated under reduced pressure and purified via silica gel chromatography (0->100% EtOAc/Hept – product eluted around ~80% EtOAc/Hept) to afford the desired product as a yellow oil (39 mg, 64 µmol, 62 % yield).

^{1}H NMR (400 MHz, d_6-DMSO, 295 K) δ: 1.17 (m, 1 H), 1.31 (s, 12 H), 1.91 (s, 1 H), 1.99 (s, 1 H), 2.50 (u), 2.82 (s, 6 H), 2.94 (q, J=5.83, 5.83, 5.83 Hz, 2 H), 3.28 (m, 4 H), 3.45 (u), 4.03 (q, J=7.17, 7.17, 7.17 Hz, 1 H), 7.24 (d, J=7.46 Hz, 1 H), 7.59 (m, 2 H), 7.74 (m(para), 2 H), 7.83 (m(para), 2 H), 8.11 (dd, J=7.34, 0.98 Hz, 1 H), 7.98 (t, J=5.81, 5.81 Hz, 1 H), 8.28 (d, J=8.68 Hz, 1 H), 8.44 (d, J=8.56 Hz, 1 H), 8.52 (t, J=5.50, 5.50 Hz, 1 H).

^{13}C NMR (150 MHz, d_6-DMSO, 300 K) δ: 24.65(4C), 39.51, 42.20, 45.03(2C), 68.72, 68.93, 69.29, 69.38, 83.89(2C), 115.05, 119.21, 123.50, 126.47(2C), 127.71, 127.97, 129.01, 129.03, 129.24, 134.21(2C), 136.32, 136.80, 151,27, 165.99.

The carbon directly bonded to boron could not be detected due to quadrupolar relaxation.

HRMS (ESI-TOF) Calcd for $C_{31}H_{43}BN_3O_7S$ $[M+Na]^+$ 634.2734; Found: 634.2737.



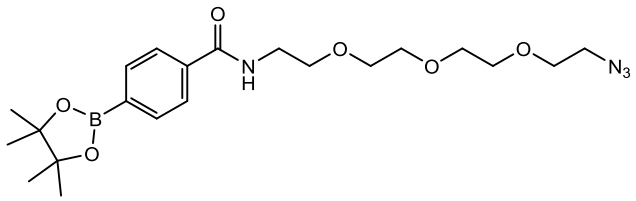
Synthesis of Boronic pinacol ester-PEG_3-azide (12)

4-carboxylphenylboronic acid pinacol ester (200 mg , 806 µmol), DIEA (422 µL, 2.42 mmol) and propylphosphonic anhydride T3P (720 µL, 1.21 mmol – 50% wt. solution in ethyl acetate) were dissolved in 500 µL of DCM and was allowed to premix at room temperature for 5 minutes before the dropwise addition of amino-PEG_3-azide (211 mg, 968 µmol) previously dissolved in 500 µL of DCM .

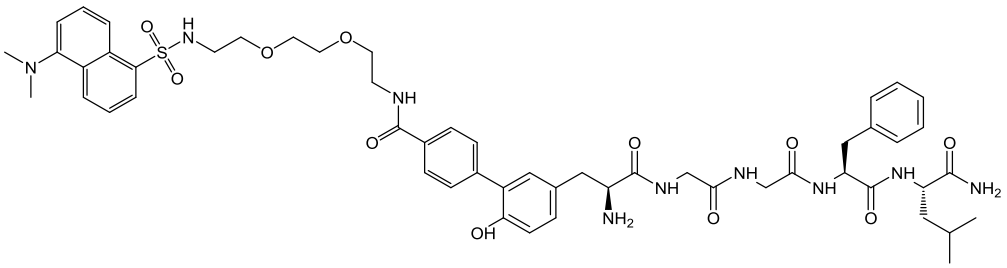
The reaction was stirred at room temperature for 10 minutes. Reaction was monitored by LC-MS. The crude was dissolved in 5 mL of DCM and 5 mL of 0.5M HCl aqueous solution, washed twice with water (2x5mL) before being dried with magnesium sulfate, filtered and evaporated under reduced pressure to afford the desired product as a white powder (308 mg, 687 µmol, 85 % yield).

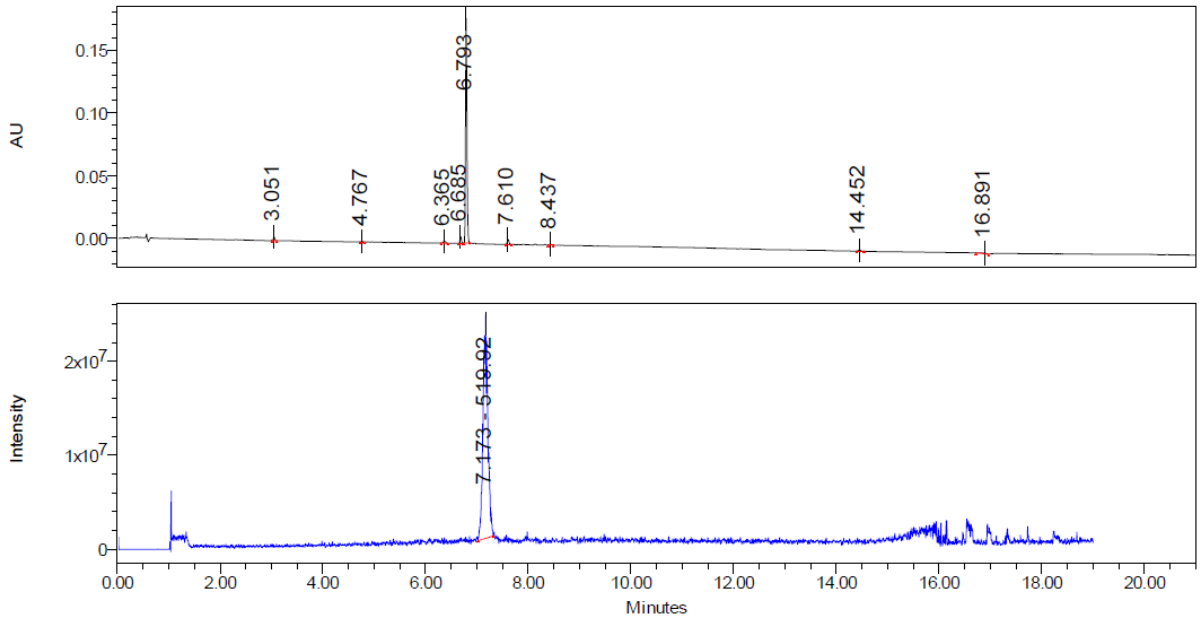
^{1}H NMR (400 MHz, d_6-DMSO, 295 K) δ: 0.94 (m, 1 H), 1.31 (s, 12 H), 1.48 (m, 1 H), 2.50 (u), 3.23 (br s, 1 H), 3.44 (br s, 1 H), 3.53 (d, J=4.65 Hz, 9 H), 3.57 (m, 4 H), 7.79 (m(para), 4 H), 8.55 (t, J=5.50, 5.50 Hz, 1 H).

^{13}C NMR (150 MHz, d_6-DMSO, 300 K) δ: 24.65(4C), 39.51, 49.95, 68.78, 69.18, 69.57, 69.64, 69.73, 69.76, 83.89(2C), 126.49(2C), 134.21(2C), 136.84, 166.00.
The carbon directly bonded to boron could not be detected due to quadrupolar relaxation.
HRMS (ESI-TOF) Calcd for $C_{21}H_{34}BN_4O_6$ $[M+Na]^+$ 471.2389; Found: 471.2388.



Synthesis of Dansyl-PEG$_2$-Leucin Enkephalinamide (10) - Suzuki-Miyaura Cross-coupling
Preparation of the Pd catalyst was performed as described by Chalker *et al*[2]. Briefly, 2-amino-4,6-dihydroxypyrimidine disodium (7.6 mg, 44 µmol) was dissolved in 440 µL of water by stirring for 2 minutes in a water bath preheated to 65°C. To the resulting solution was added $Pd(OAc)_2$ (5.0 mg, 22 µmol). The mixture was stirred vigorously at 65°C for 30 minutes to give a homogenous yellow-orange solution. After cooling to room temperature, the stir bar was removed to give the catalyst stock solution 50 mM in Pd(II).
In a 1.5 mL Eppendorf tube, [mono-Iodo]-Leucine Enkephalinamide (1.0 mg, 1.50 µmol) was dissolved in 75 µL of water and 75 µL of dioxane before the addition of glycerol (50 µL), K_2HPO_4 (1M aqueous stock solution, 45 µL, 45 µmol) and boronic pinacol ester-PEG$_2$-Dansyl (**9**) (50 mM stock solution in dioxane, 45 µL, 2.25 µmol). Prior and after the addition of $Pd(OAc)_2.L_2$ (50 mM aqueous stock solution, 75 µL, 3.75 µmol) the mixture was bubbled with argon for 10 minutes. The resulting solution was capped and stirred at 38°C for 12 hours on an Eppendorf Thermomixer (1300 rpm). The reaction was monitored by LC-MS. The crude mixture was quenched with 1 mL of a 1M HCl aqueous solution before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product Dansyl-PEG$_2$-Leucine Enkephalinamide (0.55 mg, 0.53 µmol, 35 % yield).
HRMS (ESI-TOF) Calcd for $C_{53}H_{67}N_9O_{11}S$: 1038.4754; Found: 1038.4753.
UPLC-MS rt: 6.793 min (Gradient 1).
Max Abs/Em - / 506 nm (absorption <400 nm cannot be measured with our device)

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16.891
Intensity
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1x10^7
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8.00
10.00
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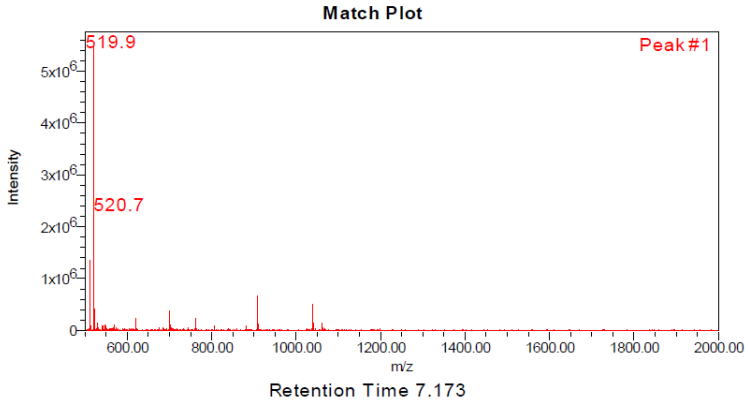
14.00
16.00
18.00
20.00
Minutes


