Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Chirality effects at each amino acid position on tripeptide self-assembly into hydrogel biomaterials

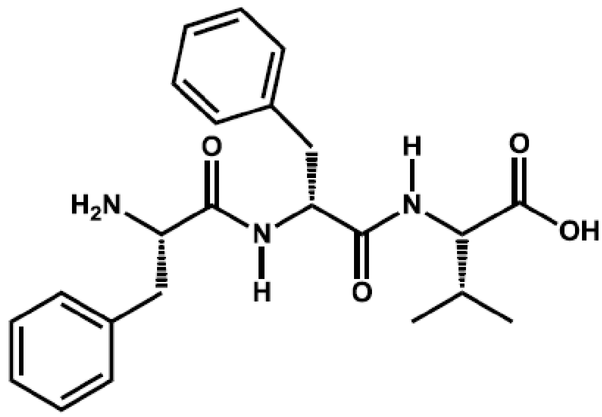
S. Marchesan*[a,b], C. D. Easton[a], K. E. Styan[a], L. J. Waddington[a], F. Kushkaki[c], L. Goodall[a], K. M. McLean[a], J. S. Forsythe[d], and P. G. Hartley[a].

a CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3053, Australia
b Present address: INSTM, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy
c La Trobe University, Department of Chemistry, La Trobe Institute of Molecular Sciences, Kingsbury Drive, Bundoora, Melbourne VIC 3086, Australia.
d Department of Materials Engineering, Monash University, Victoria 3800, Australia

* email: marchesan.silvia@gmail.com

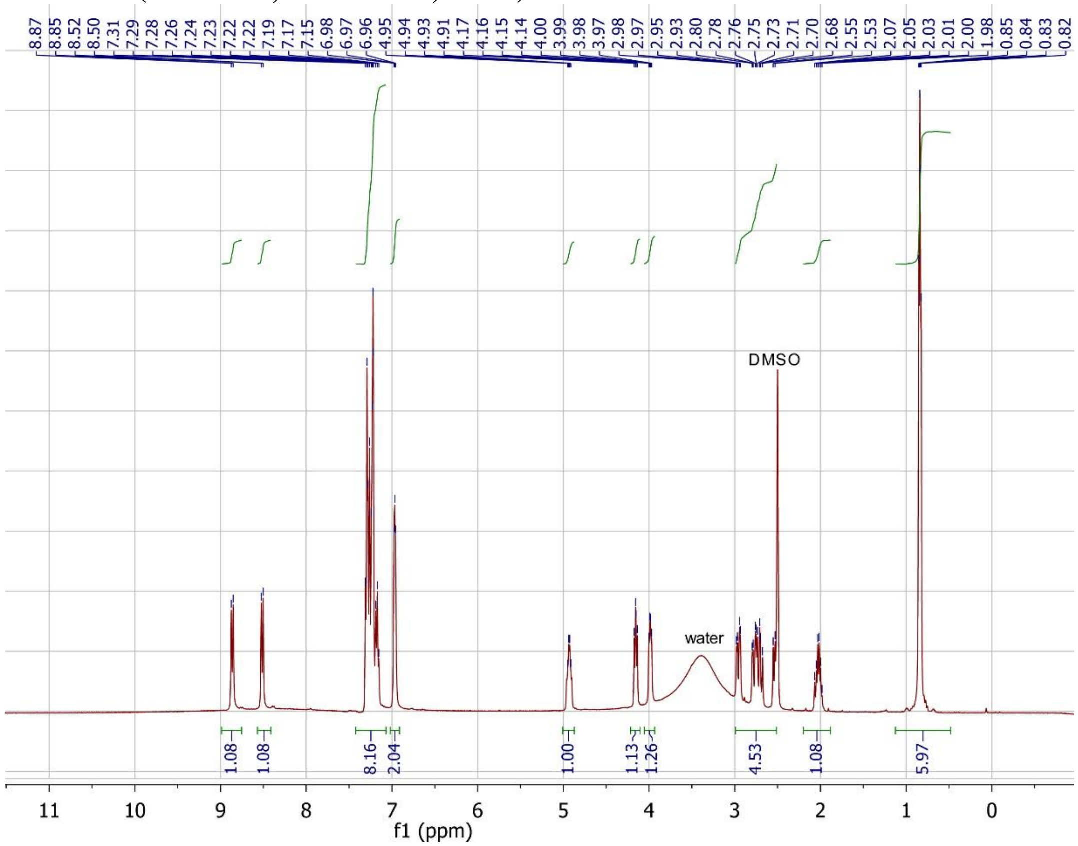
Table of Contents

1. Phe-^{D}Phe-Val

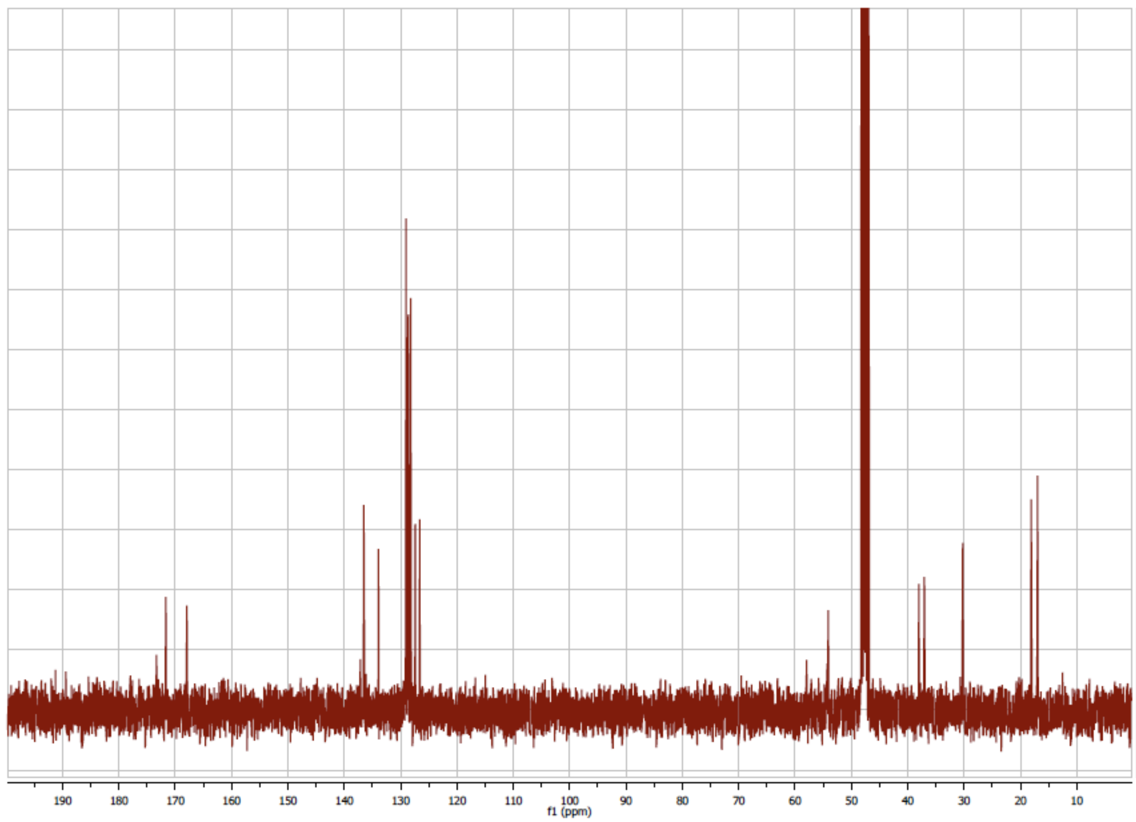


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ 8.86 (d, *J* = 8 Hz, 1H, NH), 8.51 (d, *J* = 8 Hz, 1H, NH), 7.31-7.15 (m, 8H, Ar), 6.97 (m, 2H, Ar), 4.93 (m, 1H, αCH), 4.15 (m, 1H, αCH), 3.98 (m, 1H, αCH), 2.97-2.53 (m, 4H, 2 x βCH_2), 2.00 (m, 1H, βCH), 0.84 (d, 3H, γCH_3), 0.83 (d, 3H, γCH_3). ^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS): δ (ppm) 173.3, 171.7, 167.9 (3 x CO); 136.5, 133.9, 129.1, 129.0, 128.9, 128.8, 127.6, 126.7 (Ar); 57.9, 54.3, 54.1, (3 x αC); 38.0, 37.1 (2 x βCH_2); 30.2 (βCH); 18.1, 16.9 (2 x γCH_3). MS (ESI): m/z 412.1 $(M+H)^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

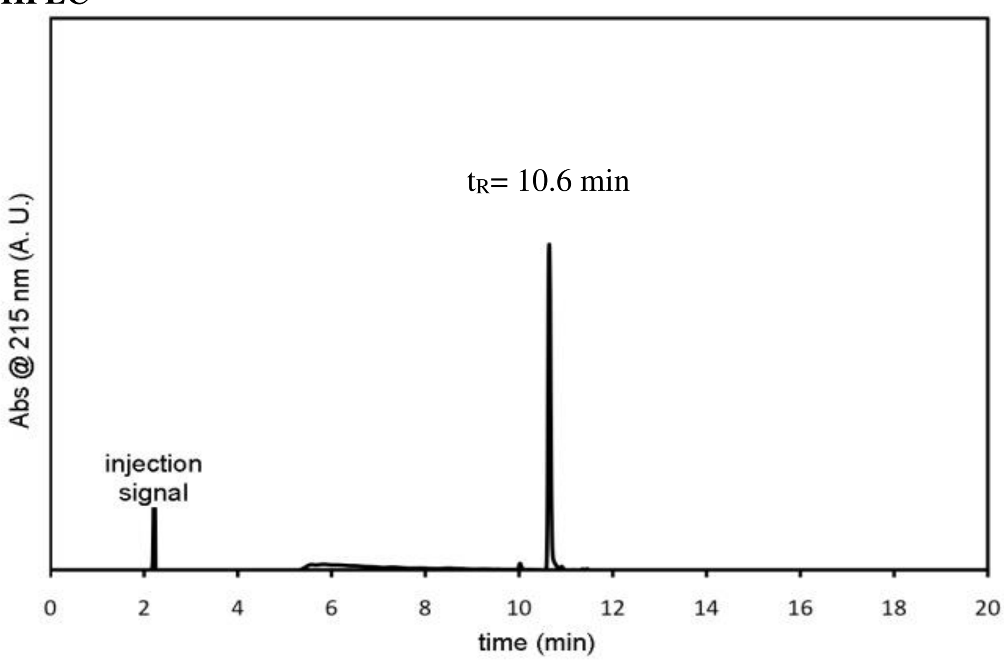
^{1}H-NMR (400 MHz, DMSO- d$_6$, TMS)



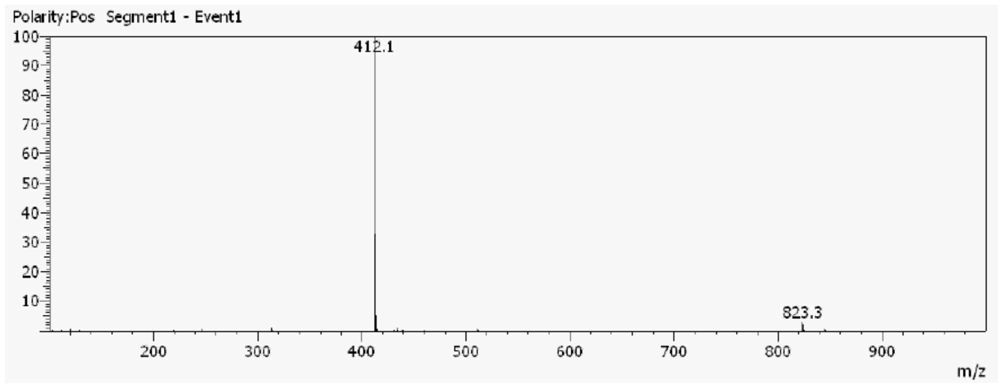
^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS)



