Supporting Information

Backbone Engineered γ-Peptide Amphitropic Gels for Immobilization of Semiconductor Quantum dots and 2D Cell Culture

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1. ORTEP Diagram of Boc-γPhe-γPhe-OEt:

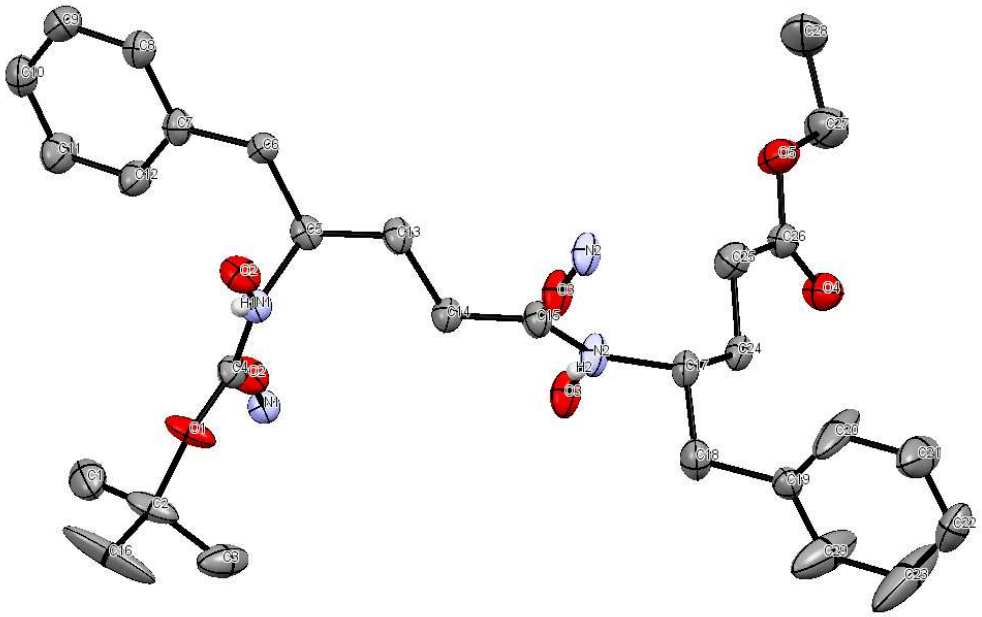


Figure S1: ORTEP diagram of peptide Boc-γPhe- γPhe-OEt (CCDC 1532237). H-atoms are omitted for clarity. Ellipsoids are drawn at 50% probability.

2. ORTEP Diagram of NH_2-γPhe- γPhe-OH:

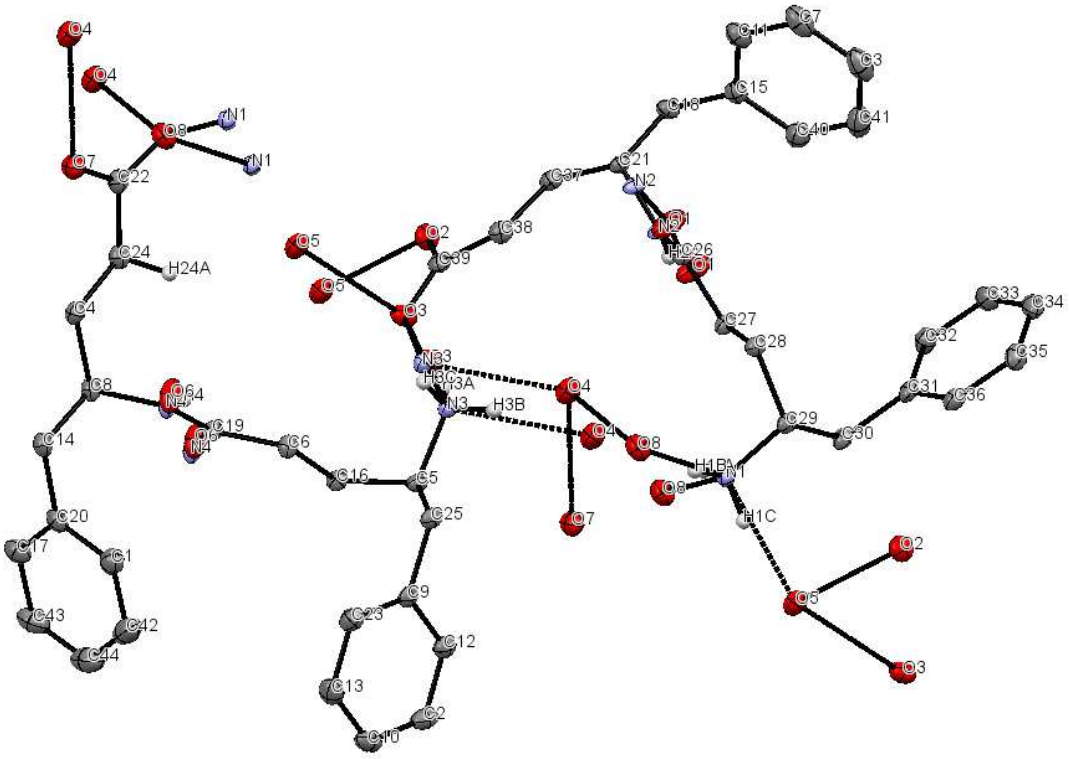


Figure S2: ORTEP diagram of peptide NH_2-γPhe-γPhe-OH (CCDC 1532238). H-atoms are omitted for clarity. Ellipsoids are drawn at 50% probability.

3. Crystallographic Information of Peptides

Crystal structure analysis of Boc-γPhe-γPhe-OEt: Crystals of **Boc-γPhe-γPhe-OEt** were grown by slow evaporation from a solution of ethyl acetate. A single crystal (0.1 × 0.06 × 0.02 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å), ω-scans (2θ = 56.66), for a total of 12748 independent reflections. Space group P 21, a = 5.172(8), b = 27.15(4), c = 9.752(14), β = 95.20(3), V = 1364(3) $Å^3$, monoclinic, Z = 2 for chemical formula C_{29} H_{38} N_2 O_5, with one molecule in asymmetric unit; ρcalcd = 1.204 gcm^{-3} , μ = 0.082 mm^{-1} , F (000) = 532. The structure was obtained by direct methods using SHELXS-97.[1] The final R value was 0.0710 (wR2 = 0.1549) 6480 observed reflections (F0 ≥ 4σ (|F0|)) and 330 variables, S = 0.897. The largest difference peak and hole were 0.316 and -0.274e$Å^3$, respectively.

Crystal structure analysis of NH_2-γPhe-γPhe-OH: Crystals of **NH_2-γPhe- γPhe-OH** were grown by slow evaporation from a solution of water. A single crystal (0.4 × 0.1 × 0.18 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å), ω-scans (2θ = 54.9), for a total of 18173 independent reflections. Space group P1, a = 5.0140(10), b = 11.531(2), c = 18.198(4), 95.463(4), V = 1047.3(4) $Å^3$, monoclinic, Z = 2 for chemical formula C_{22} H_{28} N_2 O_4, with one molecule in asymmetric unit; ρcalcd = 1.204 gcm^{-3}, μ = 0.084 mm^{-1}, F (000) = 330. The structure was obtained by direct methods using SHELXS-97.[1] The final R value was 0.0448 (wR2 = 0.1336) 7091 observed reflections (F0 ≥ 4σ (|F0|)) and 507 variables, S = 0.787. The largest difference peak and hole were 0.532and -0.268e$Å^3$, respectively.

4. General experiment details

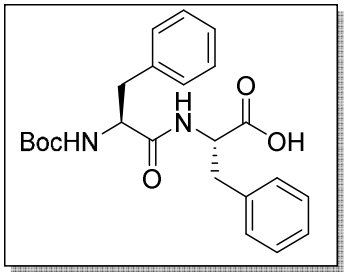
Synthesis of peptide P1-P5:

Synthesis of peptide **P1, P2, P3, P4** and **P5** were carried out by conventional solution phase methods using racemization free fragment condensation strategy. The Boc group was used for the *N*-terminal protection and the C-terminus was protected either with ethyl or methyl esters. The Boc-(*S*)-β-Phe was synthesized by Arndt–Eistert homologation of Boc-Phe and Boc-(*S*)-γ-Phe was synthesized by Wittig reaction followed by catalytic hydrogenation. Couplings were carried out using *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1- hydroxybenzotriazole (HOBt). C-terminal methyl or ethyl group was deprotected by using aqueous sodium hydroxide. The final compound was fully characterized by mass spectrometry, ^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy.

