Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


Supporting Information

# Multi-responsive Supramolecular Hydrogels Based on Merocyanine-peptide Conjugates

Wei Wang, Jing Hu, Mengmeng Zheng, Li Zheng, Huan Wang* and Yan Zhang*

E-mail: njuzy@nju.edu.cn, wanghuan@nju.edu.cn

**Table S1. Hydrogelation conditions for MC*I* substituted short peptides.**

| Peptide Conjugates | pH [a] | $C_{min}$/(mg/mL) [b] |
|---|---|---|
| **MC*I***-Ala-Ala | 3.6 | 1.4 |
| **MC*I***-Ala-Phe | 5.2 | 1.3 |
| **MC*I***-Phe-Phe | 6.4 | 4.4 |

[a] Optimal pH for hydrogelation. [b] Minimum concentration for hydrogelation under the appropriate pH.

**Table S2.** Hydrogelation conditions for **MC*II*** substituted short peptides.

| Peptide Conjugates | pH [a] | $C_{min}$/(mg/mL) [b] |
|---|---|---|
| Gly-Gly-**MC*II*** | 4.4 | 2.0 |
| Gly-Gly-Gly-**MC*II*** | 4.4 | 4.0 |
| Ala-Ala-Gly-**MC*II*** | 5.2 | 1.4 |
| Phe-Gly-Gly-**MC*II*** | 3.8 | 2.5 |
| Phe-Phe-Gly-**MC*II*** | - | - |

[a] Optimal pH for hydrogelation. [b] Minimum concentration for hydrogelation under the appropriate pH.