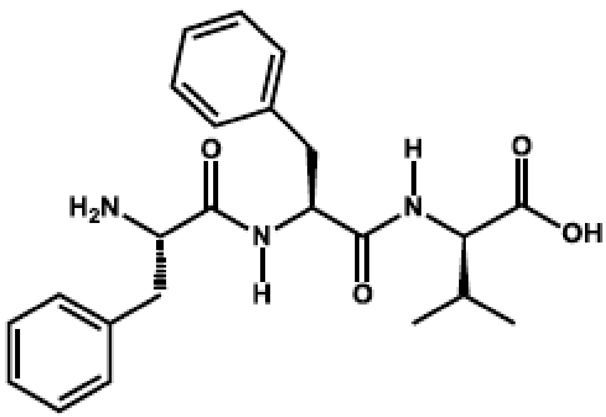
HPLC



ESI-MS

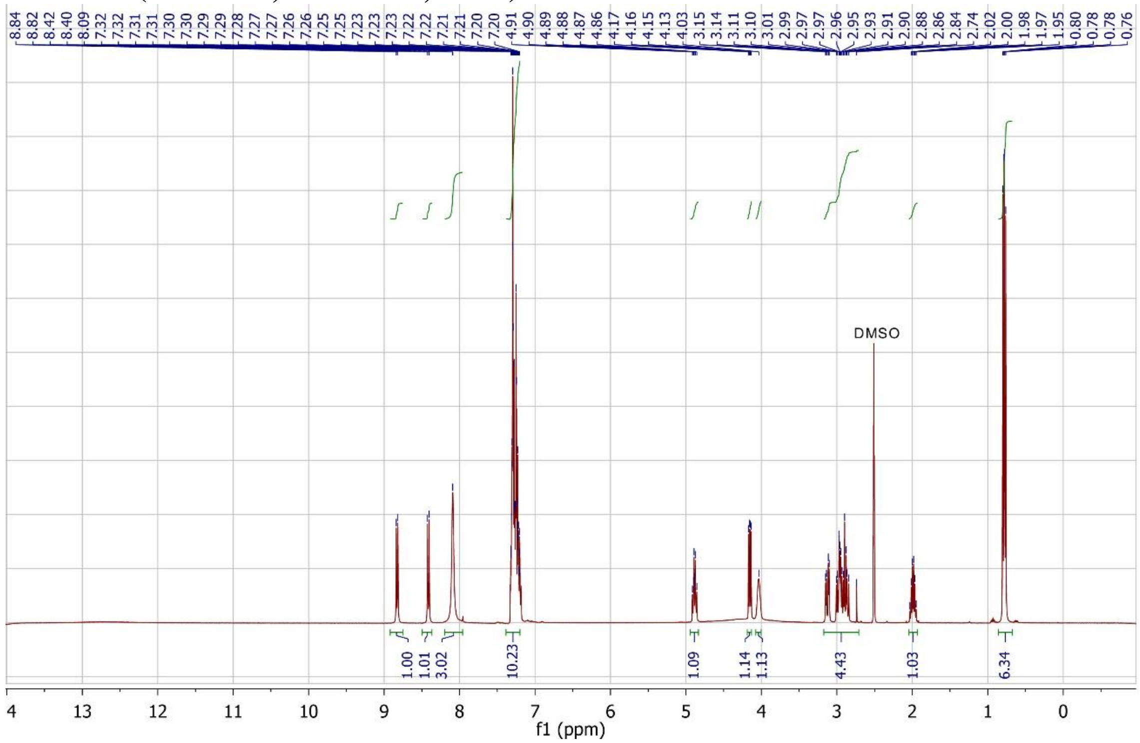


2. Phe-Phe-^{D}Val

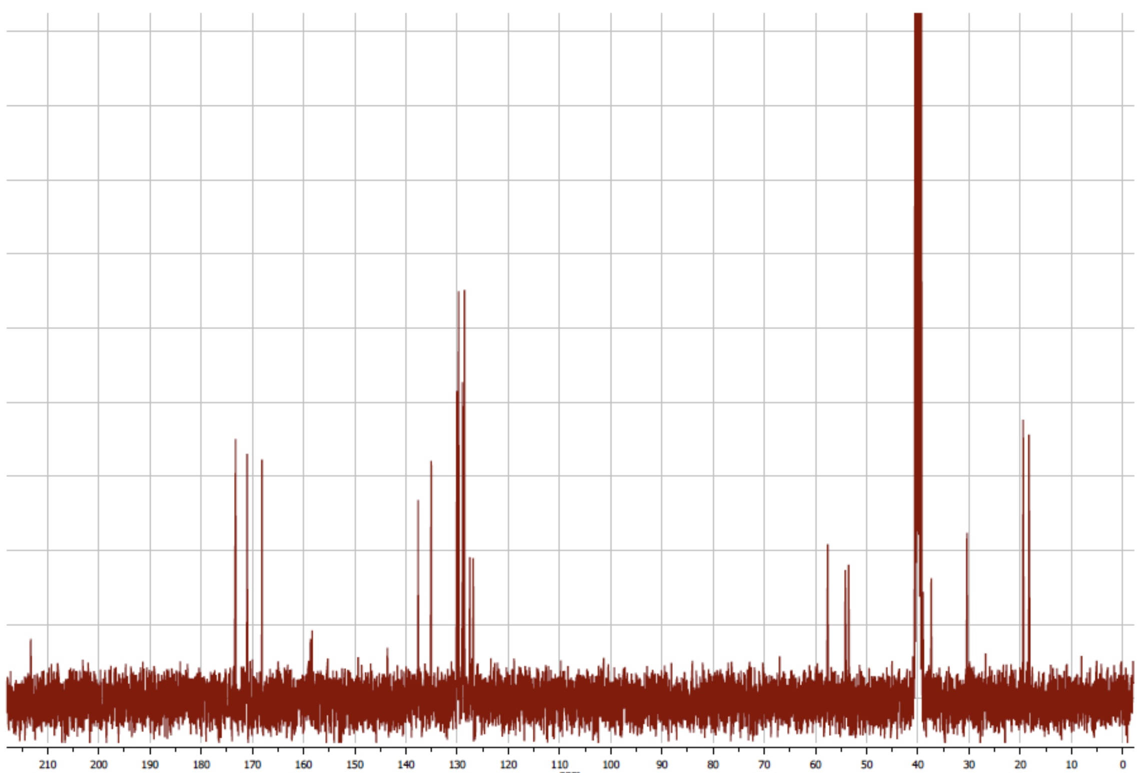


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ (ppm) 8.83 (d, *J* = 8 Hz, 1H, NH), 8.41 (d, *J* = 8 Hz, 1H, NH), 8.09 (s (br), 3H, NH_3^+), 7.32-7.20 (m, 10H, Ar), 4.88 (m, 1H, αCH), 4.17-4.13 (m, 1H, αCH), 4.03 (m, 1H, αCH), 3.07 (dd, *J* = 4 and 12 Hz,1H, βCH_2), 2.95 (dd, *J* = 4 and 16 Hz, 1H, βCH_2), 2.90 (dd, *J* = 4 and 12 Hz, 1H, βCH_2), 2.85 (m, *J* = 4 and 16 Hz, 1H, βCH_2), 1.97 (m, 1H, βCH), 0.79 (d, 3H, γCH_3), 0.77 (d, 3H, γCH_3). ^{13}C-NMR (100 MHz, DMSO- d$_6$, TMS): δ (ppm) 173.7, 171.3, 168.2 (3 x CO); 137.5, 134.9, 129.9, 129.8, 128.8, 128.6, 127.4, 126.8 (Ar); 57.6, 54.0, 53.5, (3 x αC); 39.0, 37.4 (2 x βCH_2); 30.4 (βCH); 19.4, 18.3 (2 x γCH_3). MS (ESI): m/z 412.1 $(M+H)^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

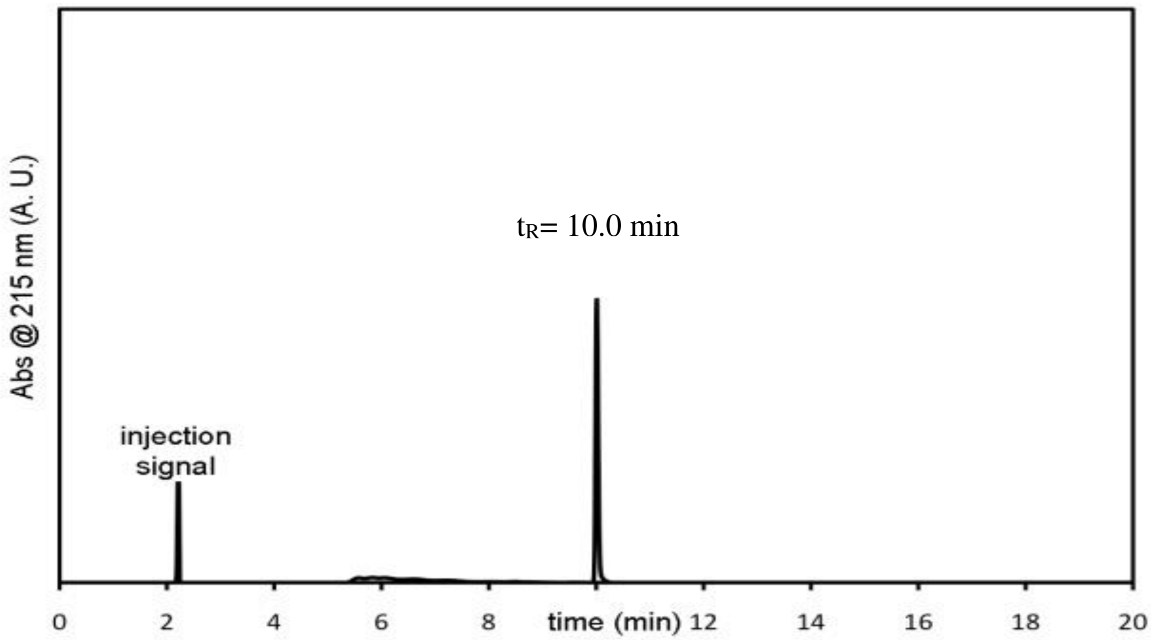
^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS)



^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS)

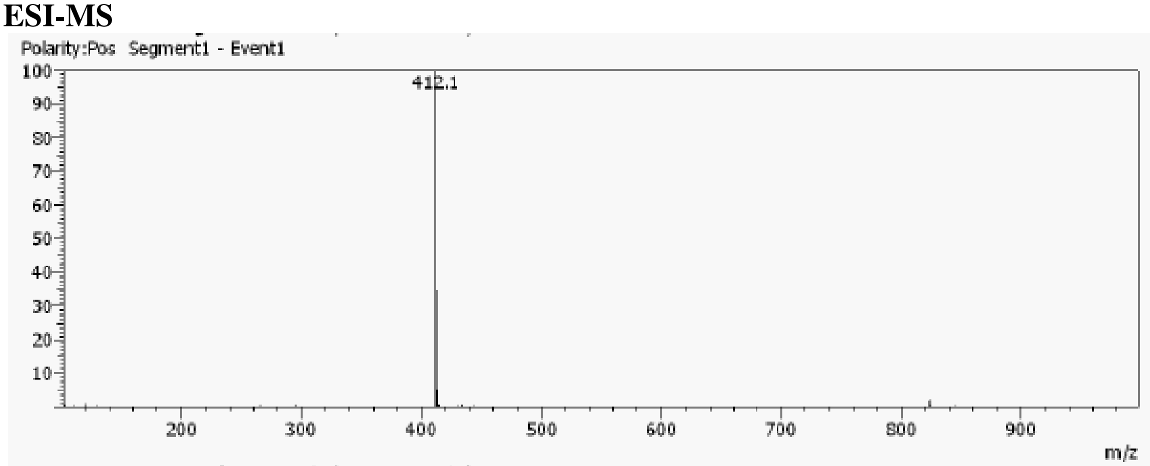


HPLC



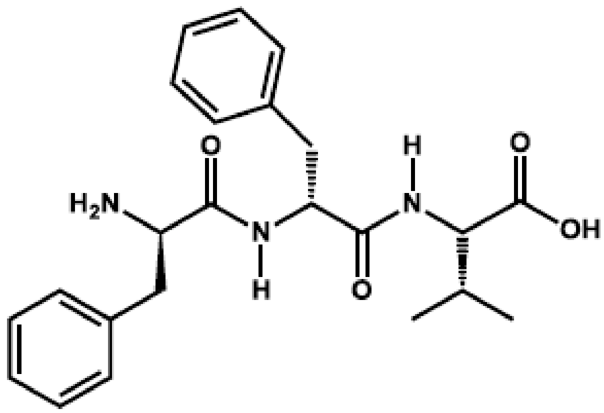
Abs @ 215 nm (A. U.)
t_R= 10.0 min
injection
signal
0
2
4
6
8
time (min)
12
14
16
18
20