5. Characterization of peptide P1-P5:

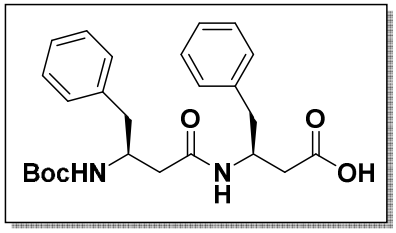
Peptide P1

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (bs, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.41 – 7.07 (m, 10H), 6.87 (d, J = 8.8 Hz, 1H), 4.48 (m, , 1H), 4.17 (m, 1H), 3.09 (m, 1H), 2.99 – 2.86 (m, 2H), 2.67 (m, 1H), 1.28 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.22, 172.10, 155.54, 138.56, 137.79, 129.68, 129.62, 128.64, 128.43, 126.91, 126.60, 78.49, 56.13, 53.78, 37.90, 37.25, 28.58. HR-MS m/z calculated for $C_{23}H_{28}N_2O_5$ is $[M+H]^+$ 413.2076 and observed 413.2076.



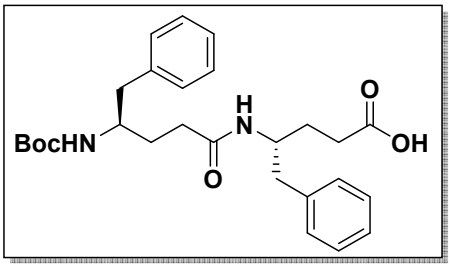
Peptide P2

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, J = 8.2 Hz, 1H), 7.32 – 6.99 (m, 10H), 6.59 (d, J = 8.5 Hz, 1H), 4.31 – 4.12 (m, 1H), 3.82 (m, 1H), 2.67 (m, , 2H), 2.28 (m, 2H), 2.11 (m, 2H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.27, 169.84, 155.22, 139.47, 139.26, 129.72, 129.64, 128.62, 128.47, 126.57, 126.34 77.95, 49.76, 47.97, 41.11, 28.73. HR-MS m/z calculated for $C_{25}H_{32}N_2O_5$ is $[M+H]^+$ 441.2389 and observed 441.2382



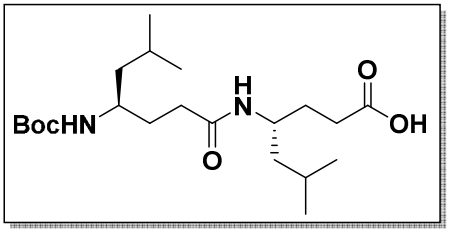
Peptide P3

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 – 11.76 (m, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.21 (m, 4H), 7.13 (m, 6H), 6.63 (d, J = 8.8 Hz, 1H), 4.04 – 3.62 (m, 1H), 3.55 – 3.41 (m, 1H), 2.60 (m,4H), 2.25 – 1.91 (m, 4H), 1.70 – 1.33 (m, 4H), 1.27 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.06, 155.77, 139.67, 139.43, 129.65, 128.57, 128.55, 77.86, 52.03, 49.81, 41.16, 40.97 33.02, 31.05, 30.86, 29.71, 28.78. HR-MS m/z calculated for $C_{27}H_{36}N_2O_5$ is $[M+H]^+$ 469.2702 and observed 469.2702



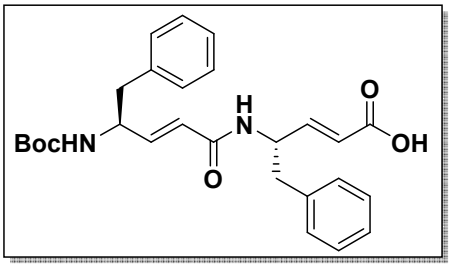
Peptide P4

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 7.45 (d, J = 8.9 Hz, 1H), 6.54 (d, J = 9.1 Hz, 1H), 3.78 (m,1H), 3.42 (m, 1H), 2.24 – 2.09 (m, 2H), 2.03 (m, 2H), 1.72 – 1.02 (m, 18H), 0.91 – 0.74 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.83, 172.05, 155.93, 77.65, 48.38, 46.07, 44.34, 32.99, 32.21, 31.00, 30.87, 28.75, 24.90, 24.88, 23.61, 23.58, 22.39. HR-MS m/z calculated for $C_{21}H_{40}N_2O_5$ is $[M+H]^+$ 401.3015 and observed 401.3015.



Peptide P5

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 – 11.97 (m, 2H), 8.24 (d, J = 8.2 Hz, 1H), 7.23 (m, , 5H), 7.20 – 7.14 (m, 6H), 7.05 (d, J = 8.8 Hz, 1H), 6.80 – 6.73 (dd, 1H), 6.51 (dd, J = 15.4, 5.6 Hz, 1H), 5.87 (dt, J = 15.0, 4.0 Hz, 1H), 5.66 (dd, J = 15.9, 1.7 Hz, 1H), 4.69 (m, J = 8.5, , 1H), 4.24 (m, 1H), 2.91 – 2.82 (m, 1H), 2.78 – 2.64 (m, 3H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.45, 164.63, 155.44, 148.43, 143.80, 138.51, 129.73, 129.62, 128.73, 128.59, 127.05, 126.85, 126.65, 123.51, 121.57, 78.27, 53.20, 51.53, 28.72. HR-MS m/z calculated value for $C_{27}H_{32}N_2O_5$ is $[M+H]^+$ 465.2389 and observed 465.2389



6. Gelation study:

Peptide **P3** was placed in a glass vial and heated on a hot air gun after the addition of 1 mL of 100 mM or 50 mM phosphate buffer (pH 7.46) to give a clear solution. The solution was sonicated and upon standing at room temperature for a few minutes (almost 10 minutes) it produced a transparent gel. This was confirmed by vial inversion experiment. The minimum gelation concentration of the formation of hydrogel was found to be 5 mg/mL. For the cell culture experiments, gel was made in PBS buffer. The solution of peptide **P3** (6 mg) in PBS buffer (1 mL) was heated using hot air gun, after some time the clear dissolved solution was pipetted out and sonicated. The solution slowly transformed into gel upon standing at room temperature. For the organogel, similar procedure was followed using different aromatic solvents such as benzene, tolune, mesytelene and xylene.

7. Details of gelation study in various solvents of peptide P1 to P5

(In the following gelation experiments, 6 mg of peptide was used in 1mL of solvent)

Solvent	P1	P2	P3	P4	P5
Phosphate buffer (100 mM)	Sol	Opaque gel[a]	**Transparent gel**	Sol	Sol
Benzene	Sol	Unstable gel	**Transparent gel**	Sol[b]	Crystaline fiber[b]
Toluene	Transparent gel	Unstable gel	**Transparent gel**	Sol[b]	Crystaline fiber[b]
Xylene	Transparent gel	Transparent gel	**Transparent gel**	Sol[b]	Crystaline fiber[b]
Mesitylene	Transparent gel	Transparent gel	**Transparent gel**	Sol[b]	Crystaline fiber[b]

[a]Difficult to solubilize completely. After prolonged heating , the amount which was dissolved formed an opaque hydrogel at room temperature. [b]After 24 hours it start aggregating.

8. Microscopy:

FE-SEM experiments were performed by drop casting a small amount of gel samples (4 μL) on SiO2/Si substrate and dried under room temperature, imaged using Zeiss DSM 950 scanning electron microscope with tungsten filament as electron source operated at 10 kV. Similarly TEM sample were prepared by depositing dilute gel sample (4μL) on copper grid, dried at room temperature and imaged using a FEI Tecnai G2 F20 X-TWIN TEM at an accelerating voltage of 200 kV.

9. Rheology:

The rheology experiments were performed on hydrogel as well as organogles (6 mg/mL) using a TAARES rheometer.

10. Spectroscopy:

Circular dichroism (CD) spectra between 200 and 300 nm were recorded in a JASCO 815 spectrometer at room temperature with different concentration of peptide in 100 mM phosphate buffer. Further the fluorescence of the peptide **P3** organogels were measured using fluorescence spectrometer, pure peptides in DMF were used as control. The samples were excited at 259 nm, and the emission spectra were collected from 269 to 500 nm. Powder XRD data of xerogel samples were collected at 3-40° using a Rigaku D/max-2500 instrument (Cu/ KR-1).

11. Biocompatibility experiment of the hydrogel P3:

Cell Culture or peptide hydrogels **P3**. Prior to 2D-culture, Thin gel was made after mixing DMEM:Hydrogel (1:1) and coating the culture plate for 3 hours. Subsequently, solution is

aspirated and plate was dried for 5-10 minutes. Cells were collected from a subconfluent monolayer by trypsin-EDTA treatment, and were re-suspended in complete medium containing DMEM, 10% FBS, and 1% Penicillin/Streptomycin solution. Cells suspension in complete medium was pipetted into each well. The 96-well plate was placed in incubator at 37 °C under 5% of CO_2 atmosphere. Cells were evenly distributed on the gel surfaces by gently pipetting the medium, and the medium was changed every other day. The cultured cells were stained by calcein and imaged by fluorescence microscope.