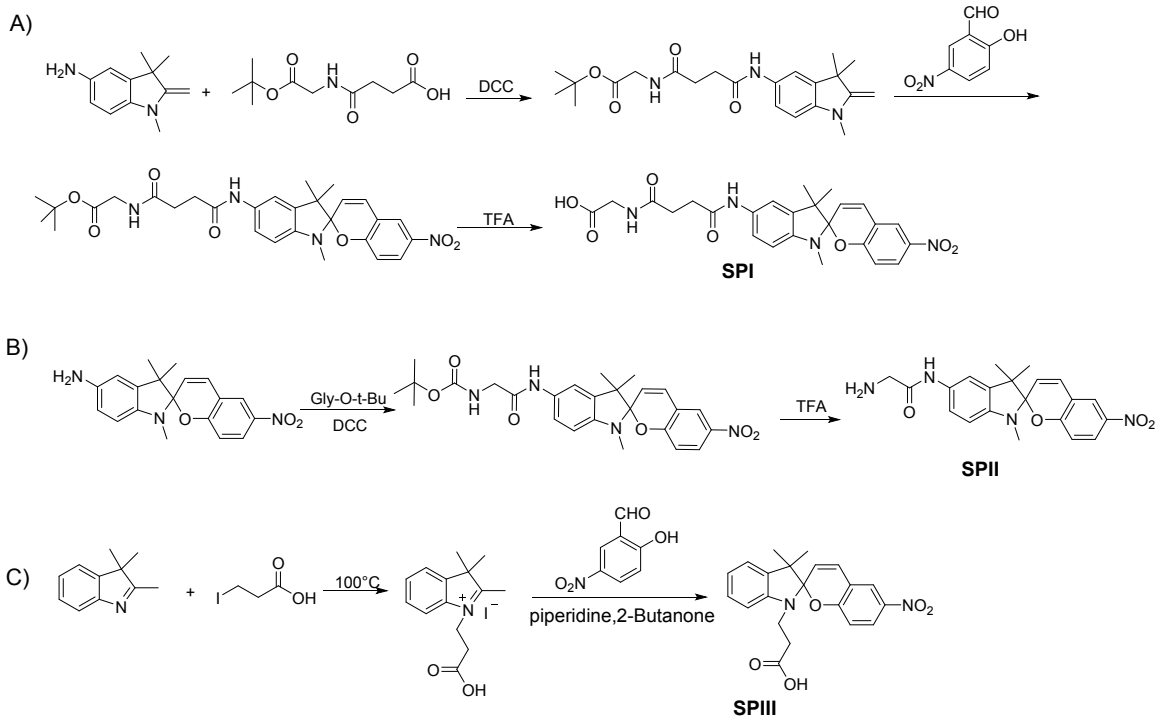



**Figure S1**. Synthesis of spiropyran building blocks.

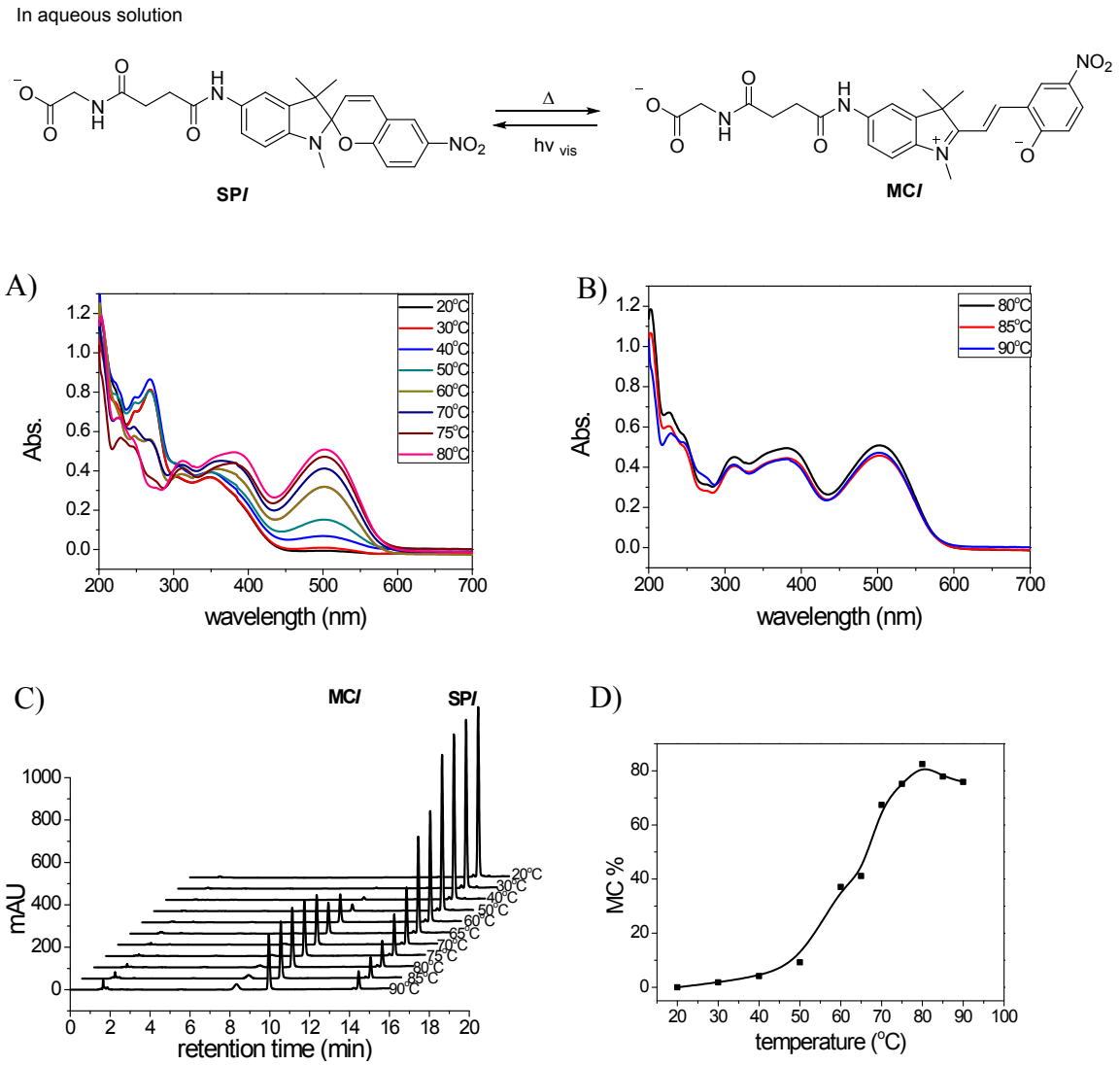



**Figure S2**. (A) Temperature-dependent changes in UV/Vis spectra of **SP*I*** (0.1 mg/mL) in PBS buffer (pH 7.4), 20 to 80 ℃; (B) Temperature-dependent changes in UV/Vis spectra of **SP*I*** (0.1 mg/mL) in PBS buffer (pH 7.4), 80 to 90 ℃; (C) HPLC monitoring of the transformation of **SP*I*** to **MC*I*** at different temperatures; (D) Temperature dependence of the MC% in the system.

