# Supporting Information

## Solid-Phase Synthesis and Fluorine-18 Radiolabeling of CycloRGDyK

Ryan A. Davis,[a-c] Kevin Lau,[a,b] Sven H. Hausner,[a-c] and Julie L. Sutcliffe[a-d*]

Radiochemistry Research and Training Facility,[a] Department of Biomedical Engineering,[b] Department of Internal Medicine, Division of Hematology and Oncology,[c] and Center for Molecular and Genomic Imaging,[d]
University of California, Davis, 2921 Stockton Blvd. Sacramento, CA 95817, USA

Email: jlsutcliffe@ucdavis.edu

## Contents

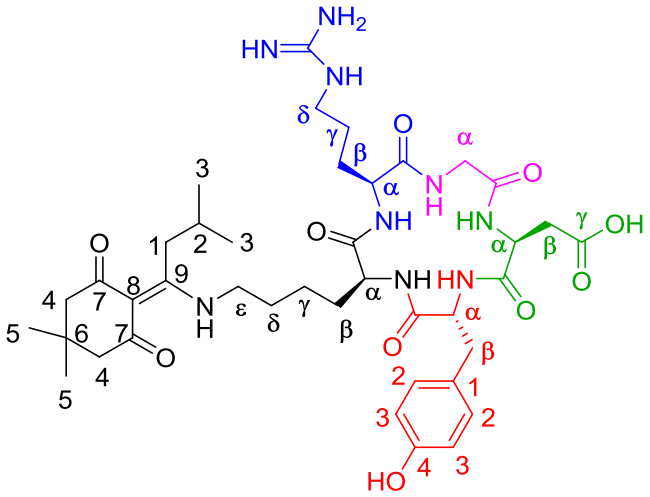
***General Experimental***. All reactions were conducted under ambient temperature and atmosphere. Solvents including *N*,*N*-dimethylformamide (DMF) 99.9%, dichloromethane ($CH_2Cl_2$) 99.9%, and methanol 99.8% were purchased as reagent grade and were used without further purification unless otherwise denoted. All other chemical reagents were commercial grade and used without further purification. NMR experiments were conducted on an 800 MHz instrument and chemical shifts are reported in parts per million (ppm) relative to the deuterated solvent peak water 4.79 ppm. High resonance mass spectrometry samples were analyzed either by electrospray ionization mass spectrometry in positive mode using flow-injection analysis. All HPLC purification and analysis was carried out using an analytical column Proteo-Jupiter-C12-column, dimensions: 250 mm x 4.6 mm x 4 micron or on a semi-prep column Proteo-Jupiter-C12-column, dimensions 250 mm x 10 mm x 10 micron. Semi-preparative HPLC for radioactive peptide was carried out on a Proteo-Jupiter-C18-column, dimensions 250 mm x 10 mm x 10 micron. The HPLC loop size was a 2 mL loop and the flow rate of 1.5 mL/min was used for analytical columns and the flow rate of 3.0 mL/min was used for semi-preparative HPLC columns. The HPLC is equipped with a UV detector and monitored at two wavelengths 220 nm and 254 nm followed by a gamma detector (~0.25 minutes apart) to monitor radioactivity. The mobile phase for HPLC runs are presented in **Table 1** and **Table 2** on the following page. **Table 1** was used for every compound except compound **4** which used the gradient system provided in **Table 2**.

**Table 1**. Standard HPLC Solvent Gradient.

| Standard Solvent Gradient for HPLC | | |
|---|---|---|
| Time (minutes) | %Acetonitrile | % Water with 0.05% TFA |
| 0 | 9% | 91% |
| 2 | 9% | 91% |
| 32 | 81% | 19% |

**Table 2**. HPLC Solvent Gradient for Compound **4**.

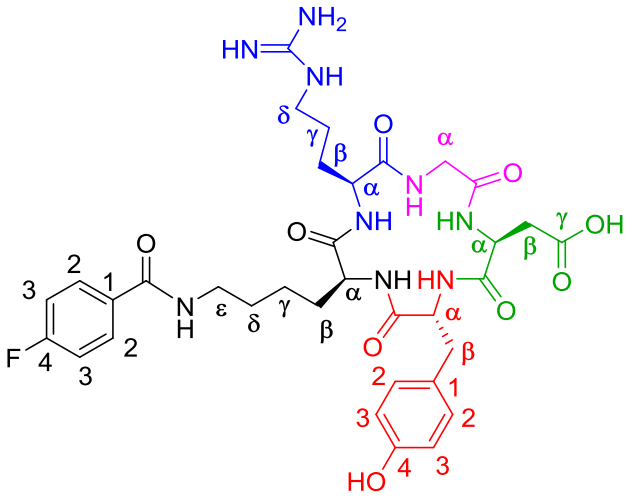
| HPLC Solvent Gradient for Compound **4** | | |
|---|---|---|
| Time (minutes) | %Acetonitrile | % Water with 0.05% TFA |
| 0 | 0% | 100% |
| 2 | 0% | 100% |
| 7 | 10% | 90% |
| 10 | 10% | 90% |
| 15 | 20% | 80% |
| 20 | 20% | 80% |
| 35 | 81% | 19% |



(**7**)

**cRGDyK(ivDde) (7).** Linear peptide sequence yKRGD was built using standard Fmoc chemistry on apartic acid preloaded via its side chian to NovaSynTGA resin (400 mg, Loading capacity 0.19 mmol/g). The aspartic acid carboxylate was protected as an allyl ester, while the side chain of arganine was protected with a Pbf group, lysine with an ivDde group, and tyrosine as a *t*-butyl ether. Each coupling was perfomed with 3 equivalents of amino acid (0.228 mmol) and 2.8 equivalents of dimethylamino(triazolo[4,5-b]pyridin-3-yloxy)methylidene]-dimethylazanium hexafluorophosphate (HATU) (0.213 mmol, 80 mg) in the presence of 6 equivalents of diisopropylethylamine (DIPEA) (0.456 mmol, 80 uL) in 3 mL of DMF for 2 hours. Between each coupling the reaction solution was removed and the resin was washed three times with DMF, followed by removal of Fmoc for 7 minutes using 20% piperidine in DMF (3 mL). After the removal of Fmoc, the resin was rinsed three times with DMF, three times with methanol, and again three more times with DMF. Upon completion of the fully protected linear sequence, the allyl ester was converted to the free carboxylic acid using tetrakis(triphenylphosphine)palladium(0) (0.5 equiv) and triphenylsilane (20 equiv) in dichloromethane for 1 hour. Then the palladium solution was removed and the resin was washed with DMF three times, followed by 5% diethylthiocarbamic acid solution in DMF (5 x 2 mL), 1% DIPEA in DMF (3 x 1 mL), and finally again with 100% DMF three times, to remove any residual palladium metal. Upon removal of the allyl group, the *N*-terminal Fmoc was removed using 20% piperidine in DMF ( 3 mL) for 7 minutes. Cyclization was then carried out with a single equivalent of HATU in the presence of 2 equivalents of DIPEA. Several scales of cyclization was perfromed (10mg, 30mg, 50mg, and 100 mg). The reaction time for smaller amounts of resin 10 mg was complete after 5 minutes, while 30 mg and 50 mg reactions took between 10-15 minutes. The larger scale cyclizations using 100 mg of resin needed a time of 30 minutes to reach completeion.

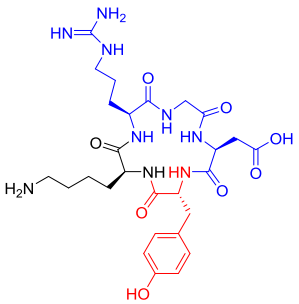
Once cyclized on resin, the resin was rinsed with DMF (3x) followed by methanol (3x), and finally again with DMF (3x). The resin was then dried under vacuum for 1 hour and the peptide was either stored or deprotected and cleaved from the resin using TIPS (75 uL), water (75 uL), and TFA ( 3 mL) for 3 hours. The TFA solution was evaporated by steady flow of air over the solution. HPLC purification was carried out using reverse stationary phase on a semiprepartive-HPLC column using the gradient solvent system in **Table 1**. The flow rate was 3.0 mL/min and gave a HPLC retention time of the product of 18.64 minutes. The purified compound was analyzed by HPLC with an anlytical column at a flow rate of 1.5 mL/min using the same gradient solvent system in **Table 1**, which afforded a HPLC retention time of 15.39 min for the product. Upon lyophilization the material was obtained as a white foam 90% ( 4.5 mg, 0.0054 mmol from a scale of 32 mg resin (theoretical yield 0.006 mmol, 5 mg). The NMR chemical shifts were references to the deterium oxide peak for the proton NMR being set at 4.79 ppm or TSP in the carbon where the trimethylsilyl protons were set to 0 ppm. $^{1}$H NMR (800 $MH_Z$, $D_2O$): δ 0.85-0.98 (m, Lys-γ, 2H), 0.94 (dd, *J* = 2.6, 6.6 Hz, ivDde-3, 6H), 1.00 (s, ivDde-5, 6H), 1.45-1.51 (m, Lys-β, Arg-γ, Lys-δ, 5H), 1.61-1.65 (m, Arg-β, 1H), 1.66-1.73 (m, Lys-β', 1H), 1.80-1.89 (m, ivDde-2, Arg-β', 2H), 2.39 (s, ivDde-4, 4H), 2.51 (dd, *J* = 7.4, 15.5 Hz, Asp-β, 1H), 2.65 (dd, *J* = 7.4, 15.5 Hz, Asp-β', 1H), 2.83 (app. t, *J* = 12.8 Hz, Tyr-β, 1H), 2.95 (dd, *J* = 9.3, 12.8 Hz, ivDde-1, 1H), 3.00 (dd, *J* = 5.6, 12.8 Hz, Tyr-β', 1H), 3.02 (dd, *J* = 9.3, 12.8 Hz, ivDde-1', 1H), 3.13-3.20 (m, Arg-δ, 2H), 3.44 (d, *J* = 14.6 Hz, Gly-α, 1H), 3.44-3.51 (m, Lys-ε, 2H), 3.85 (dd, *J* = 3.6, 11.3 Hz, Lys-α, 1H), 4.20 (d, *J* = 14.6 Hz, Gly-α', 1H), 4.40 (dd, *J* = 6.1, 8.6 Hz, Arg-α, 1H), 4.49 (dd, *J* = 5.6, 11.2 Hz, Tyr-α, 1H), 4.68 (app. t, *J* = 7.4 Hz, Asp-α, 1H), 6.78 (d, *J* = 8.4 Hz, Tyr-aromatic-3, 2H), 7.10 (d, *J* = 8.4 Hz, Tyr-aromatic-2, 2H). $^{13}$C NMR (200 MHz, $D_2O$): δ 203.8 (ivDde-CO-7), 180.7 (ivDde-C=C-9), 177.1 (Asp-CO-γ), 176.6 (Lys-CO), 175.4 (Tyr-CO), 175.3 (Asp-CO), 174.1 (Arg-CO), 159.5 (Gly-CO), 157.4 (Arg-C=NH), 133.3 (Tyr-aromatic-2), 130.4 (Tyr-aromatic-4), 119.9 (Tyr-aromatic-1), 118.3 (Tyr-aromatic-3), 110.2 (ivDde-C=C-8), 58.6 (Tyr-α), 57.9 (Lys-α), 55.0 (Arg-α), 54.6 (ivDde-4), 53.7 (Asp-α), 46.6 (Gly-α), 46.4 (Lys-ε), 43.3 (Arg-δ), 40.7 (Asp-β), 40.2 (ivDde-1), 38.7 (Tyr-β), 32.7 (Lys-β), 32.3 (ivDde-2), 31.6 (Arg-β), 30.3 (Lys-δ), 30.2 (ivDde-6), 29.8 (ivDde-5), 27.1 (Arg-γ), 25.5 (Lys-γ), 24.5 (ivDde-3), 24.46 (ivDde-3'). HRMS (ESI-Ion Trap) *m/z*: $[M + H]^+$ calcd for $C_{40}H_{90}N_9O_{10}$ 826.4458; found 826.4460.



(**9**)

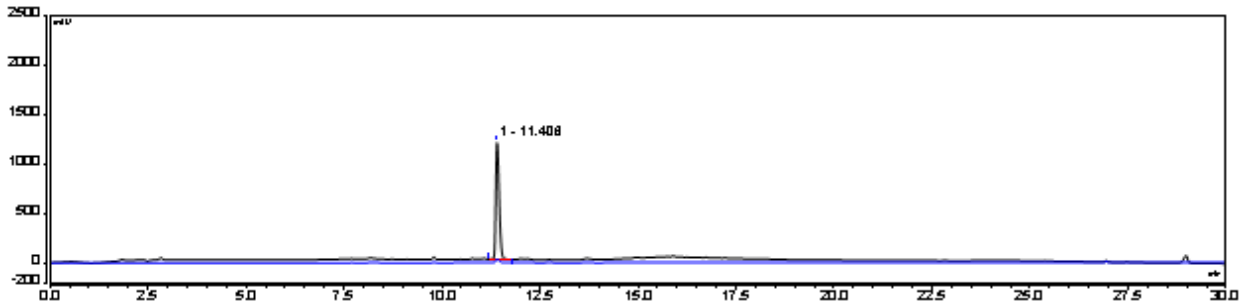
**cRGDyK([$^{19}$F]FBA) (9).** After cyclizing the peptide's lysine ivDde protecting group was removed using 2%-hydrazine in DMF (20 uL $NH_2NH_2$, 980 uL of DMF) for 1 hour. The reaction solution was removed and the resin rinsed with DMF (3x), methanol (3x), and again with DMF (3x). The cold standard was then prepared by coupling 4-[$^{19}$F]-fluorobenzoic acid (FBA) (10 equiv, 0.095 mmol, 15 mg), HATU (9.5 equiv, 0.09 mmol, 34 mg), and DIPEA (30 equiv, 0.285 mmol, 50 uL) for 1 hour and 30 minutes. The 4-[$^{19}$F]-fluorobenzoic acid solution was removed and the resin was rinsed with DMF (3x), methanol (3x), and again with DMF (3x). The resin was dried under vacuum for 1 hour and then the peptide was deprotected and cleaved from the resin using TIPS (25 uL), water (25 uL), and TFA ( 1 mL) for 3 hours. The TFA solution was evaporated by steady flow of air over the solution. HPLC purification was carried out using reverse phase analytical column and the gradient solvent system found in **Table 1**. The flow rate was 1.5 mL/min which afforded a HPLC retention time of 12.93 min for the product. Upon lyophilization the material was obtained as a white powder in 80% (5.6 mg, 0.0076 mmol from a scale of 50 mg resin (theoretical yield 0.0095 mmol, 7 mg). The NMR chemical shifts were references to the deterium oxide peak for the proton NMR being set at 4.79 ppm or TSP in the carbon where the trimethylsilyl protons were set to 0 ppm. $^1$H NMR (800 $MH_Z$, $D_2O$): δ 0.87-0.89 (m, Lys-γ, 1H), 0.97-0.98 (m, Lys-γ', 1H), 1.45-1.49 (m, Lys-β, Lys-δ, Arg-γ, 5H), 1.60-1.61 (m, Arg-β, 1H), 1.66-1.67 (m, Lys-β', 1H), 1.82-1.84 (m, Arg-β', 1H), 2.51 (dd, *J* = 7.2, 15.5 Hz, Asp-β, 1H), 2.65 (dd, *J* = 7.2, 15.5 Hz, Asp-β', 1H), 2.82 (app. t, *J* = 12.8 Hz, Tyr-β, 1H), 3.00 (dd, *J* = 5.0, 12.8 Hz, Tyr-β', 1H), 3.05-3.09 (m, Arg-δ, 1H), 3.10-3.13 (m, Arg-δ', 1H), 3.26-3.31 (m, Lys-ε, 2H), 3.44 (d, *J* = 14.6 Hz, Gly-α, 1H), 3.81 (dd, *J* = 4.0, 10.4 Hz, Lys-α, 1H), 4.19 (d, *J* = 14.6 Hz, Gly-α', 1H), 4.39 (dd, *J* = 5.9, 8.5 Hz, Arg-

α, 1H), 4.47 (dd, *J* = 5.0, 10.8 Hz, Tyr-α, 1H), 4.68 (app. t, *J* = 7.2 Hz, Asp-α, 1H), 6.77 (d, *J* = 8.2 Hz, Tyr-aromatic-3, 2H), 7.06 (d, *J* = 8.2 Hz, Tyr-aromatic-2, 2H), 7.23 (app. t, *J* = 8.6 Hz, FBA-3, 2H), 7.79 (dd, *J* = 5.3, 8.6 Hz, FBA-2, 2H). $^{13}$C NMR (200 MHz, $D_2O$): δ 177.3 (Asp-CO-γ), 176.4 (Lys-CO), 175.3 (FBA-CO), 174.1 (Tyr-CO), 173.9 (Asp-CO), 172.6 (Arg-CO), 163.1 (FBA-4), 159.4 (Gly-CO), 157.3 (Arg-C=NH), 133.3 (Tyr-aromatic-2), 132.8 (FBA-1), 132.4 (FBA-2), 132.3 (Tyr-aromatic-4), 118.6 (FBA-3), 118.5 (Tyr-aromatic-1), 118.3 (Tyr-aromatic-3), 58.6 (Tyr-α), 58.2 (Lys-α), 55.0 (Arg-α), 53.6 (Asp-α), 46.6 (Gly-α), 43.2 (Arg-δ), 42.1 (Lys-ε), 40.6 (Asp-β), 38.7 (Tyr-β), 32.7 (Lys-β), 30.5 (Lys-δ), 30.2 (Arg-β), 27.0 (Arg-γ), 25.4 (Lys-γ). HRMS (ESI-Ion Trap) *m/z*: $[M + H]^+$ calcd for $C_{34}H_{45}FN_9O_9$ 742.3319; found 742.3319.



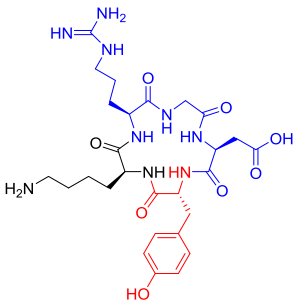

**4**-cRGDyK

HPLC Trace of crude **4**.