Match Plot
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1200.00
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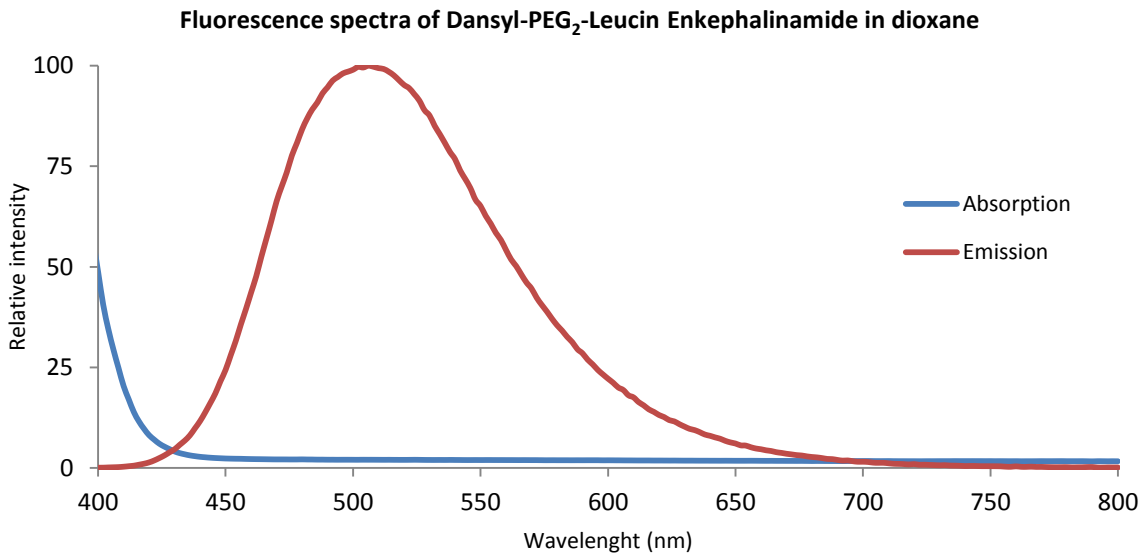
1600.00
1800.00
2000.00
m/z
Retention Time 7.173

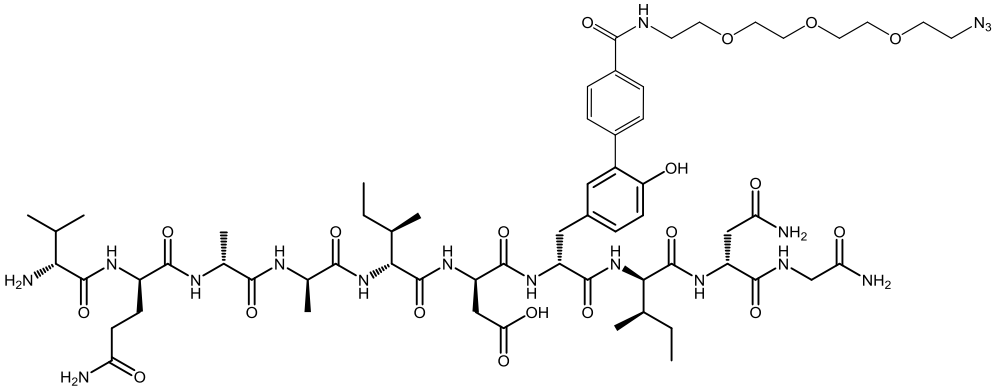


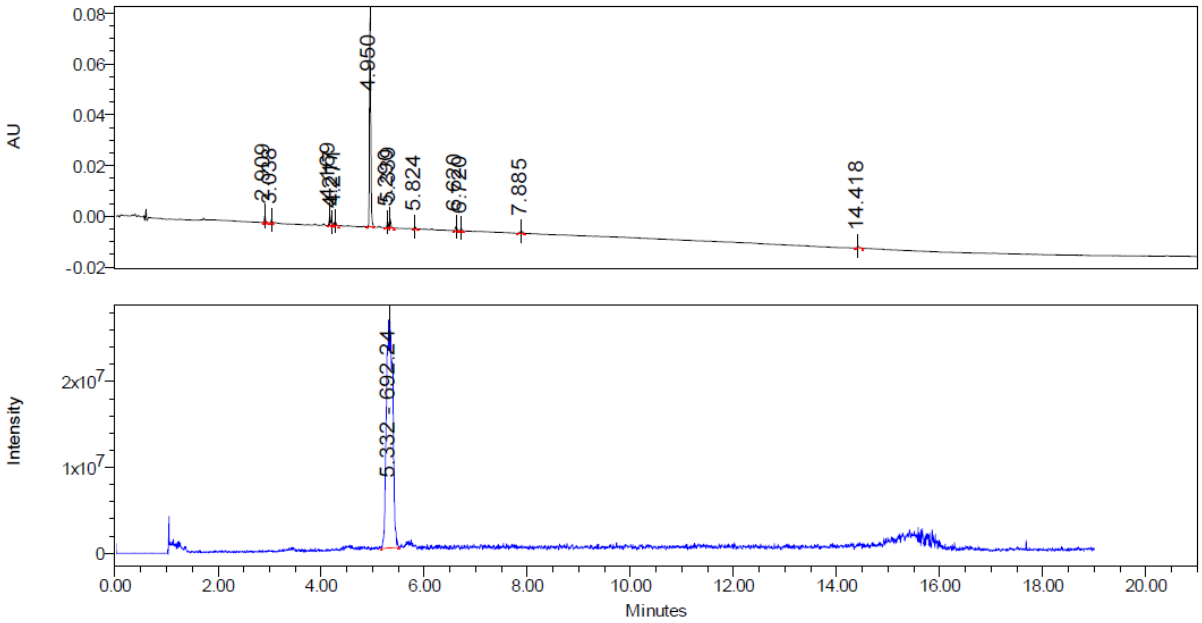
Synthesis of Azido-PEG$_3$-ACP fragment (65-74) - Suzuki-Miyaura Cross-coupling

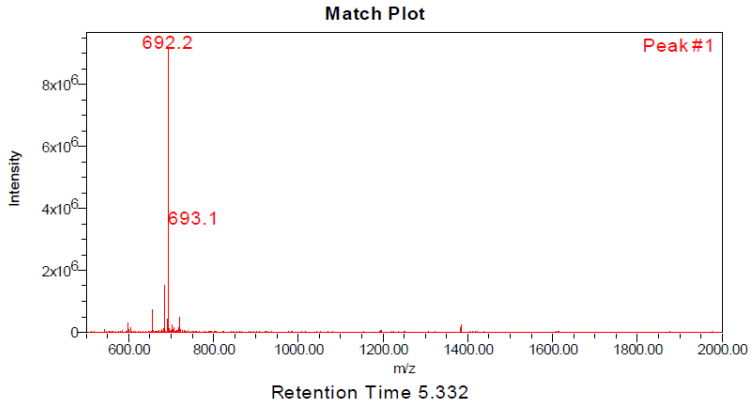
In a 1.5 mL Eppendorf tube, [mono-Iodo]-ACP fragment (65-74) (2.43 mg, 2.05 µmol) was dissolved in 100 µL of water and 100 µL of dioxane before the addition of glycerol (50 µL), K_2HPO_4 (1M aqueous stock solution, 50 µL, 50 µmol) and boronic pinacol ester-PEG$_3$-azide (**12**) (50 mM stock solution in dioxane, 75 µL, 3.75 µmol). Prior and after the addition of $Pd(OAc)_2.L_2$ (50 mM aqueous stock solution, 80 µL, 4.0 µmol) the mixture was bubbled with argon for 10 minutes. The resulting solution was capped and stirred at 38 °C for 12 hours on an Eppendorf Thermomixer (1300 rpm). The reaction was monitored by LC-MS. The crude mixture was quenched with 1mL of a 1M HCl aqueous solution before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product Azido-PEG$_3$- ACP fragment (65-74) (1.68 mg, 1.22 µmol, 59 % yield).

HRMS (ESI-TOF) Calcd for $C_{62}H_{95}N_{17}O_{19}$: 1380.6917; Found: 1380.6899.

UPLC-MS rt: 4.950 min (Gradient 1).







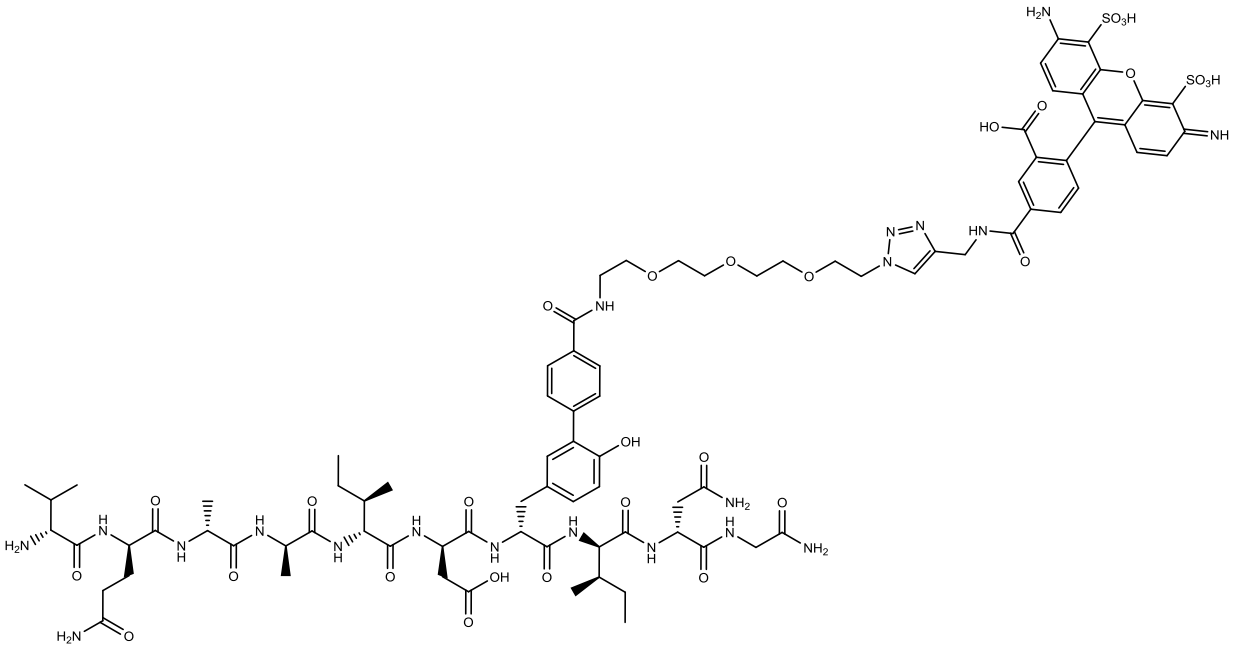
Synthesis of Alexa Fluor 488-PEG$_3$-ACP fragment (65-74) (13) – CuAAC