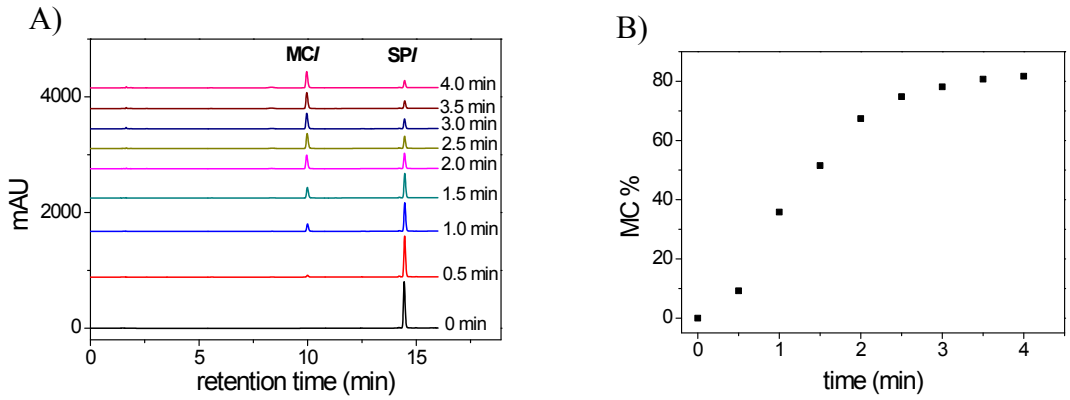



**Figure S3**. (A) Time-dependent transformation of **SP*l*** to **MC*l*** at 70 ℃ monitored by HPLC; (B) Accumulation of the MC during heat treatment at 70 ℃.

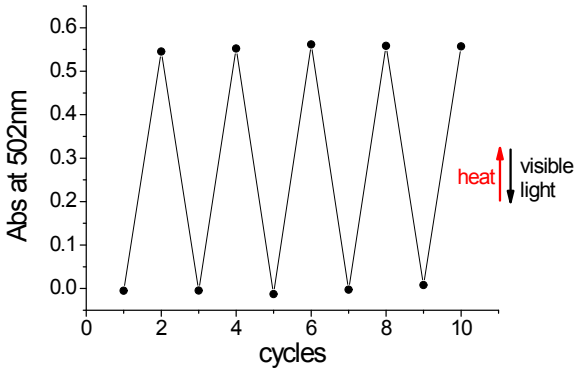



**Figure S4.** Switching between SP form and MC form of **SP*l*** (0.1 mg/mL) by either heating the solution at 70 ℃ in PBS buffer, pH 7.4 for 3 min or exposure it under sunlight for 0.5 min.