ESI-MS



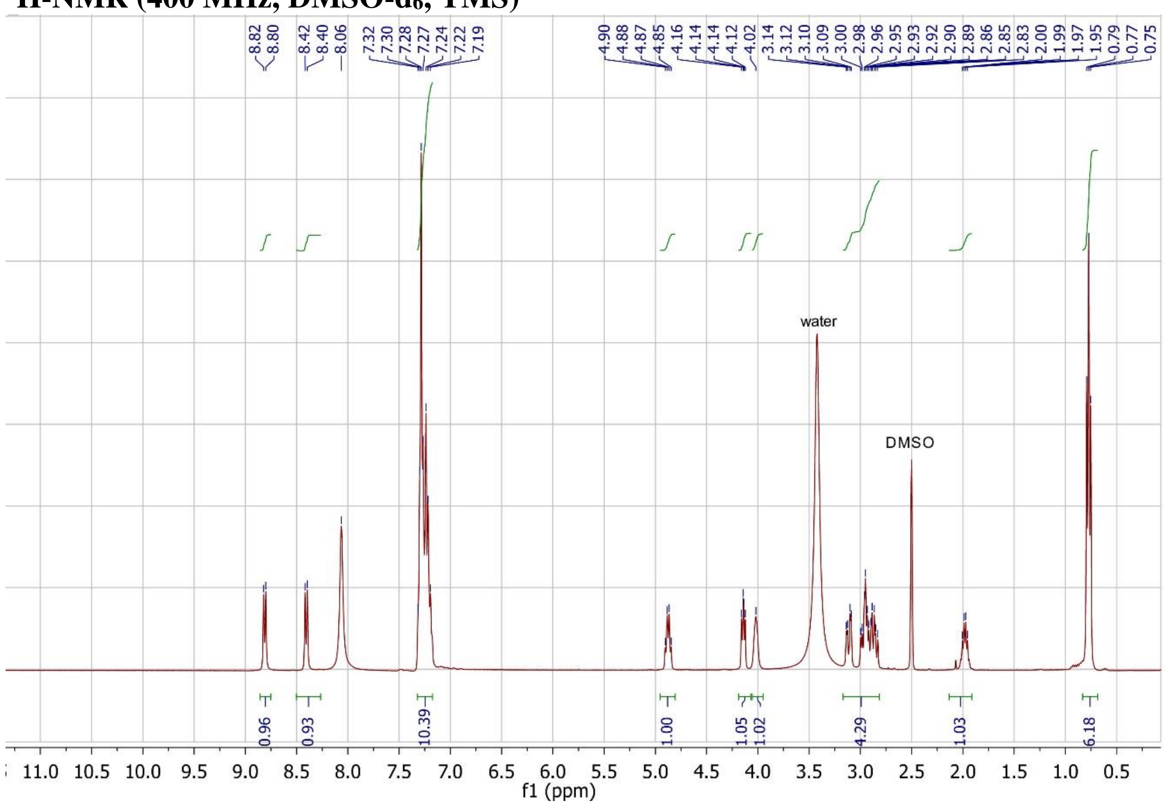
Polarity:Pos Segment1 - Event1
412.1
100
90
80
70
60
50
40
30
20
10
200
300
400
500
600
700
800
900
m/z

3. ^{D}Phe-^{D}Phe-Val

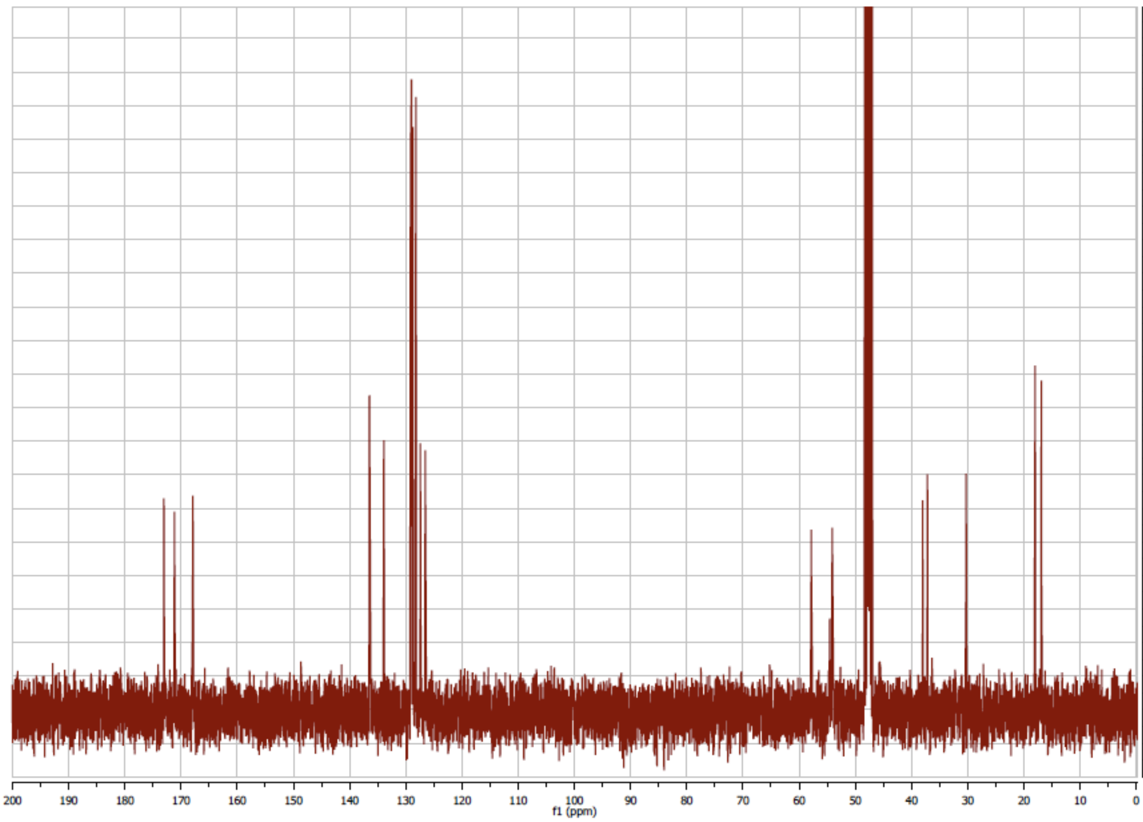


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ (ppm) 8.77 (d, J = 8 Hz, 1H, NH), 8.37 (d, J = 8 Hz, 1H, NH), 8.03 (s (br), 3H, NH_3^+), 7.25-7.16 (m, 10H, Ar), 4.84 (m, 1H, αCH), 4.12-4.09 (m, 1H, αCH), 3.98 (m, 1H, αCH), 3.07 (dd, J = 4 and 12 Hz,1H, βCH$_2$), 2.93 (dd, J = 4 and 16 Hz, 1H, βCH$_2$), 2.89 (dd, J = 4 and 12 Hz, 1H, βCH$_2$), 2.82 (m, J = 4 and 16 Hz, 1H, βCH$_2$), 1.94 (m, 1H, βCH), 0.75 (d, 3H, γCH$_3$), 0.72 (d, 3H, γCH$_3$). ^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS): δ (ppm) 173.3, 171.0, 168.1 (3 x CO); 137.6, 135.0, 130.0, 129.7, 129.0, 128.6, 127.5, 126.9 (Ar); 57.6, 54.2, 53.5, (3 x αC); 39.0, 37.4 (2 x βCH$_2$); 30.4 (βCH); 19.4, 18.3 (2 x γCH$_3$). MS (ESI): m/z 412.1 (M+H)$^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

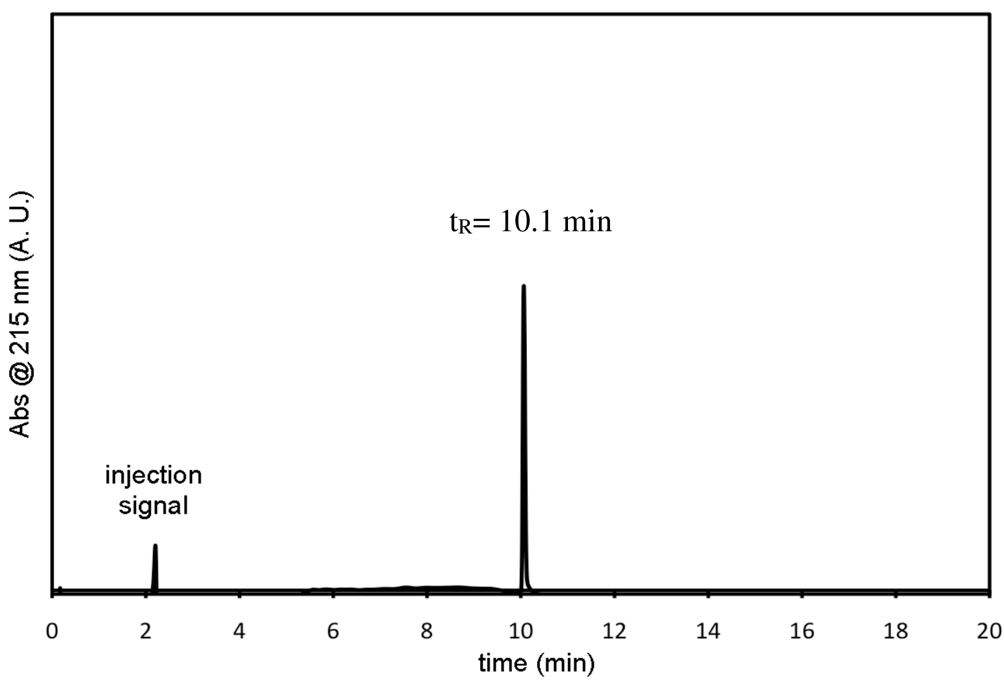
^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS)



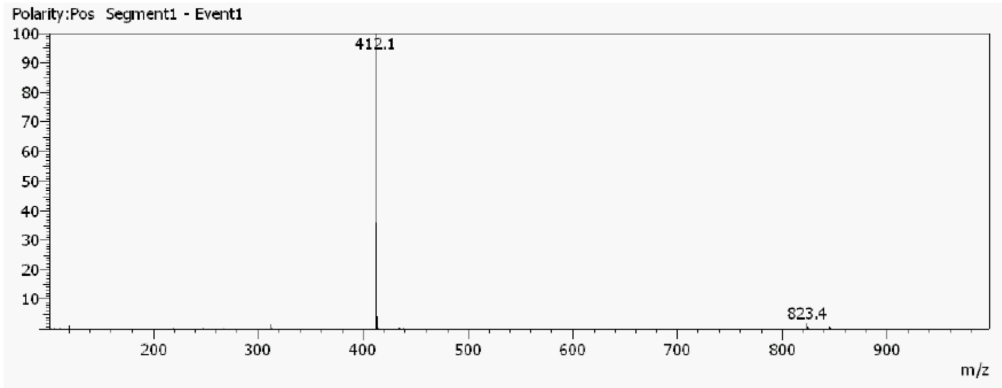
^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS)