12. Gelation test of the compound P1 to P5 in aromatic solvent

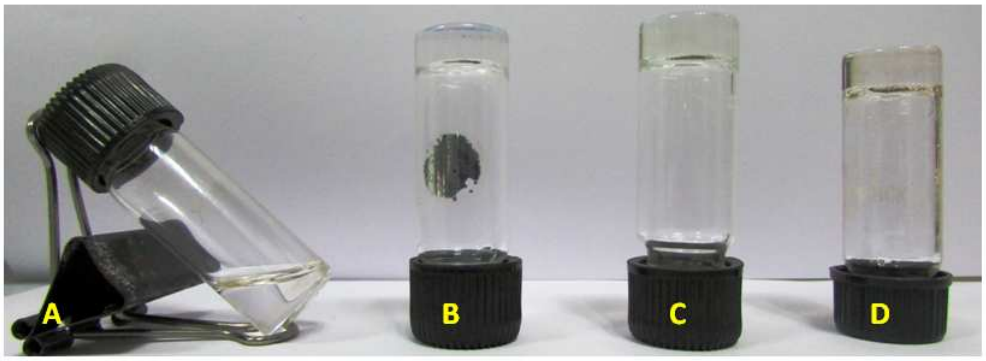


Figure S3: Inverted sample vial test to confirm the gel formation for the peptide **P1** in A) Benzene failed to gelate B) Toluene, C) Xylene, D) Mesitylene .

Figure S4: Low strength gel formation for the peptide **P2** in A) Benzene B) Toluene, C) Xylene, D) Stable gel formed Mesitylene.

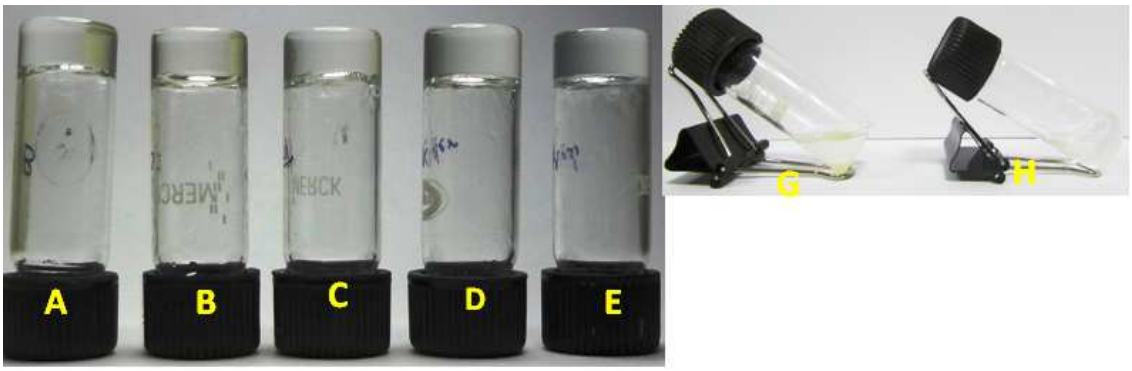


Figure S5: Inverted sample vial experiment to confirm the gel formation for the peptide **P3** in A) Benzene; B) Chlorobenzene; C) Toluene; D) Xylene; E) Mesitylene; G) Compound **P5** failed to gelate after 6 hours in toluene; H) Compound **P4** failed to gelate toluene after 6 hours.

13. Florescence spectra of peptide P3 in gel state and in solution.

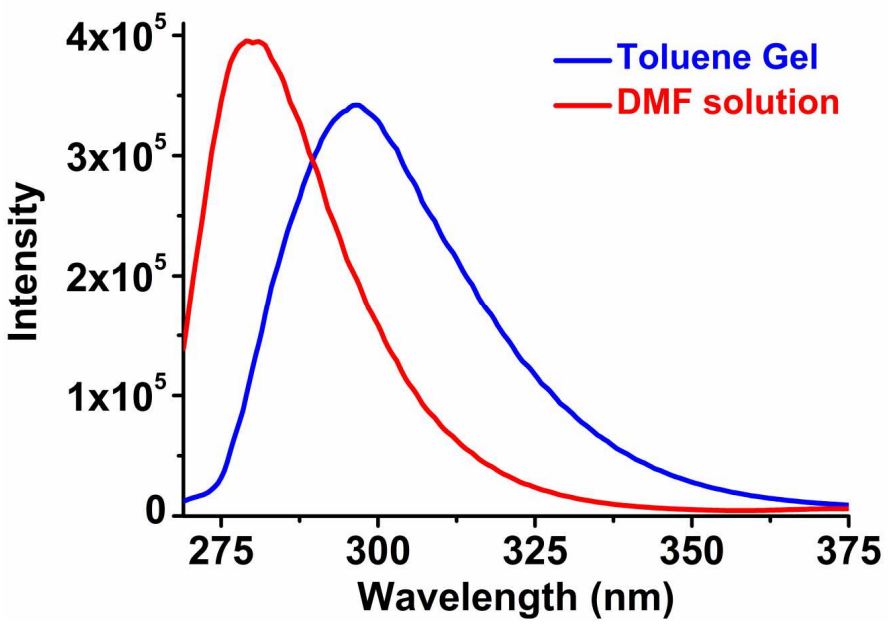


Figure S6: Florescence emission spectra (λ = excitation 259 nm) of peptide **P3** in DMF solution and organogel in toluene at fixed concentration.

14. Step-strain rehology experiment of peptide hydrogel (P3) at 6mg/mL

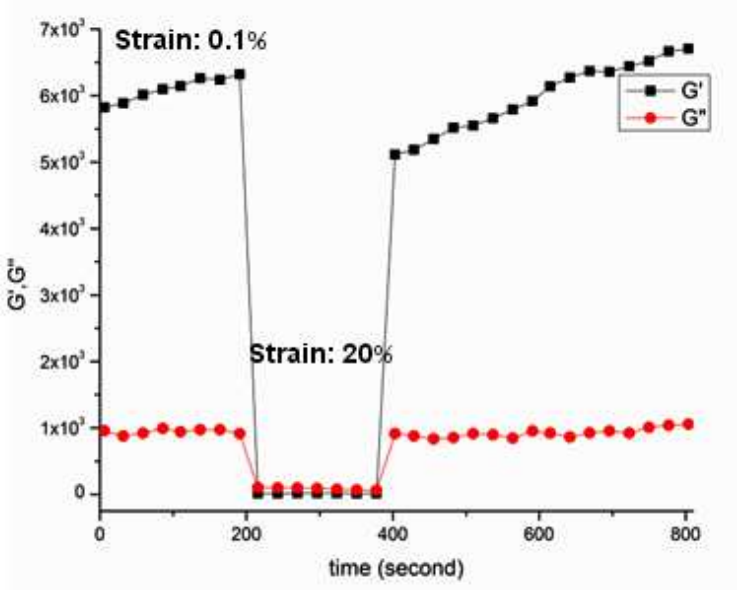


Figure S7: Step-strain rehology experiment of peptide hydrogel (**P3**) at 6mg/mL

15. Quantification of cell viability in peptide hydrogel system

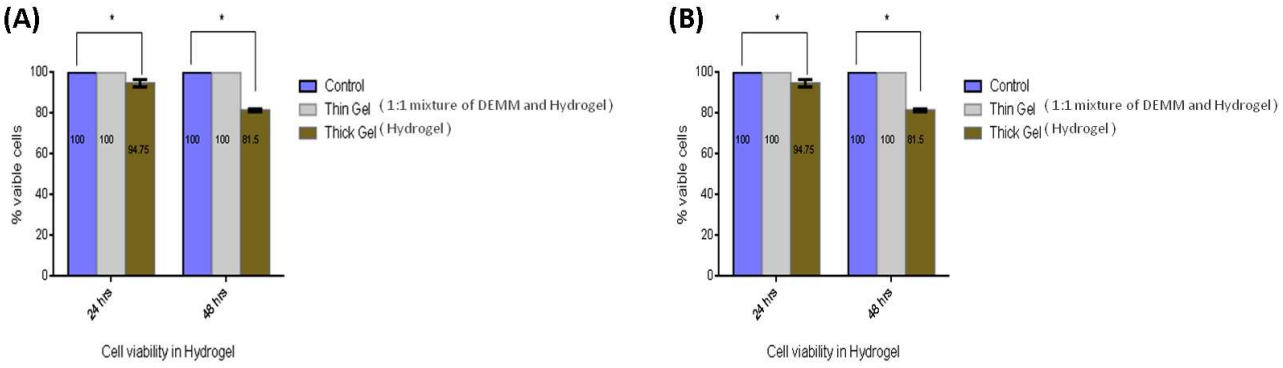


Figure S8: Quantification of cell viability in peptide hydrogel system, data is represented as mean+ SD from three independent experiments. Magnification X63.