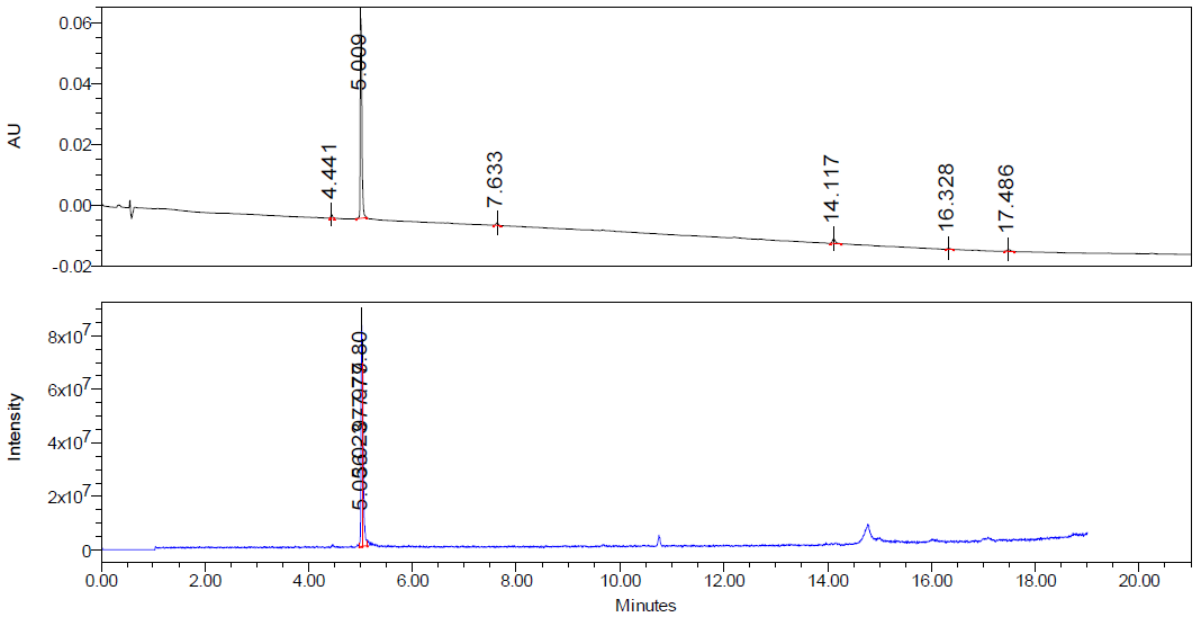
Azido-PEG$_3$- ACP fragment (65-74) (1.68 mg, 1.22 µmol) was dissolved in 200 µL of water and 100 µL of tBuOH before the addition of Alkyne-Alexa Fluor 488 (1.0 mg, 1.29 µmol). Copper sulfate (50 mM stock solution in water, 3 µL, 0.15 µmol), Cu-ligand THPTA[3] (200 mM stock solution in water, 3 µL, 0.60 µmol) and ascorbic acid (100 mM stock solution in water, 7.5 µL, 0.75 µmol) were premixed together before being added to the reaction mixture. The resulting solution was stirred at 38°C in a Thermomixer (1300 rpm) for 12 hours. The reaction was monitored by LC-MS. The crude mixture was dissolved in water/ACN before being submitted to HPLC (purification performed on Agilent Zorbax Rx C18, gradient: see general methods). The collected fractions were lyophilized to afford the desired product as an orange powder Alexa Fluor488-PEG$_3$-ACP fragment (65-74) (1.48 mg, 0.76 µmol, 62 % yield).

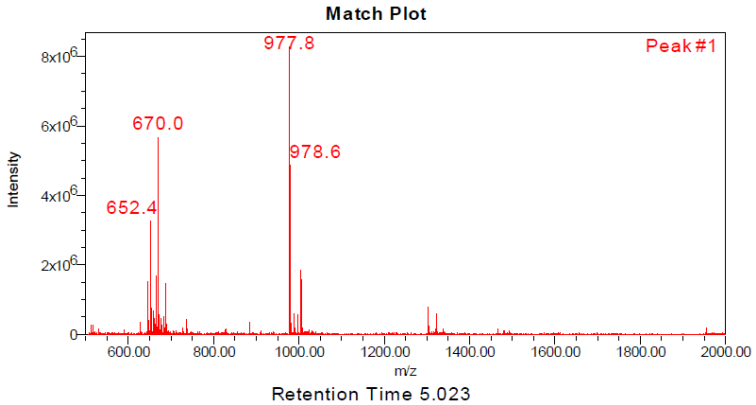
HRMS (ESI-TOF) Calcd for $C_{86}H_{112}N_{20}O_{29}S_2$ $[M+2H]^{2+}$ 977.8760; Found: 977.8803.

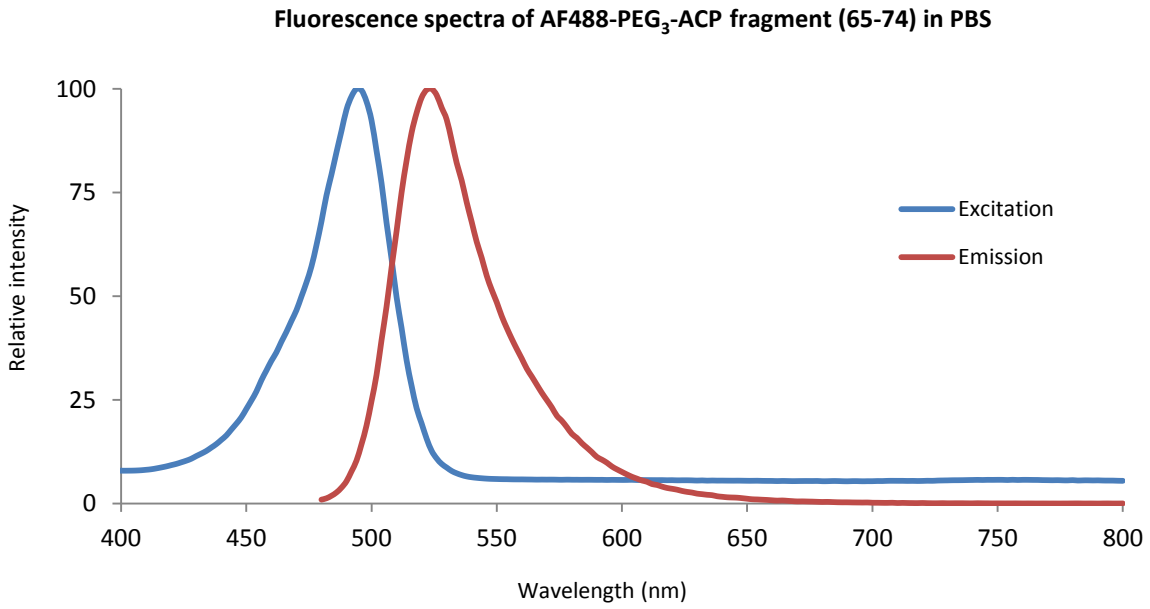
UPLC-MS rt: 5.009 min (Gradient 1).

Max Abs/Em 494/524 nm.



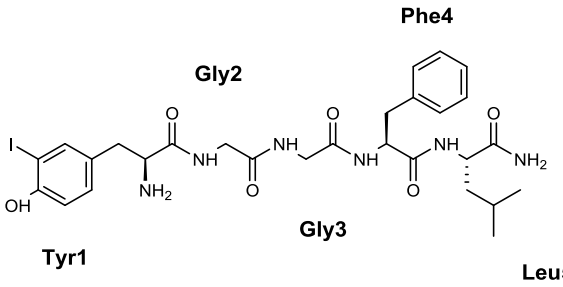

AU
0.06
0.04
0.02
0.00
-0.02
5.009
4.441
7.633
14.117
16.328
17.486
Intensity
8x10^7
6x10^7
4x10^7
2x10^7
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes


Match Plot
977.8
Peak#1
670.0
978.6
652.4
Intensity
8x10^6
6x10^6
4x10^6
2x10^6
0
600.00
800.00
1000.00
1200.00
1400.00
1600.00
1800.00
2000.00
m/z
Retention Time 5.023


Fluorescence spectra of AF488-PEG3-ACP fragment (65-74) in PBS
Relative intensity
100
75
50
25
0
400
450
500
550
600
650
700
750
800
Wavelength (nm)
Excitation
Emission

NMR Spectra and proton assignment of mono-iodinated peptides

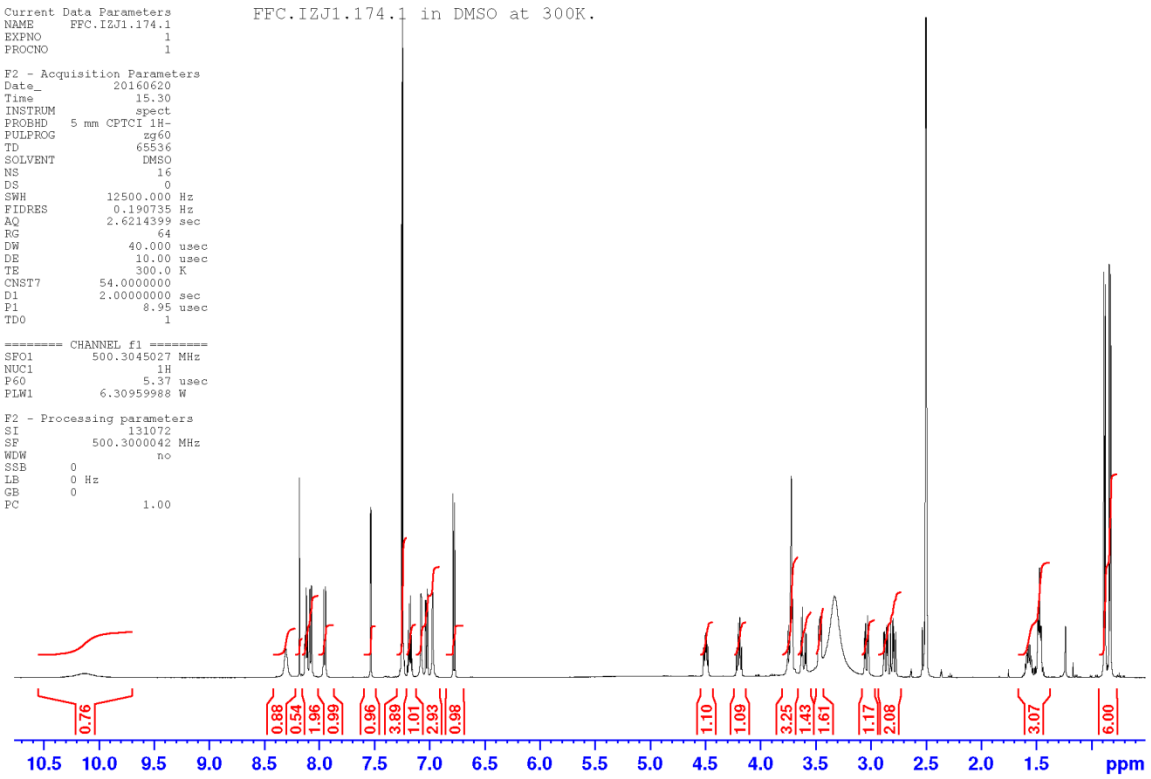
[mono-iodo] Leucine Enkephalinamide



^{1}H NMR (500 MHz, d_6-DMSO, 300 K) and ^{13}C NMR (125 MHz, d_6-DMSO, 300 K).