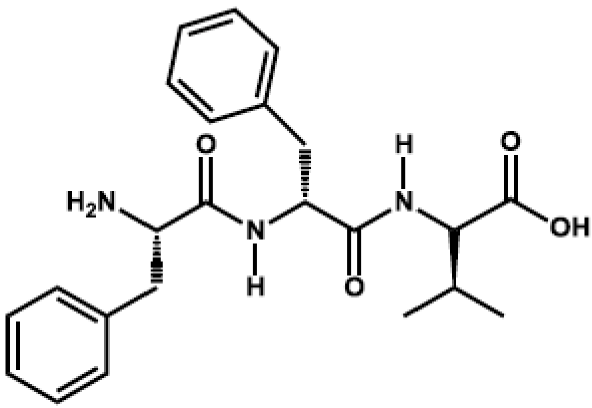
HPLC



ESI-MS

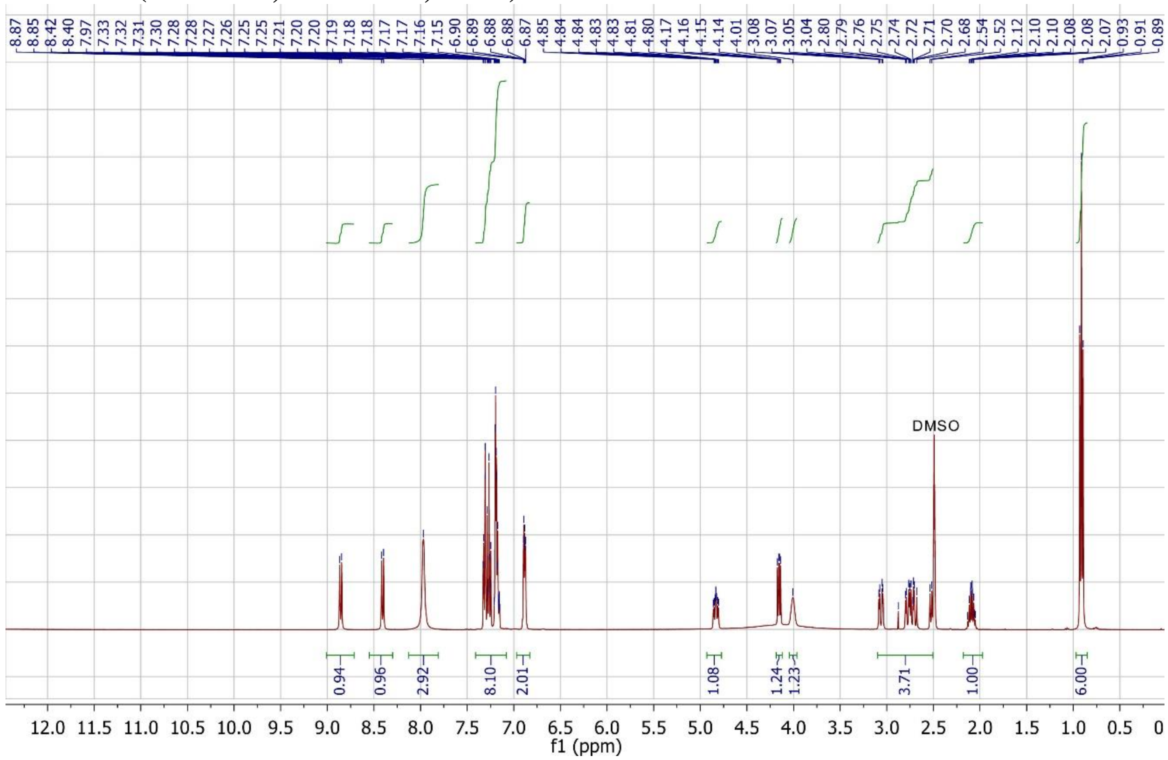


4. Phe-^{D}Phe-^{D}Val

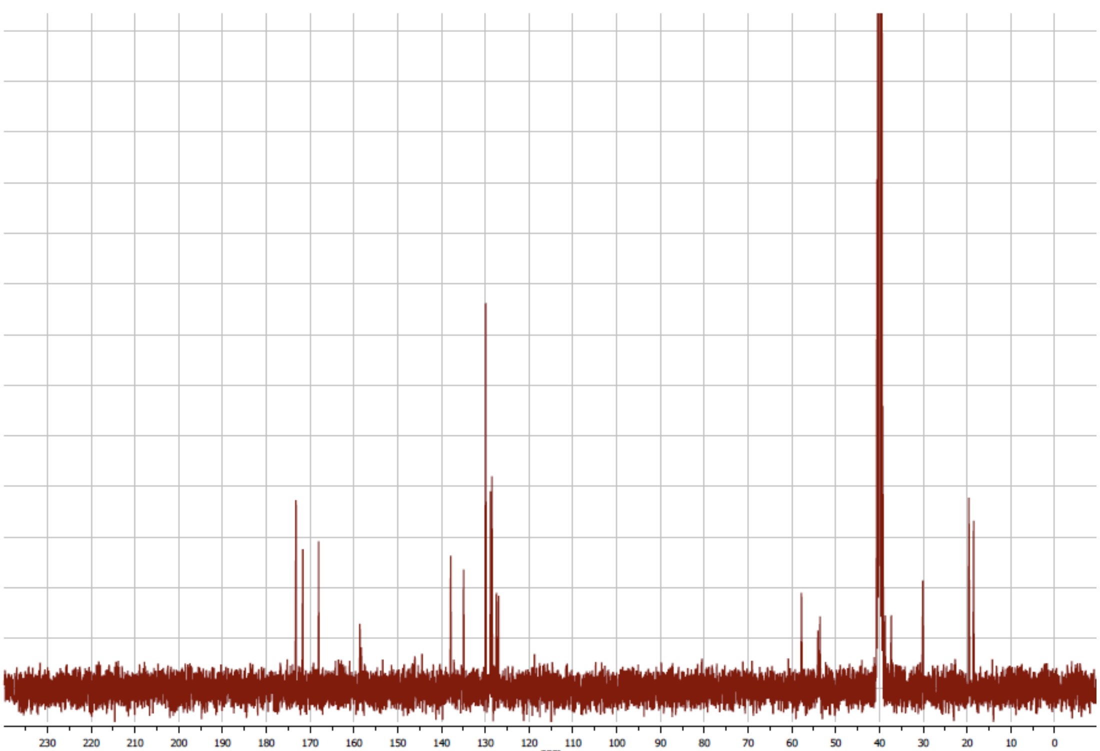


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ (ppm) 8.86 (d, *J* = 8 Hz, 1H, NH), 8.41 (d, *J* = 8 Hz, 1H, NH), 7.97 (s (br), 3H, NH_3^+), 7.33-7.15 (m, 8H, Ar), 6.88 (m, 2H, Ar), 4.83 (m, 1H, αCH), 4.17-4.14 (m, 1H, αCH), 4.01 (m, 1H, αCH), 3.06 (dd, *J* = 4 and 12 Hz, 1H, βCH_2), 2.78 (dd, *J* = 4 and 12 Hz, 1H, βCH_2), 2.71 (dd, *J* = 8 and 12 Hz, 1H, βCH2), 2.51 (dd, *J* = 8 and 12 Hz, 1H, βCH_2), 2.09 (m, 1H, βCH), 0.92 (d, 3H, γCH_3), 0.90 (d, 3H, γCH_3). ^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS): δ (ppm) 173.2, 171.7, 168.0 (3 x CO); 137.9, 135.0, 129.9, 128.8, 128.5, 127.5, 127.0 (Ar); 57.8, 54.1, 53.6, (3 x αC); 38.7, 37.3 (2 x βCH_2); 30.1 (βCH); 19.6, 18.5 (2 x γCH_3). MS (ESI): m/z 412.1 $(M+H)^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

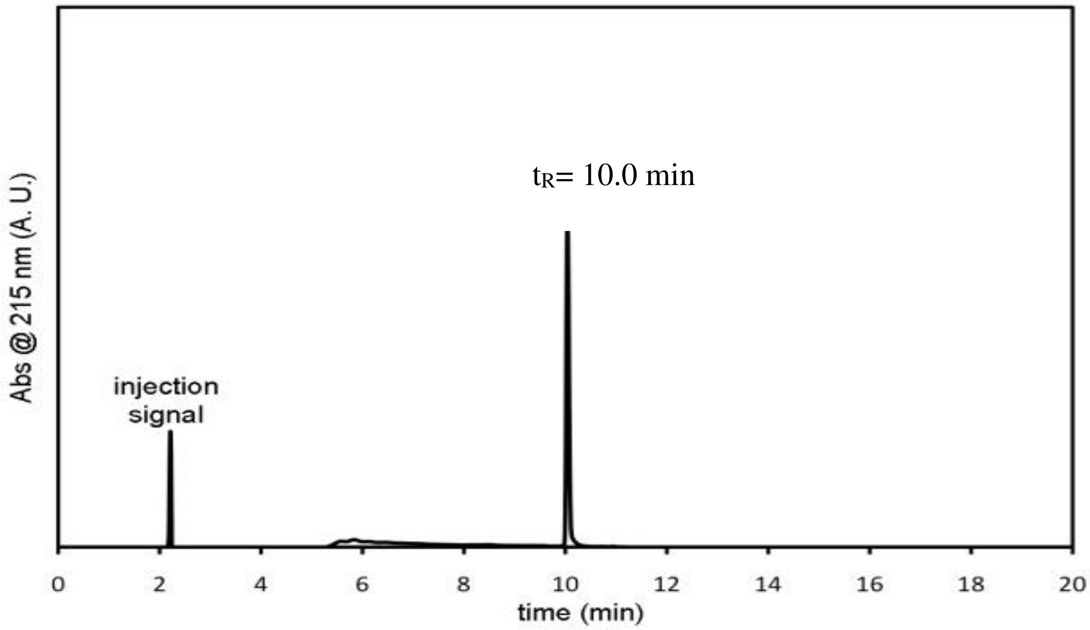
[1]H-NMR (400 MHz, DMSO-d$_6$, TMS)



[13]C-NMR (100 MHz, DMSO-d$_6$, TMS)

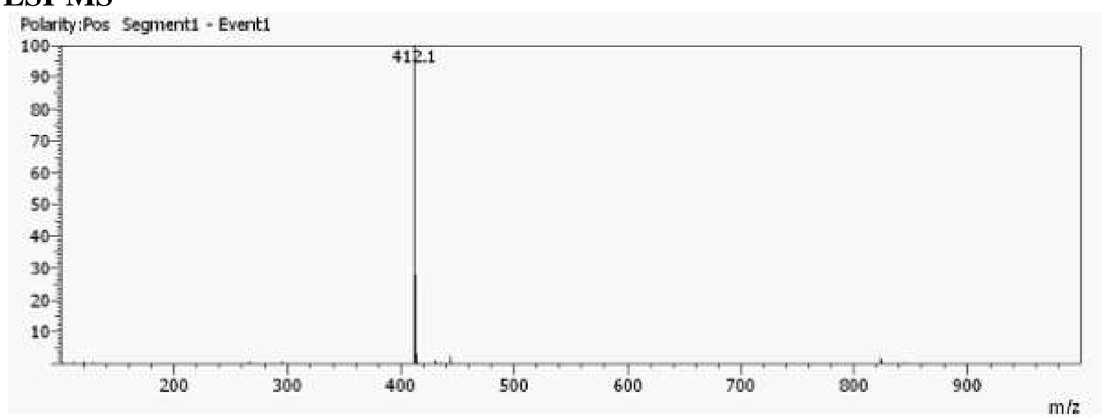


HPLC



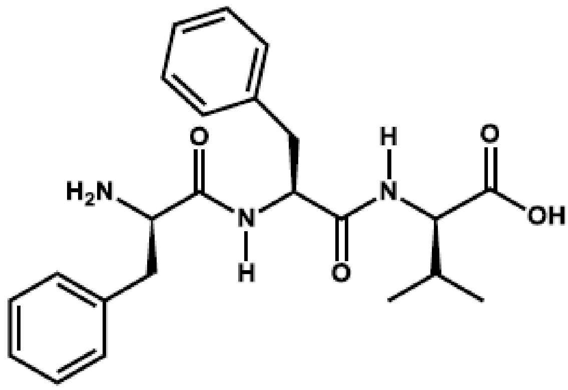
t_R= 10.0 min
injection signal
Abs @ 215 nm (A. U.)
0
2
4
6
8
10
12
14
16
18
20
time (min)

ESI-MS



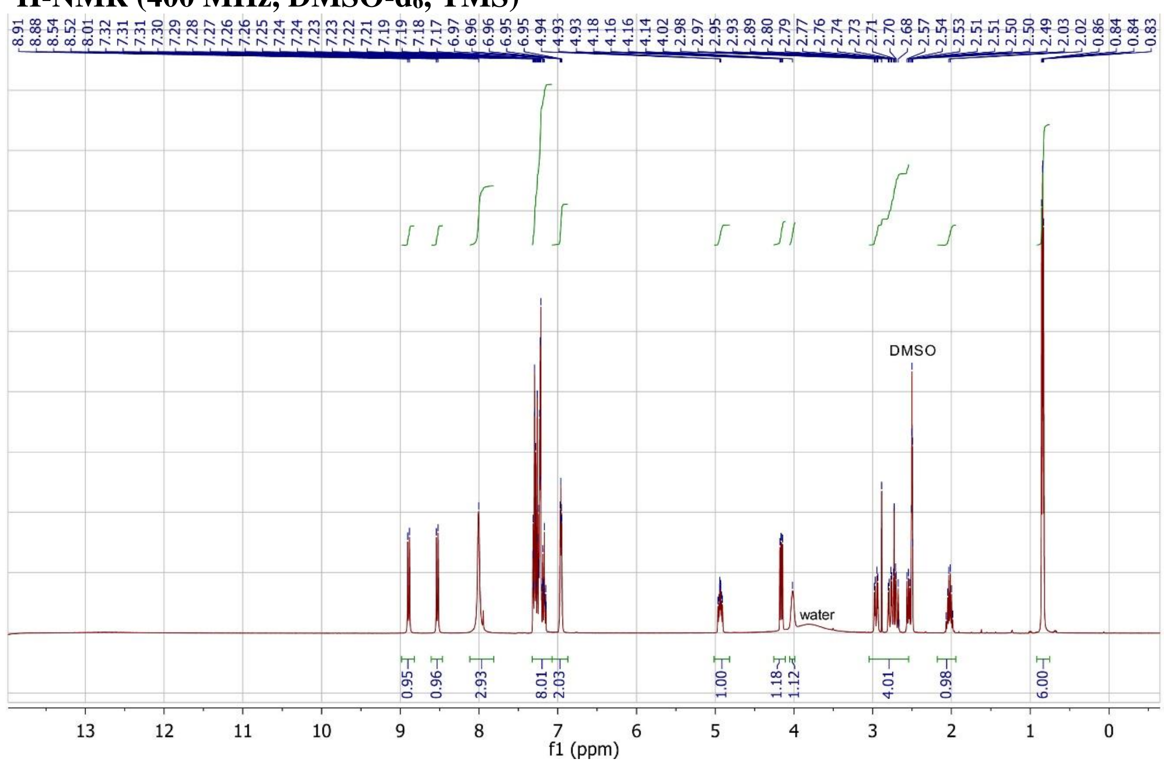
Polarity:Pos Segment1 - Event1
412.1
100
90
80
70
60
50
40
30
20
10
200
300
400
500
600
700
800
900
m/z