16. SEM images of peptide P1, P4, at minimum gelation condition(6mg/mL).

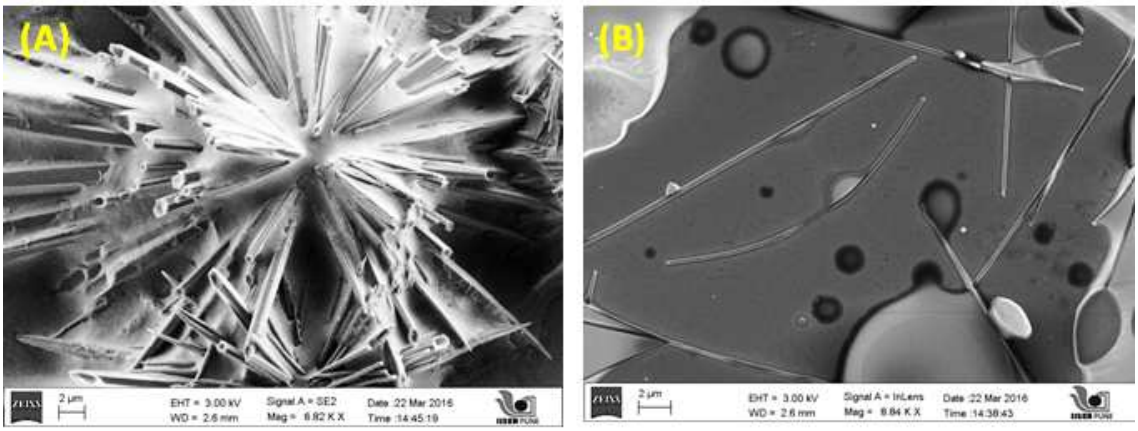
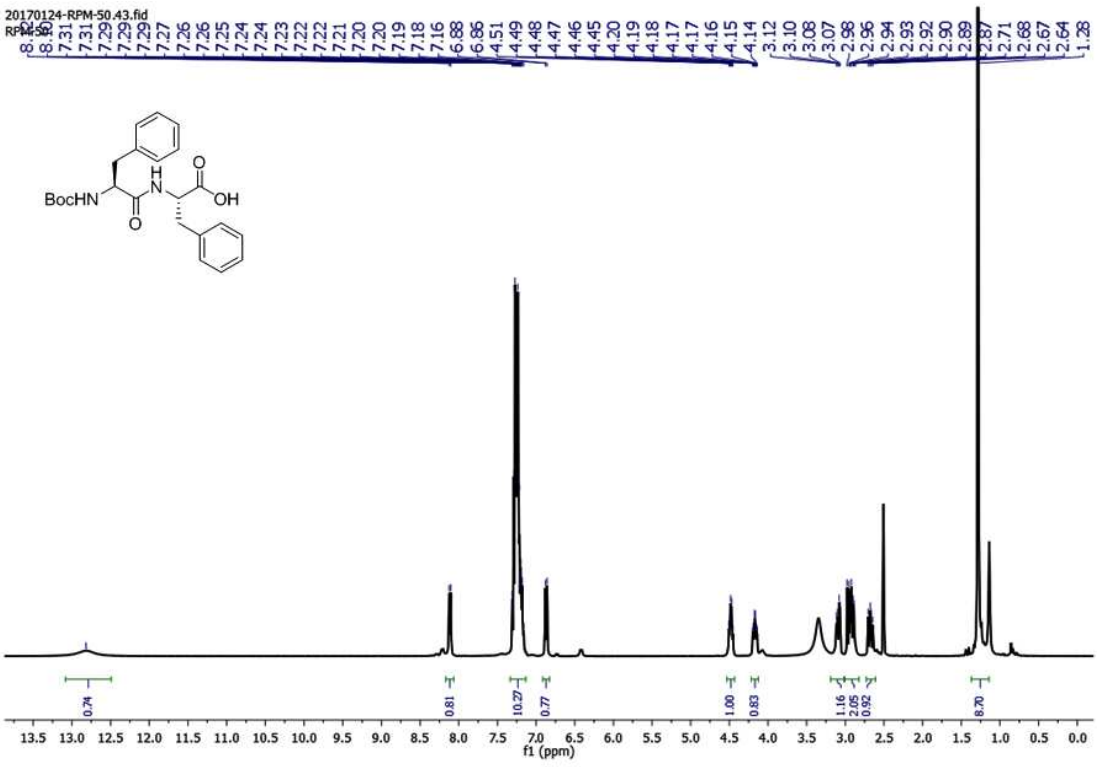
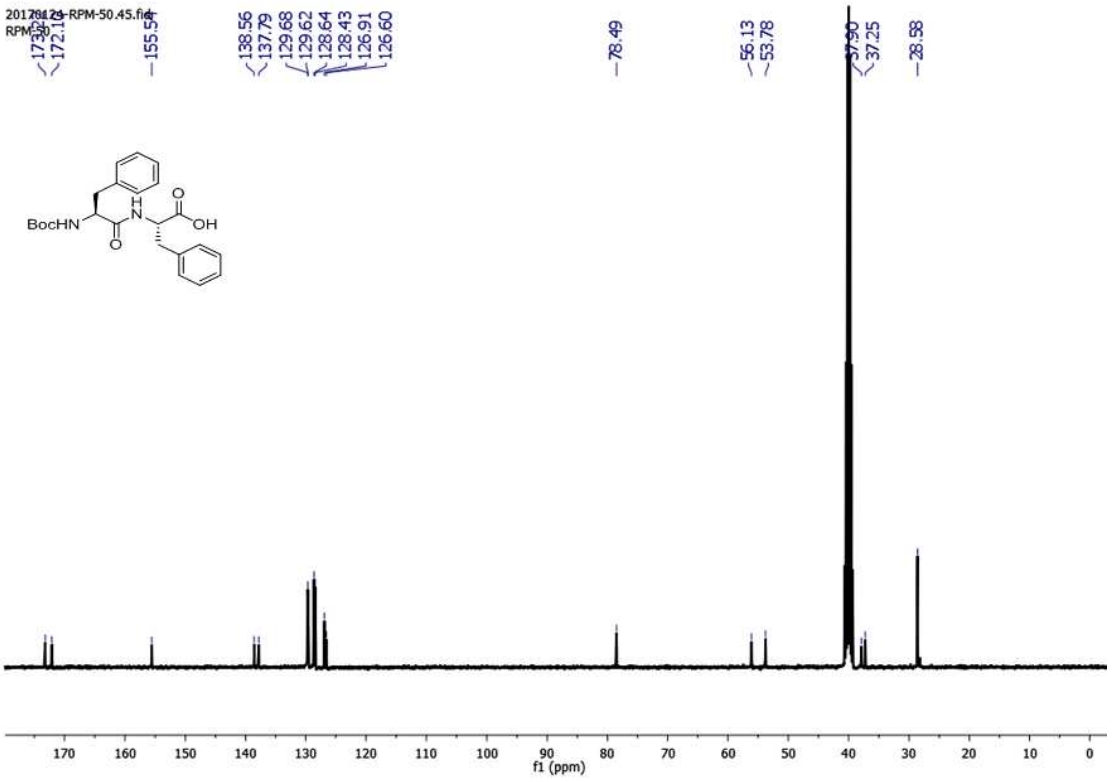


Figure S9: High resolution SEM images peptide **P1**, **P4**, at minimum gelation condition (6mg/mL).