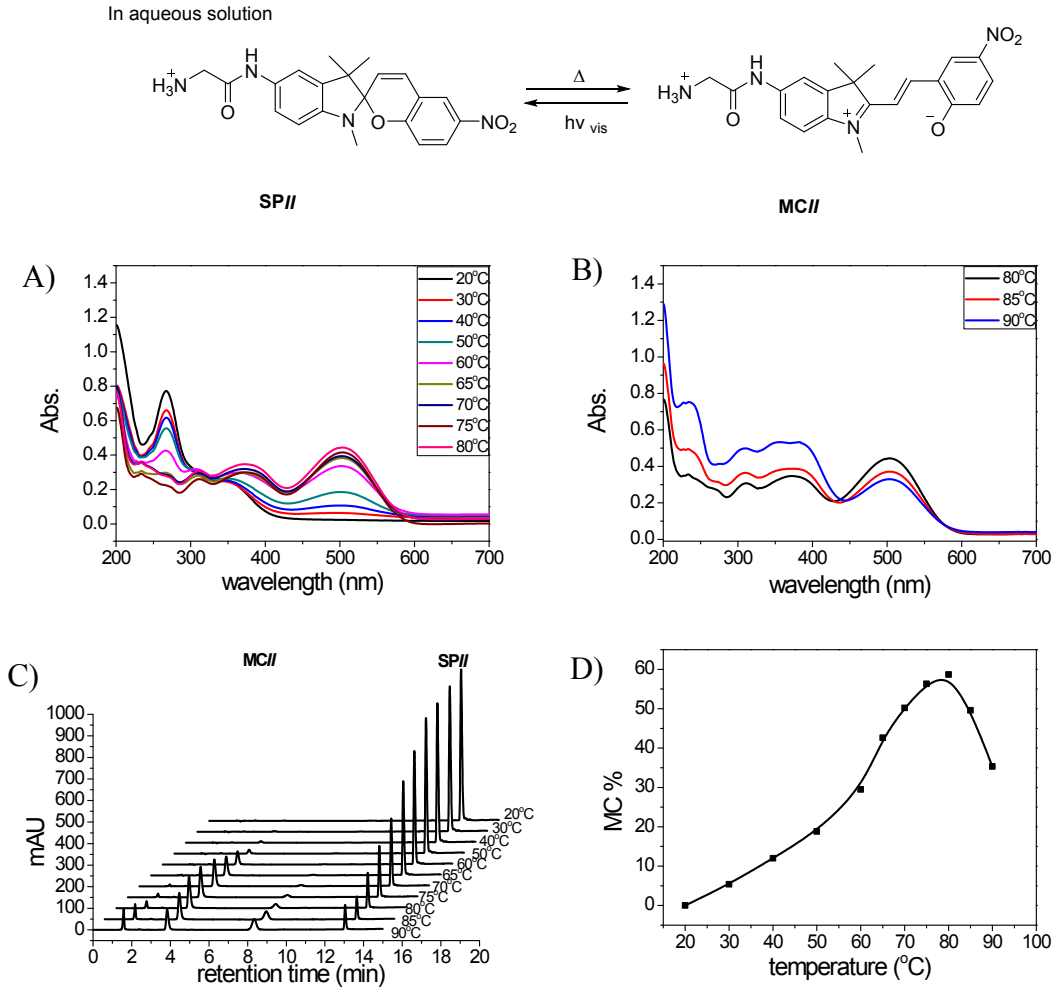



**Figure S5**. (A) Temperature-dependent changes in UV/Vis spectra of **SP*II*** (0.1 mg/mL) in PBS buffer (pH 7.4), 20 to 80 ℃; (B) Temperature-dependent changes in UV/Vis spectra of **SP*II*** (0.1 mg/mL) in PBS buffer (pH 7.4), 80 to 90 ℃; (C) The transformation of **SP*II*** to **MC*II*** at different temperatures monitored by HPLC; (D) Temperature-dependent formation of MC.