5. ^{D}Phe-Phe-^{D}Val

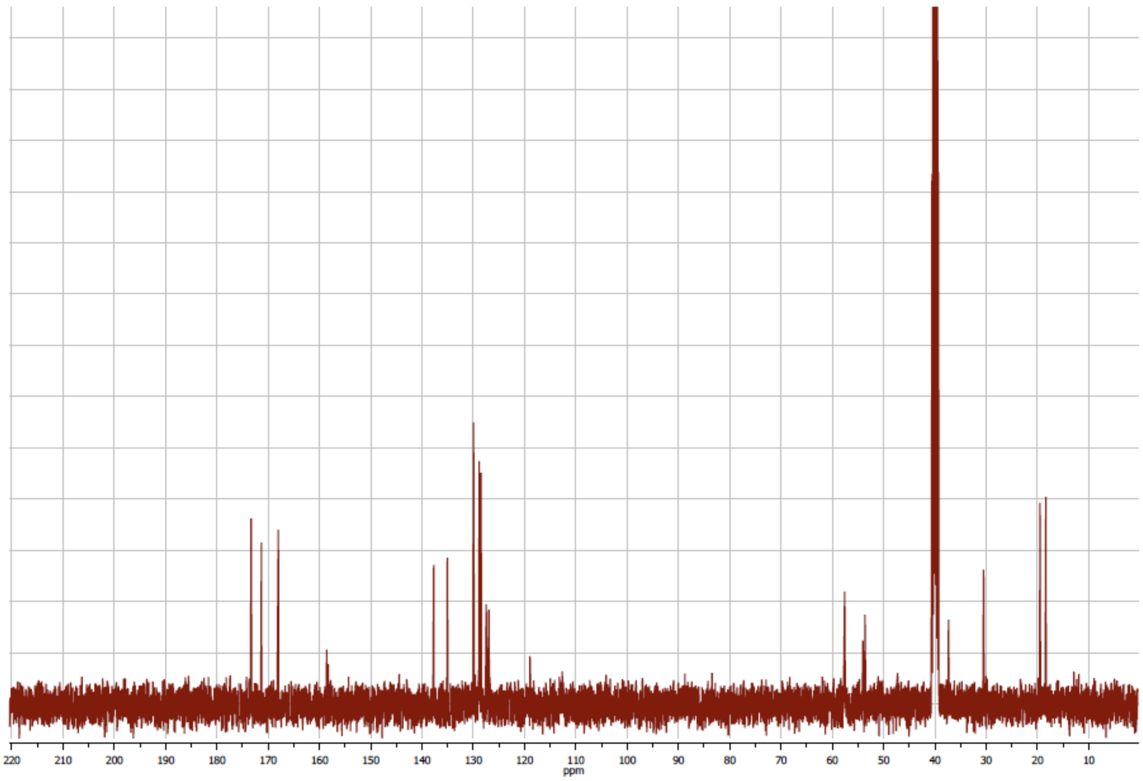


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ (ppm) 8.90 (d, J = 8 Hz, 1H, NH), 8.53 (d, J = 8 Hz, 1H, NH), 8.01 (s (br), 3H, NH_3^+), 7.32-7.17 (m, 8H, Ar), 6.96 (m, 2H, Ar), 4.93 (m, 1H, αCH), 4.18-4.14 (m, 1H, αCH), 4.02 (m, 1H, αCH), 2.96 (dd, J = 4 and 12 Hz, 1H, βCH$_2$), 2.80-2.66 (m, 2H, βCH$_2$), 2.53 (dd, J = 4 and 16 Hz, 1H, βCH$_2$), 2.02 (m, 1H, βCH), 0.85 (d, 3H, γCH$_3$), 0.83 (d, 3H, γCH$_3$). ^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS): δ (ppm) 173.3, 171.3, 168.0 (3 x CO); 137.7, 135.0, 130.0, 129.9, 128.8, 128.5, 127.5, 127.0 (Ar); 57.6, 54.0, 53.6, (3 x αC); 39.3, 37.3 (2 x βCH$_2$); 30.5 (βCH); 19.5, 18.4 (2 x γCH$_3$). MS (ESI): m/z 412.1 $(M+H)^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

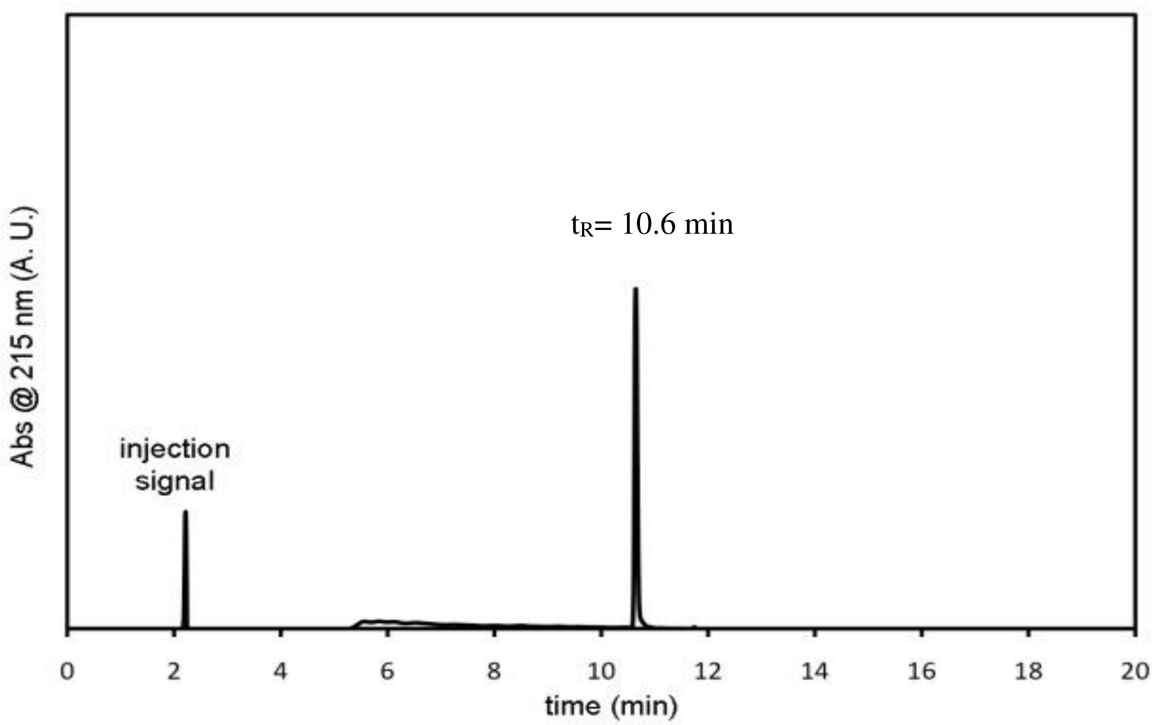
^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS)



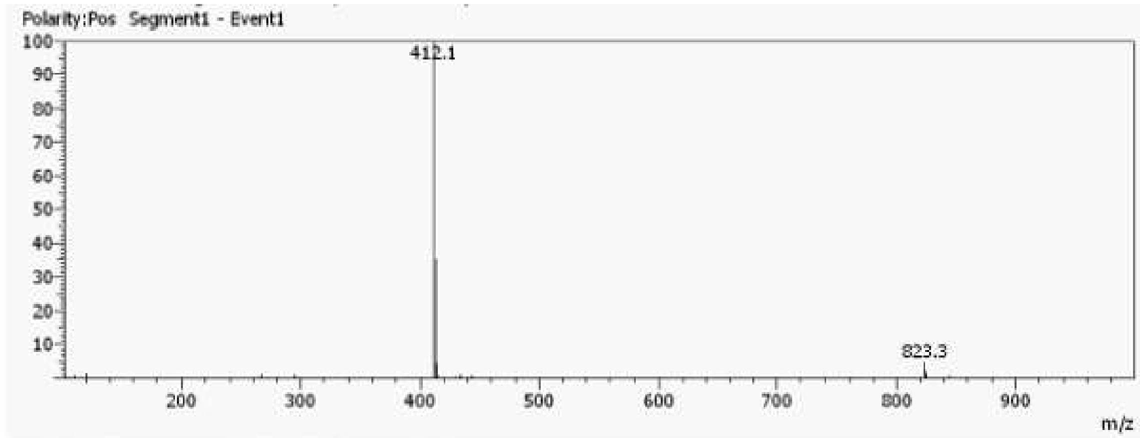
^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS)