11. ^{1}H, ^{13}C and mass spectra of compounds P1 to P5

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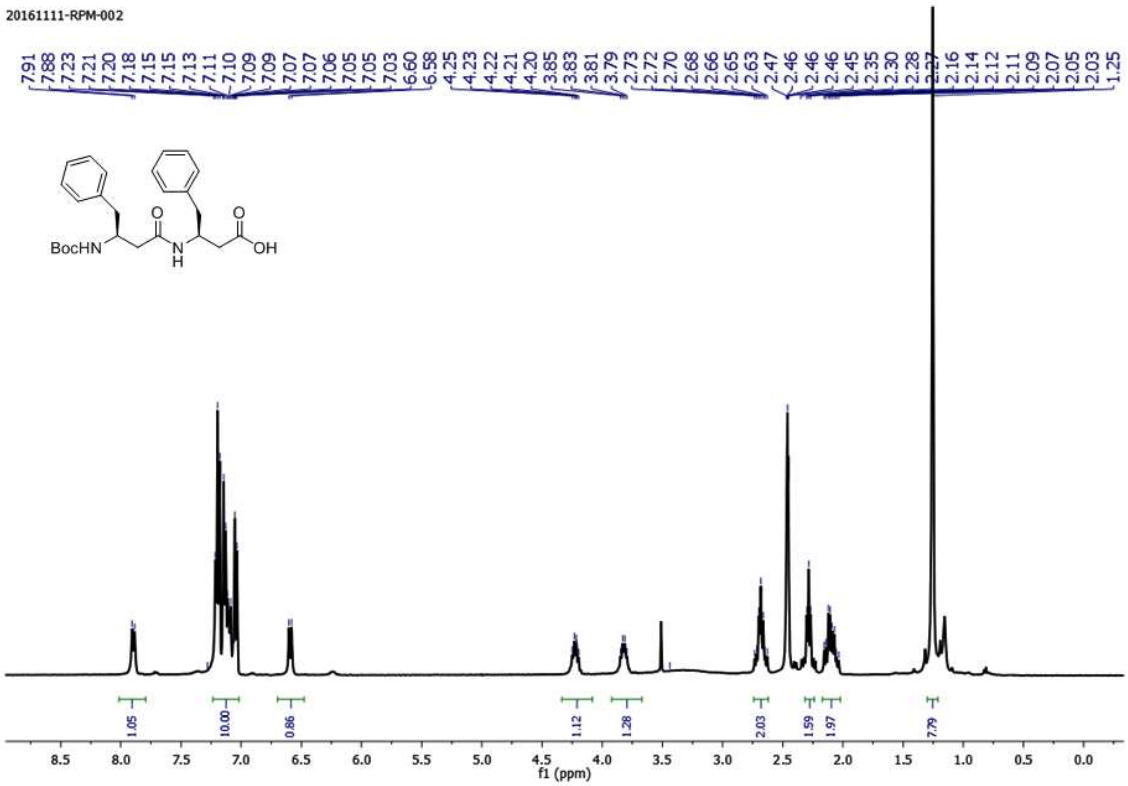
f1 (ppm)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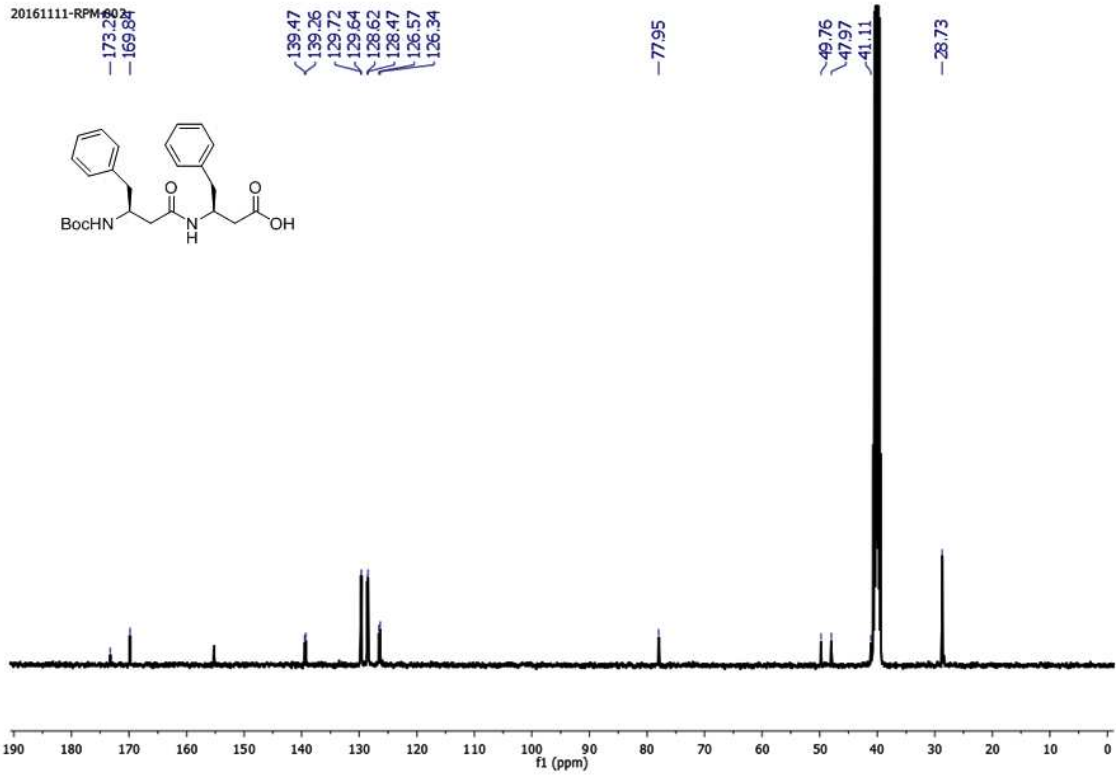
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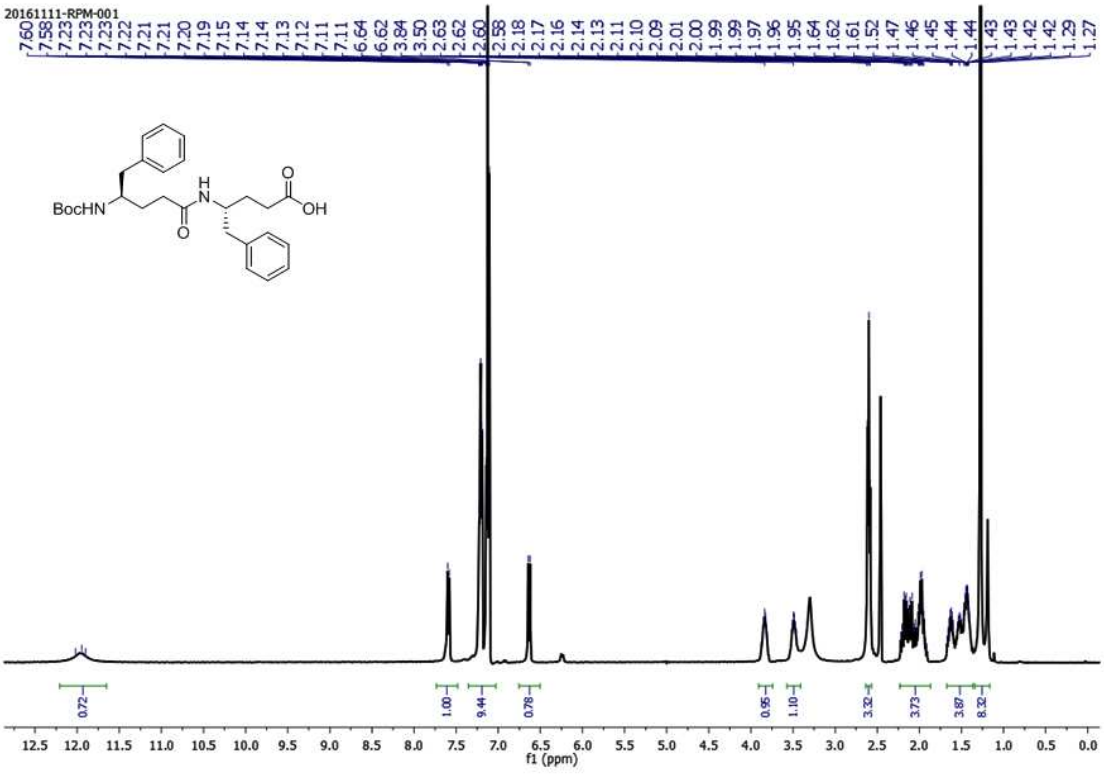