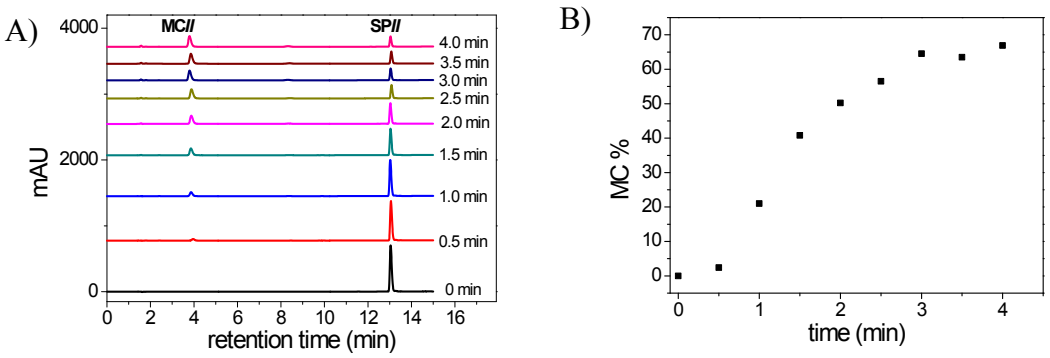



**Figure S6.** (A) Temperature-dependent transformation of **SP*II*** to **MC*II*** monitored by HPLC; (B) Accumulation of the MC during heat treatment at 70℃.