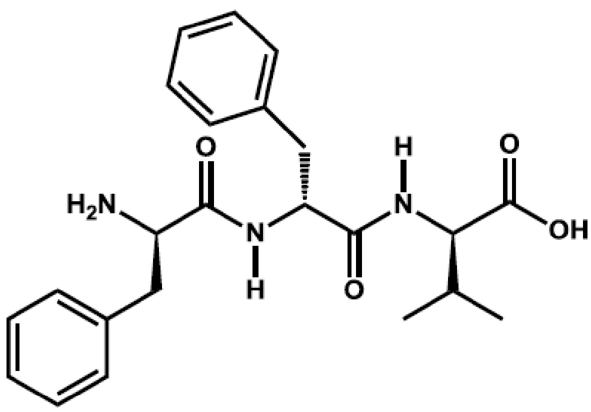
HPLC



ESI-MS

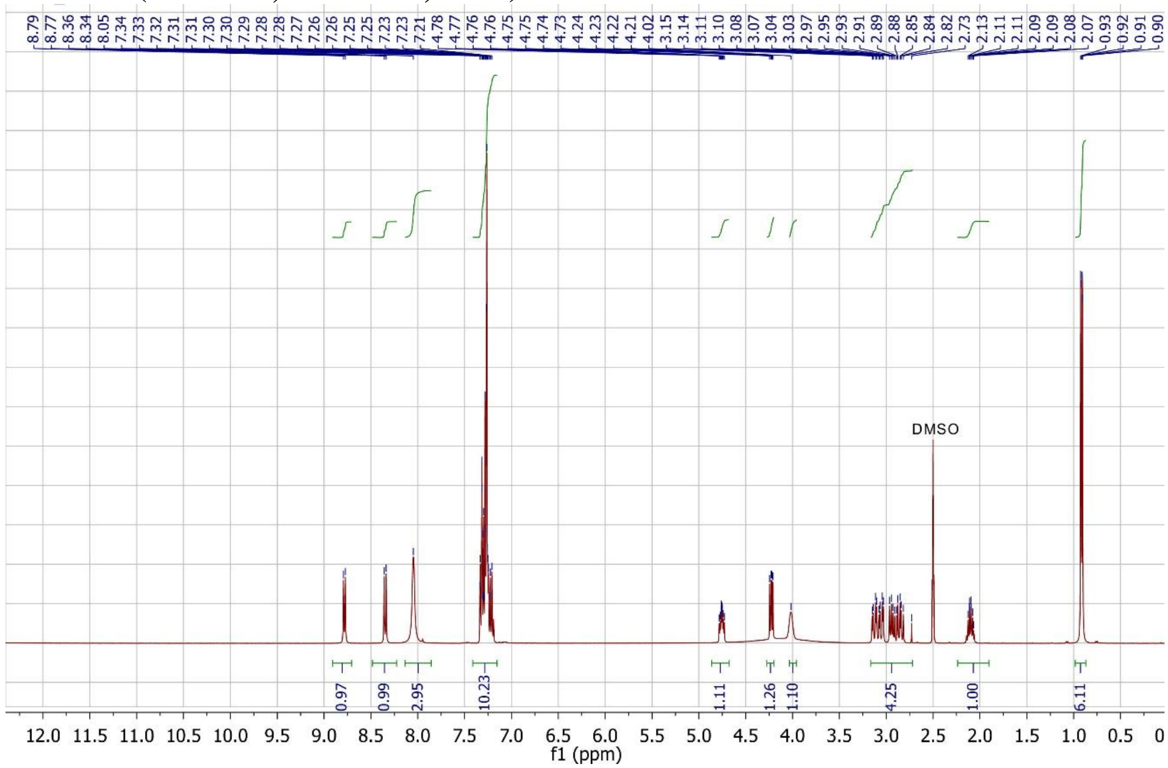


6. ^{D}Phe-^{D}Phe-^{D}Val

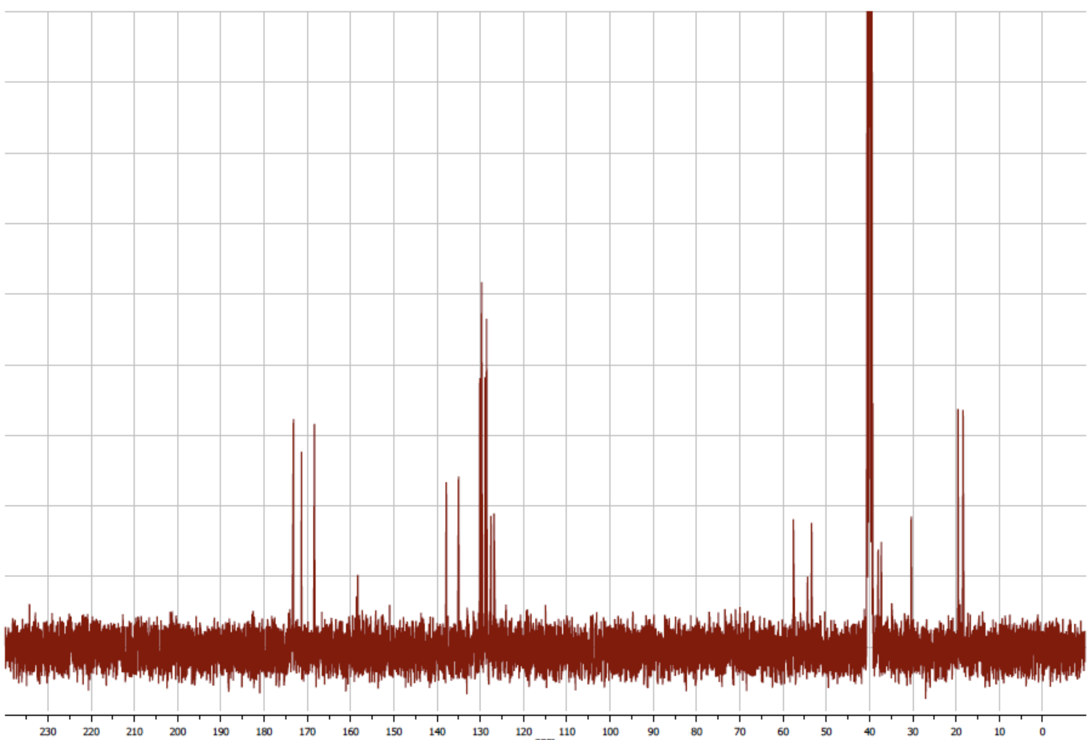


^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS): δ (ppm) 8.78 (d, *J* = 8 Hz, 1H, NH), 8.35 (d, *J* = 8 Hz, 1H, NH), 8.05 (s (br), 3H, NH_3^+), 7.34-7.21 (m, 10H, Ar), 4.78-4.73 (m, 1H, αCH), 4.24-4.21 (m, 1H, αCH), 4.02 (m, 1H, αCH), 3.12 (dd, *J* = 4 and 16 Hz, 1H, βCH_2), 3.05 (dd, *J* = 4 and 16 Hz, 1H, βCH_2), 2.94 (dd, *J* = 8 and 16 Hz, 1H, βCH_2), 2.85 (dd, *J* = 8 and 16 Hz, 1H, βCH_2), 2.10 (m, 1H, βCH), 0.92 (d, 3H, γCH_3), 0.90 (d, 3H, γCH_3). ^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS): δ (ppm) 173.3, 171.3, 168.0 (3 x CO); 137.7, 135.0, 130.0, 129.9, 128.8, 128.5, 127.5, 127.0 (Ar); 57.6, 54.0, 53.6, (3 x αC); 39.3, 37.3 (2 x βCH_2); 30.5 (βCH); 19.5, 18.4 (2 x γCH_3). MS (ESI): m/z 412.1 $(M+H)^+$, $C_{23}H_{29}N_3O_4$ requires 412.2.

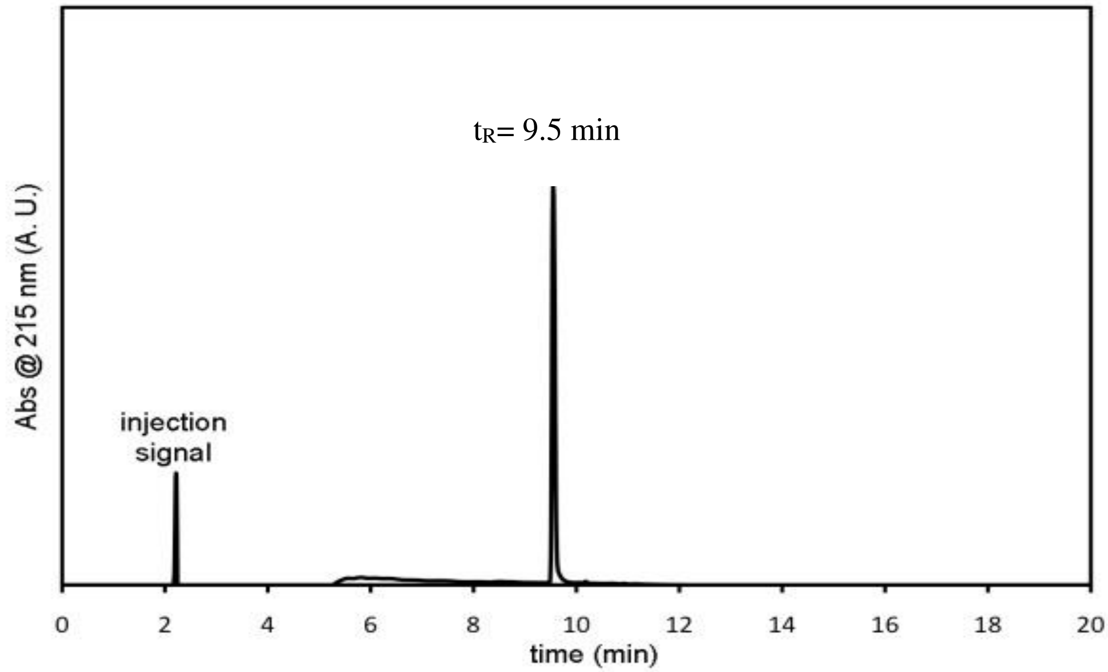
^{1}H-NMR (400 MHz, DMSO-d$_6$, TMS)



^{13}C-NMR (100 MHz, DMSO-d$_6$, TMS)

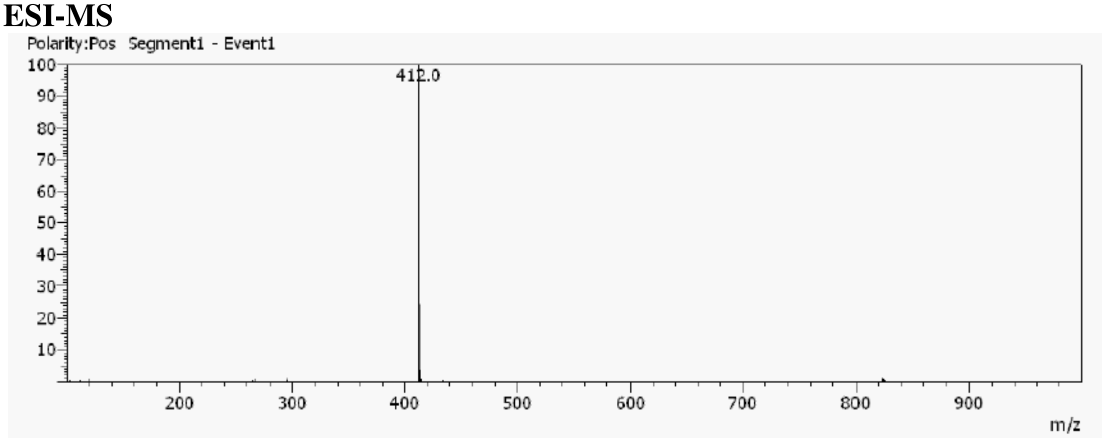


HPLC



tR= 9.5 min
Abs @ 215 nm (A. U.)
injection signal
0
2
4
6
8
10
12
14
16
18
20
time (min)

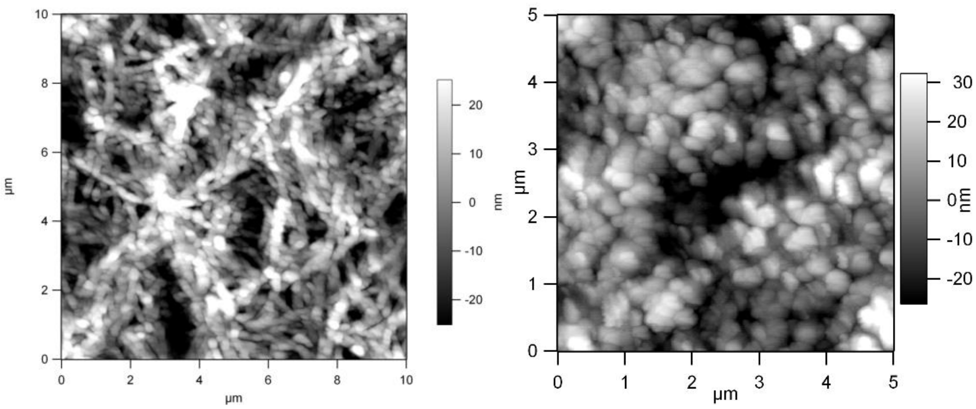
ESI-MS



Polarity:Pos Segment1 - Event1
412.0
100
90
80
70
60
50
40
30
20
10
200
300
400
500
600
700
800
900
m/z

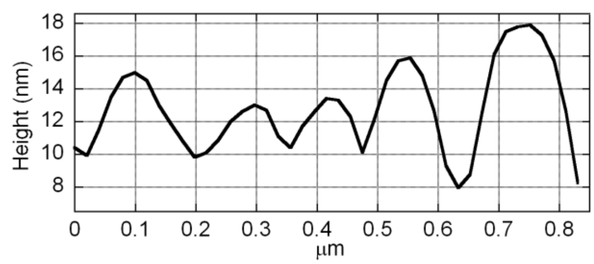
7. AFM analysis of homochiral tripeptides.

AFM images for ^{D}Phe-^{D}Phe-^{D}Val (left) and Phe-Phe-Val (right). The image on the right is reproduced from “S. Marchesan *et al.*, *Chem. Commun.* **2012**, *48* (16), 2195” with permission from The Royal Society of Chemistry.

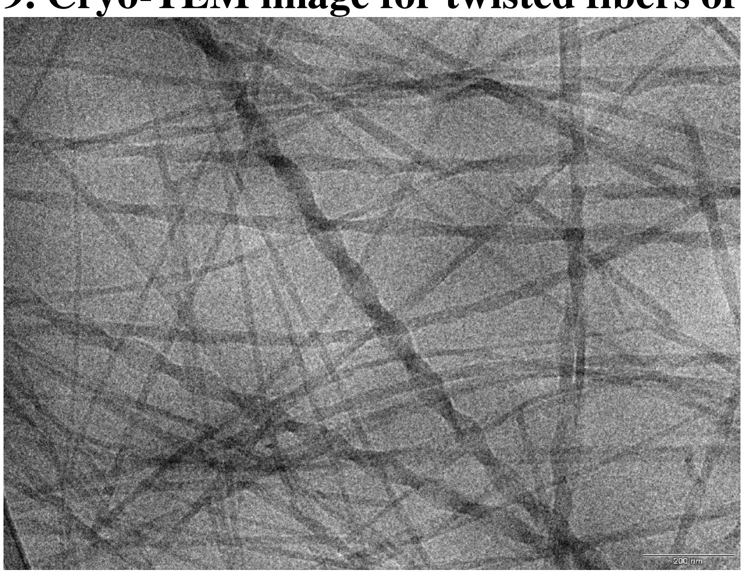


8. AFM line scan confirms twisted fibers for Phe-^{D}Phe-^{D}Val

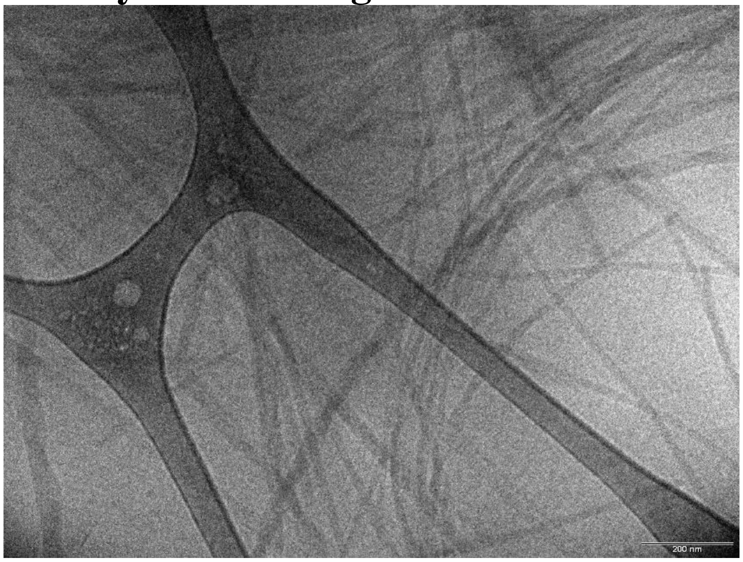
AFM analysis of Phe-^{D}Phe-^{D}Val confirmed twisted fibers as observed for its enantiomer ^{D}Phe-Phe-Val (see S. Marchesan *et al.*, *Chem. Commun.* **2012**, *48* (16), 2195).