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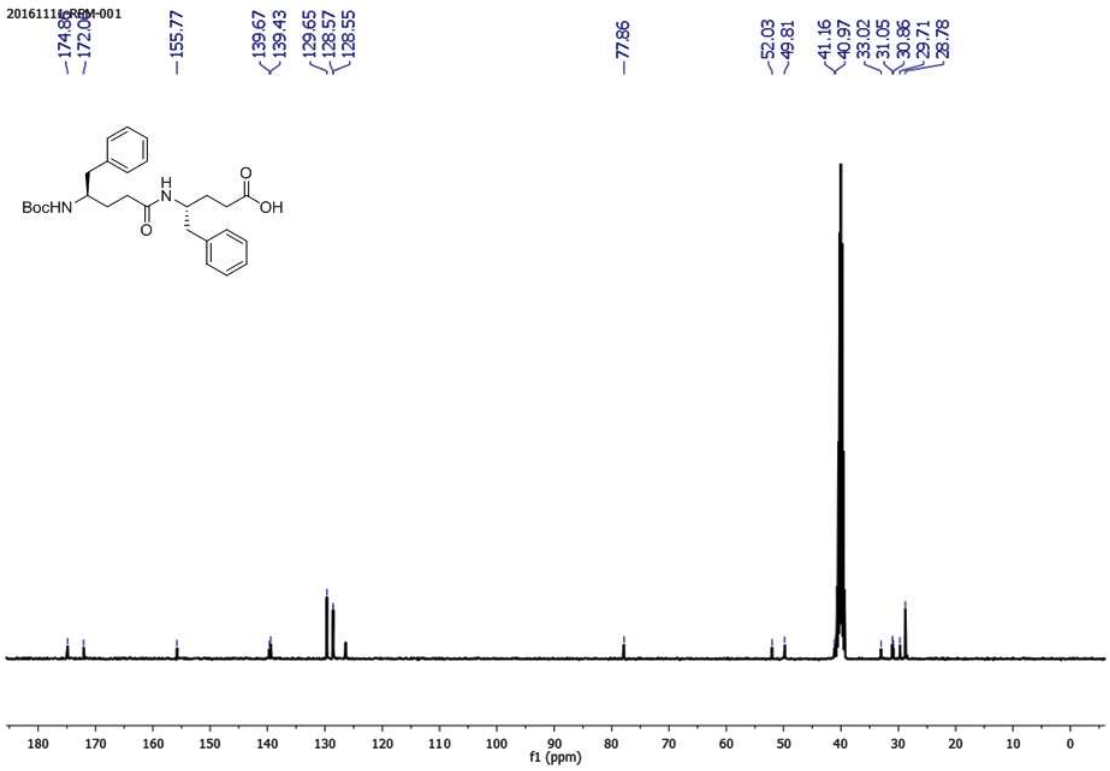
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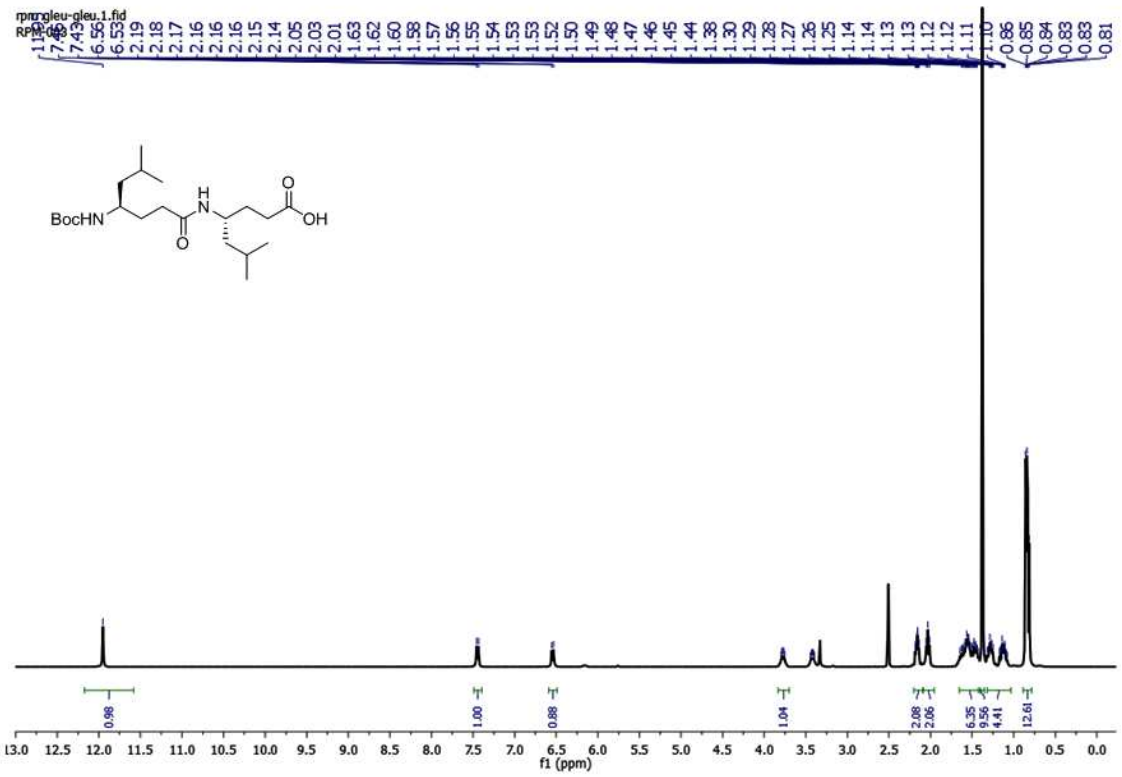
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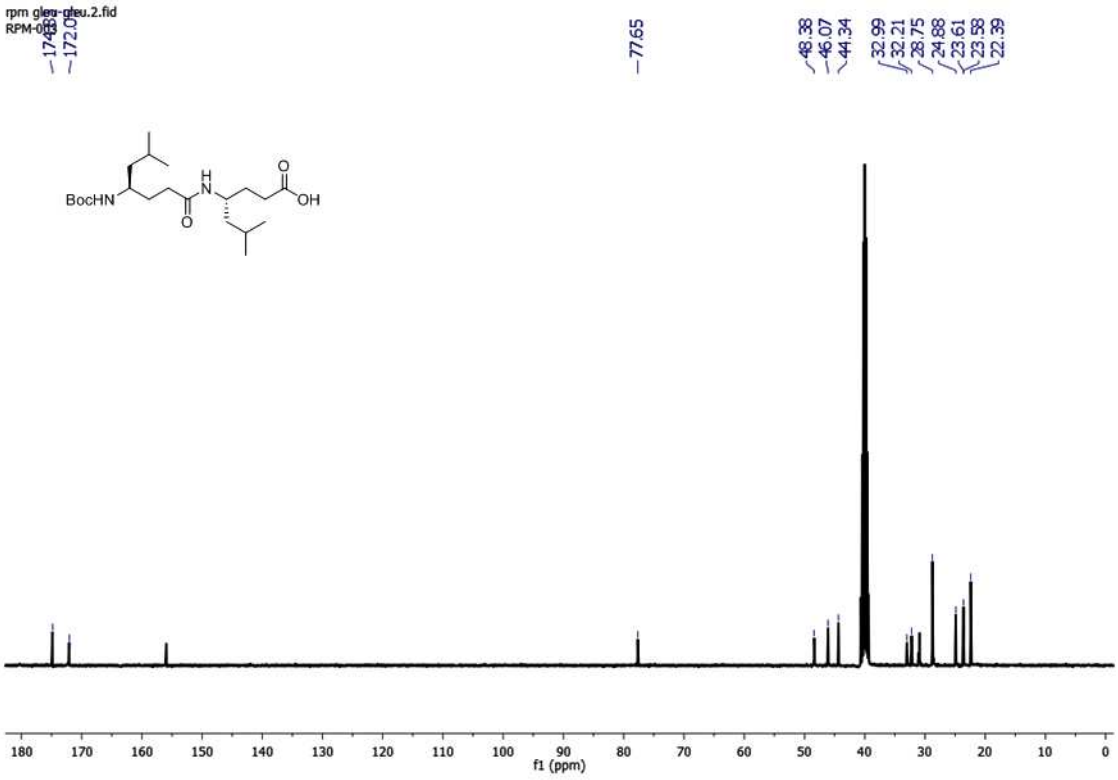
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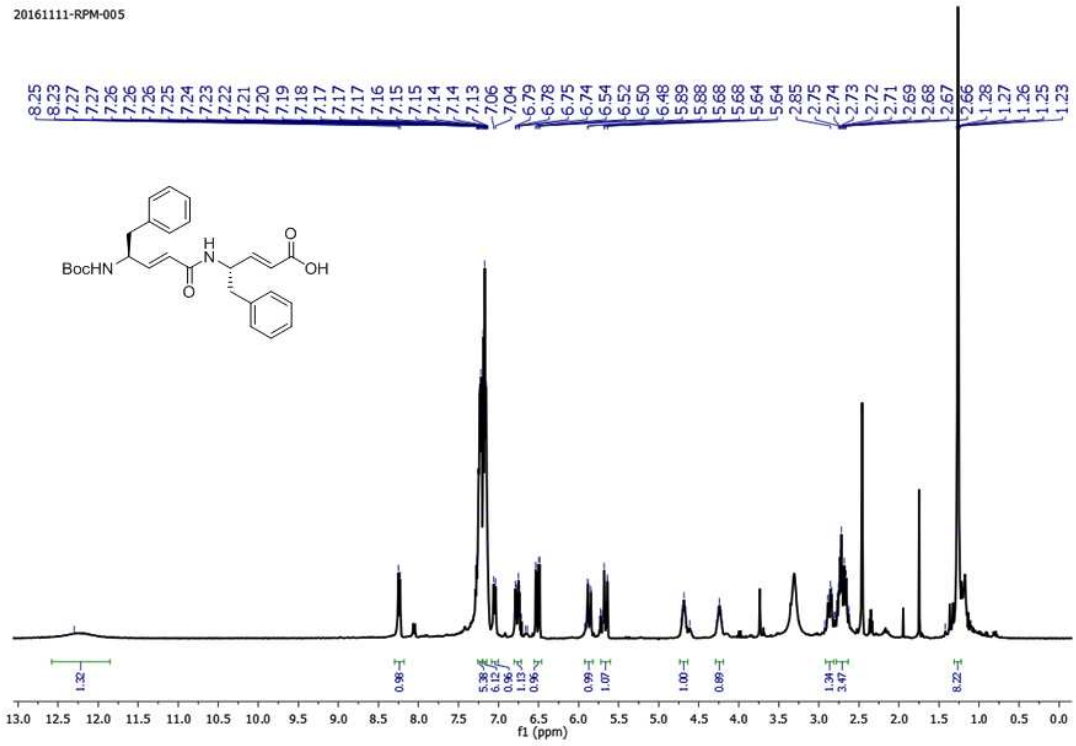