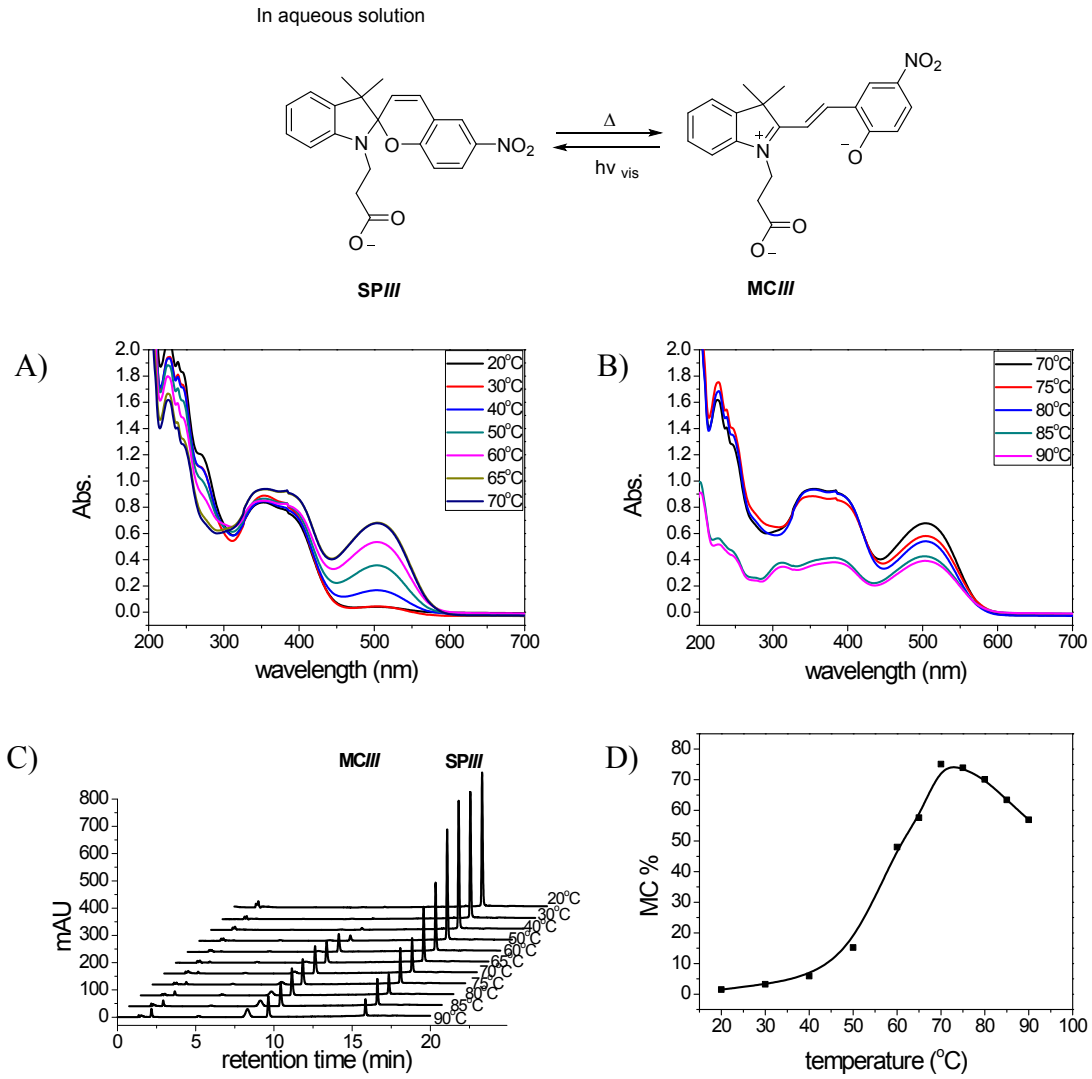



**Figure S7**. (A) Temperature-dependent changes in UV/Vis spectra of **SP*III*** (0.1 mg/mL) in PBS buffer (pH 7.4), 20 to 70℃; (B) Temperature-dependent changes in UV/Vis spectra of **SP*III*** (0.1 mg/mL) in PBS buffer (pH 7.4), 70 to 90 ℃; (C) Temperature-dependent transformation of **SP*III*** to **MC*III*** monitored by HPLC; (D) Temperature-dependent formation of MC.

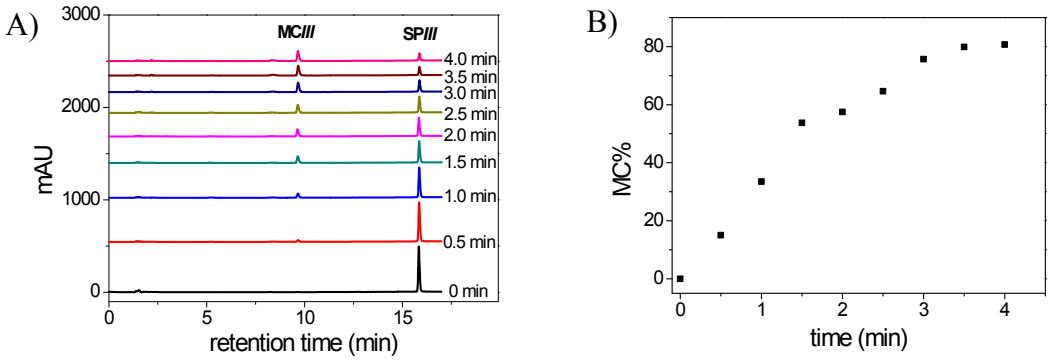



**Figure S8**. (A) Temperature-dependent transformation of **SP*III*** to **MC*III*** monitored by HPLC; (B) Accumulation of the MC during heat treatment at 65℃.

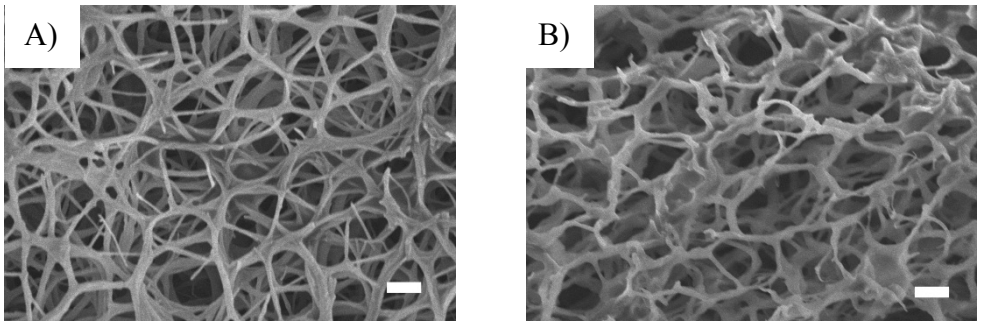



**Figure S9.** The scanning electron micrographs of the hydrogels formed by **MC*I*-**dipeptides: (A) **MC*I*-**Ala-Ala (2.0 mg/mL, pH 3.6); (B) **MC*I*-**Phe-Phe (5.0 mg/mL, pH 6.4). Scale bar = 1 μm.