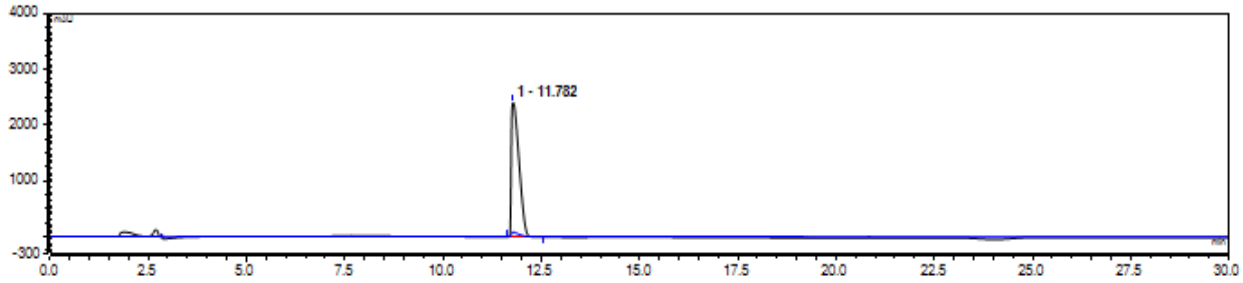

Retention Time:  11.41 minutes

Black 220 nm and Blue 254 nm




**4**-cRGDyK

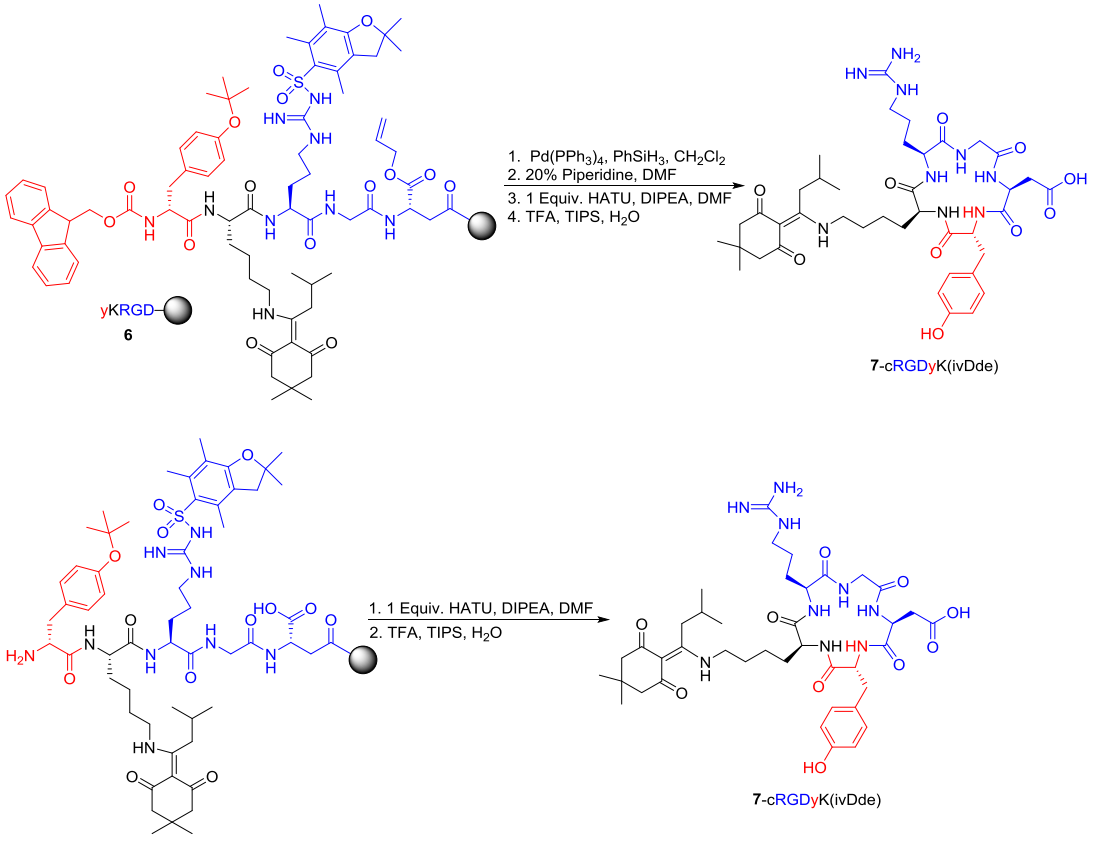
HPLC Trace of purified **4**.



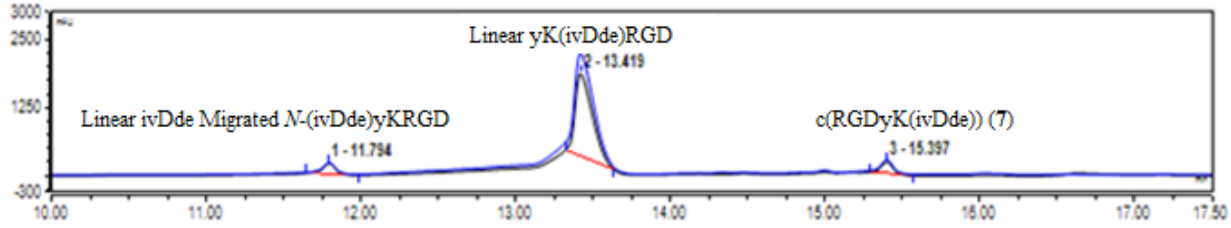

Retention Time: 11.78 minutes

Black 220 nm and Blue 254 nm

Cyclization Kinetics performed on 2 mg resin scale per time point




Time = 2 minutes



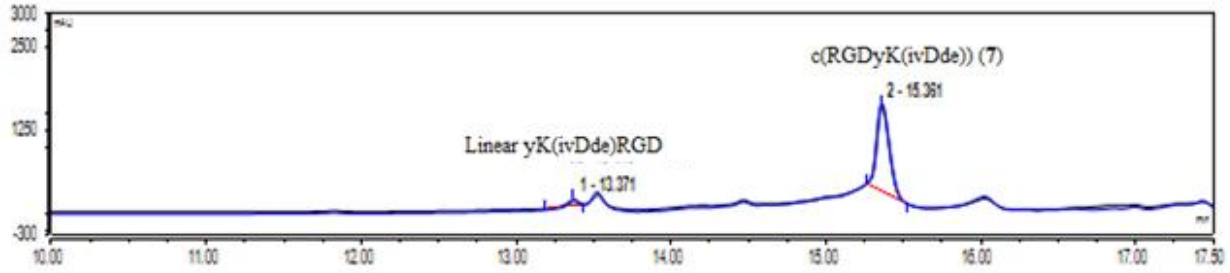

Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.79 minutes, 7.7%.

Retention Time for Linear yK(ivDde)RGD was 13.42 minutes, 84.7%.

Retention Time for cRGDyK(ivDde) (**7**) was 15.4 minutes, 7.6%.

Time = 5 minutes

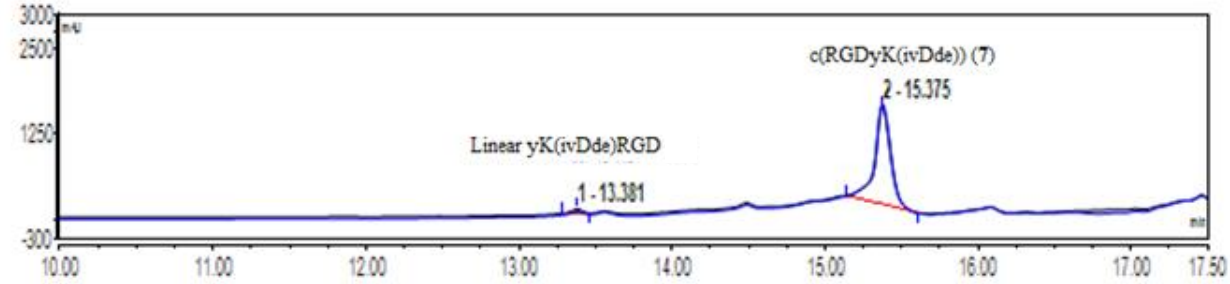



Black 220 nm and Blue 254 nm

Retention Time for Linear yK(ivDde)RGD was 13.37 minutes, 5.7%.

Retention Time for cRGDyK(ivDde) (**7**) was 15.36 minutes, 94.3%.

Time = 15 minutes

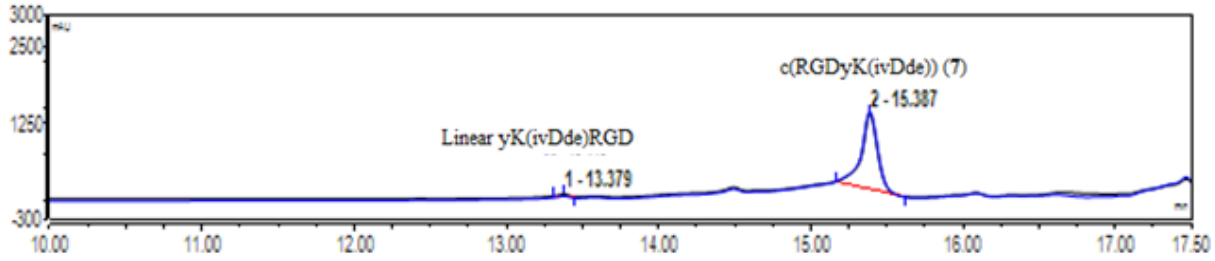



Black 220 nm and Blue 254 nm

Retention Time for Linear yK(ivDde)RGD was 13.38 minutes, 2.9%.

Retention Time for cRGDyK(ivDde) (**7**) was 15.38 minutes, 97.1%.

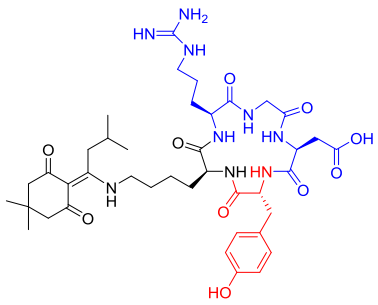
Time = 20 minutes




Black 220 nm and Blue 254 nm

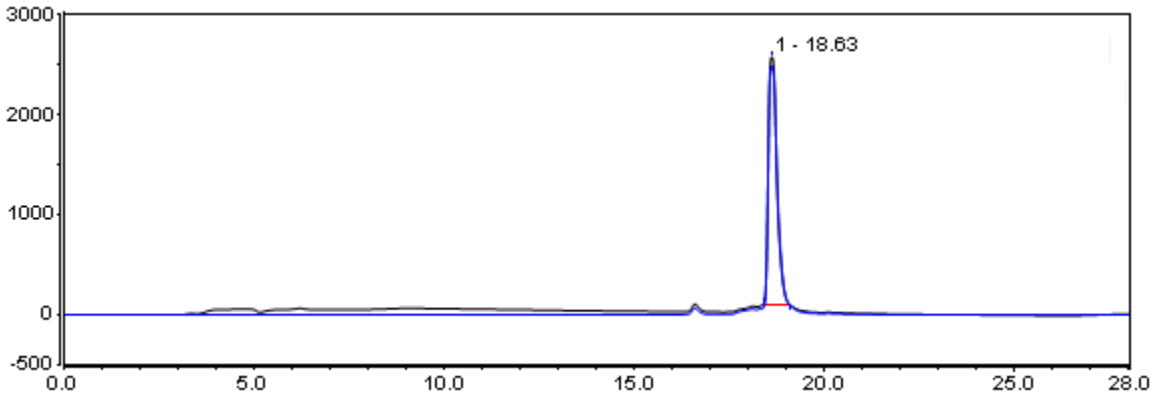
Retention Time for Linear yK(ivDde)RGD was 13.38 minutes, 1.6%.

Retention Time for cRGDyK(ivDde) (**7**) was 15.39 minutes, 98.4%.



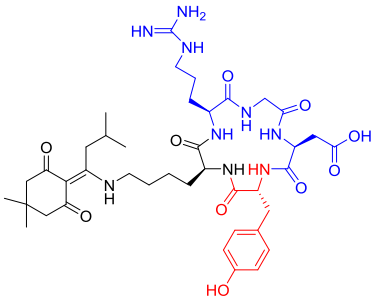
**7**-cRGDyK(ivDde)

HPLC Trace of crude **7** on large scale semi-preparative HPLC column.



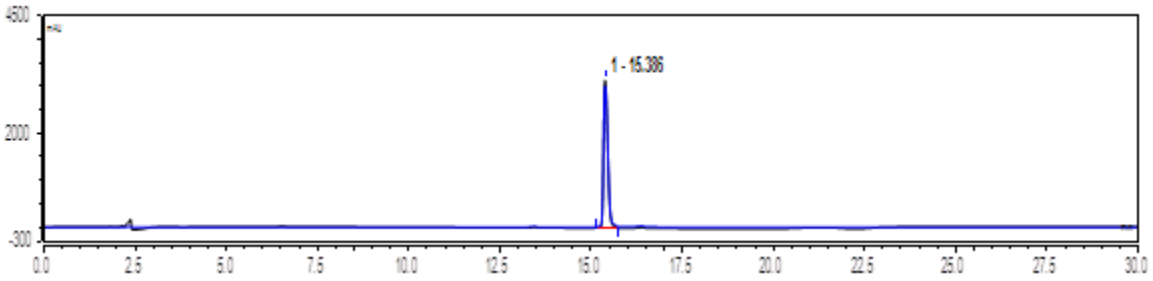

Black 220 nm and Blue 254 nm

Retention Time 18.6 minutes




**7**-cRGDyK(ivDde)

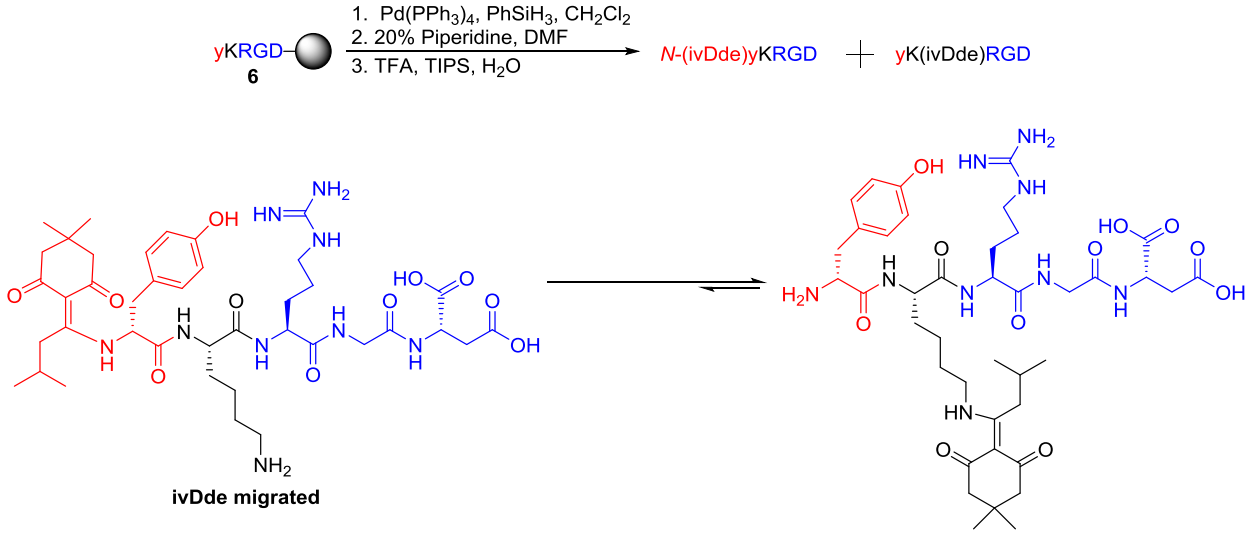
HPLC Trace of purified **7**.



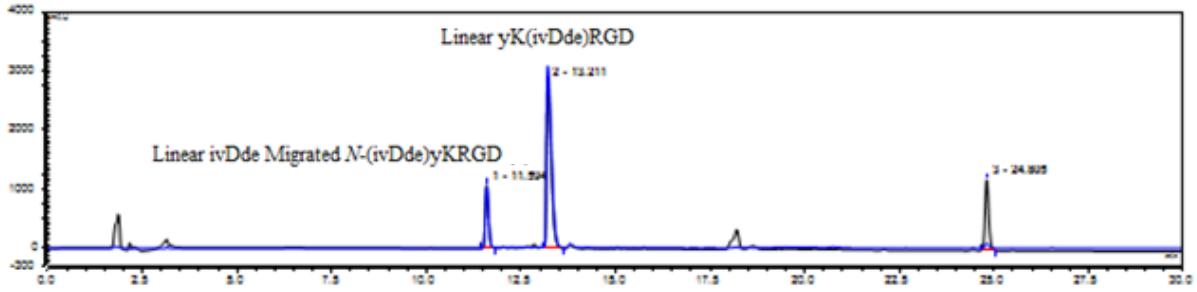

Black 220 nm and Blue 254 nm

Retention Time 15.4 minutes

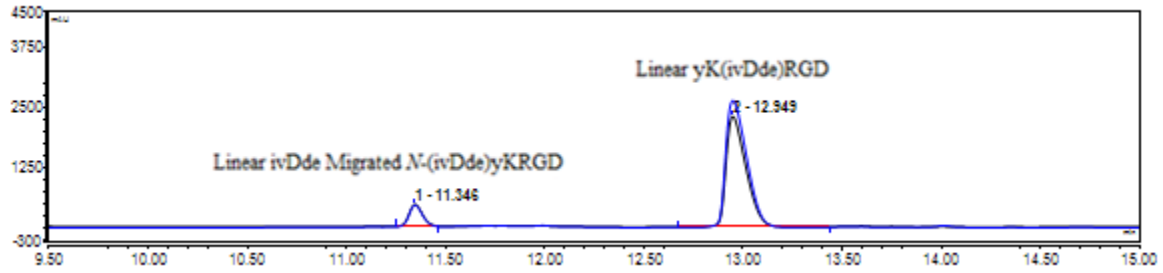
The linear yK(ivDde)RGD peptide exhibited ivDde- migration to the *N*-terminus (linear ivDde migrated) upon removal of the allyl ester, Fmoc group, and cleavage of the peptide. Upon cleavage a ratio of ~1:9 of linear ivDde migrated to non-ivDde migrated linear as observed by HPLC. The migration event was more pronounced in acidic solutions promoting ivDde-carbocation formation and the $S_N1$ attack by the *N*-terminal amine. After HPLC purification of linear ivDde migrated *N*-ivDde-yKRGD and linear yK(ivDde)RGD and re-injection to HPLC, linear yK(ivDde)RGD was observed to be stable for over a 24 hour period of time containing ≤1% of the ivDde migrated linear *N*-ivDde-yKRGD. This stability was related to the basicity of the two competing amine groups with lysine's side-chain being the most basic. The *N*-terminally protected ivDde exhibited rapid ivDde-migration back to the original lysine side chain protected amine non-ivDde migrated linear. Re-injection of the *N*-terminal ivDde protected migrated ivDde linear *N*-ivDde-yKRGD within a 4 hour period showed a shift to linear yK(ivDde)RGD as the major product and even after just 6 min ≥14% of the material was converted back to its original position of linear yK(ivDde)RGD. The ivDde migration was less than 12% upon deprotection prior to cyclization, but after cyclization appeared non-existent as no major byproduct was observed.




HPLC Trace of crude linear material.



Zoom in of crude linear material

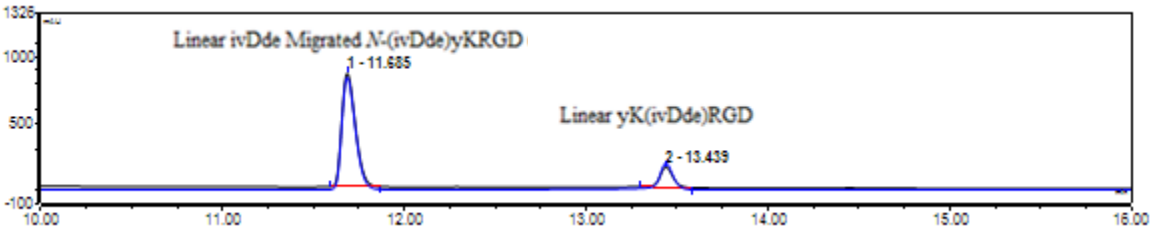



Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.35 minutes, 11.6%.

Retention Time for Linear yK(ivDde)RGD was 12.95 minutes, 88.4%.

Re-injection of Linear ivDde Migrated *N*-(ivDde)yKRGD 6 minutes later.

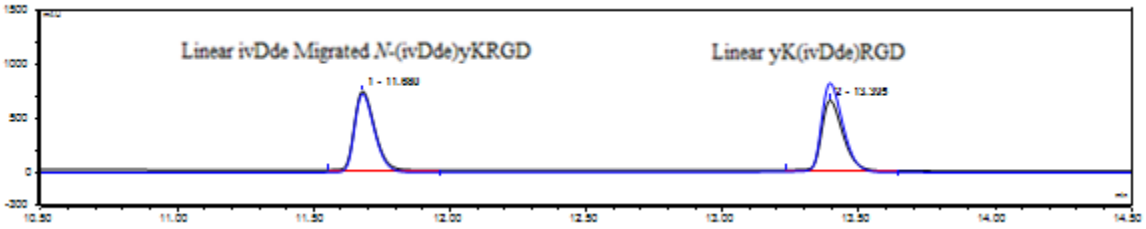



Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.69 minutes, 85.6%.

Retention Time for Linear yK(ivDde)RGD was 13.44 minutes, 14.4%.

Re-injection of Linear ivDde Migrated *N*-(ivDde)yKRGD 3 hours later.