f1 (ppm)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H
N
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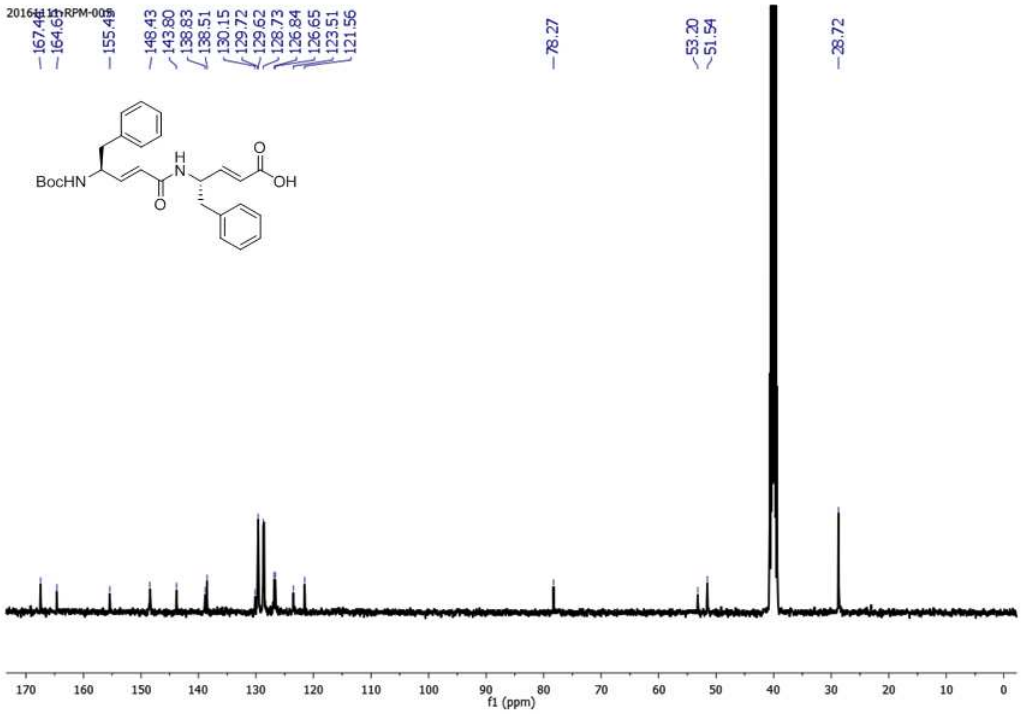
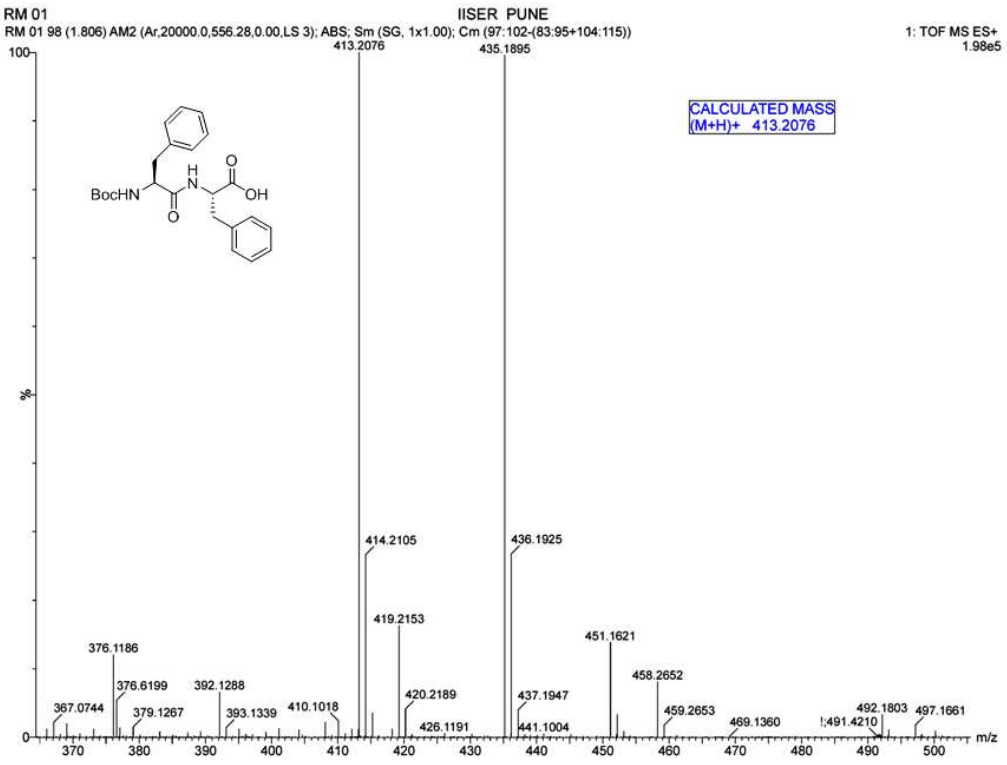
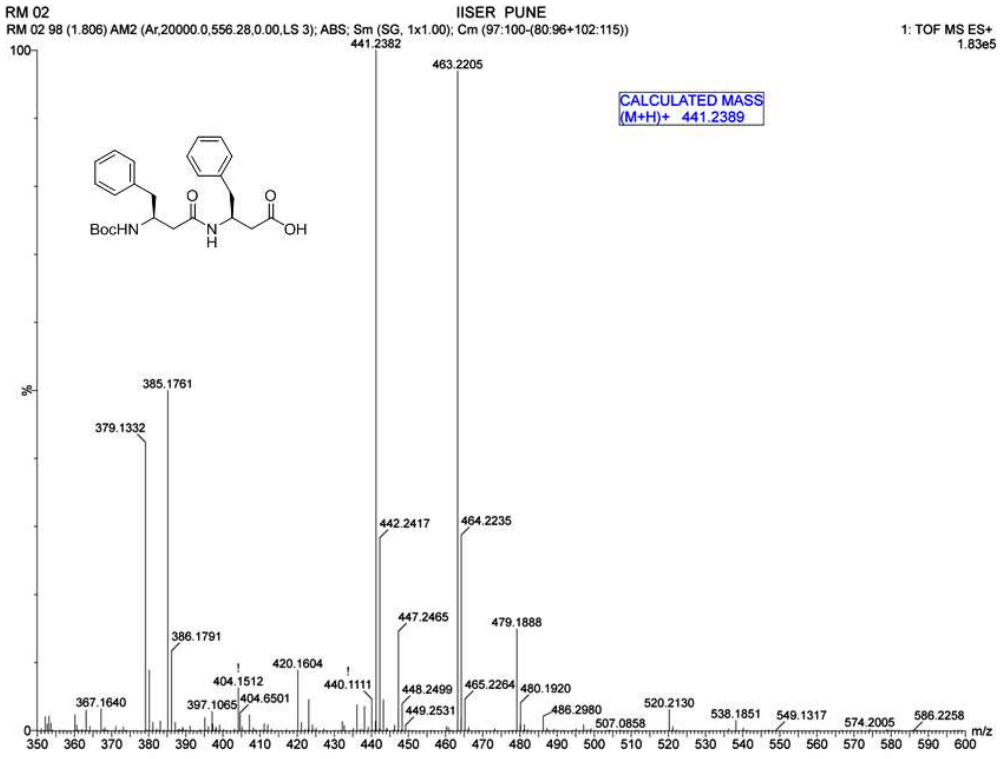
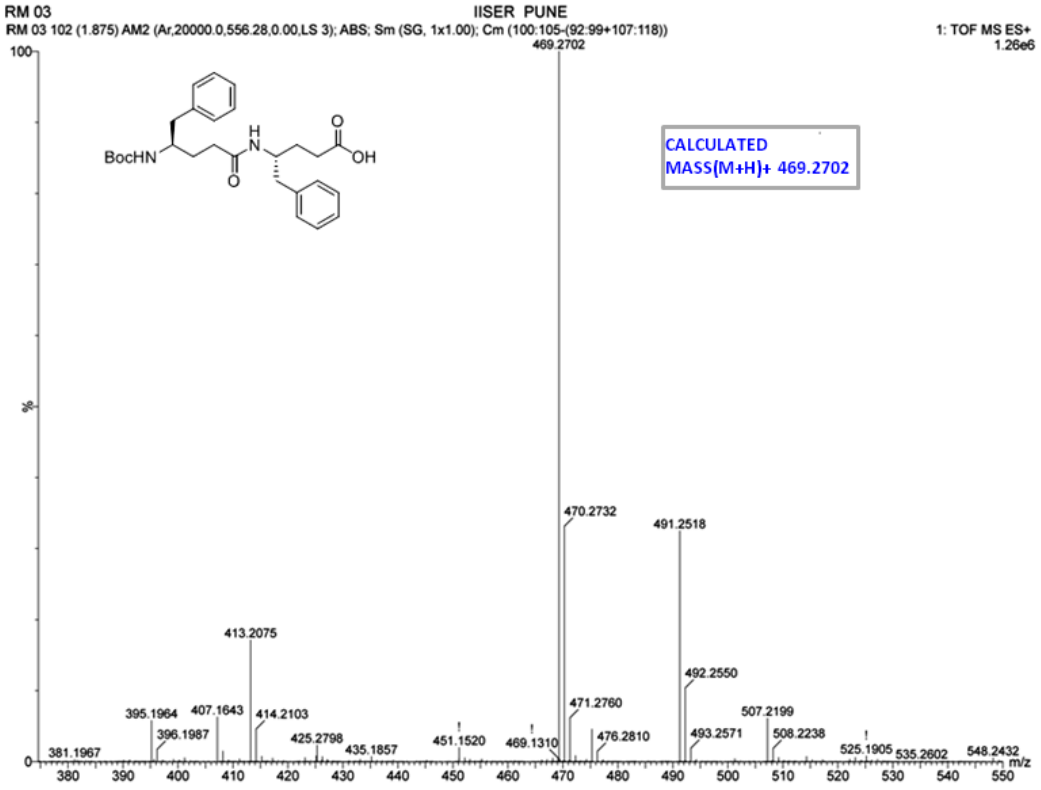
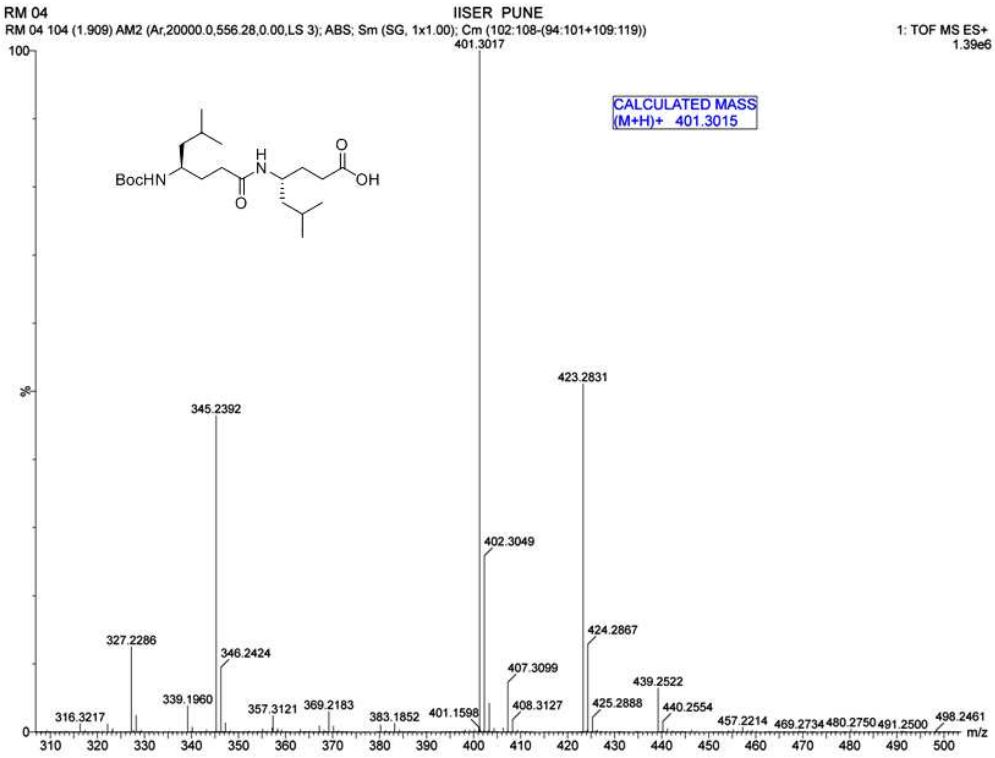
OH
f1 (ppm)