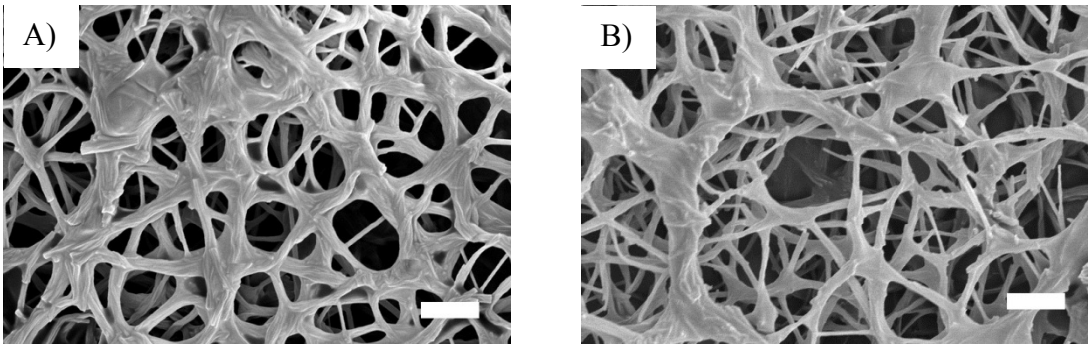



**Figure S10.** The scanning electron micrographs of the hydrogels of compounds: (A) Gly-Gly-**MC*II*** (4.0 mg/mL, pH 4.4); (B) Phe-Gly-**MC*II*** (5.0 mg/mL, pH 4.8). Scale bar = 1 μm.

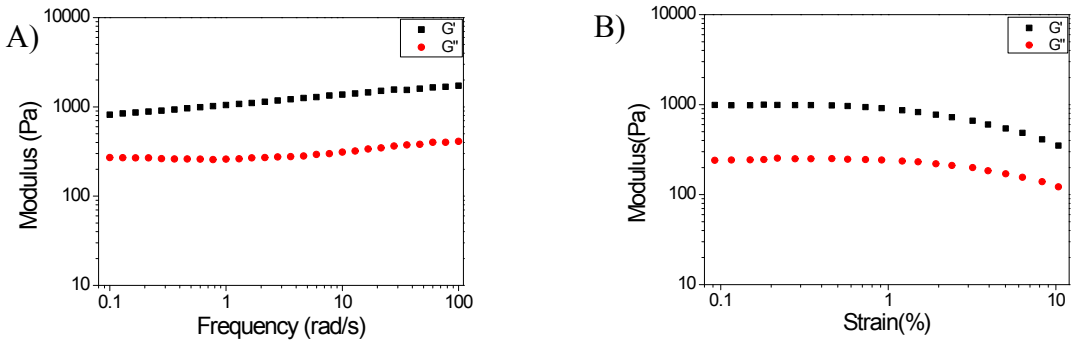



**Figure S11.** Rheology data of the hydrogel of **MC*I*-**Ala-Ala (5.0 mg/mL, pH 3.6): (A) frequency sweep test; (B) strain sweep test.