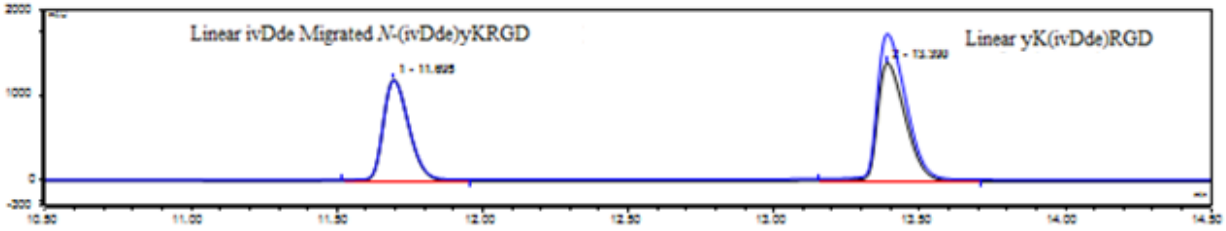


Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.68 minutes, 51.6%.

Retention Time for Linear yK(ivDde)RGD was 13.4 minutes, 48.4%.

Re-injection of Linear ivDde Migrated *N*-(ivDde)yKRGD 4 hours later.

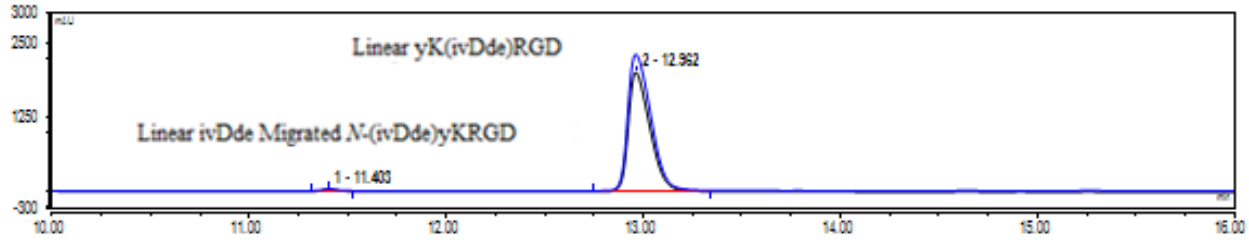



Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.69 minutes, 44.0%.

Retention Time for Linear yK(ivDde)RGD was 13.39 minutes, 56.0%.

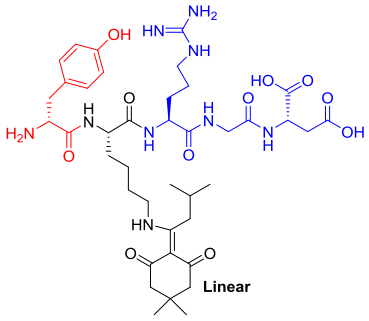
Re-injection of Linear ivDde Migrated *N*-(ivDde)yKRGD 24 hours later.

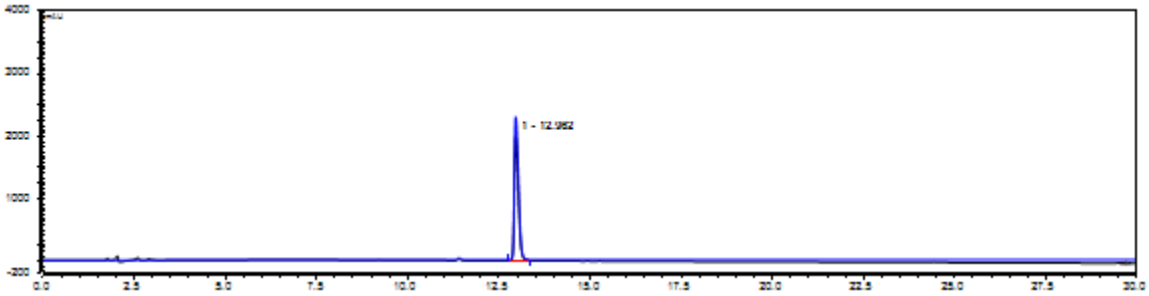



Black 220 nm and Blue 254 nm

Retention Time for Linear ivDde Migrated *N*-(ivDde)yKRGD was 11.4 minutes, 1.0%.

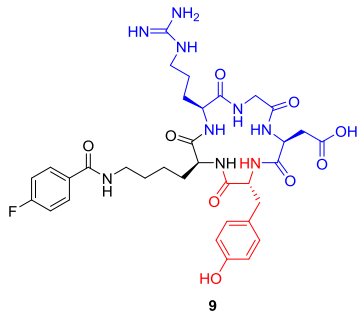
Retention Time for Linear yK(ivDde)RGD was 12.96 minutes, 99.0%.



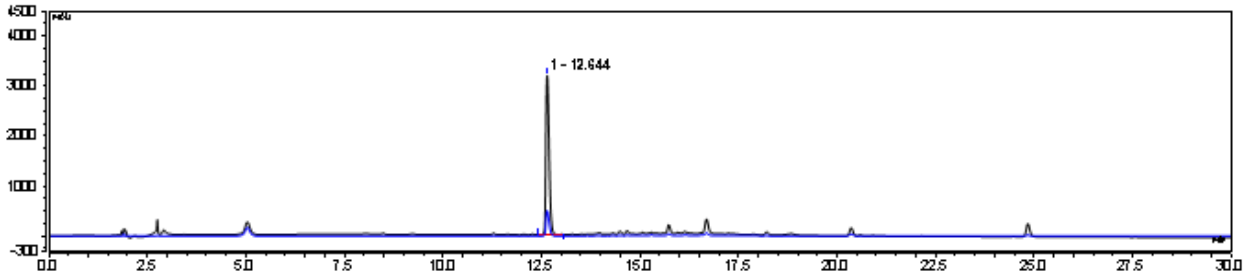

Black 220 nm and Blue 254 nm

Retention Time for Linear yK(ivDde)RGD was 12.96 minutes



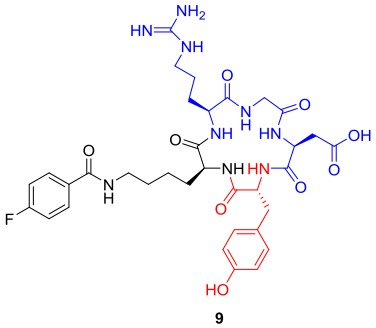
**9**

HPLC Trace of crude **9**.



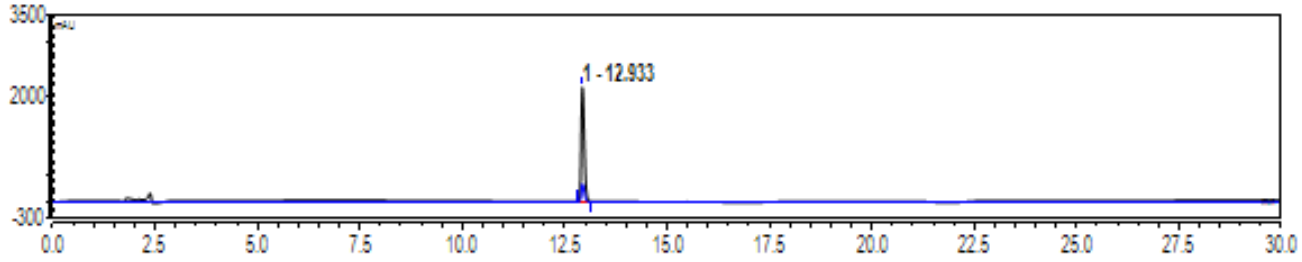

Black 220 nm and Blue 254 nm

Retention Time of **9** was 12.64 minutes.



**9**

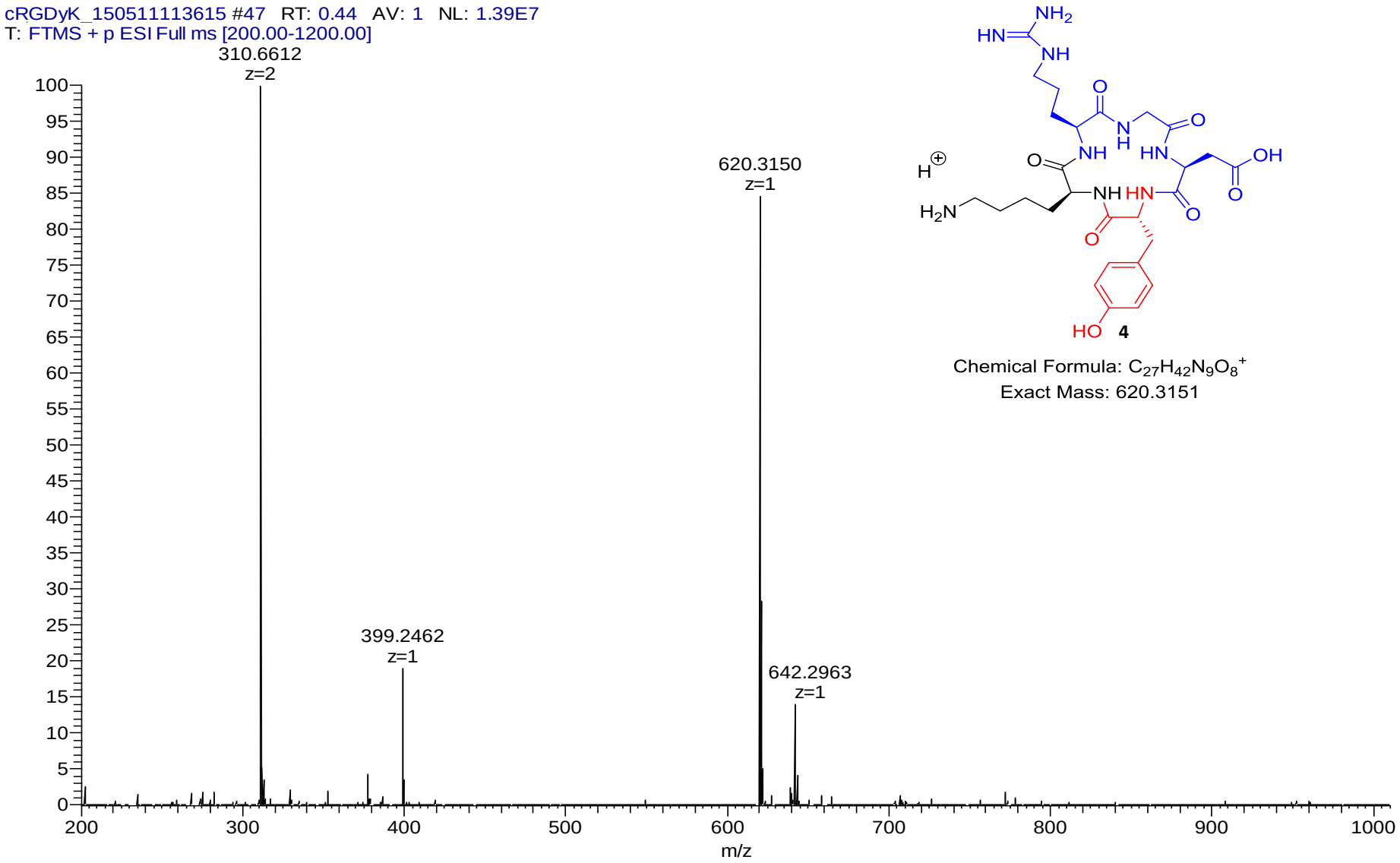
HPLC Trace of purified **9**.

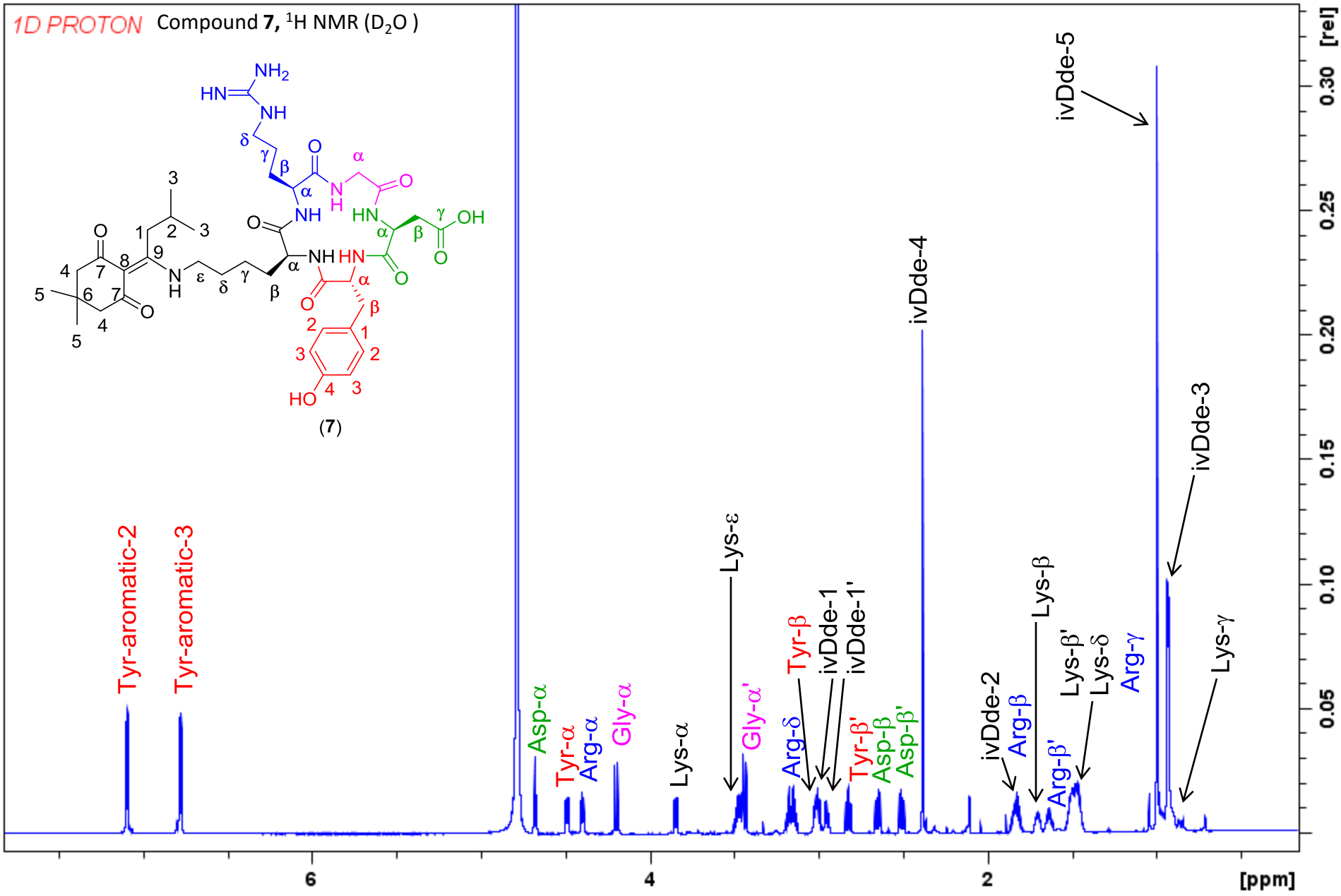


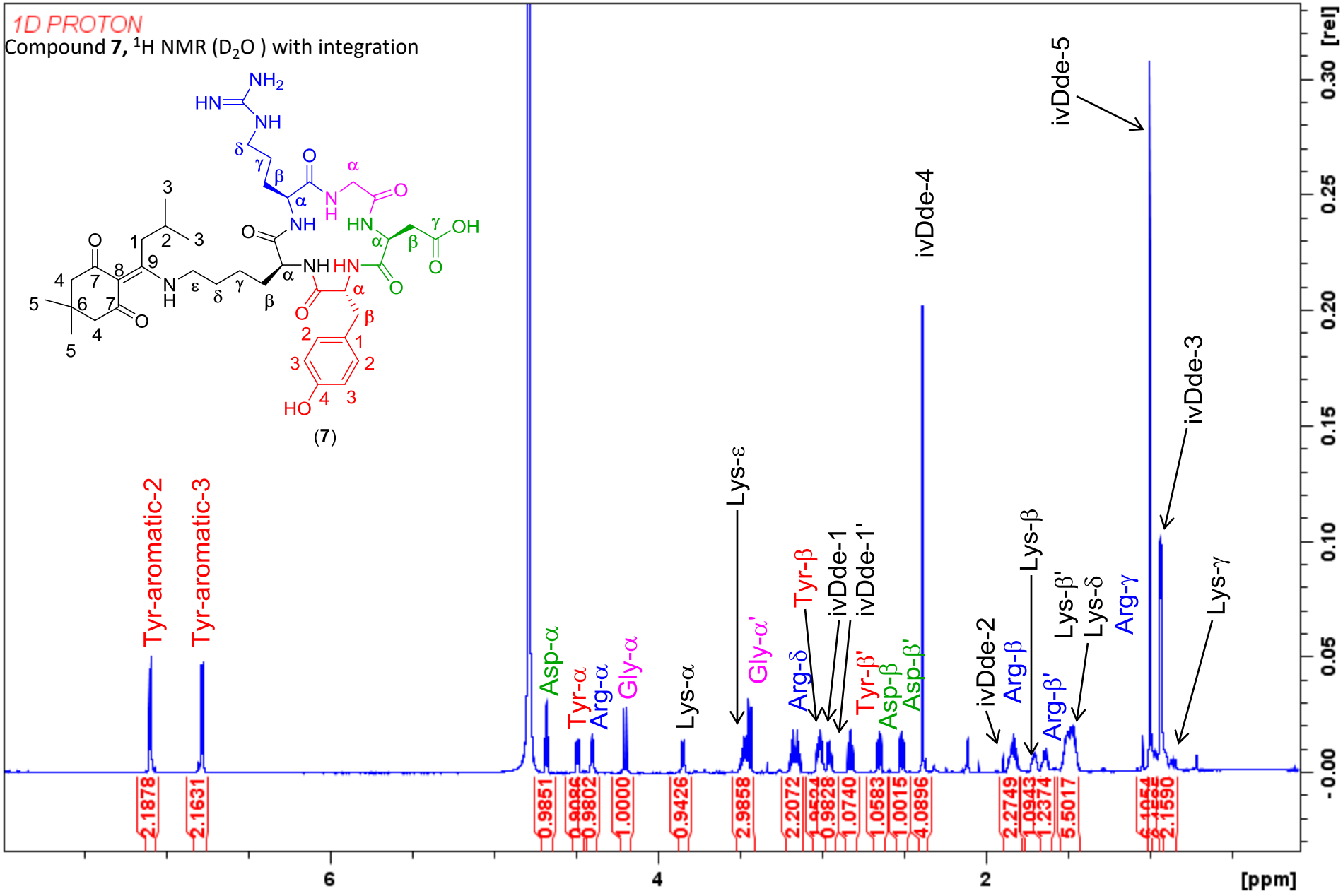

Black 220 nm and Blue 254 nm

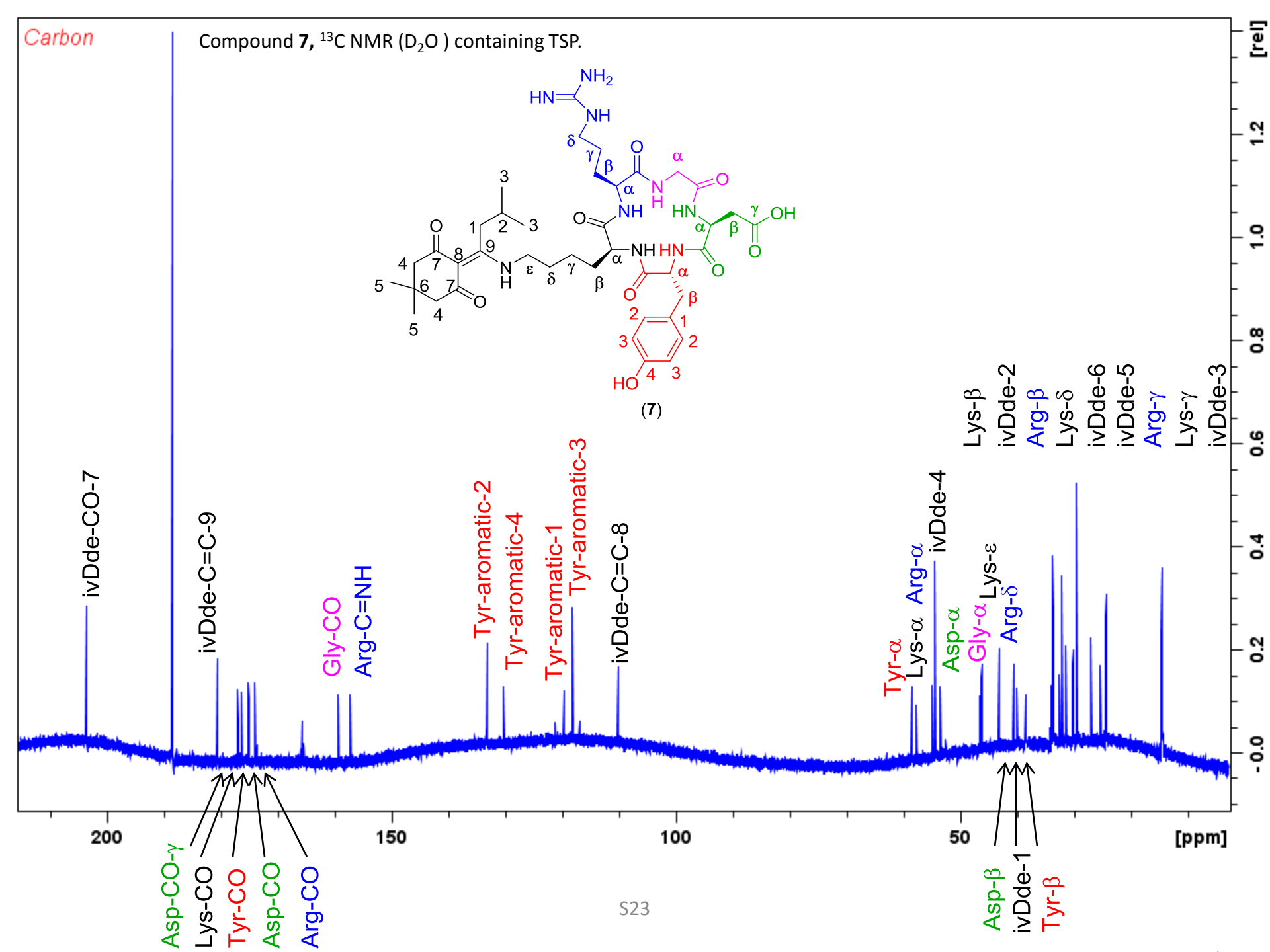
Retention Time of **9** was 12.93 minutes.