9. Cryo-TEM image for twisted fibers of Phe-^{D}Phe-^{D}Val

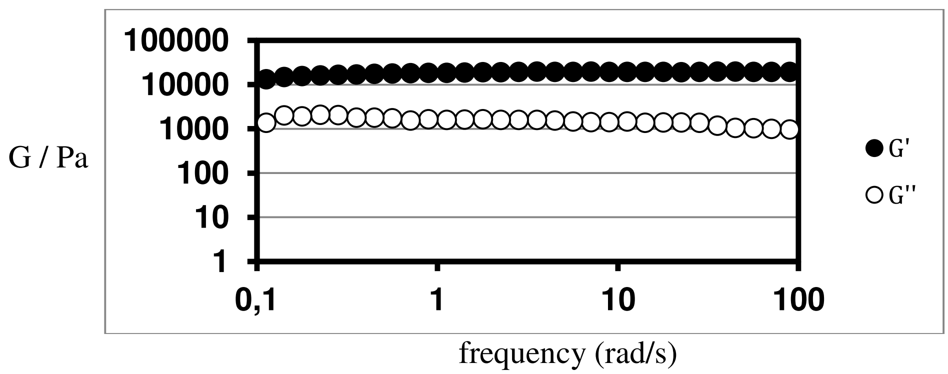


10. Cryo-TEM image for twisted fibers of ^{D}Phe-Phe-Val



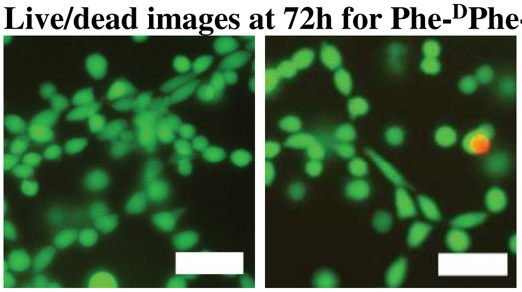
11. Rheometry

Frequency sweeps for Phe-^{D}Phe-^{D}Val gels confirmed independence of the viscoelastic modulus from the frequency applied, as expected for hydrogels, and as observed for its enantiomer ^{D}Phe-Phe-Val (see S. Marchesan *et al.*, *Chem. Commun.* **2012**, *48* (16), 2195).



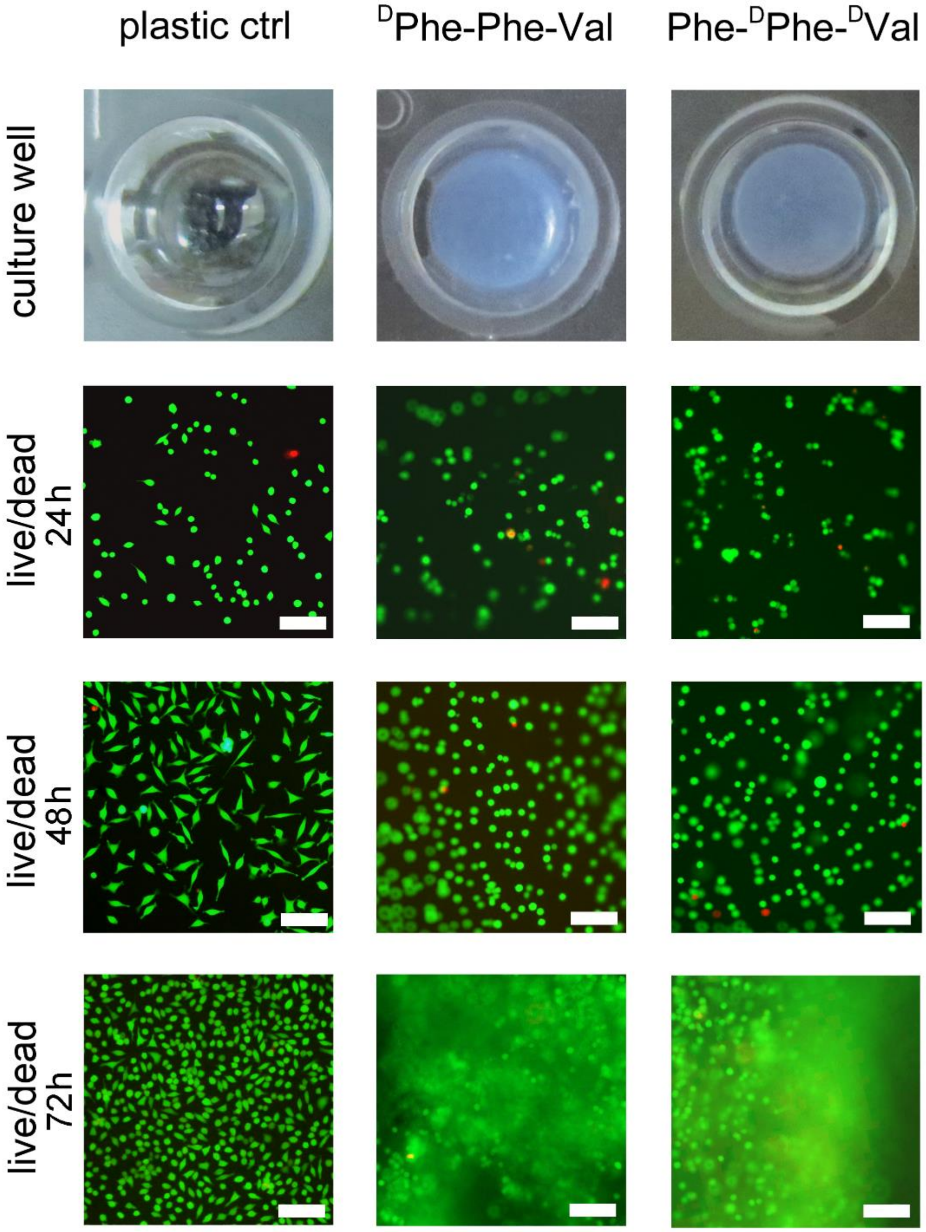
12. Live/dead cell culture images

Live/dead images at 72h for Phe-^{D}Phe-^{D}Val showing details of spreading cells.

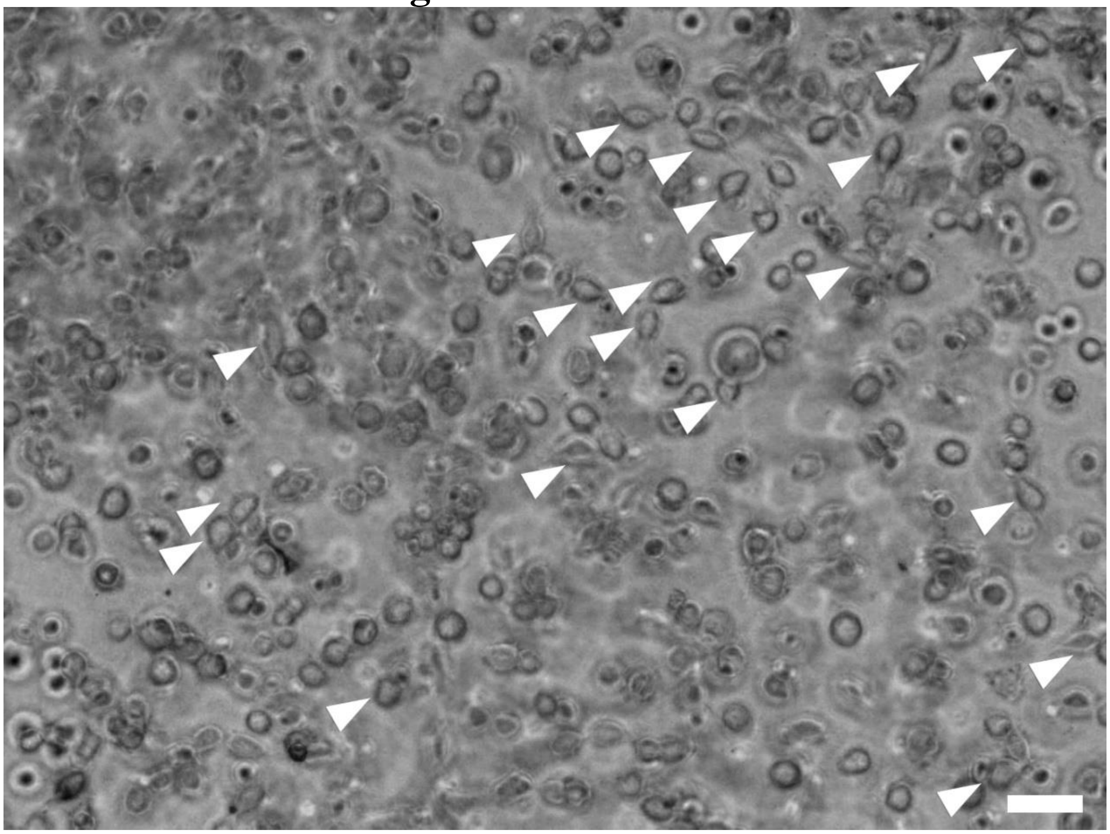


Scale bar = 50 microns.

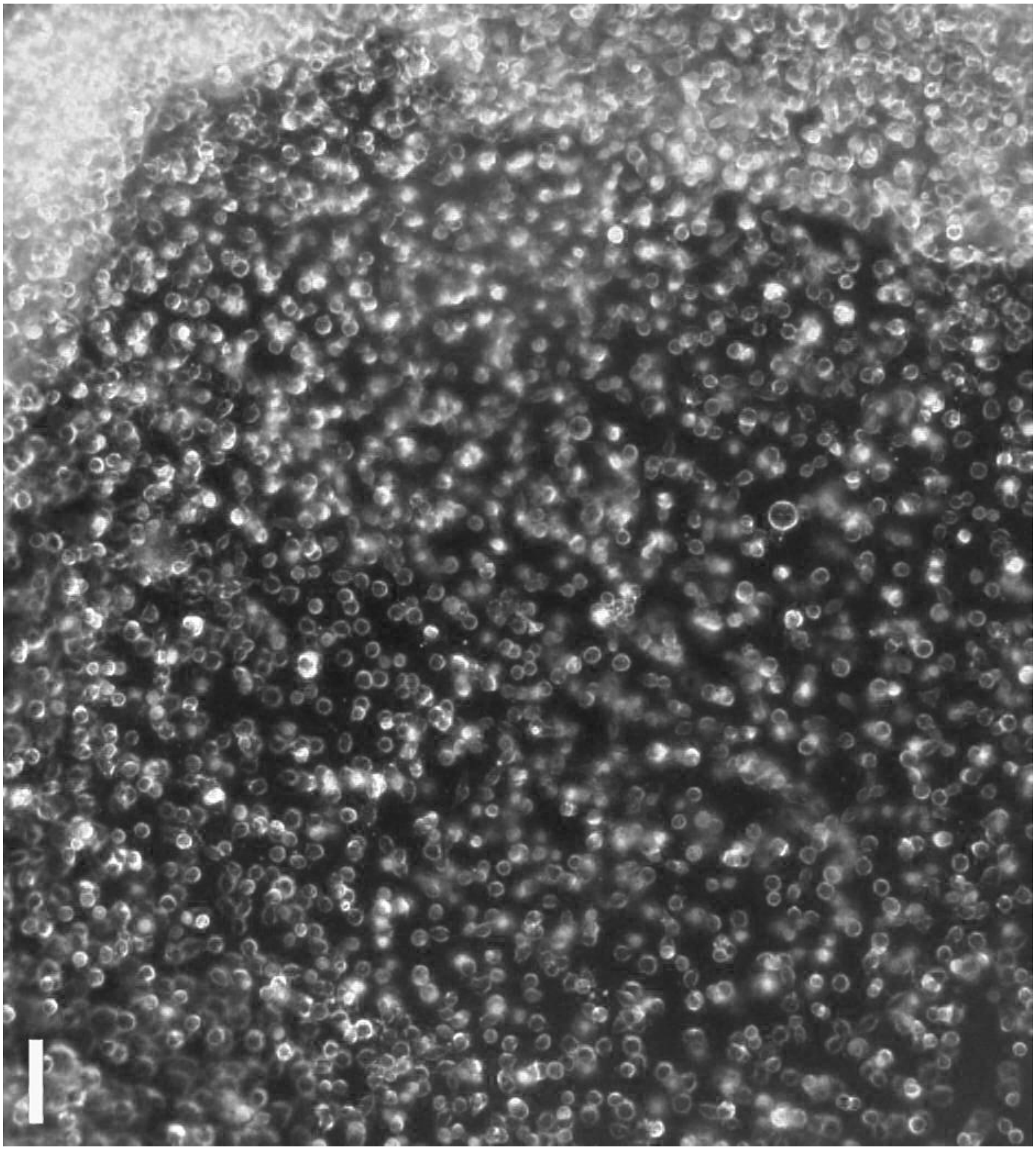
Enlargement of Fig.8.