		^{1}H	^{13}C
Tyr-1	NH_3^+	broad	-
	α	3.46	55.77
	β	2.86/2.51	38.53
	γ	-	130.62
	δ1	7.53	139.22
	δ2	7.03	130.40
	ε1	-	84.36
	ε2	6.78	114.64
	ζ	-	155.08
	ζ-OH	~ 10.1 (broad)	-
	C’	-	173.76
Gly-2	NH	8.30	-
	α	3.73	42.02
	C’	-	169.10
Gly-3	NH	8.12	-
	α	3.73/3.61	41.90

	C′	-	168.75
Phe-4	NH	8.08	-
	α	4.50	54.09
	β	3.04/2.80	37.28
	γ	-	137.78
	δ	7.25	129.17
	ε	7.25	128.04
	ζ	7.18	126.26
	C′	-	170.69
Leu-5	NH	7.95	-
	α	4.20	50.99
	β	1.47	40.80
	γ	1.57	24.19
	δ	0.88	23.02
	δ′	0.83	21.56
	C′	-	173.88
	NH_2	7.08/6.97	-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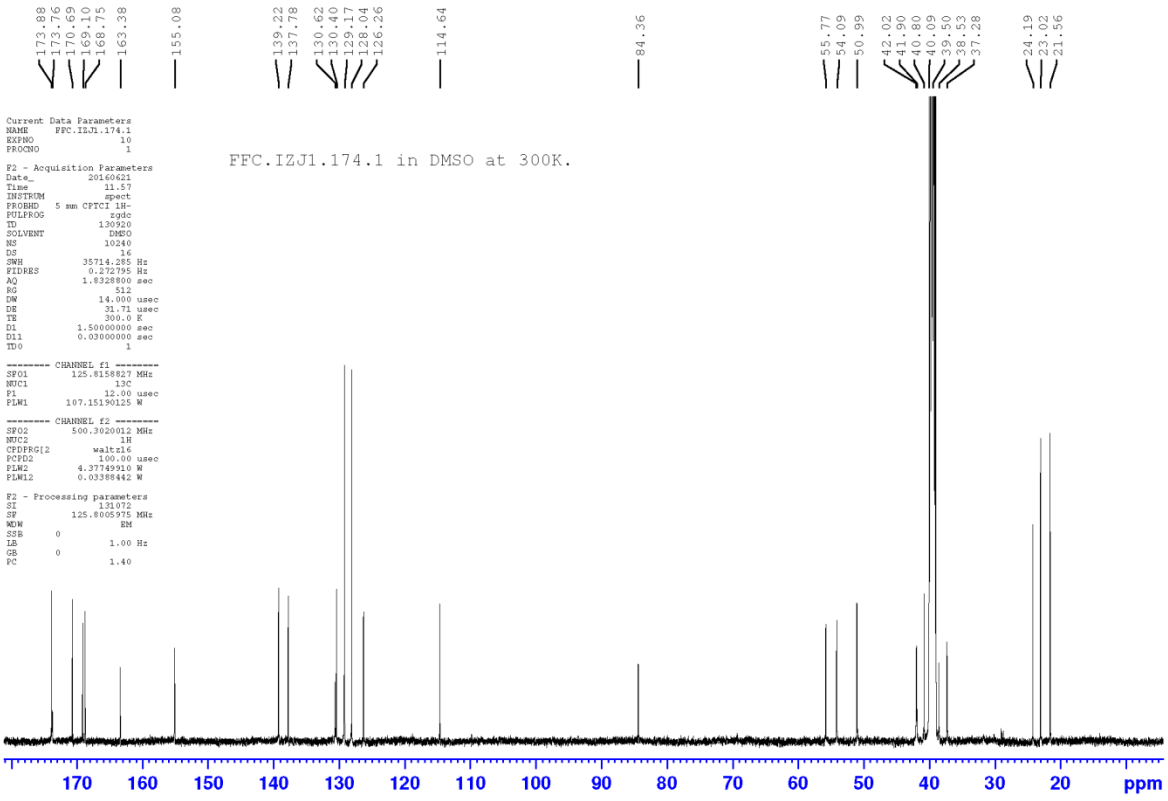
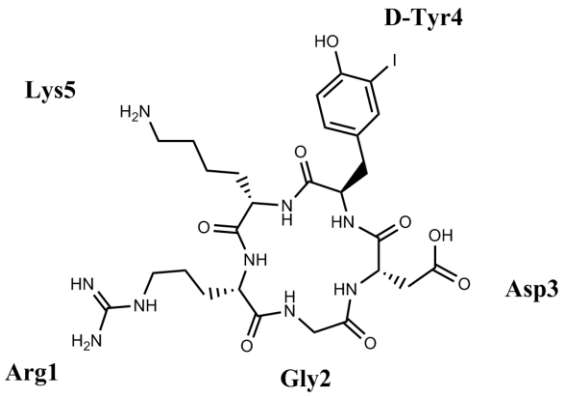
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160620
Time 15.30
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 0
SWH 12500.000 Hz
FIDRES 0.190735 Hz
AQ 2.6214399 sec
RG 64
DW 40.000 usec
DE 10.00 usec
TE 300.0 K
CNST7 54.0000000
D1 2.00000000 sec
P1 8.95 usec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3045027 MHz
NUC1 1H
P60 5.37 usec
PLW1 6.30959988 W
F2 - Processing parameters
SI 131072
SF 500.3000042 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
FFC.IZJ1.174.1 in DMSO at 300K.
0.76
0.88
0.54
1.96
0.99
0.96
3.89
1.01
2.93
0.98
1.10
1.09
3.25
1.43
1.61
1.17
2.08
3.07
6.00
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm



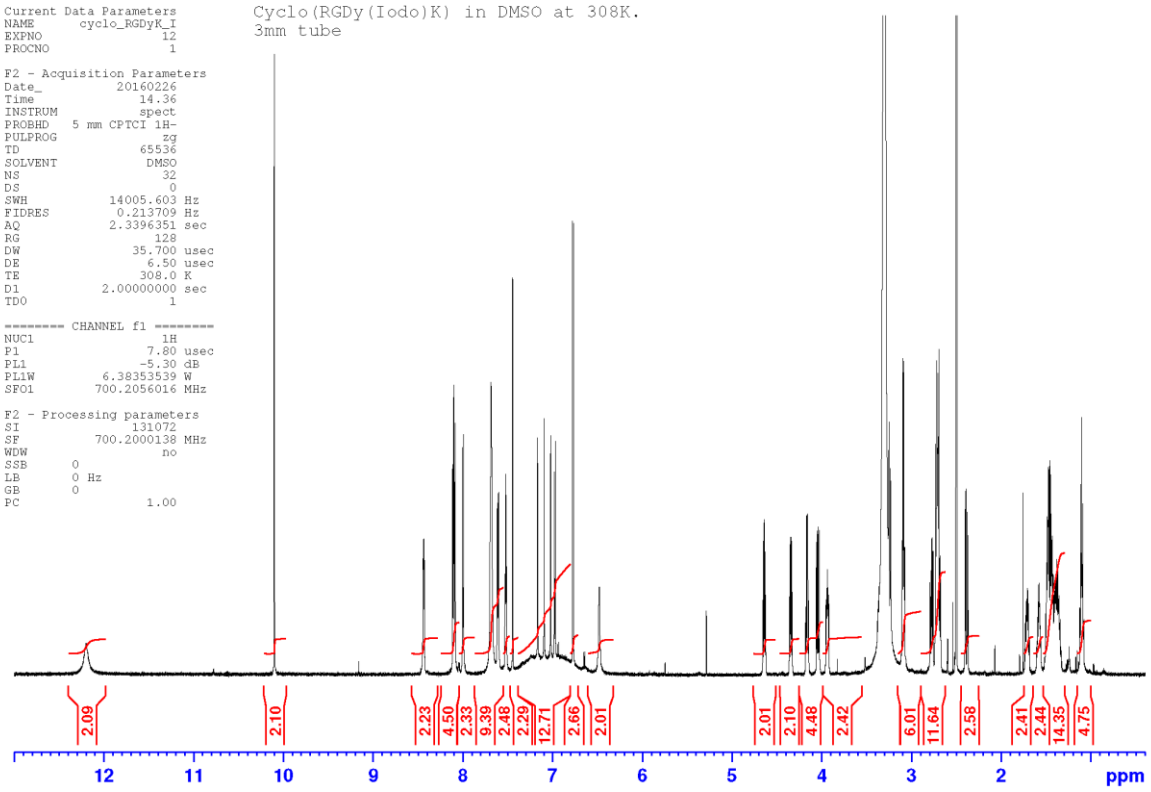
Cyclo(RGD[mono-iodo]yK)

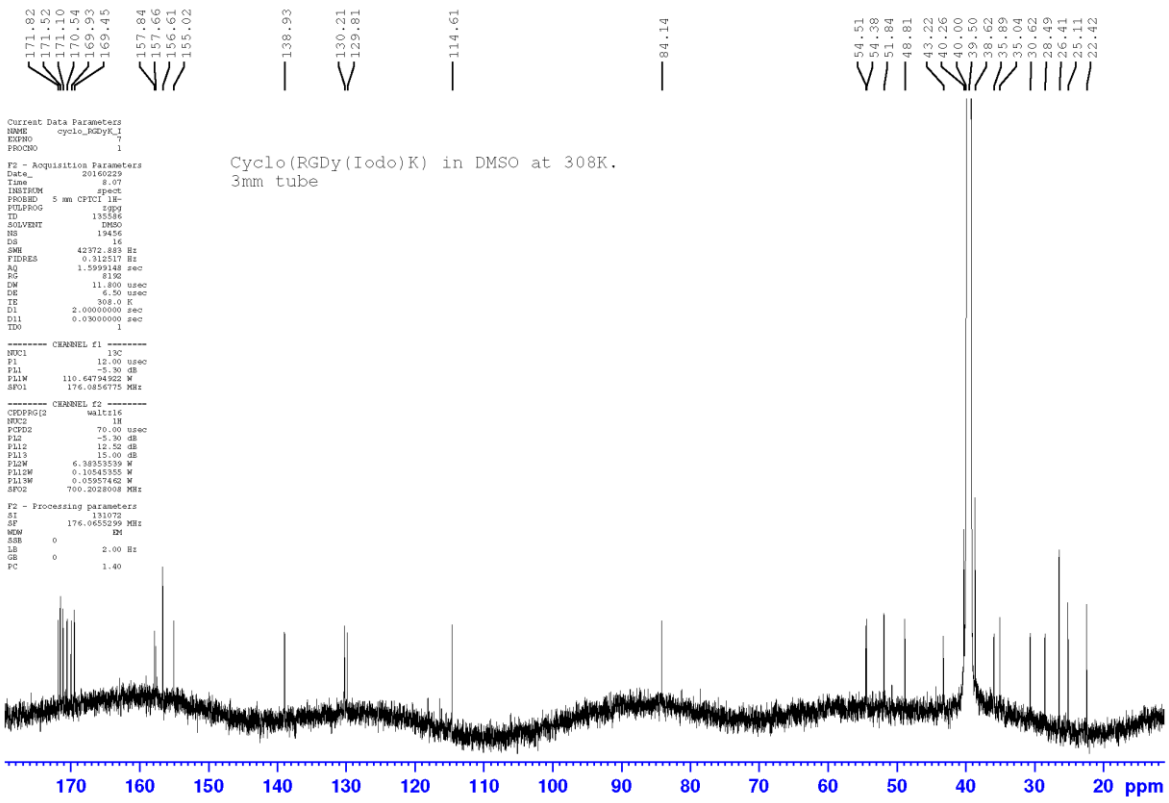


^{1}H NMR (700 MHz, d_6-DMSO, 308 K) and ^{13}C NMR (175 MHz, d_6-DMSO, 308 K).
Chemical shifts are referenced to the solvent signals (^{1}H: 2.50 ppm, ^{13}C: 39.50 ppm). At 308K a better dispersion of the amide signals (Asp-3 and Lys-5) has been obtained.