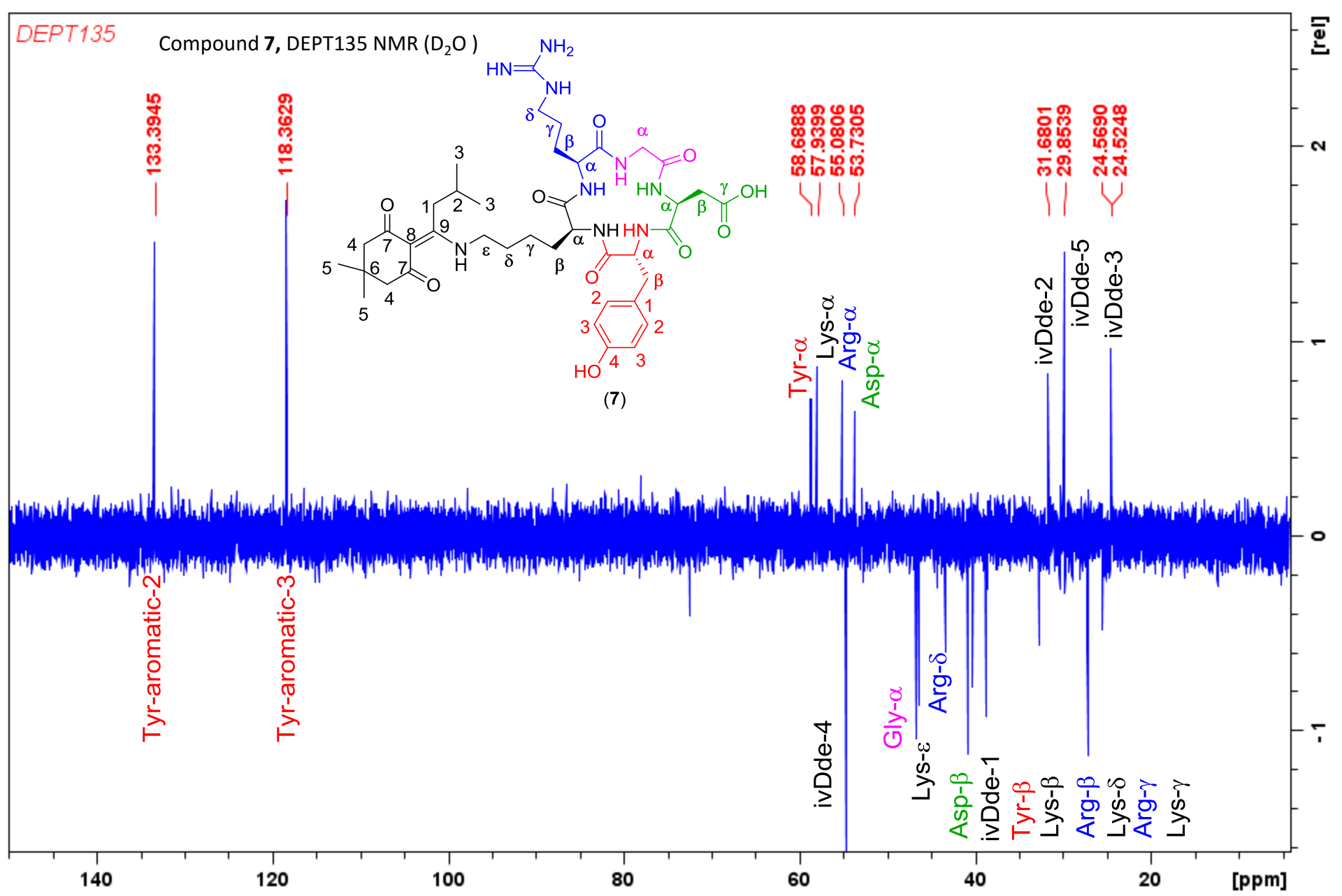
Compound **4**, ESI-(FTMS-ion trap)

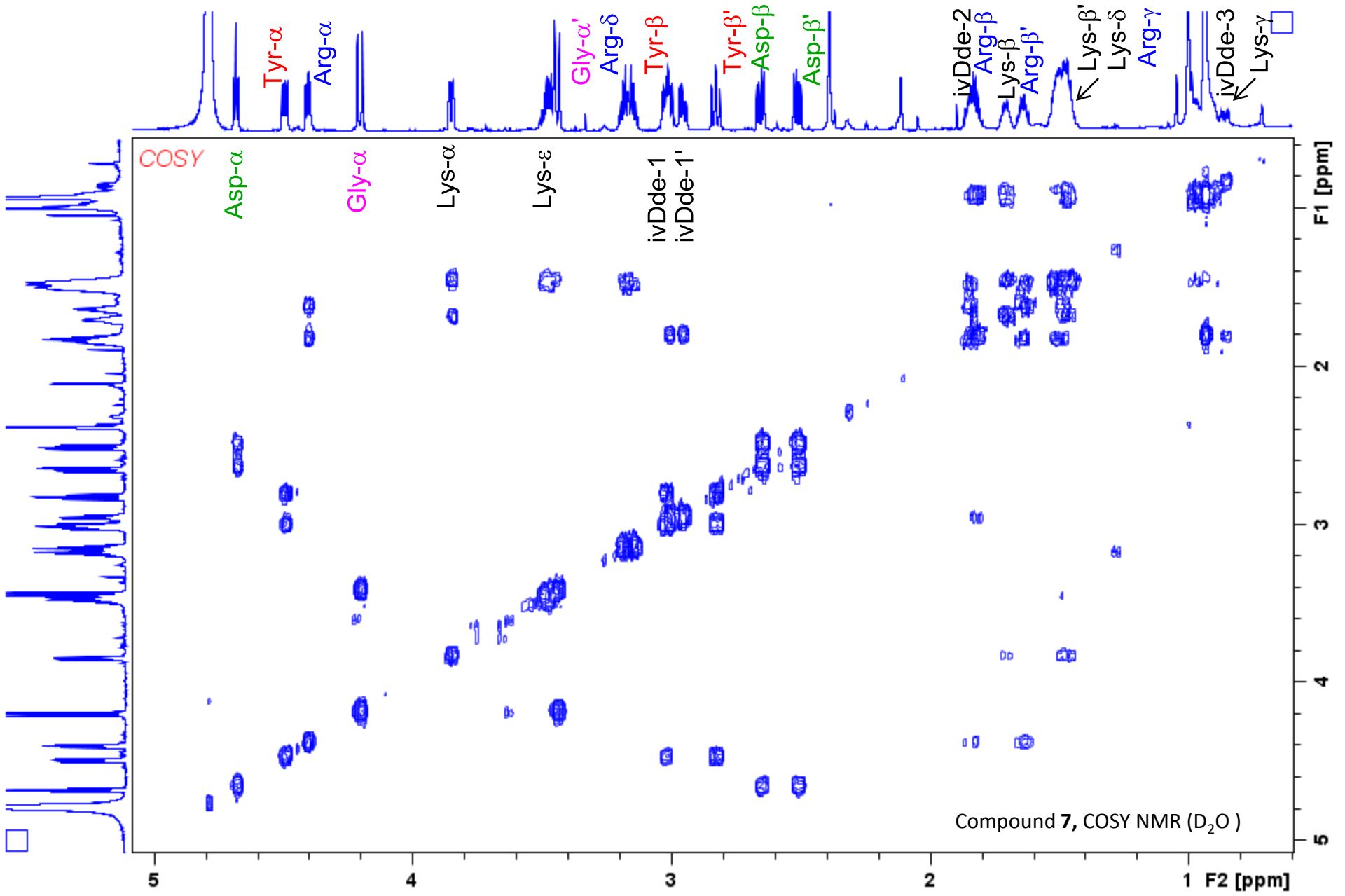


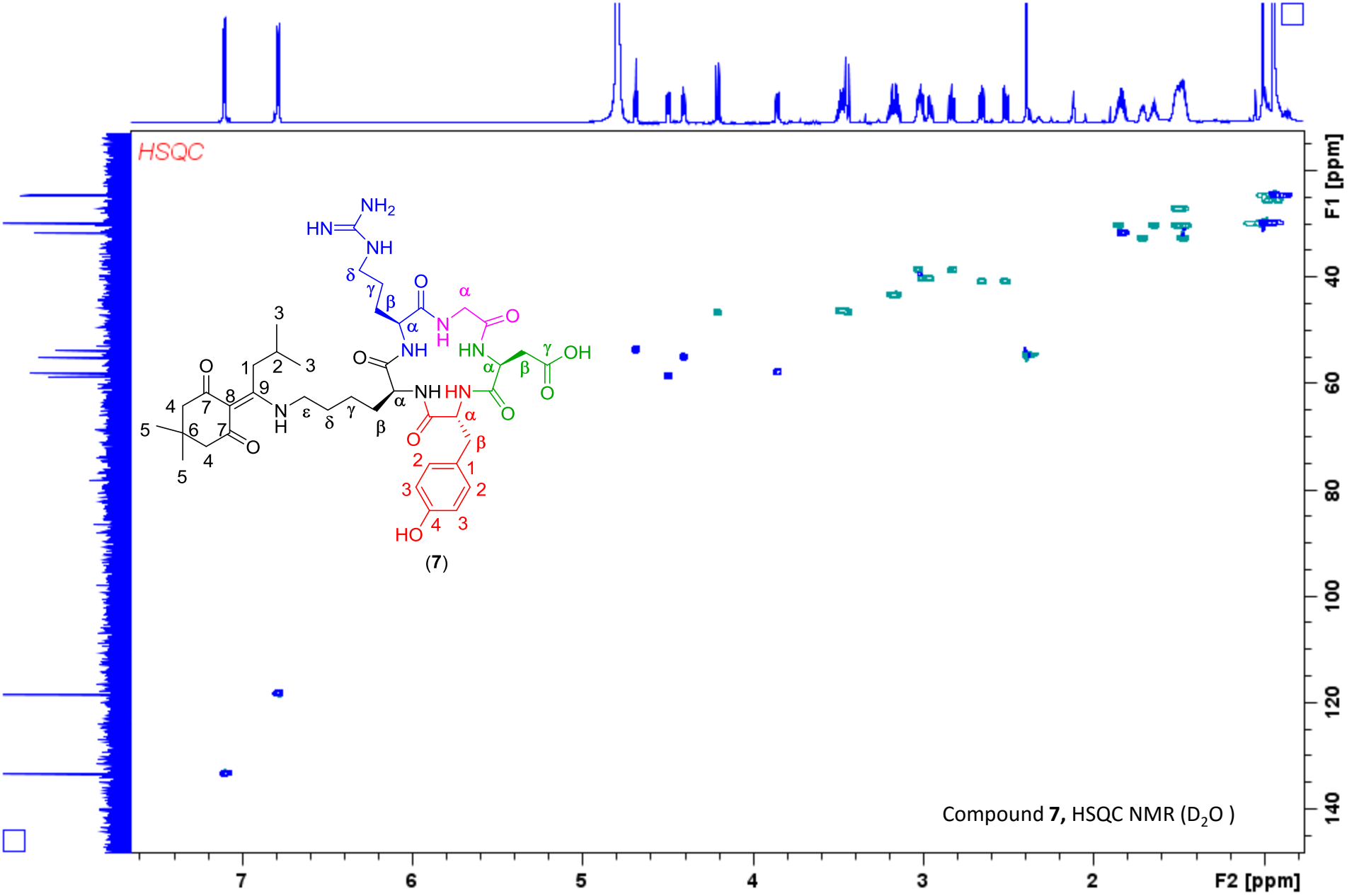

1D PROTON
Compound 7, 1H NMR (D2O )
(7)
Tyr-aromatic-2
Tyr-aromatic-3
Asp-α
Tyr-α
Arg-α
Gly-α
Lys-α
Lys-ε
Gly-α'
Arg-δ
Tyr-β
ivDde-1
ivDde-1'
Tyr-β'
Asp-β
Asp-β'
ivDde-4
ivDde-2
Arg-β
Arg-β'
Lys-β
Lys-β'
Lys-δ
Arg-γ
ivDde-5
ivDde-3
Lys-γ
6
4
2
[ppm]
0.30
0.25
0.20
0.15
0.10
0.05
[rel]


1D PROTON
Compound 7, $^1$H NMR ($D_2O$ ) with integration
(7)
Tyr-aromatic-2
Tyr-aromatic-3
Asp-α
Tyr-α
Arg-α
Gly-α
Lys-α
Lys-ε
Gly-α'
Arg-δ
Tyr-β
ivDde-1
ivDde-1'
Tyr-β'
Asp-β
Asp-β'
ivDde-4
ivDde-2
Arg-β
Lys-β
Arg-β'
Lys-β'
Lys-δ
Arg-γ
ivDde-5
ivDde-3
Lys-γ
2.1878
2.1631
0.9851
0.9802
1.0000
0.9426
2.9858
2.2072
0.9828
1.0740
1.0583
1.0015
4.0896
2.2749
1.0943
1.2374
5.5017
2.1590
6
4
2
[ppm]
[rel]
0.30
0.25
0.20
0.15
0.10
0.05
-0.00


Carbon
Compound 7, $^{13}$C NMR ($D_2O$ ) containing TSP.
(7)
ivDde-CO-7
ivDde-C=C-9
Gly-CO
Arg-C=NH
Tyr-aromatic-2
Tyr-aromatic-4
Tyr-aromatic-1
Tyr-aromatic-3
ivDde-C=C-8
Tyr-α
Lys-α
Arg-α
ivDde-4
Asp-α
Gly-α
Lys-ε
Arg-δ
Lys-β
ivDde-2
Arg-β
Lys-δ
ivDde-6
ivDde-5
Arg-γ
Lys-γ
ivDde-3
Asp-CO-γ
Lys-CO
Tyr-CO
Asp-CO
Arg-CO
Asp-β
ivDde-1
Tyr-β
200
150
100
50
[ppm]
[rel]
1.2
1.0
0.8
0.6
0.4
0.2
-0.0

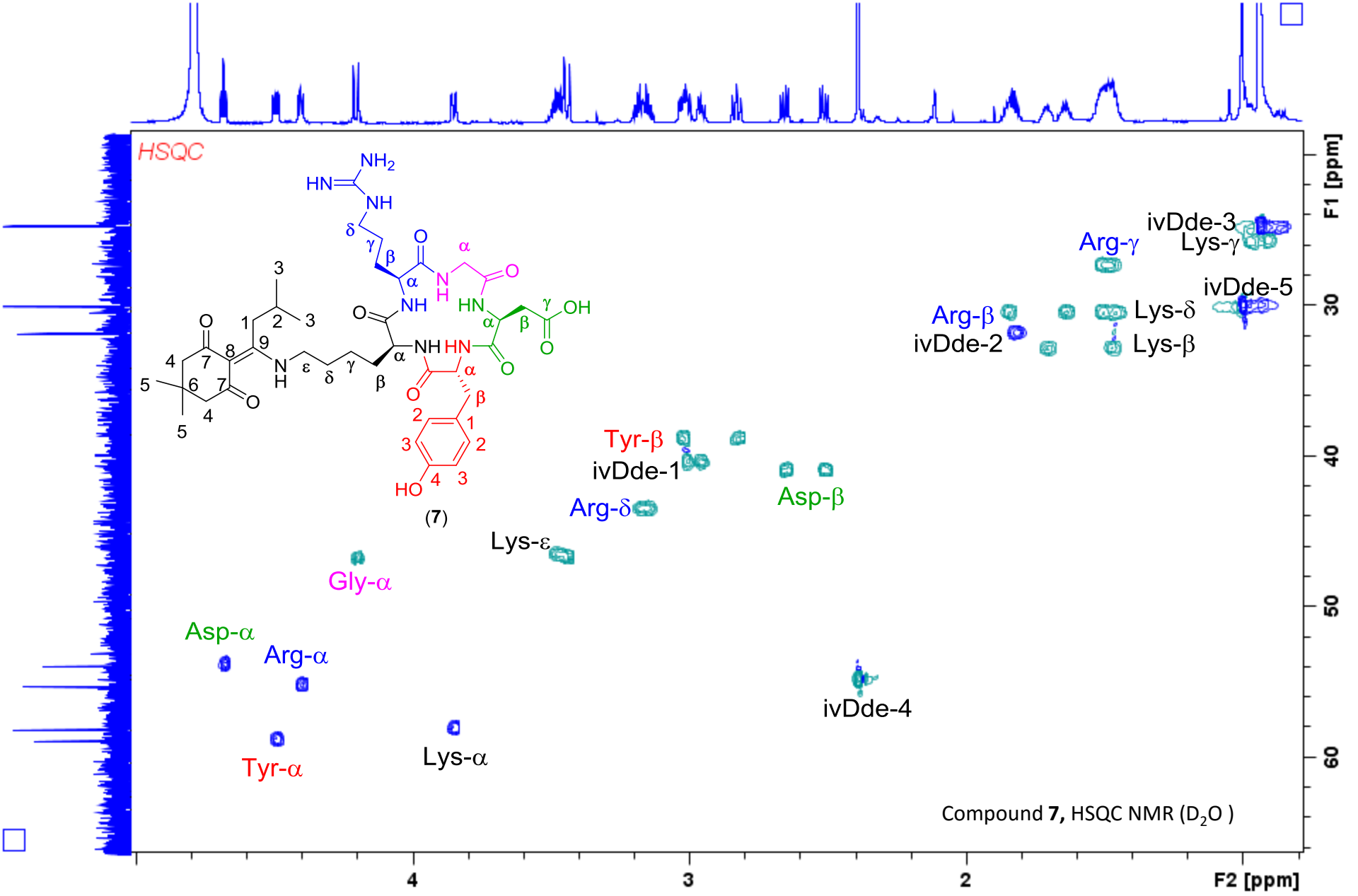

DEPT135
Compound 7, DEPT135 NMR (D2O )
133.3945
118.3629
58.6888
57.9399
55.0806
53.7305
31.6801
29.8539
24.5690
24.5248
Tyr-aromatic-2
Tyr-aromatic-3
Tyr-α
Lys-α
Arg-α
Asp-α
ivDde-2
ivDde-5
ivDde-3
ivDde-4
Gly-α
Lys-ε
Arg-δ
Asp-β
ivDde-1
Tyr-β
Lys-β
Arg-β
Lys-δ
Arg-γ
Lys-γ
(7)
140
120
100
80
60
40
20
[ppm]
[rel]
2
1
0
-1