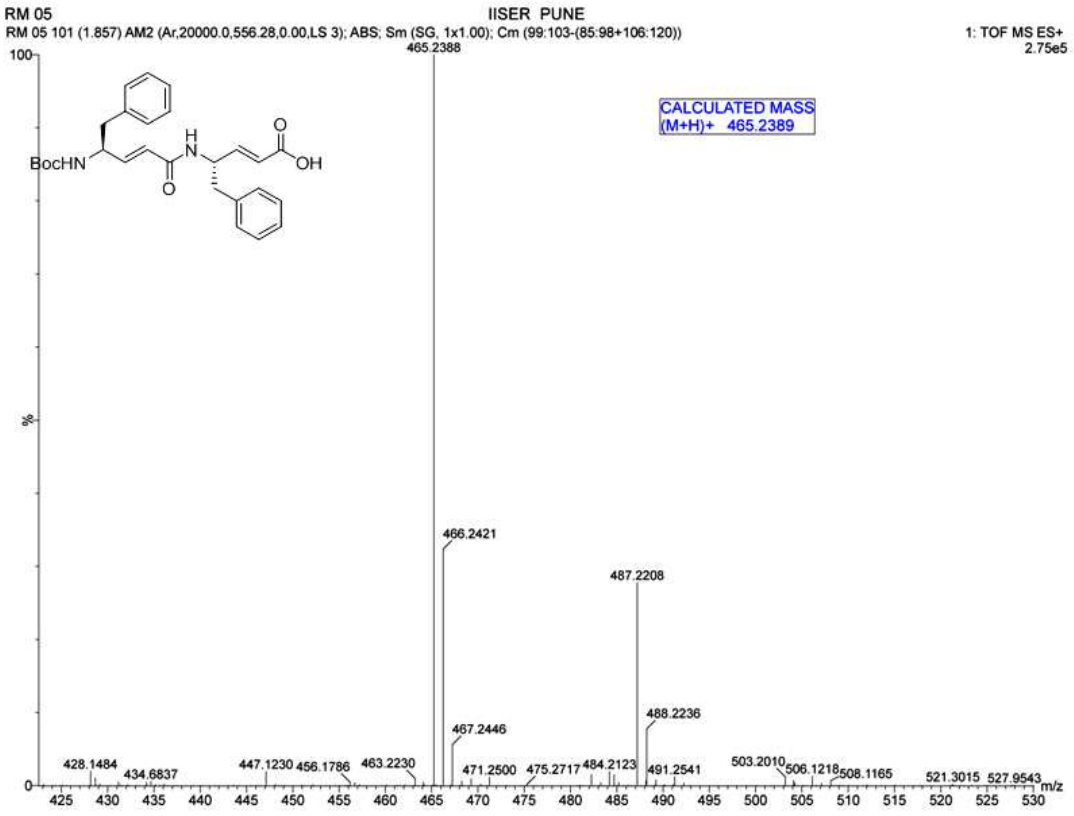

167.46
164.65
155.49
148.43
143.80
138.83
138.51
130.15
129.72
129.62
128.73
126.84
126.65
123.51
121.56
78.27
53.20
51.54
28.72
BocHN
H
N
O
O
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


RM 01
IISER PUNE
RM 01 98 (1.806) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (97:102-(83:95+104:115))
1: TOF MS ES+
1.98e5
CALCULATED MASS
(M+H)+ 413.2076
BocHN
H
N
O
O
OH
O
%
m/z
413.2076
435.1895
414.2105
436.1925
419.2153
451.1621
376.1186
458.2652
376.6199
392.1288
420.2189
437.1947
367.0744
379.1267
393.1339
410.1018
426.1191
441.1004
459.2653
469.1360
492.1803
497.1661
491.4210


RM 02
IISER PUNE
RM 02 98 (1.806) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (97:100-(80:96+102:115))
1: TOF MS ES+
1.83e5
CALCULATED MASS
(M+H)+ 441.2389
BocHN
O
N
H
O
OH
441.2382
463.2205
385.1761
379.1332
442.2417
464.2235
447.2465
479.1888
386.1791
420.1604
404.1512
440.1111
448.2499
465.2264
480.1920
367.1640
397.1065
404.6501
449.2531
486.2980
507.0858
520.2130
538.1851
549.1317
574.2005
586.2258
%
m/z


RM 03
IISER PUNE
RM 03 102 (1.875) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (100:105-(92:99+107:118))
1: TOF MS ES+
1.26e6
BocHN
OH
CALCULATED
MASS(M+H)+ 469.2702
469.2702
470.2732
491.2518
413.2075
492.2550
471.2760
507.2199
395.1964
407.1643
414.2103
396.1987
493.2571
508.2238
425.2798
476.2810
381.1967
435.1857
451.1520
469.1310
525.1905
535.2602
548.2432
%
m/z


RM 04
IISER PUNE
RM 04 104 (1.909) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (102:108-(94:101+109:119))
1: TOF MS ES+
1.39e6
CALCULATED MASS
(M+H)+ 401.3015
BocHN
H
N
O
O
OH
O
%
100
0
401.3017
423.2831
345.2392
402.3049
424.2867
327.2286
346.2424
407.3099
439.2522
339.1960
408.3127
425.2888
440.2554
316.3217
357.3121
369.2183
383.1852
401.1598
457.2214
469.2734
480.2750
491.2500
498.2461
310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490 500
m/z


RM 05
IISER PUNE
RM 05 101 (1.857) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (SG, 1x1.00); Cm (99:103-(85:98+106:120))
1: TOF MS ES+
2.75e5
465.2388
CALCULATED MASS
(M+H)+ 465.2389
BocHN
H
N
O
O
OH
%
466.2421
487.2208
467.2446
488.2236
428.1484
434.6837
447.1230
456.1786
463.2230
471.2500
475.2717
484.2123
491.2541
503.2010
506.1218
508.1165
521.3015
527.9543
m/z

12. Reference

(1) SHELXS-97: Sheldrick, G. M. *Acta Crystallogr. Sect A*. **1990**, *4*6, 467,

(2) Sheldrick,G. M. SHELXL-97, Universität Göttingen (Germany) **1997**