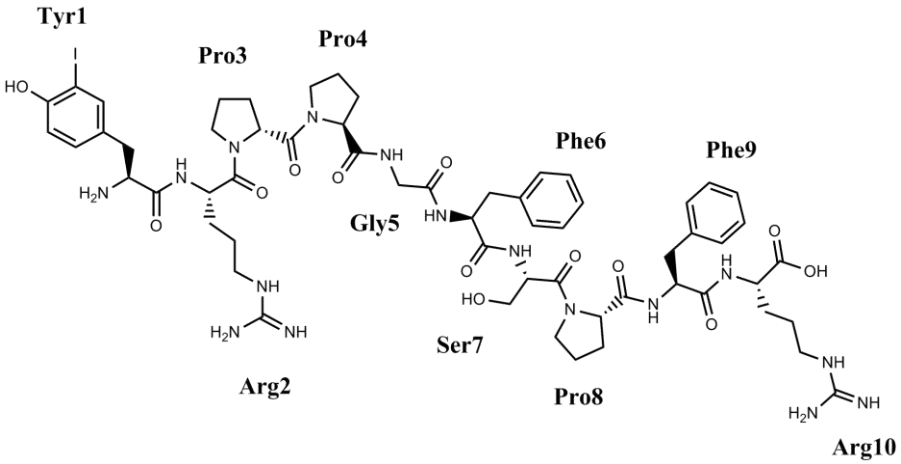
	^{1}H	^{13}C
Arg-1		
NH	7.61	-
α	4.16	51.84
β	1.71/1.48	28.48
γ	1.37	25.11
δ	3.09	40.26
ε	7.52	-
ζ	-	156.61
C′	-	171.10
Gly-2		
NH	8.44	-
α	4.04/3.24	43.22
C′	-	169.45
Asp-3		
NH	8.09	-
α	4.64	48.81
β	2.71/2.39	35.04
γ	(OH: ~12.2)	171.52
C′	-	169.93
D-Tyr-4		
NH	8.00	-
α	4.34	54.51
β	2.78/2.69	35.89
γ	-	129.81
δ1	7.44	138.93

δ2	6.97	130.21
ε1	-	84.14
ε2	6.77	114.61
ζ	-	155.02
ζ-OH	10.10	-
C’	-	170.54
Lys-5		
NH	8.11	-
α	3.94	54.38
β	1.58/1.43	30.62
γ	1.10	22.42
δ	1.46	26.41
ε	2.71	38.62
ζ	7.69	-
C’	-	171.82


Current Data Parameters
NAME cyclo_RGDyK_I
EXPNO 12
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160226
Time 14.36
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14005.603 Hz
FIDRES 0.213709 Hz
AQ 2.3396351 sec
RG 128
DW 35.700 usec
DE 6.50 usec
TE 308.0 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 7.80 usec
PL1 -5.30 dB
PL1W 6.38353539 W
SFO1 700.2056016 MHz
F2 - Processing parameters
SI 131072
SF 700.2000138 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
Cyclo(RGDy(Iodo)K) in DMSO at 308K.
3mm tube
2.09
2.10
2.23
4.50
2.33
9.39
2.48
2.29
12.71
2.66
2.01
2.01
2.10
4.48
2.42
6.01
11.64
2.58
2.41
2.44
14.35
4.75
12
11
10
9
8
7
6
5
4
3
2
ppm


171.82
171.52
171.10
170.54
169.93
169.45
157.84
157.66
156.61
155.02
138.93
130.21
129.81
114.61
84.14
54.51
54.38
51.84
48.81
43.22
40.26
40.00
39.50
38.62
35.89
35.04
30.62
28.49
26.41
25.11
22.42
Cyclo(RGDy(Iodo)K) in DMSO at 308K.
3mm tube
Current Data Parameters
NAME cyclo_RGDyK_I
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160229
Time 8.07
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg
TD 135586
SOLVENT DMSO
NS 19456
DS 16
SWH 42372.883 Hz
FIDRES 0.312517 Hz
AQ 1.5999148 sec
RG 8192
DW 11.800 usec
DE 6.50 usec
TE 308.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 -5.30 dB
PL1W 110.64794922 W
SFO1 176.0856775 MHz
======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -5.30 dB
PL12 12.52 dB
PL13 15.00 dB
PL2W 6.38353539 W
PL12W 0.10545355 W
PL13W 0.05957462 W
SFO2 700.2028008 MHz
F2 - Processing parameters
SI 131072
SF 176.0655299 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

[mono-Iodo-Tyr^{0}]-Bradykinin



^{1}H NMR (500 MHz, d_6-DMSO, 300 K) and ^{13}C NMR (125 MHz, d_6-DMSO, 300 K).

	^{1}H	^{13}C
Tyr-1		
NH_3^+	8.08	-
α	3.99	53.12
β	2.89/2.79	35.44
γ	-	126.90
δ1	7.51	139.39
δ2	7.00	130.71
ε1	-	84.78
ε2	6.78	114.66
ζ	-	155.84
ζ-OH	10.31	-
C′	-	167.52
Arg-2		
NH	8.66	-
α	4.49	49.98

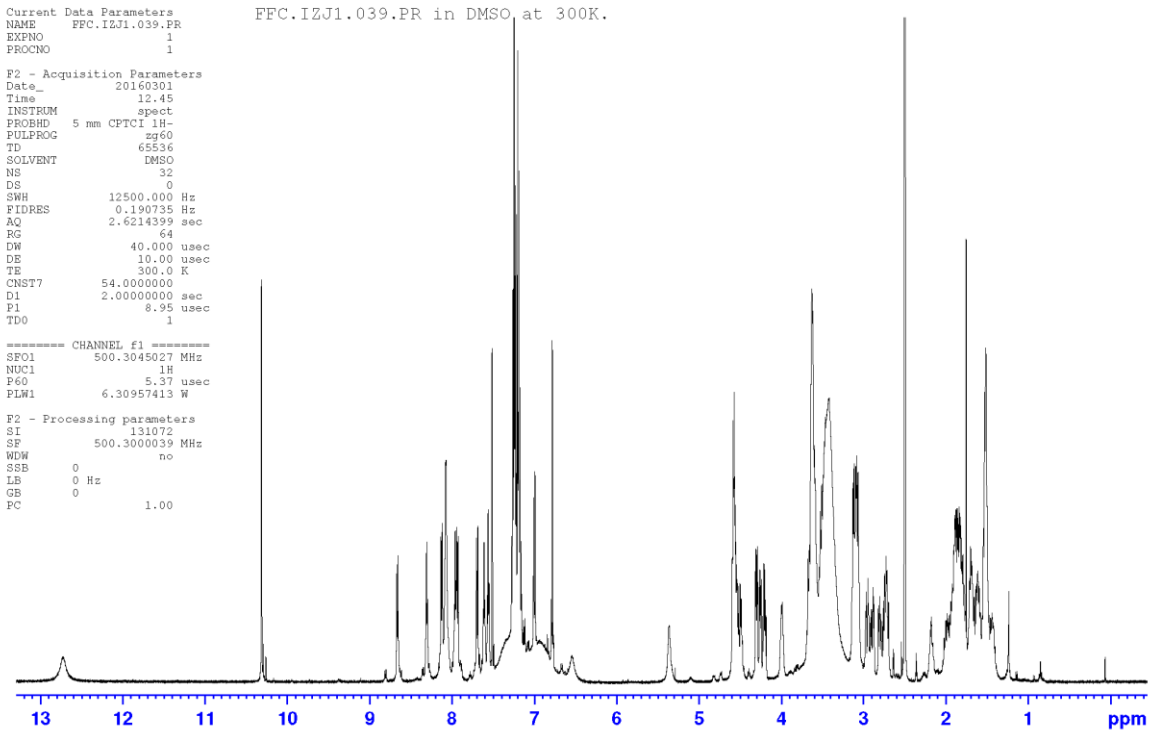
β	1.69/1.50	28.54
γ	1.53	24.41
δ	3.08	40.47
ε	7.61	-
ζ	-	156.73
C′	-	(a)
Pro-3		
α	4.58	57.63
β	2.18/1.84	27.87
γ	1.87	24.49
δ	3.60/3.48	46.90
C′	-	169.63
Pro-4		
α	4.26	59.44
β	2.00/1.81	29.05
γ	1.93/1.89	24.41
δ	3.66/3.58	46.81
C′	-	171.77
Gly-5		
NH	7.96	-
α	3.63	41.67
C′	-	168.48 (or 168.50) (a)
Phe-6		
NH	7.93	-
α	4.57	53.38
β	2.96/2.73	37.68

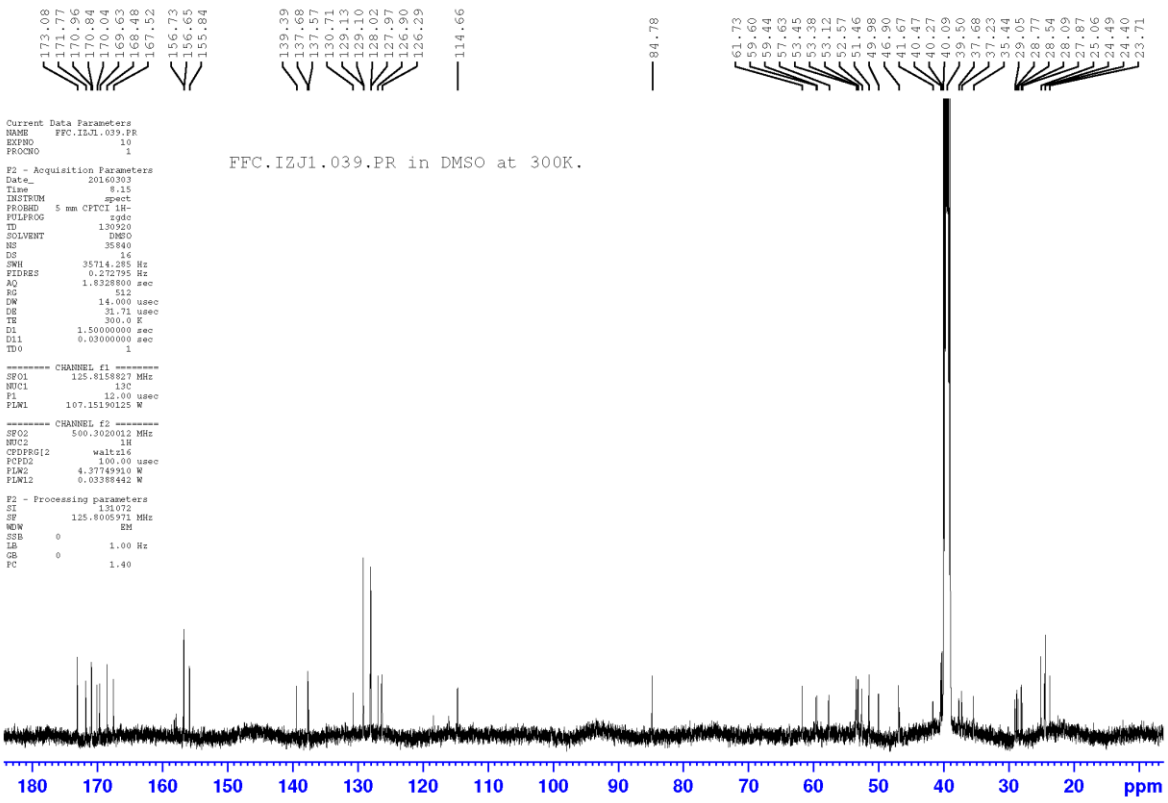
γ	-	137.57
δ	7.19	129.13
ε	7.24	128.02
ζ	7.18	126.29
C′	-	170.96 (b)
Ser-7		
NH	8.31	-
α	4.58	
β	3.63	61.73
β-OH	5.36	-
C′	-	(a)
Pro-8		
α	4.30	59.60
β	1.88/1.60	28.77
γ	1.70/1.43	23.72
δ	3.61/3.51	46.90
C′	-	170.84
Phe-9		
NH	7.70	-
α	4.54	53.45
β	3.08/2.72	37.23
γ	-	137.68
δ	7.24	129.10
ε	7.24	127.97
ζ	7.18	126.26
C′	-	170.95 (b)