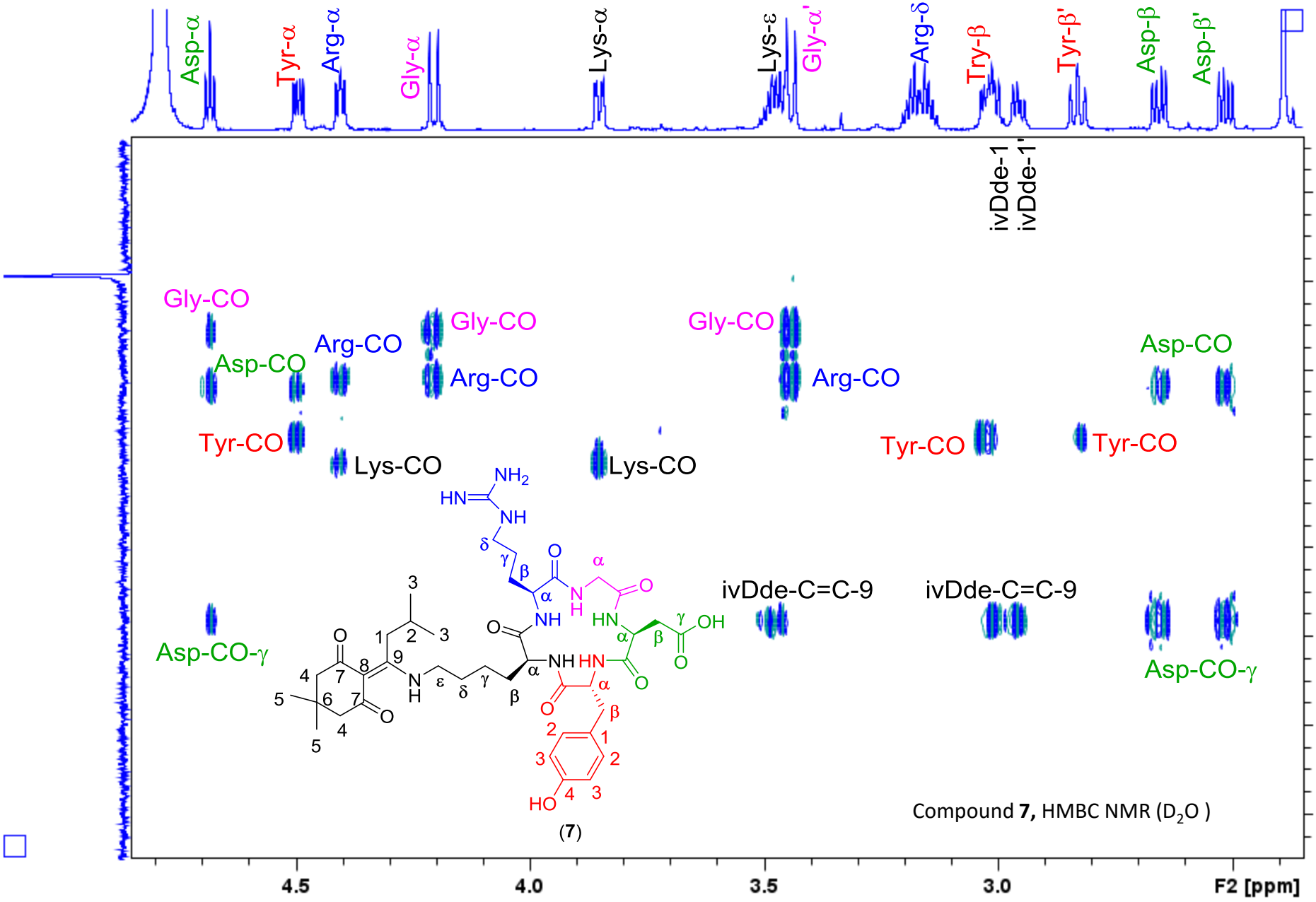

COSY
Tyr-α
Arg-α
Gly-α'
Arg-δ
Tyr-β
Tyr-β'
Asp-β
Asp-β'
ivDde-2
Arg-β
Lys-β
Arg-β'
Lys-β'
Lys-δ
Arg-γ
ivDde-3
Lys-γ
Asp-α
Gly-α
Lys-α
Lys-ε
ivDde-1
ivDde-1'
F1 [ppm]
F2 [ppm]
Compound 7, COSY NMR (D2O )




(**7**)

Compound **7,** HSQC NMR ($D_2O$ )

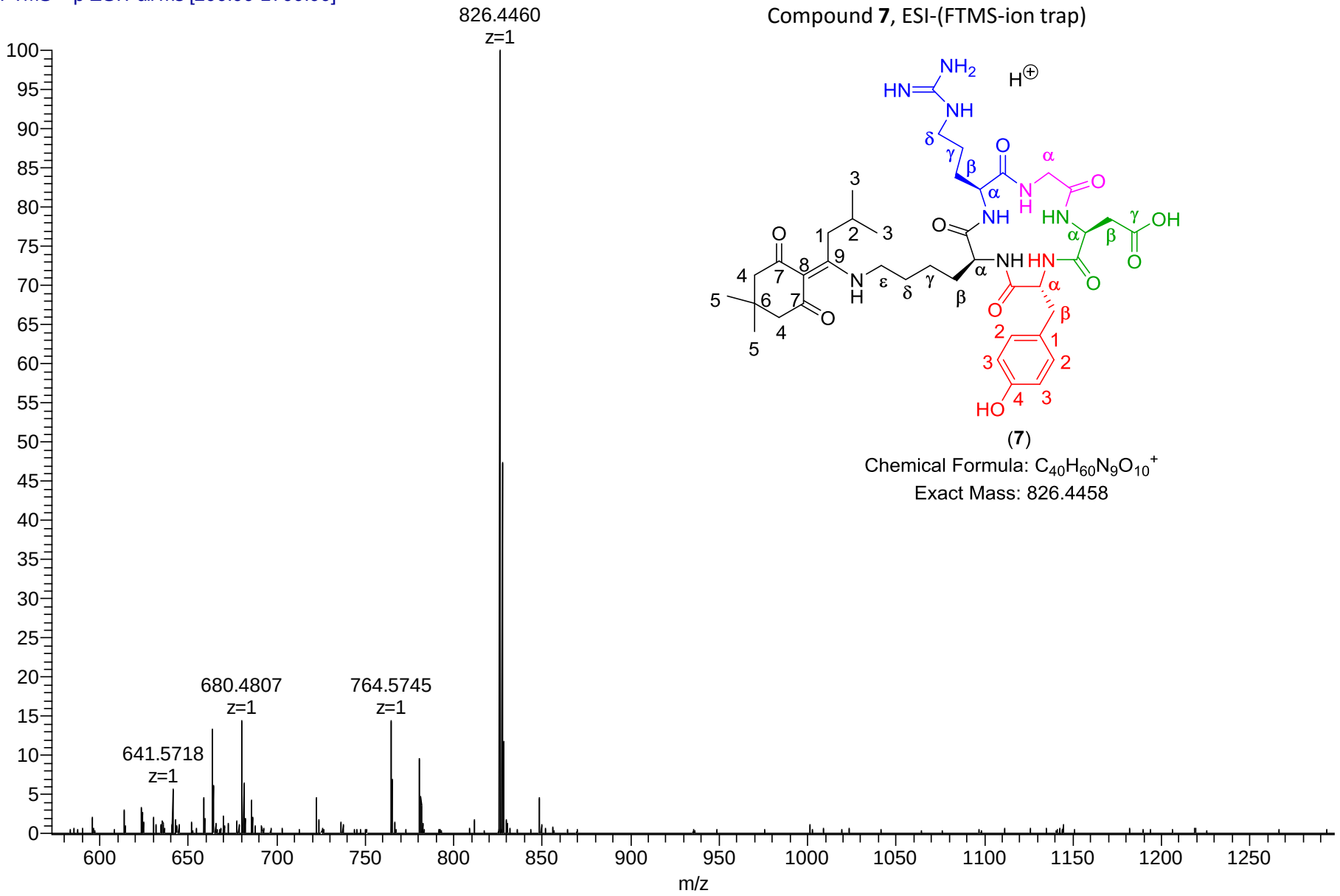

HSQC
(7)
Arg-γ
ivDde-3
Lys-γ
ivDde-5
Arg-β
ivDde-2
Lys-δ
Lys-β
Tyr-β
ivDde-1
Asp-β
Arg-δ
Lys-ε
Gly-α
Asp-α
Arg-α
ivDde-4
Tyr-α
Lys-α
F1 [ppm]
F2 [ppm]
Compound 7, HSQC NMR (D2O )




(7)

Compound **7,** HMBC NMR ($D_2O$ )

cRGDyK(ivDde)-15KS #35 RT: 0.32 AV: 1 NL: 6.36E5
T: FTMS + p ESI Full ms [200.00-1700.00]




Compound **7**, ESI-(FTMS-ion trap)

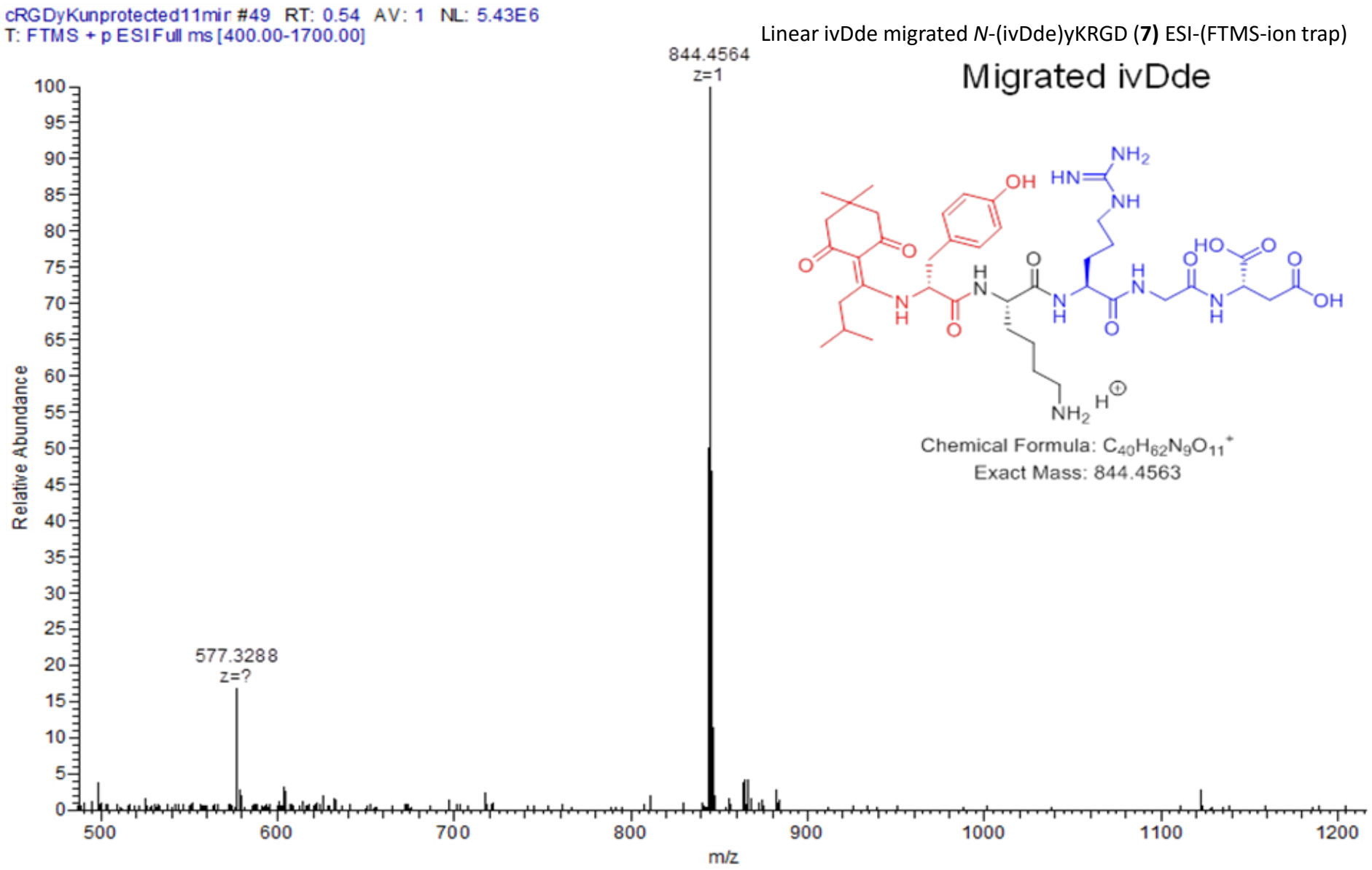


(**7**)

Chemical Formula: $C_{40}H_{60}N_9O_{10}^+$

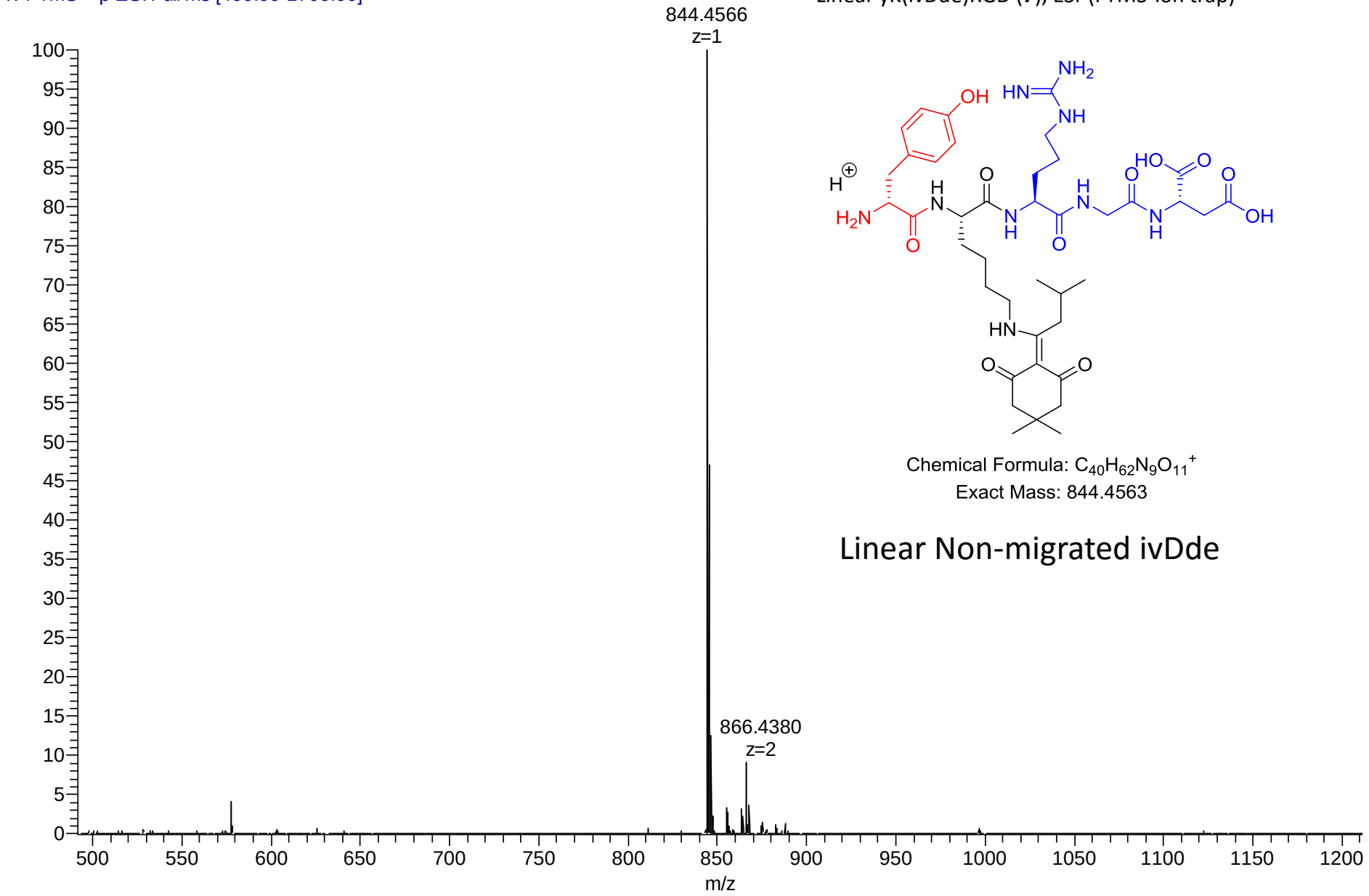
Exact Mass: 826.4458

Linear ivDde migrated *N*-(ivDde)yKRGD (**7**) ESI-(FTMS-ion trap)



cRGDyKunprotected13min #68 RT: 0.73 AV: 1 NL: 7.31E6
T: FTMS + p ESI Full ms [400.00-1700.00]

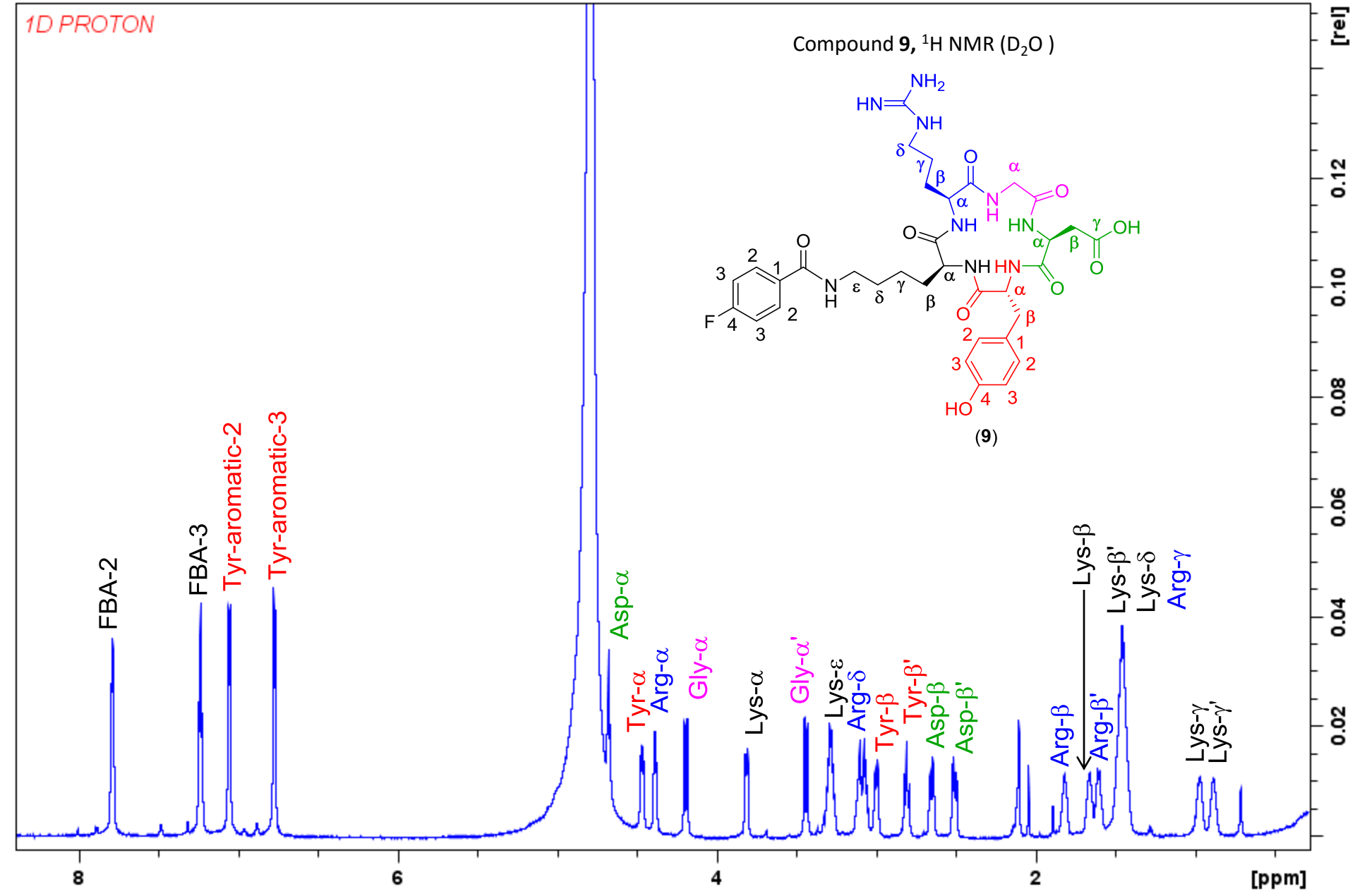
Linear yK(ivDde)RGD (**7**), ESI-(FTMS-ion trap)