13. BF cell culture images at 72h for Phe-^{D}Phe-^{D}Val

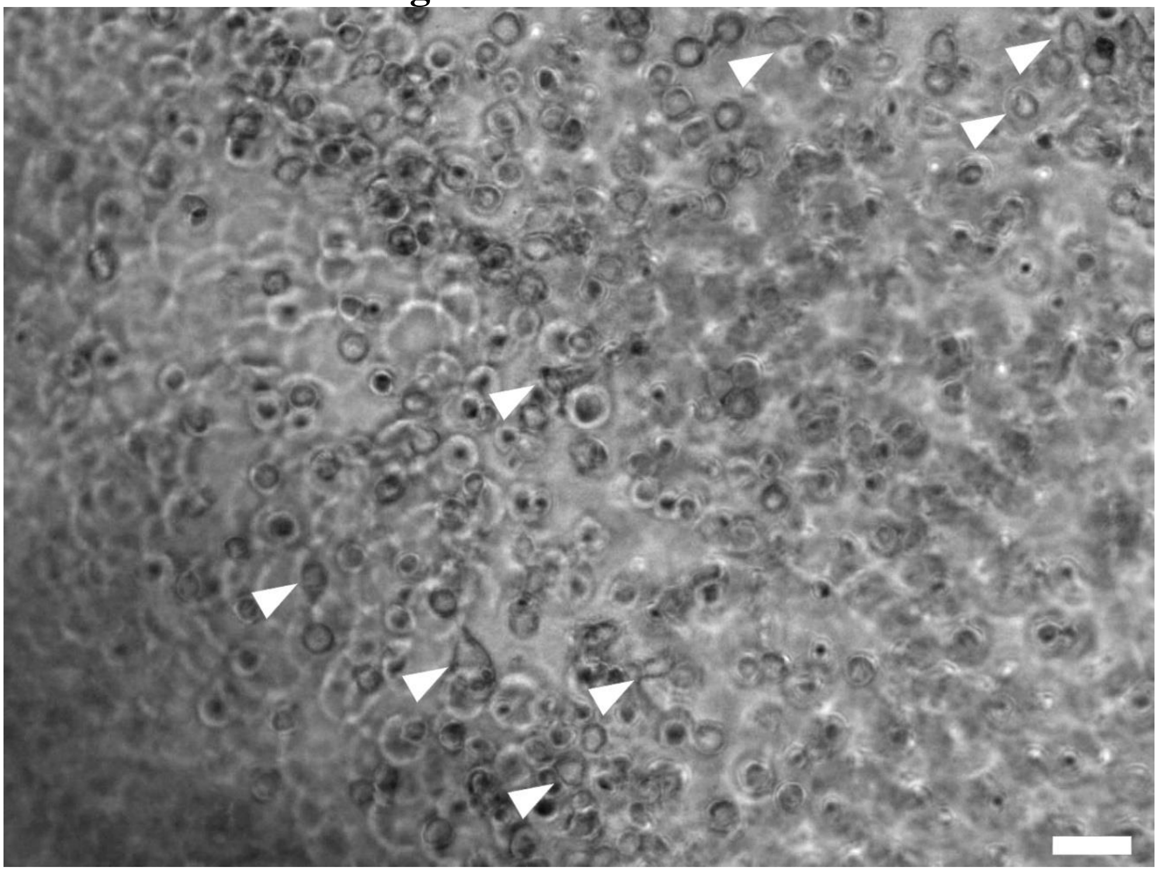


BF image. Scale bar = 50 microns.
White arrowheads point to spreading cells

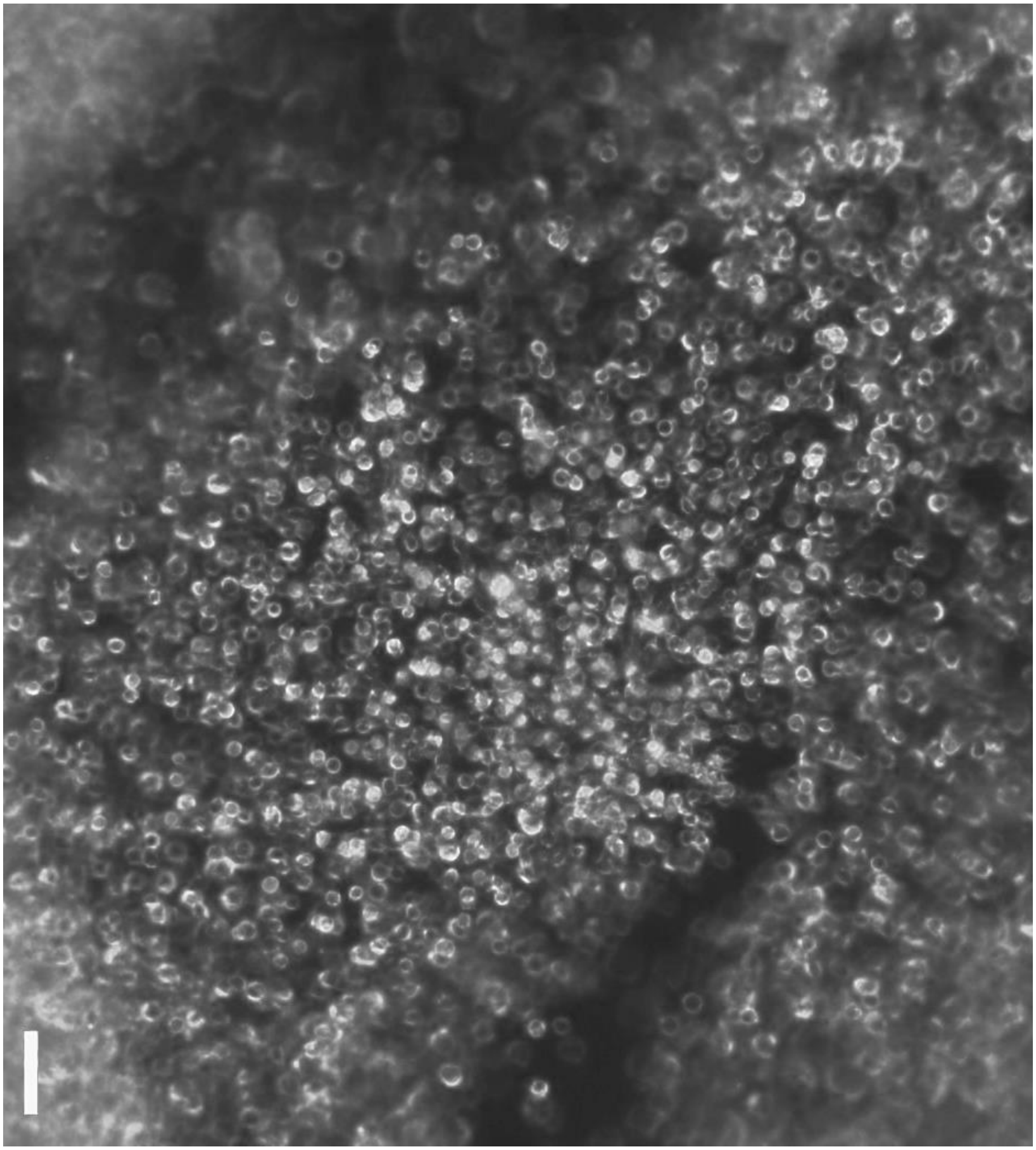


BF image. Scale bar = 100 microns.

14. BF cell culture images at 72h for ^{D}Phe-Phe-Val

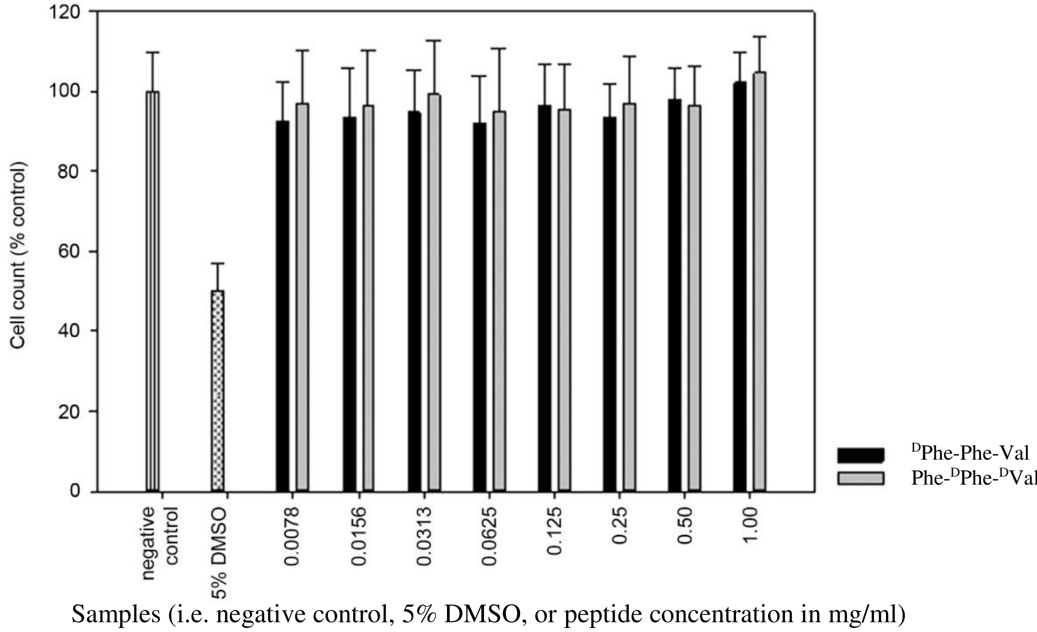


Scale bar = 50 microns.
White arrowheads point to spreading cells.

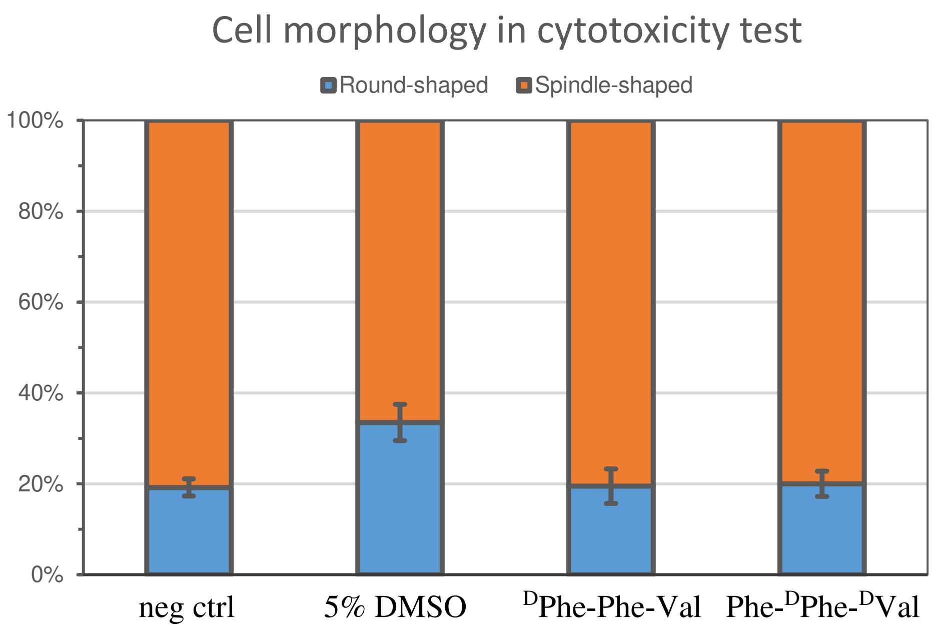


BF image. Scale bar = 100 microns.
Note: black areas represent gel fractures.

15. Cytotoxicity assay for the two gelling peptides in solution



Cytotoxicity assay according to ISO 10993 shows that the dissolved tripeptides are non-toxic to fibroblast cells. Average values ± SD are shown.



Quantification of round-shaped versus spindle-shaped cells as visualized by BF microscopy in the cytotoxicity test. Measurements were done on microscopy images (n = 6) showing an average of >500 cells each. Average values ± SD are shown. No significant difference was seen between the negative control and samples with either peptide at the concentration of 1mg/ml (peptide solubility limit). Samples with 5% DMSO were the only ones with significantly lower numbers of cells per surface area.