Arg-10		
NH	8.13	-
α	4.20	51.46
β	1.79/1.64	28.09
γ	1.51	25.06
δ	3.12	40.27
ε	7.56	-
ζ	-	156.65
C’	-	173.08

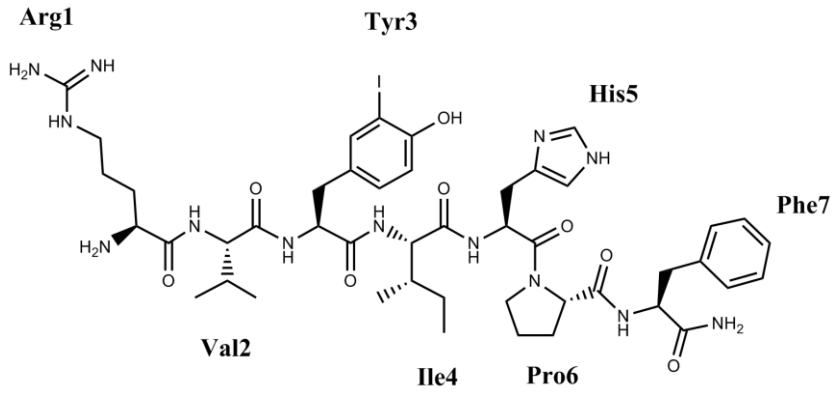
(a) Could not be assigned

(b) Might be interchanged




173.08
171.77
170.96
170.84
170.04
169.63
168.48
167.52
156.73
156.65
155.84
139.39
137.68
137.57
130.71
129.13
129.10
128.02
127.97
126.90
126.29
114.66
84.78
61.73
59.60
59.44
57.63
53.45
53.38
53.12
52.57
51.46
49.98
46.90
41.67
40.47
40.27
40.09
39.50
37.68
37.23
35.44
29.05
28.77
28.54
28.09
27.87
25.06
24.49
24.40
23.71
Current Data Parameters
NAME FFC.IZJ1.039.PR
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160303
Time 8.15
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgdc
TD 130920
SOLVENT DMSO
NS 35840
DS 16
SWH 35714.285 Hz
FIDRES 0.272795 Hz
AQ 1.8328800 sec
RG 512
DW 14.000 usec
DE 31.71 usec
TE 300.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8158827 MHz
NUC1 13C
P1 12.00 usec
PLW1 107.15190125 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 100.00 usec
PLW2 4.37749910 W
PLW12 0.03388442 W
F2 - Processing parameters
SI 131072
SF 125.8005971 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
FFC.IZJ1.039.PR in DMSO at 300K.
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

[mono-Iodo]-Angiotensin III



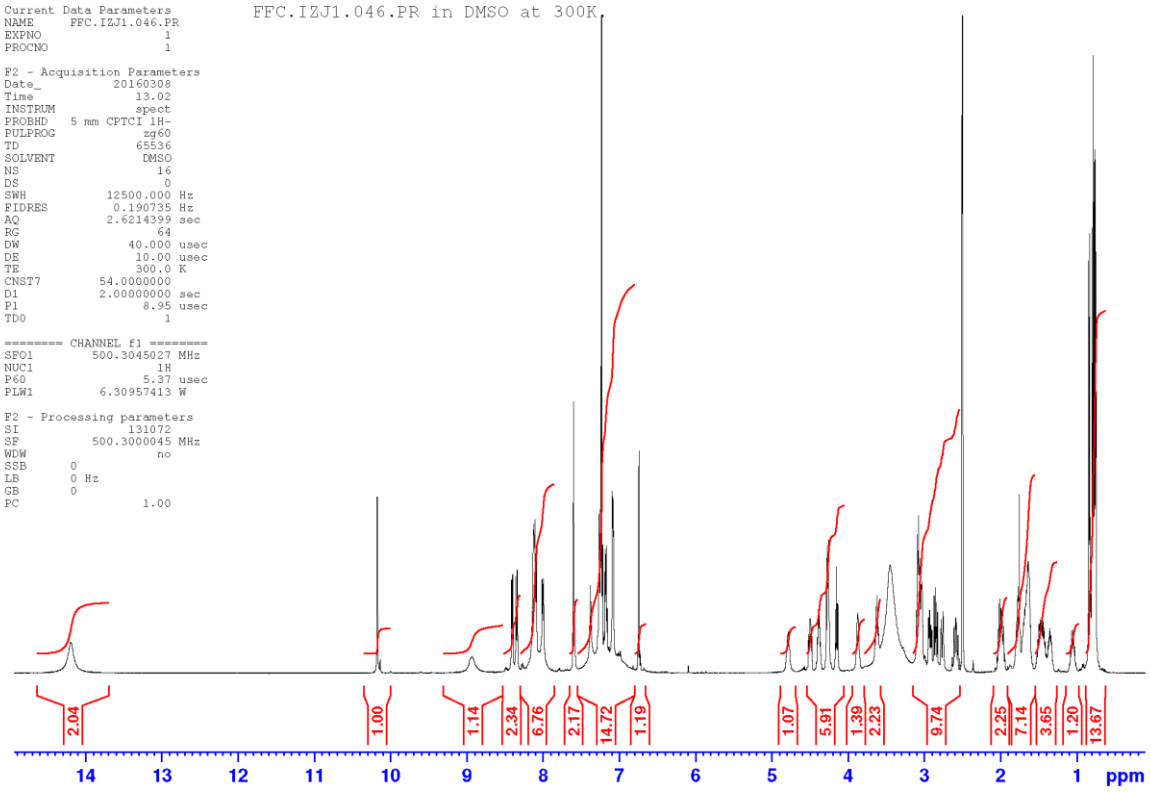
^{1}H NMR (500 MHz, d_6-DMSO, 300 K) and ^{13}C NMR (125 MHz, d_6-DMSO, 300 K).

	^{1}H	^{13}C
Arg-1		
NH_3^+	8.12	-
α	3.87	51.72
β	1.64	28.62
γ	1.46	24.28
δ	3.09	40.12
ε	7.60	-
ζ	-	156.71
C′	-	168.22
Val-2		
NH	8.35	-
α	4.27	57.12
β	2.02	31.11
γ	0.84	19.19
γ'	0.79	17.57

C’	-	170.48
Tyr-3		
NH	8.10	-
α	4.50	53.93
β	2.77/2.59	35.77
γ	-	130.29
δ1	7.60	138.97
δ2	7.09	130.36
ε1	-	84.27
ε2	6.75	114.41
ζ	-	155.02
ζ-OH	10.17	-
C’	-	170.97
Ile-4		
NH	8.00	-
α	4.15	56.65
β	1.66	36.62
β-Me	0.75	15.19
γ	1.36/1.05	24.10
δ	0.77	10.85
C’	-	170.85
His-5		
NH	8.41	-
α	4.79	~ 47.7 (broad)
β	3.05/2.93	~ 26.3 (broad)
γ	-	(a)

δ	7.38 (broad)	~ 117.1 (broad)
ε	~ 8.9 (very broad)	~ 133.8 (broad)
ε-NH_2^+	~ 14.2 (broad)	-
C′	-	(a)
Pro-3		
α	4.26	59.87
β	1.98/1.69	29.13
γ	1.77	24.25
δ	3.63/3.46	46.99
C′	-	171.43
Phe-9		
NH	~ 8.01 (very broad)	-
α	4.38	53.83
β	3.05/2.85	37.01
γ	-	137.88
δ	7.23	129.17
ε	7.23	128.00
ζ	7.17	126.20
C′	-	172.55
NH_2	7.26/7.09	-

(a) Could not be assigned due to extreme line broadening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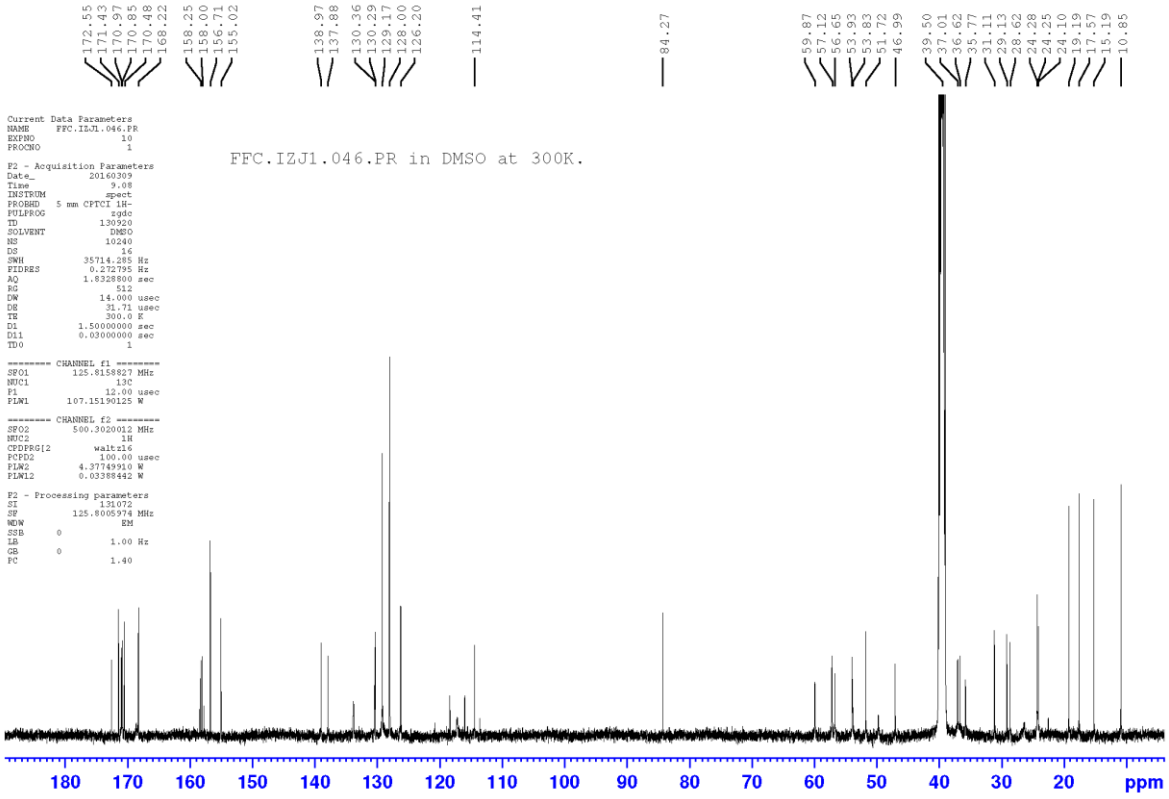
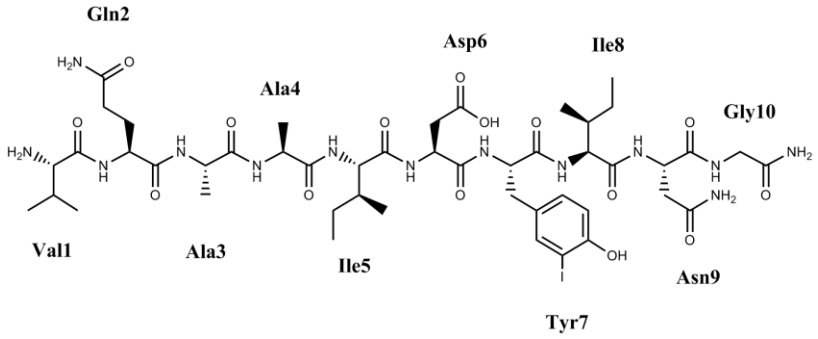
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160308
Time 13.02
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 0
SWH 12500.000 Hz
FIDRES 0.190735 Hz
AQ 2.6214399 sec
RG 64
DW 40.000 usec
DE 10.00 usec
TE 300.0 K
CNST7 54.0000000
D1 2.00000000 sec
P1 8.95 usec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3045027 MHz
NUC1 1H
P60 5.37 usec
PLW1 6.30957413 W
F2 - Processing parameters
SI 131072
SF 500.3000045 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
FFC.IZJ1.046.PR in DMSO at 300K
2.04
1.00
1.14
2.34
6.76
2.17
14.72
1.19
1.07
5.91
1.39
2.23
9.74
2.25
7.14
3.65
1.20
13.67
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm


172.55
171.43
170.97
170.85
170.48
168.22
158.25
158.00
156.71
155.02
138.97
137.88
130.36
130.29
129.17
128.00
126.20
114.41
84.27
59.87
57.12
56.65
53.93
53.83
51.72
46.99
39.50
37.01
36.62
35.77
31.11
29.13
28.62
24.28
24.25
24.10
19.19
17.57
15.19
10.85
Current Data Parameters
NAME FFC.IZJ1.046.PR
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160309
Time 9.08
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgdc
TD 130920
SOLVENT DMSO
NS 10240
DS 16
SWH 35714.285 Hz
FIDRES 0.272795 Hz
AQ 1.8328800 sec
RG 512
DW 14.000 usec
DE 31.71 usec
TE 300.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8158827 MHz
NUC1 13C
P1 12.00 usec
PLW1 107.15190125 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 100.00 usec
PLW2 4.37749910 W
PLW12 0.03388442 W
F2 - Processing parameters
SI 131072
SF 125.8005974 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
FFC.IZJ1.046.PR in DMSO at 300K.
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

[mono-Iodo]-ACP fragment 65-74

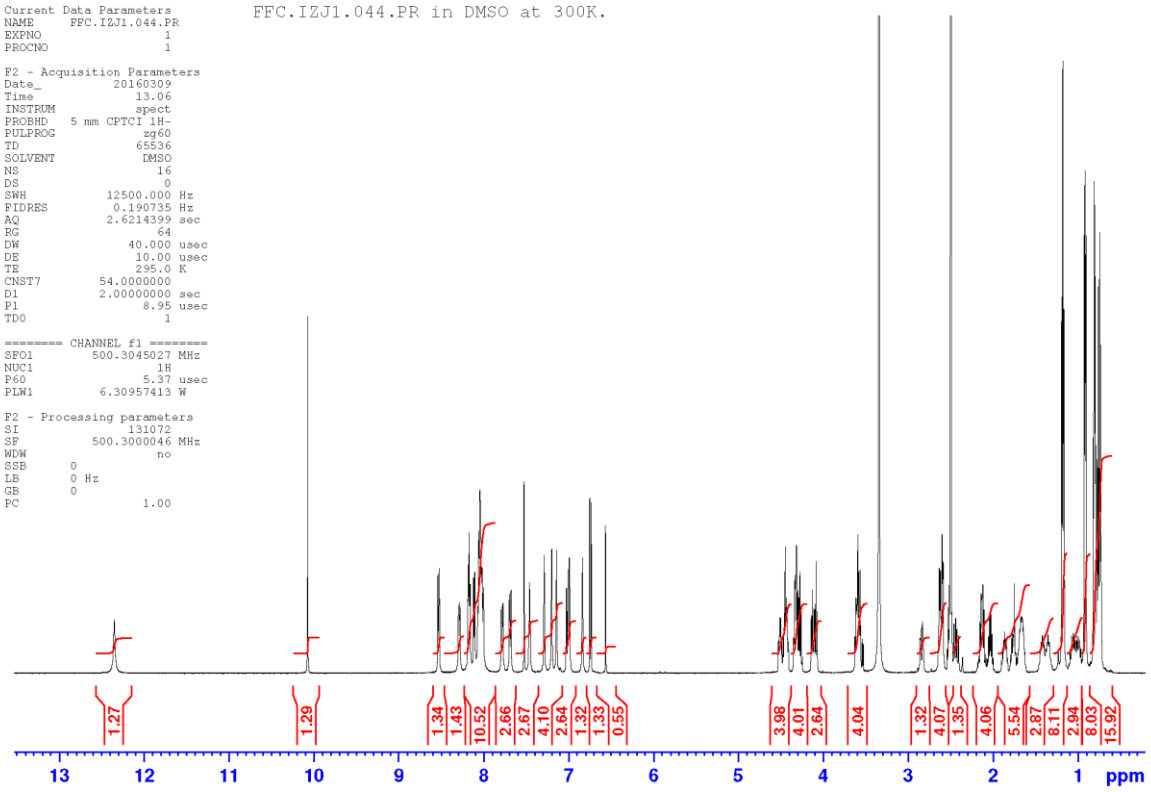


^{1}H NMR (500 MHz, d_6-DMSO, 295 K) and ^{13}C NMR (125 MHz, d_6-DMSO, 295 K).

		^{1}H	^{13}C
Val-1	NH_3^+	8.04	-
	α	3.60	57.25
	β	2.03	29.89
	γ	0.92	18.33
	γ′	0.91	17.70
	C′	-	167.63
Gln-2	NH	8.53	-
	α	4.33	52.07
	β	1.87/1.76	28.02
	γ	2.13	31.29
	δ	-	173.73
	δ-NH_2	7.29/6.84	-
	C′	-	170.29
Ala-3	NH	8.18	-
	α	4.28	47.97
	β	1.18	18.22
	C′	-	171.86

Ala-4	NH	8.11	-
	α	4.32	48.02
	β	1.17	17.79
	C′	-	171.91
Ile-5	NH	7.69	-
	α	4.13	56.60
	β	1.65	36.99
	β-Me	0.73	15.27
	γ	1.35/1.00	24.01
	δ	0.76	11.11
	C′	-	170.60
Asp-6	NH	8.17	-
	α	4.51	49.54
	β	2.62/2.43	36.27
	γ	-	171.65
	C′	-	170.47

The amide protons of Ala3 and Asp6 overlap at 300K.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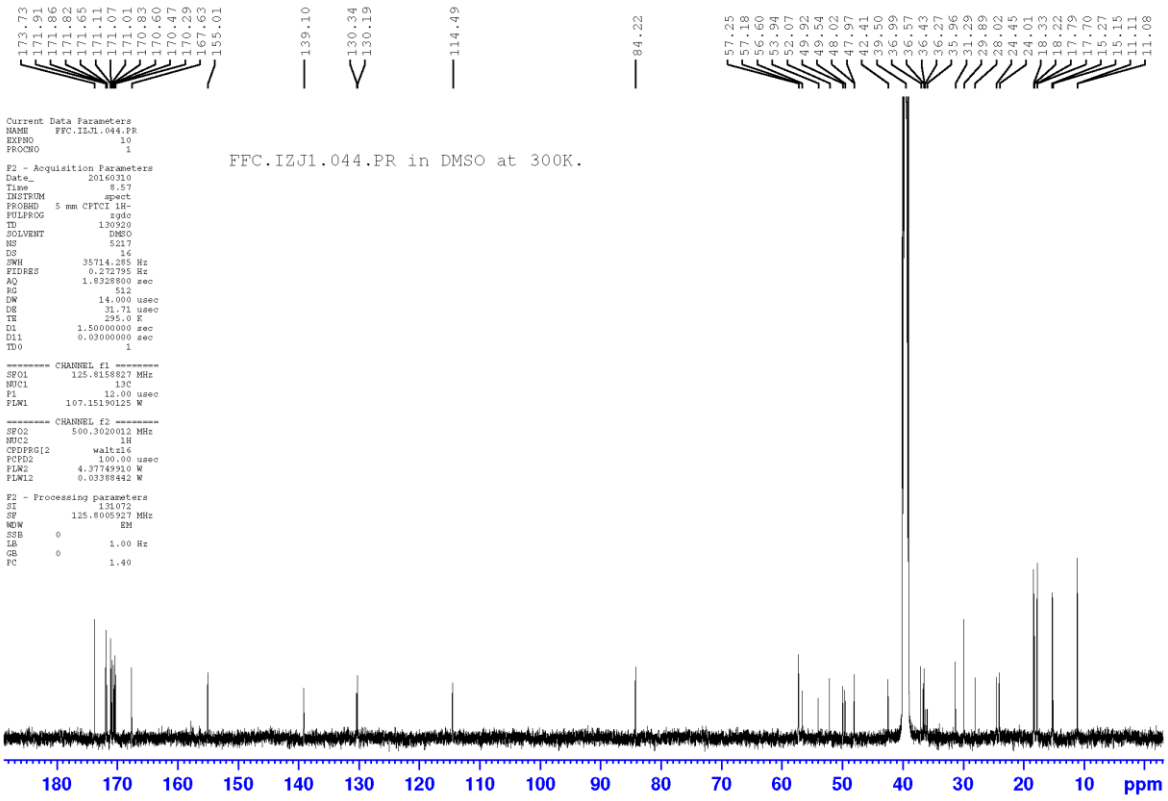
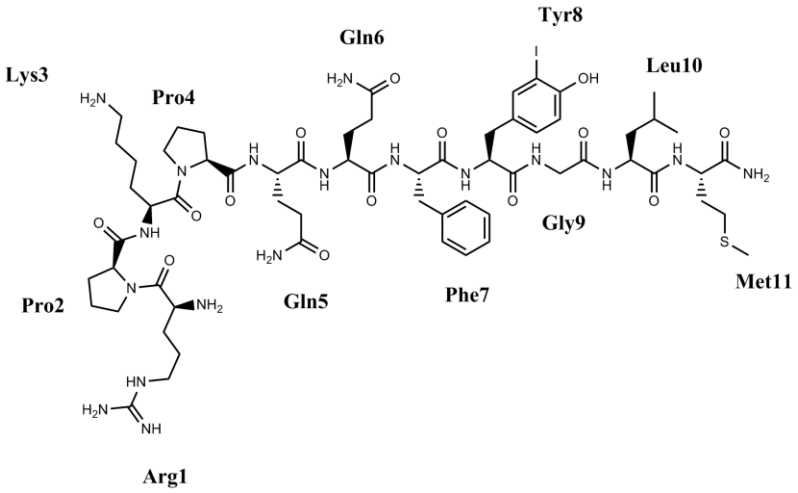
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160309
Time 13.06
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 0
SWH 12500.000 Hz
FIDRES 0.190735 Hz
AQ 2.6214399 sec
RG 64
DW 40.000 usec
DE 10.00 usec
TE 295.0 K
CNST7 54.0000000
D1 2.00000000 sec
P1 8.95 usec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3045027 MHz
NUC1 1H
P60 5.37 usec
PLW1 6.30957413 W
F2 - Processing parameters
SI 131072
SF 500.3000046 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
FFC.IZJ1.044.PR in DMSO at 300K.
1.27
1.29
1.34
1.43
10.52
2.66
2.67
4.10
2.64
1.32
1.33
0.55
3.98
4.01
2.64
4.04
1.32
4.07
1.35
4.06
5.54
2.87
8.11
2.94
8.03
15.92
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm