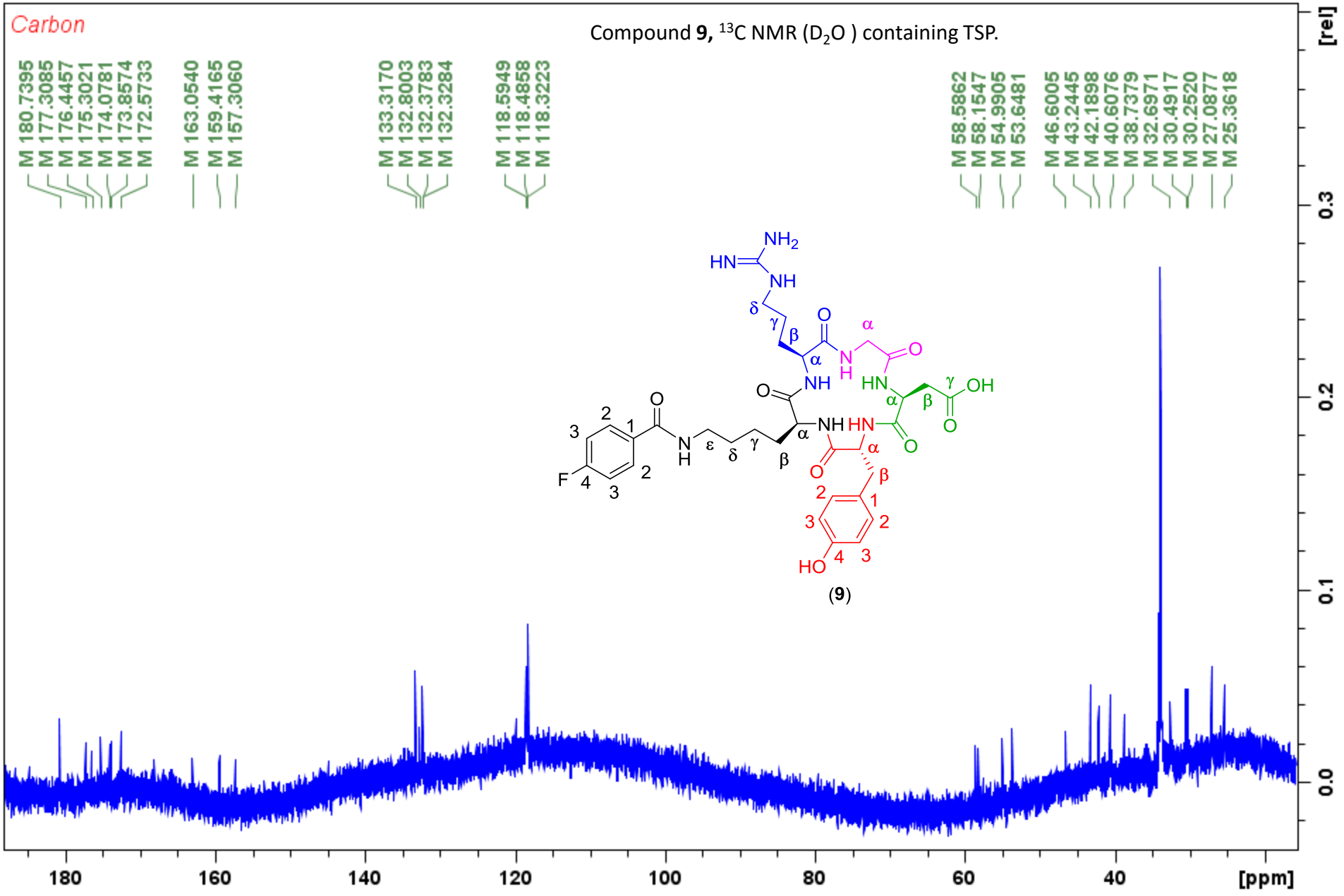





Chemical Formula: $C_{40}H_{62}N_9O_{11}^+$
Exact Mass: 844.4563

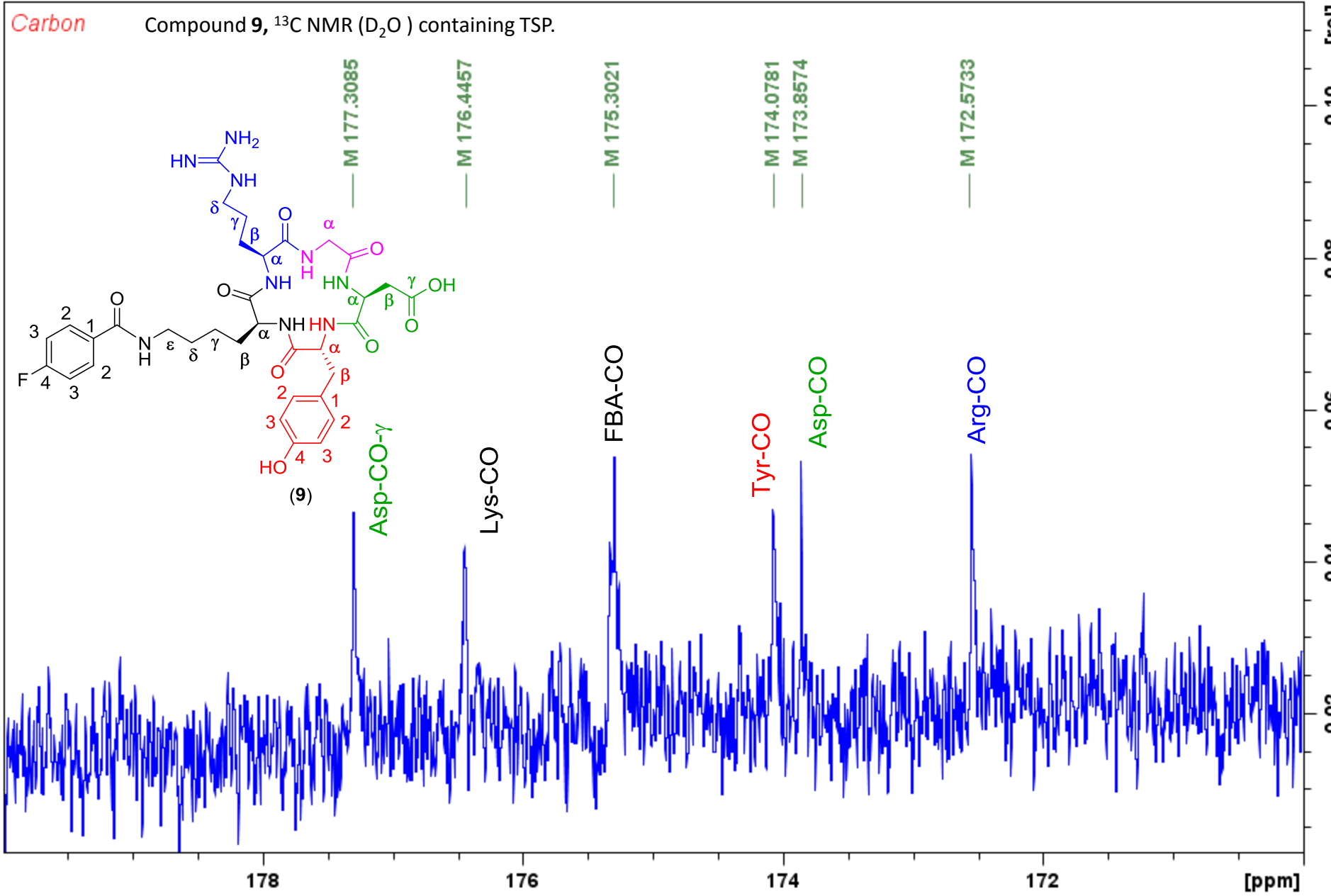
## Linear Non-migrated ivDde

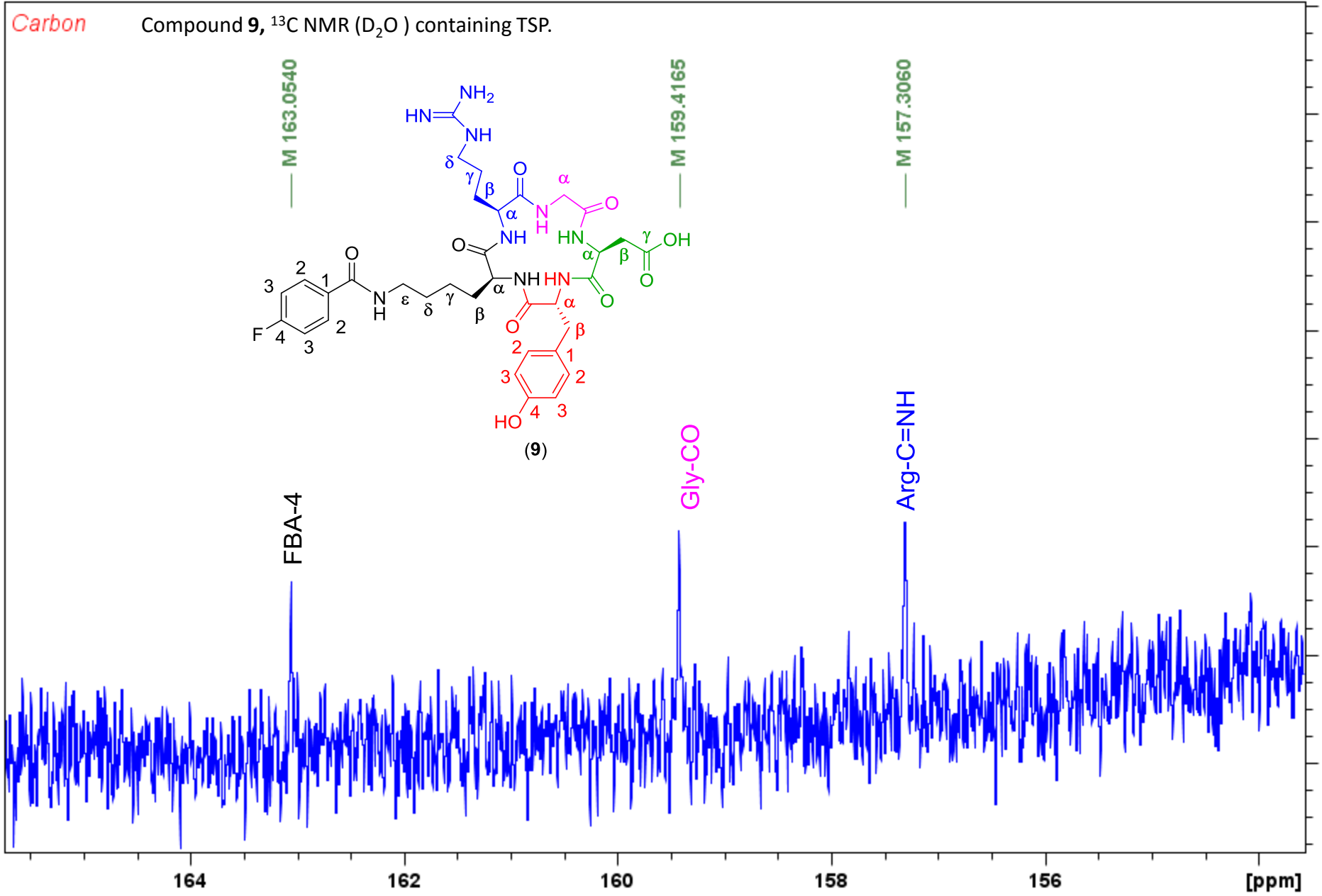

1D PROTON
Compound 9, $^1$H NMR ($D_2O$ )
(9)
FBA-2
FBA-3
Tyr-aromatic-2
Tyr-aromatic-3
Asp-α
Tyr-α
Arg-α
Gly-α
Lys-α
Gly-α'
Lys-ε
Arg-δ
Tyr-β
Tyr-β'
Asp-β
Asp-β'
Arg-β
Arg-β'
Lys-β
Lys-β'
Lys-δ
Arg-γ
Lys-γ
Lys-γ'
8
6
4
2
[ppm]
[rel]
0.12
0.10
0.08
0.06
0.04
0.02

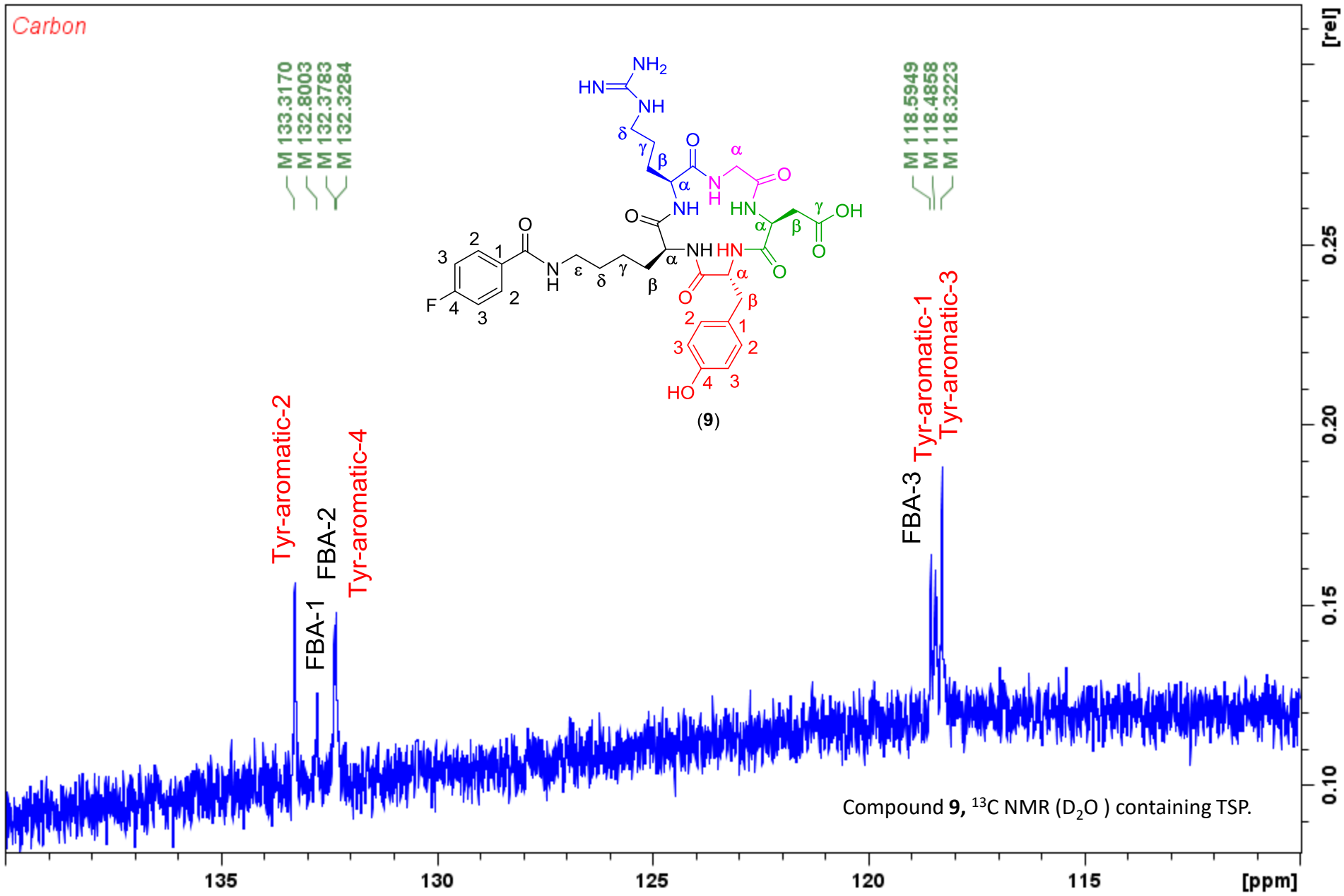



Compound **9,** $^{13}C$ NMR ($D_2O$ ) containing TSP.

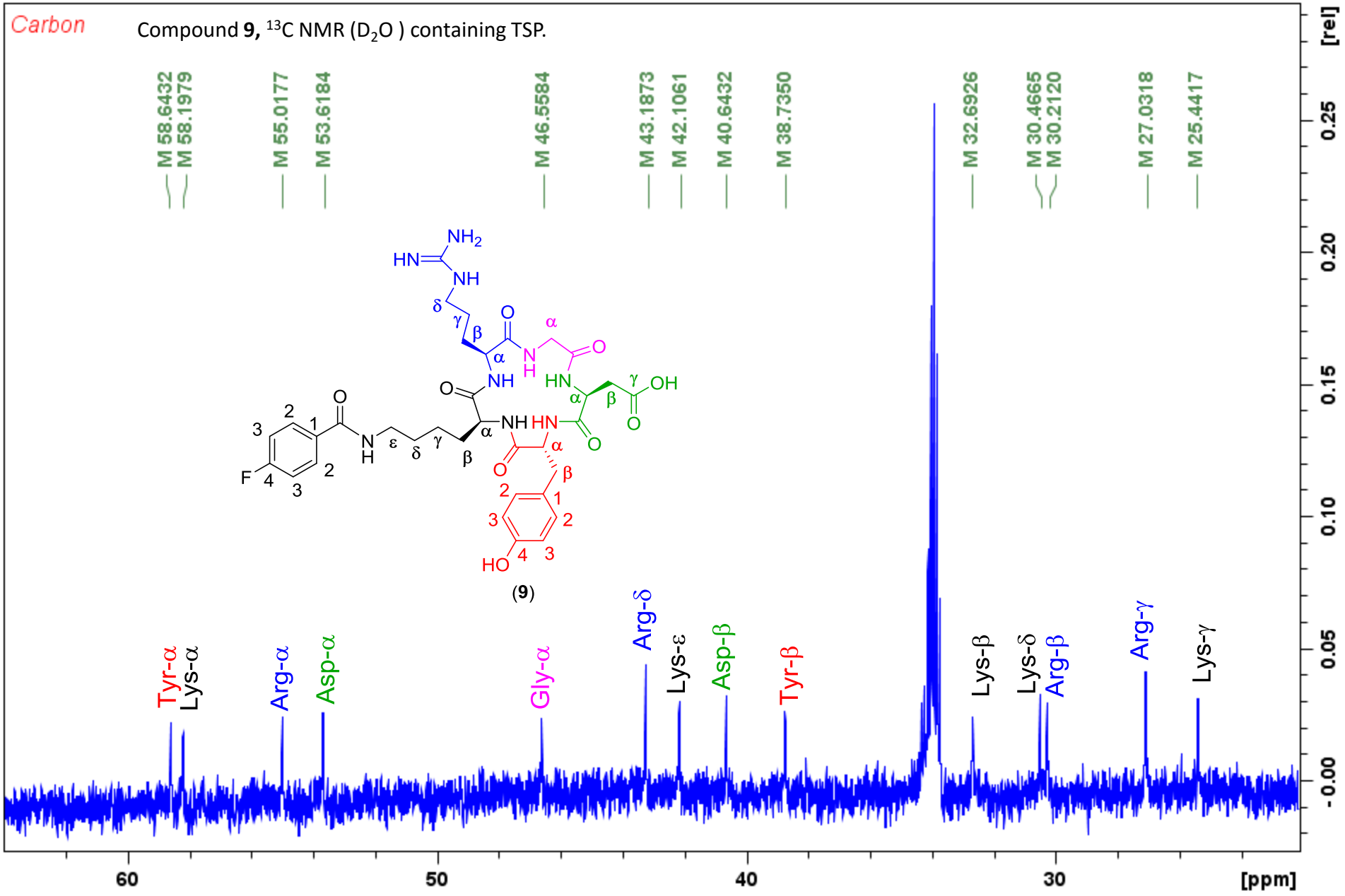
(**9**)

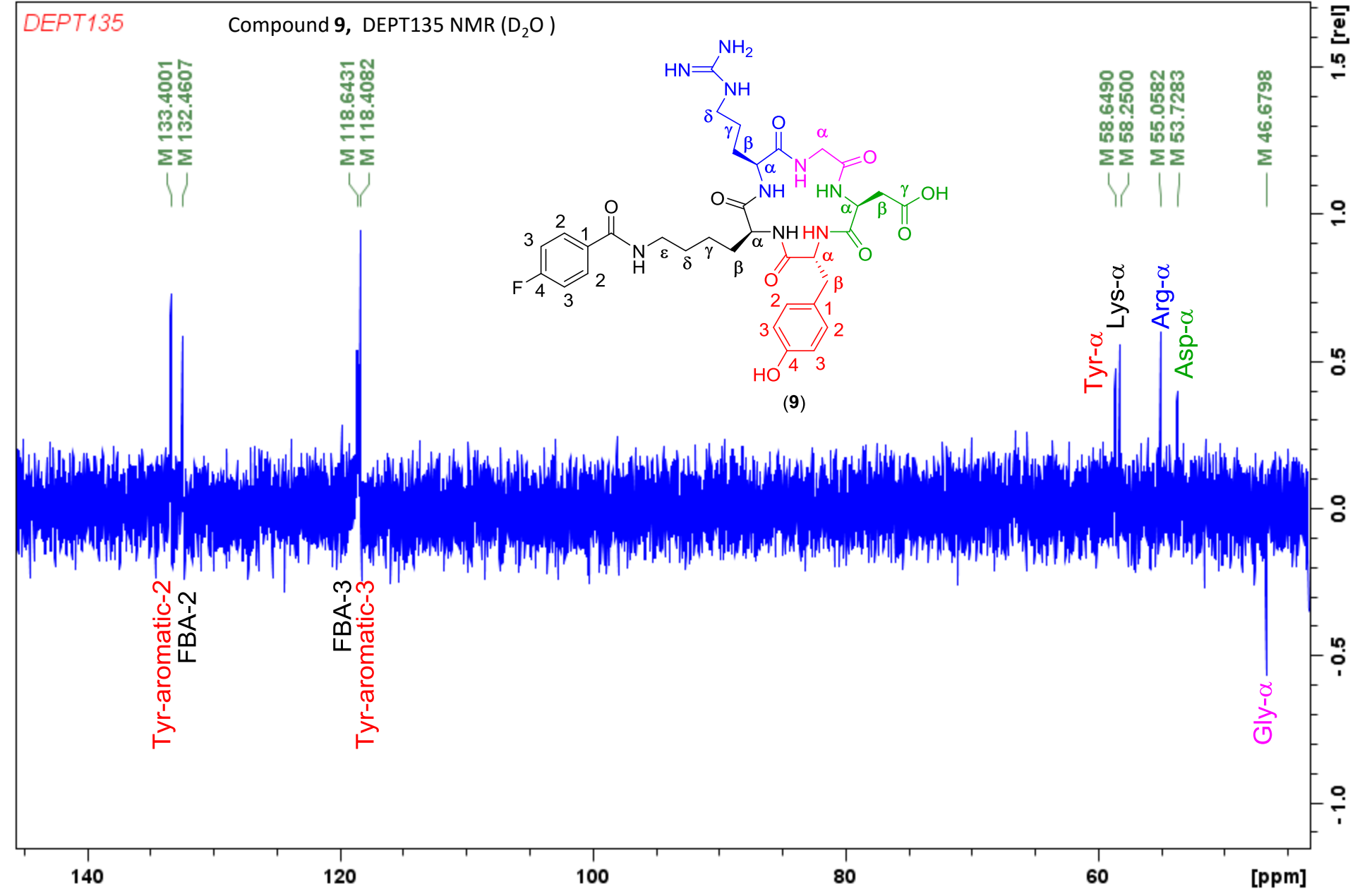

Carbon
Compound 9, 13C NMR (D2O ) containing TSP.
M 177.3085
M 176.4457
M 175.3021
M 174.0781
M 173.8574
M 172.5733
Asp-CO-γ
Lys-CO
FBA-CO
Tyr-CO
Asp-CO
Arg-CO
(9)
178
176
174
172
[ppm]
0.10
0.08
0.06
0.04
0.02
[rel]

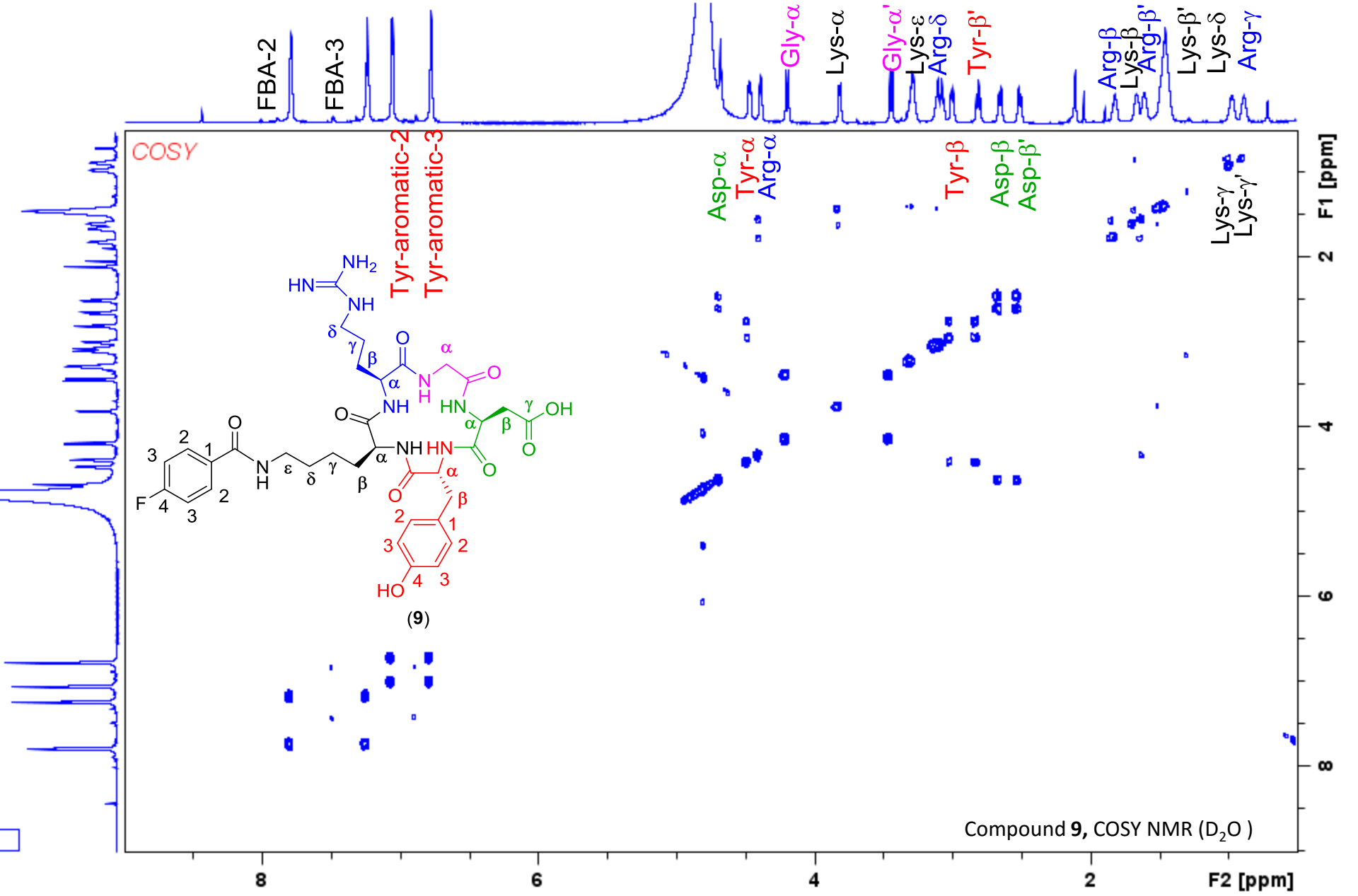

Carbon
Compound 9, 13C NMR (D2O ) containing TSP.
M 163.0540
M 159.4165
M 157.3060
FBA-4
Gly-CO
Arg-C=NH
(9)
164
162
160
158
156
[ppm]
[rel]
0.18
0.16
0.14
0.12
0.10
0.08
0.06

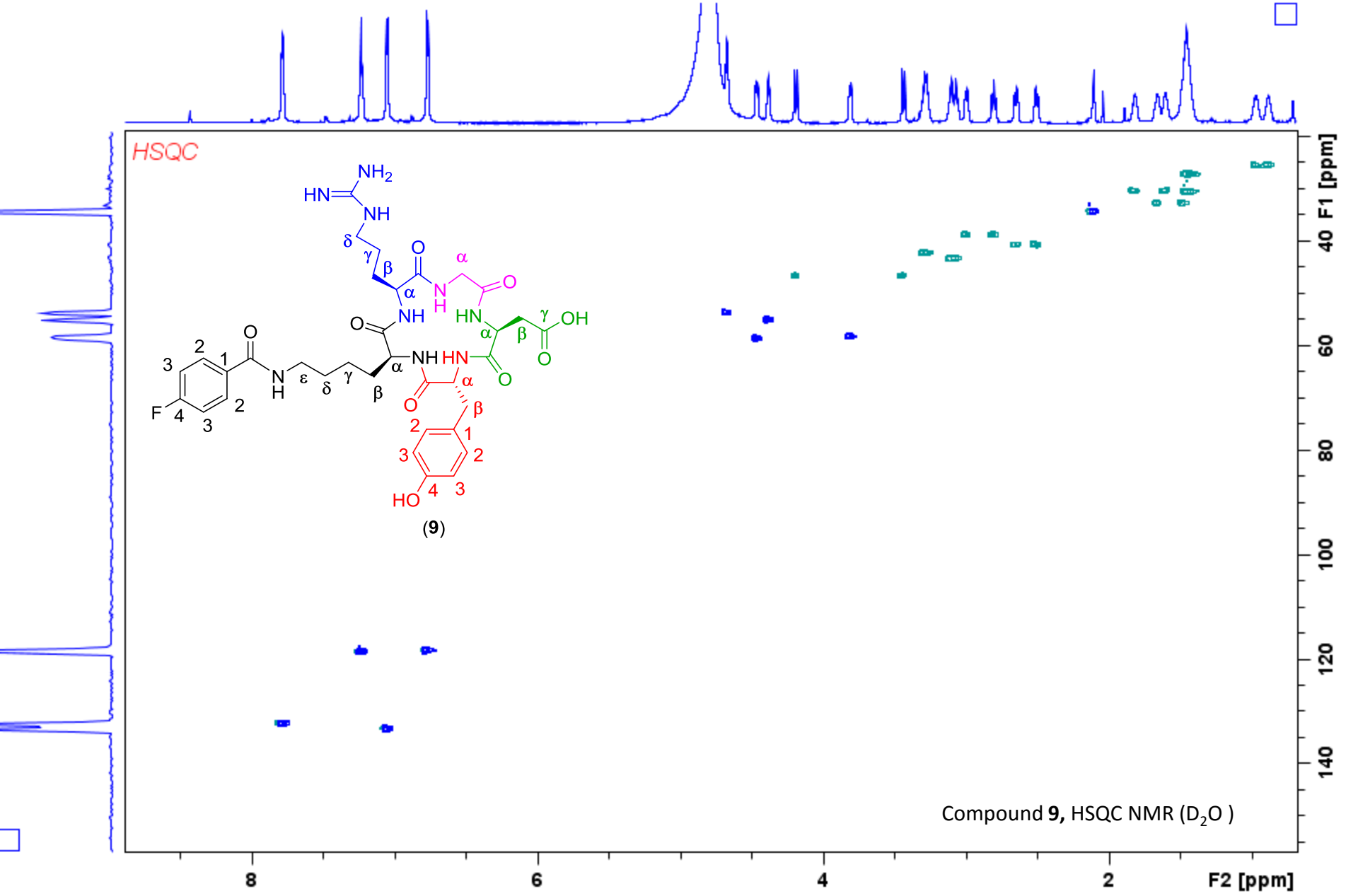



Compound **9,** $^{13}C$ NMR ($D_2O$ ) containing TSP.

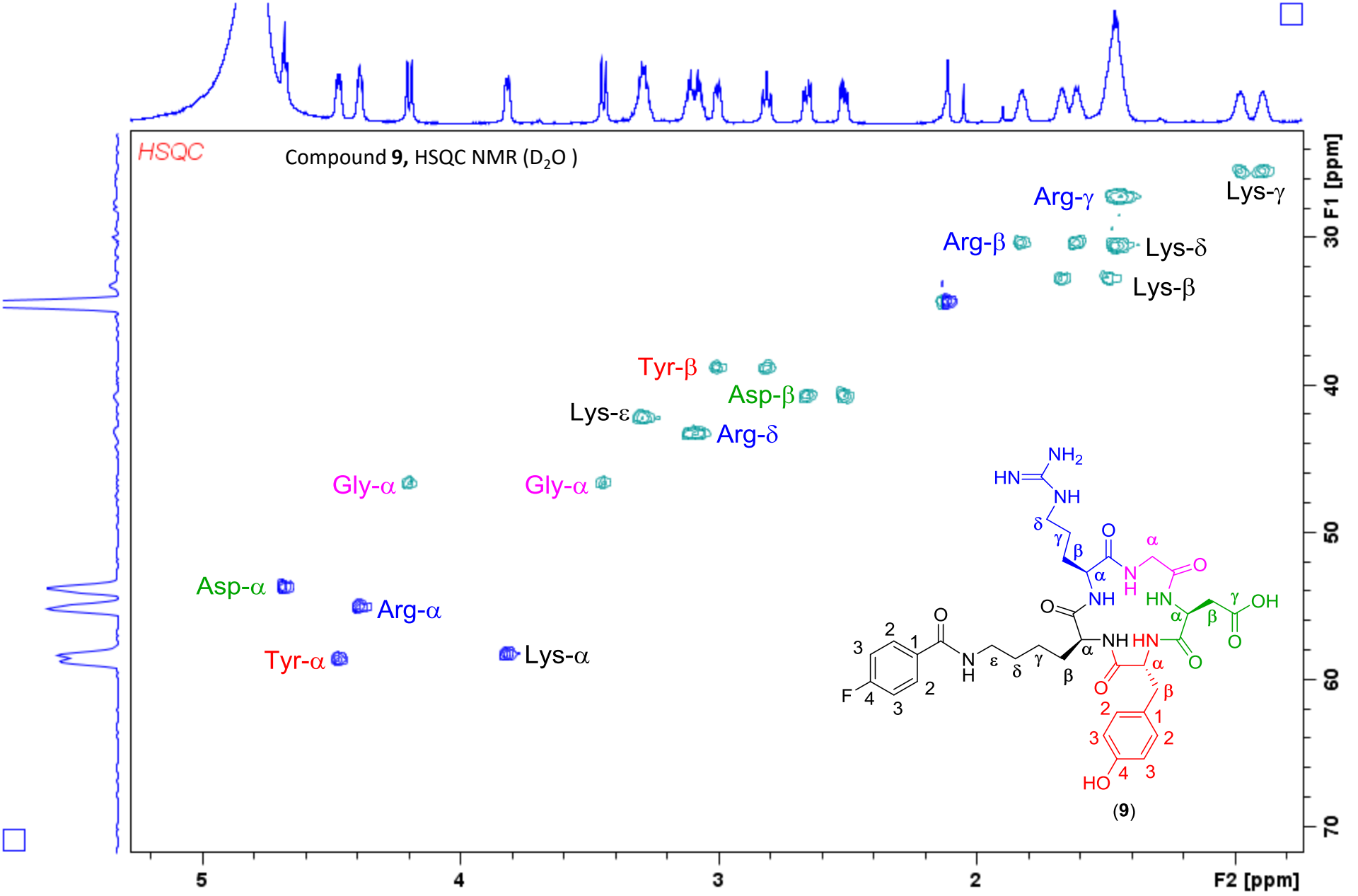

Carbon
Compound 9, $^{13}$C NMR ($D_2O$ ) containing TSP.
M 58.6432
M 58.1979
M 55.0177
M 53.6184
M 46.5584
M 43.1873
M 42.1061
M 40.6432
M 38.7350
M 32.6926
M 30.4665
M 30.2120
M 27.0318
M 25.4417
(9)
Tyr-α
Lys-α
Arg-α
Asp-α
Gly-α
Arg-δ
Lys-ε
Asp-β
Tyr-β
Lys-β
Lys-δ
Arg-β
Arg-γ
Lys-γ
60
50
40
30
[ppm]
[rel]
0.25
0.20
0.15
0.10
0.05
-0.00

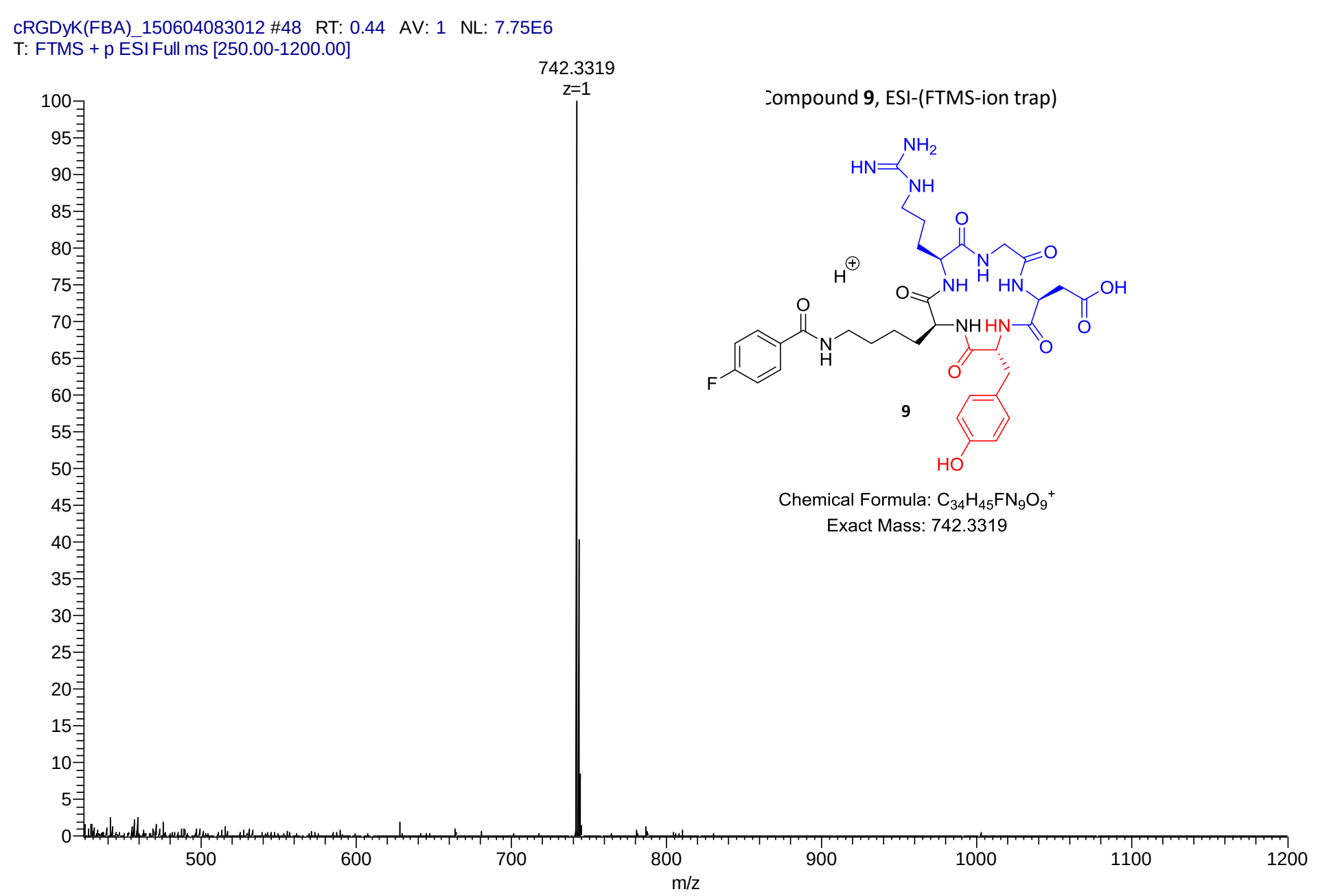

DEPT135
Compound 9, DEPT135 NMR (D2O )
M 133.4001
M 132.4607
M 118.6431
M 118.4082
M 58.6490
M 58.2500
M 55.0582
M 53.7283
M 46.6798
Tyr-aromatic-2
FBA-2
FBA-3
Tyr-aromatic-3
Tyr-α
Lys-α
Arg-α
Asp-α
Gly-α
(9)
1.5 [rel]
1.0
0.5
0.0
-0.5
-1.0
140
120
100
80
60
[ppm]