173.73
171.91
171.86
171.82
171.65
171.11
171.07
171.01
170.83
170.60
170.47
170.29
167.63
155.01
139.10
130.34
130.19
114.49
84.22
57.25
57.18
56.60
53.94
52.07
49.92
49.54
48.02
47.97
42.41
39.50
36.99
36.57
36.43
36.27
35.96
31.29
29.89
28.02
24.45
24.01
18.33
18.22
17.79
17.70
15.27
15.15
11.11
11.08
Current Data Parameters
NAME FFC.IZJ1.044.PR
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160310
Time 8.57
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgdc
TD 130920
SOLVENT DMSO
NS 5217
DS 16
SWH 35714.285 Hz
FIDRES 0.272795 Hz
AQ 1.8328800 sec
RG 512
DW 14.000 usec
DE 31.71 usec
TE 295.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8158827 MHz
NUC1 13C
P1 12.00 usec
PLW1 107.15190125 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 100.00 usec
PLW2 4.37749910 W
PLW12 0.03388442 W
F2 - Processing parameters
SI 131072
SF 125.8005927 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
FFC.IZJ1.044.PR in DMSO at 300K.
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

[mono-Iodo-Tyr^{8}]-Substance P



^{1}H NMR (700 MHz, d_6-DMSO, 295 K) and ^{13}C NMR (175 MHz, d_6-DMSO, 295 K).

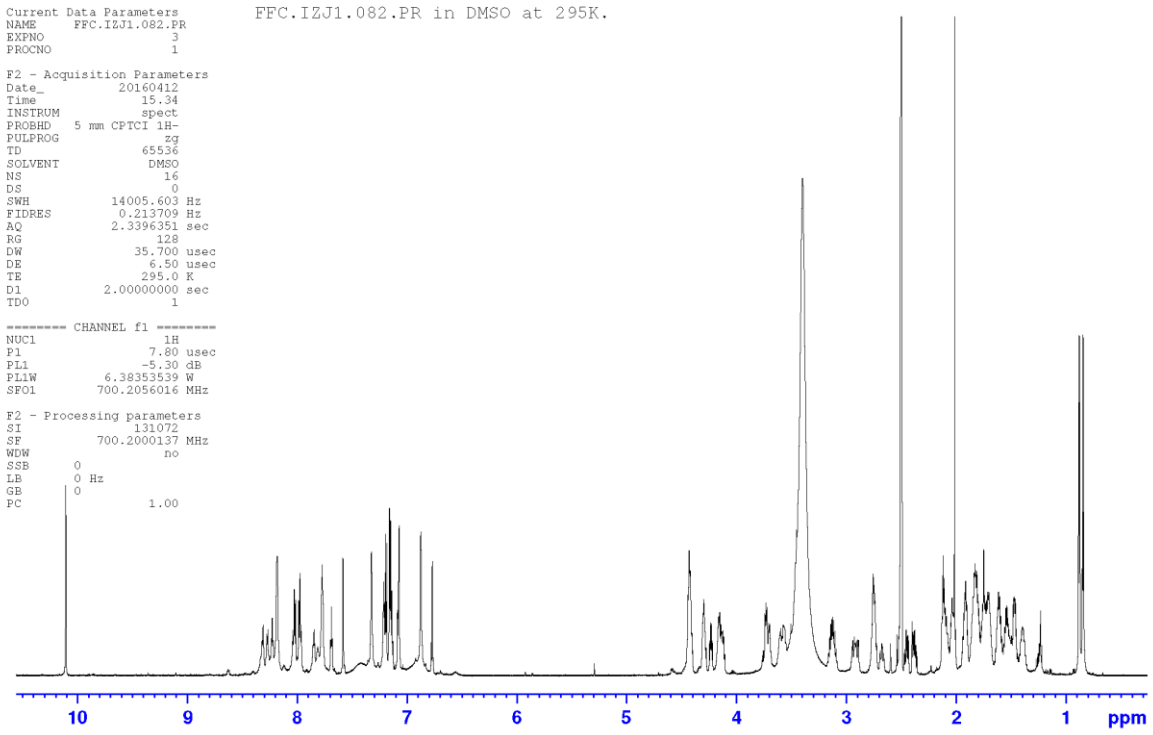
		^{1}H	^{13}C
Arg-1	NH_3^+	8.18	-
	α	4.16	50.48 (a)
	β	1.76/1.70	27.11
	γ	1.62	23.62
	δ	3.13	40.22
	ε	7.69	-
	ζ	-	156.79
	C’	-	166.84
Pro-2	α	4.43	59.24
	β	2.11/1.75	29.22
	γ	1.91/1.83	24.66
	δ	3.70/3.46	47.04
	C’	-	170.98
Lys-3	NH	8.31	-
	α	4.43	50.43 (a)

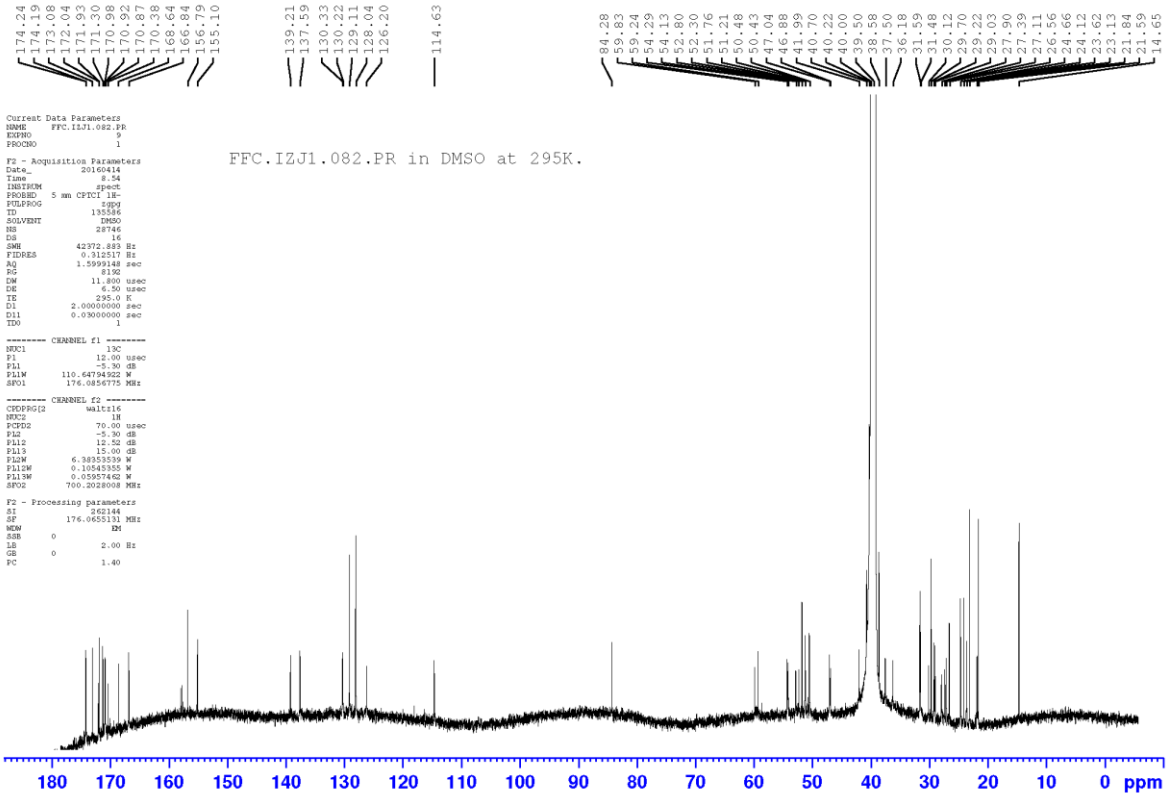
	β	1.70/1.51	30.12
	γ	1.39	21.84
	δ	1.54	26.56
	ε	2.75	38.58
	ζ	7.77	-
	C′	-	170.38
Pro-4	α	4.30	59.83
	β	2.09/1.82	29.03
	γ	1.92/1.85	24.66
	δ	3.60/3.57	46.88
	C′	-	172.04
Gln-5	NH	8.27	-
	α	4.12	52.80
	β	1.85/1.73	27.39
	γ	2.12	31.48
	δ	-	174.24
	NH_2	7.32/6.87	-
	C′	-	174.24
Gln-6	NH	7.85	-
	α	4.15	52.30
	β	1.81/1.71	27.90
	γ	2.04	31.57
	δ	-	174.19
	NH_2	7.33/6.87	-
	C′	-	170.87
Phe-7	NH	7.97	-

	α	4.42	54.13
	β	2.93/2.74	37.50
	γ	-	137.59
	δ	7.15	129.11
	ε	7.19	128.04
	ζ	7.14	126.20
	C’	-	170.92
Tyr8	NH	8.03	-
	α	4.42	54.29
	β	2.90/2.67	36.18
	γ	-	130.22
	δ1	7.58	139.21
	δ2	7.08	130.33
	ε1	-	84.28
	ε2	6.77	114.63
	ζ	-	155.10
	ζ-OH	10.09	-
	C’	-	171.30
Gly-9	NH	8.23	-
	α	3.73	41.99
	C’	-	168.64
Leu-10	NH	8.02	-
	α	4.29	51.21
	β	1.47	40.70
	γ	1.61	24.12
	δ	0.88	23.13

	δ′	0.84	21.59
	C′	-	171.93
Met-11	NH	7.98	-
	α	4.23	51.76
	β	1.92/1.81	31.59
	γ	2.45/2.38	29.70
	δ	2.02	14.65
	C′	-	173.08
	NH_2	7.21/7.07	-

(a) Might be interchanged





Supporting References

1. J. M. Collins, K. A. Porter, S. K. Singh and G. S. Vanier, *Organic letters*, 2014, **16**, 940-943.
2. J. M. Chalker, C. S. Wood and B. G. Davis, *J. Am. Chem. Soc.*, 2009, **131**, 16346-16347.
3. V. Hong, S. I. Presolski, C. Ma and M. Finn, *Angewandte Chemie International Edition*, 2009, **48**, 9879-9883.