FBA-2
FBA-3
Gly-α
Lys-α
Gly-α'
Lys-ε
Arg-δ
Tyr-β'
Arg-β
Lys-β
Arg-β'
Lys-β'
Lys-δ
Arg-γ
COSY
Tyr-aromatic-2
Tyr-aromatic-3
Asp-α
Tyr-α
Arg-α
Tyr-β
Asp-β
Asp-β'
Lys-γ
Lys-γ'
(9)
Compound 9, COSY NMR (D2O )
F1 [ppm]
F2 [ppm]
2
4
6
8




Compound **9,** HSQC NMR ($D_2O$ )


HSQC
Compound 9, HSQC NMR (D2O )
Lys-γ
Arg-γ
Arg-β
Lys-δ
Lys-β
Tyr-β
Asp-β
Lys-ε
Arg-δ
Gly-α
Gly-α
Asp-α
Arg-α
Tyr-α
Lys-α
(9)
30 F1 [ppm]
40
50
60
70
5
4
3
2
F2 [ppm]


cRGDyK(FBA)_150604083012 #48 RT: 0.44 AV: 1 NL: 7.75E6
T: FTMS + p ESI Full ms [250.00-1200.00]
742.3319
z=1
Compound 9, ESI-(FTMS-ion trap)
9
Chemical Formula: C34H45FN9O9+
Exact Mass: 742.3319
m/z

## ELISA Competition Binding Assay.

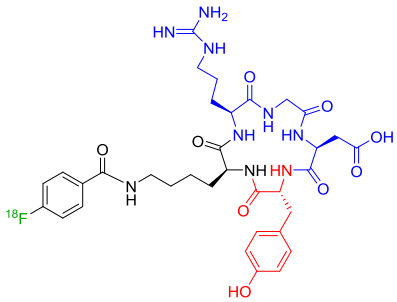
The potencies of compounds **4** and **9** were determined by competitive binding ELISA. The peptides were allowed to compete for 1 hour for binding to integrin $\alpha_v\beta_3$ in the presence of the biotinylated natural ligand vitronectin. A 96 well Nurco Immuno maxisorp plate was first coated with the capturing antibody P2W7 (50 μL per well, 5μg/mL) by incubation at 37 ˚C for 1 hour and washed three times with PBS. The wells were then blocked to prevent non-specific binding for 3 hours at room temperature with PBS containing 5% bovine serum albumin (BSA) (w/v) and 1% tween20 (v/v). The plate was then washed with a wash buffer that consisted of 2 mmol/L of Tris buffer (pH = 7.6), 150 mmol/L sodium chloride, 1 mmol/L manganese chloride, and 0.1% tween20 (v/v) in deionized water (3x). Once blocked the respective peptides were competed with equal volumes of biotinylated vitronectin in conjugate buffer consisting of 1% BSA added to the wash buffer. The peptides were serial diluted from a peptide stock of 2 mmol/L in 10% DMSO (v/v) into PBS and premixed with the same concentration of biotinylated vitronectin and placed onto the plate in triplicate for each peptide concentration. The competition was allowed to incubate at 1 hour at room temperature before being washed three times with wash buffer. Extravidin-horseradishperoxidase antibody was then added (50 μL/well, 1:1000 dilution in conjugate buffer) at room temperature and allowed to incubate for 1 hour, and again washed with wash buffer (3x). Then TMB substrate (50 μL/well) was added and incubated for 15-30 minutes at room temperature and terminated by adding 1N sulfuric acid (50 μL/well) and the absorbance was measured at 450 nm on a ThermoMultiskan Ascent plate reader. The calculated IC50 values are based on the data analysis with GraphPadPrism by fitting the data by nonlinear regression.

Positive control: Was the same procedure except no peptide was added to the wells, i.e. plated down integrin and bound vitronectin under no competition.

Negative control 1: Same as positive control 1 but no natural ligand vitronectin was added.

Negative control 2: Contains only conjugate buffer.

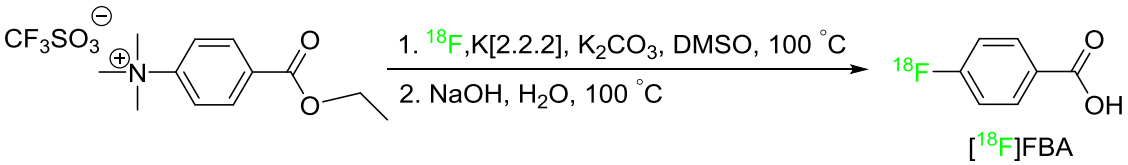
Radiochemical Experimental Compound **10.**



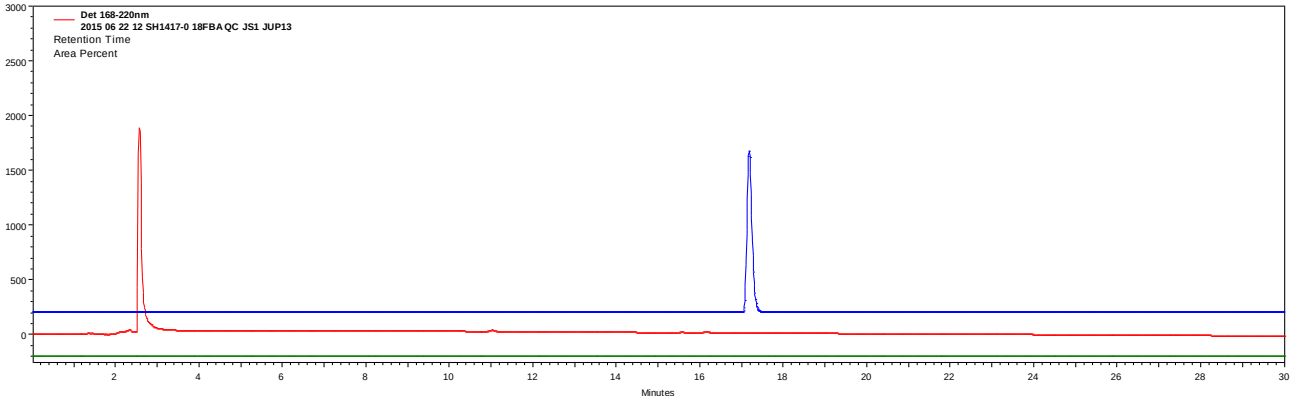

**10**-[$^{18}$F]-FBA-cRGDyK, 14%

**General Radiochemical Experimental**.

Upon cyclization and removal of the ivDde group to give **8**, the resin was then lyophilized and 5 mg of resin was used for the radiolabeling reactions with $^{18}$F-fluorobenzoic acid ($^{18}$F-FBA). Briefly, $^{18}$F-fluoride was produced via the $^{18}$O (p,n) $^{18}$F reaction by bombardment of [$^{18}$O]-water target with a 11 MeV proton negative ion cyclotron. The $^{18}$F was then captured on a fluoride separation column cartridge (Chromafix, 30-PS-$HCO_3^-$) and eluted with a solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (K2.2.2, 15 mg) and potassium carbonate(3 mg) in acetonitrile/di-water (2 mL, 6% v/v). The $^{18}$F-fluoride was dried via azeotropic distillation using an additional 2 mL of acetonitrile. A solution of ethyl-4-(trimethylammoniumtriflate)benzoate (5 mg) in anhydrous DMSO (500 uL) was added to the dried $^{18}$F- fluoride source and heated at 100℃ for 15 minutes. Aqueous NaOH (0.5 M, 1 mL) was added and heated at 100℃ for 10 minutes, upon which the reaction was quenched with HCl (1 M, 2 mL). The reaction was diluted with di-water (6 mL) and crude $^{18}$F-FBA was passed through a C18-SepPak column cartridge. The product is washed with di-water (5 mL) and dried with air. The product was eluted with acetonitrile (2 mL) and concentrated. The [$^{18}$F]FBA diluted in DMF (140 uL) was added to preswelled resin. The DMF-swelled resin and [$^{18}$F]FBA is then activated by drawing up HATU (5 mg in 30 µL DMF), DIPEA (10 uL in 20 µL DMF) solution and allowing it to react for 30 min. at 30℃. The resin is then washed with DMF (3 x 0.5 mL) and methanol (3 x 0.5 mL). The radiolabeled peptide was then cleaved from the resin using a TIPS:water:TFA cocktail (2.5:2.5:95, 2 x 0.25 mL, 10 min. each). The product was concentrated and dissolved in a 3:1 ratio of di-water containing 0.05%TFA and acetonitrile. The product was purified using reverse-phase semi-preparative HPLC with a Phenomenex Jup-C18 column (250 x 10 mm, 10 µm) using the standard HPLC solvent gradient (Table 1, S4) and a flow rate of 3 mL/min. The retention time was 16.1 ± 0.2 min (n = 4) with an average isolated decay corrected yield of 14% (n = 4) with a purity > 99% and a synthesis time of ~90 min.

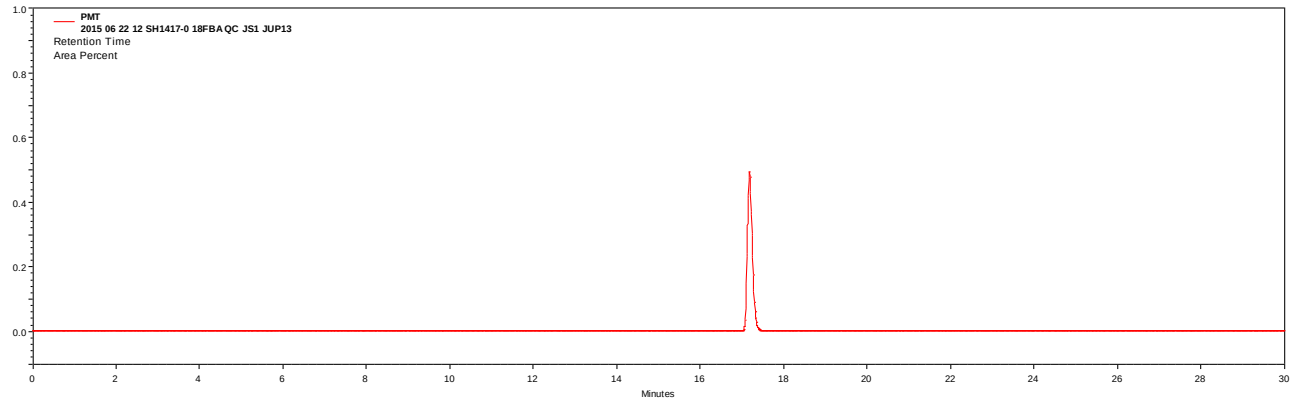



**$^{18}$F-FBA-HPLC Trace**



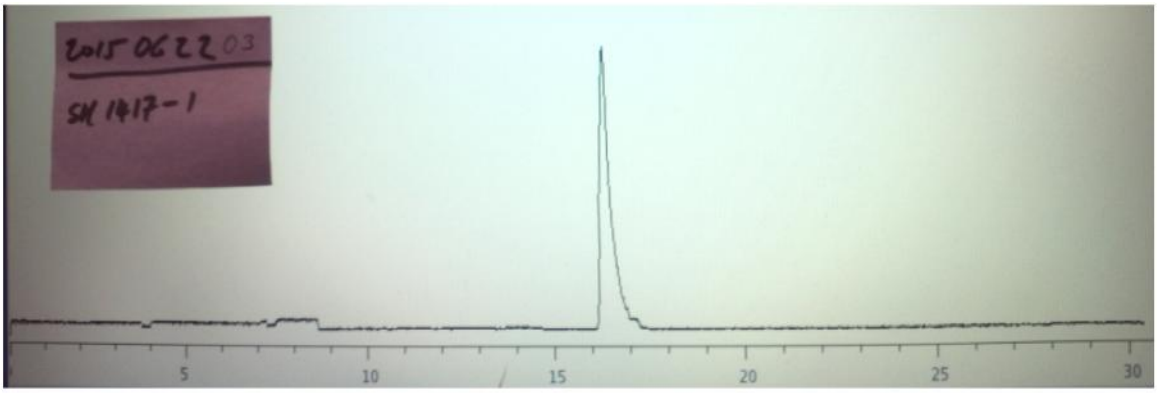

Red UV-220nm
Grn UV-254nm
Blu RA-(PMT)

**HPLC-trace for the gamma-detector only for $^{18}$F-FBA**

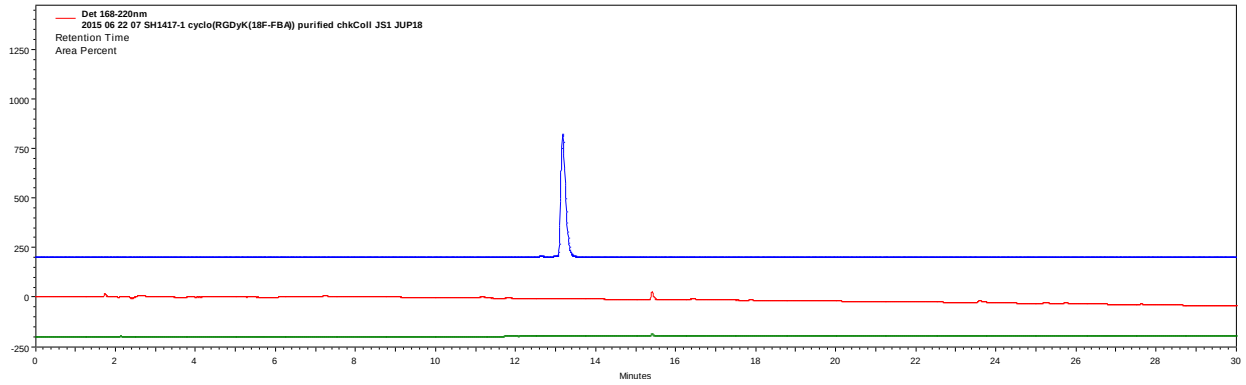



Red - RA (PMT)
RA: 17.2 min. Sharp single peak

Representative semi-preparative HPLC trace (PMT-detector) of compound **10** (n = 4).

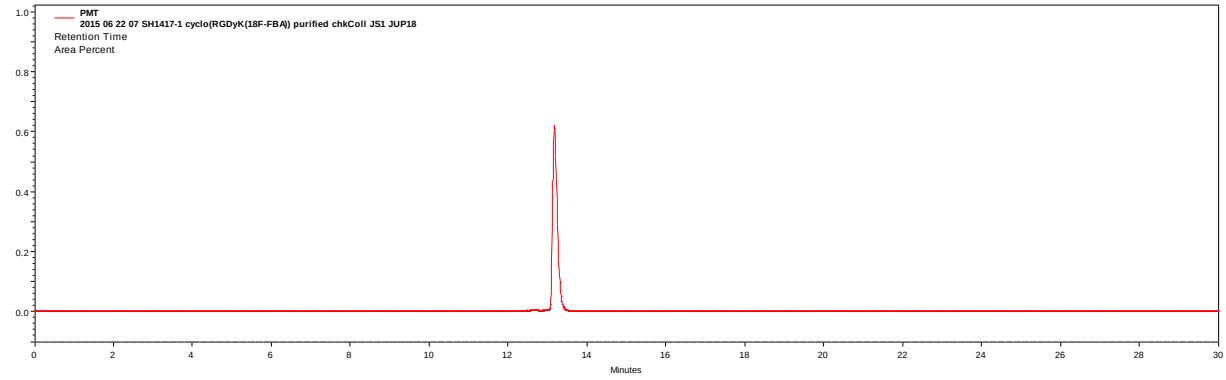



Representative analytical-HPLC trace of Compound **10** (n = 4)



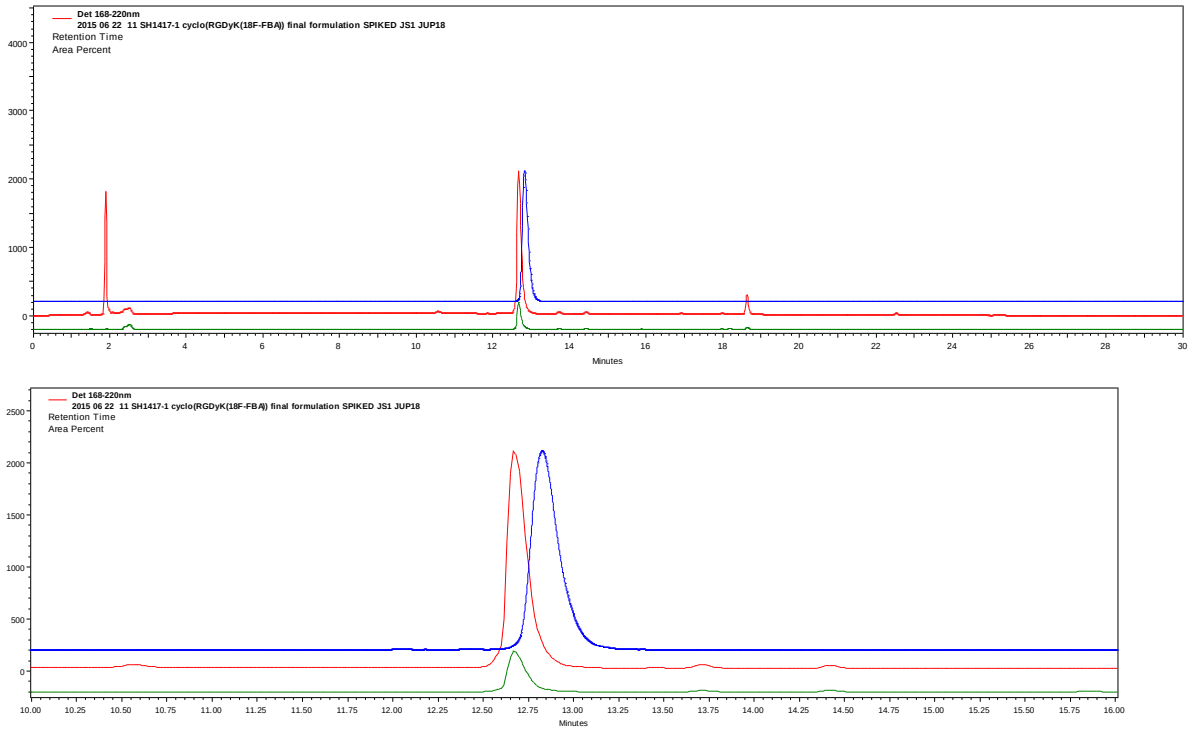

Red UV-220nm
Grn UV-254nm
Blu RA-(PMT)

Representative analytical-HPLC trace of Compound **11** showing just the gamma-detector signal (n = 4)



Red: RA (PMT)
RA: 13.2 min > 99%

Co-injection of cold standard **9** and radiolabeled compound **10** (n = 4).




Red UV-220nm
Grn UV-254nm
Blu RA-(PMT)