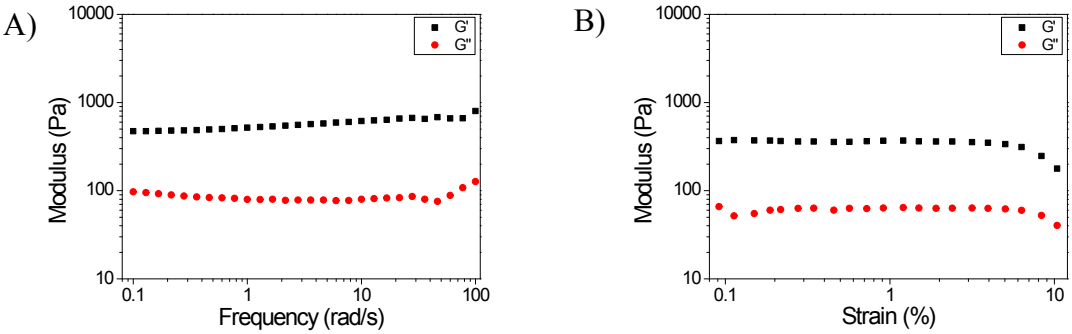



**Figure S12.** Rheology data of the hydrogel of Ala-Ala-**MC*II*** (5.0 mg/mL, pH 3.6): (A) frequency sweep test; (B) strain sweep test.

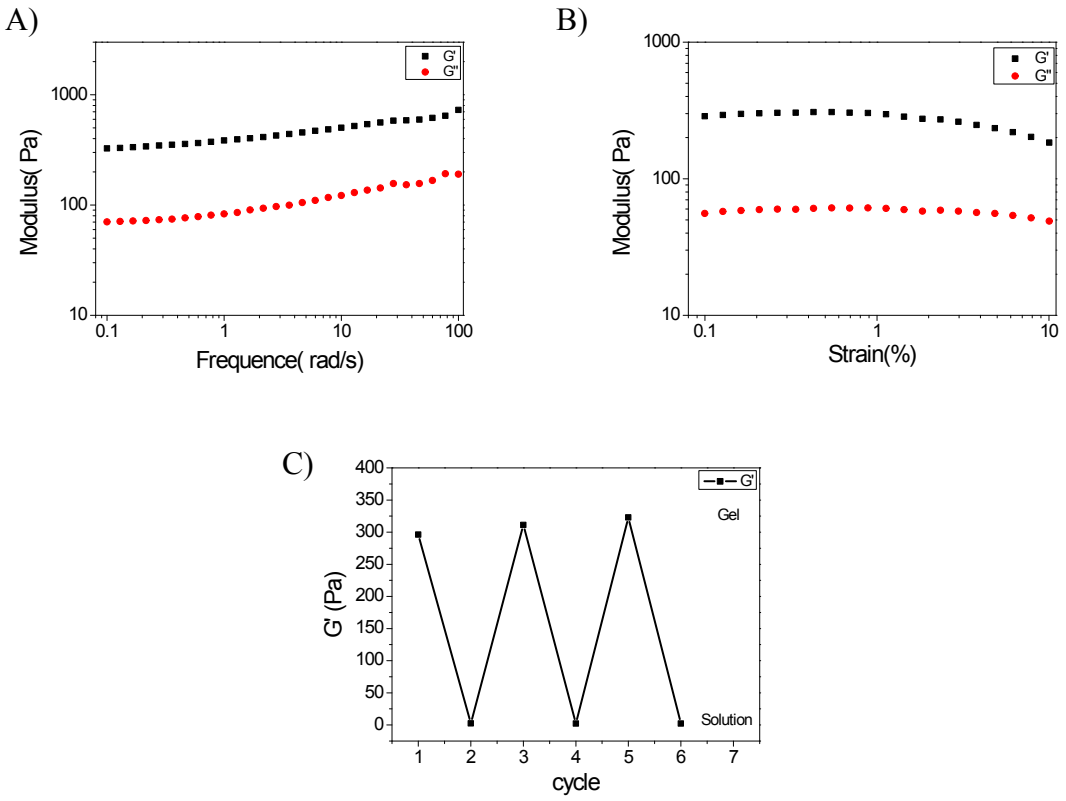



**Figure S13.** Rheology data of the hydrogel of **MC*I***-RGD (4.0 mg/mL, pH 5.2): (A) frequency sweep test; (B) strain sweep test; (C) reversible changes of storage modulus in the gel-sol phase-transition cycles.

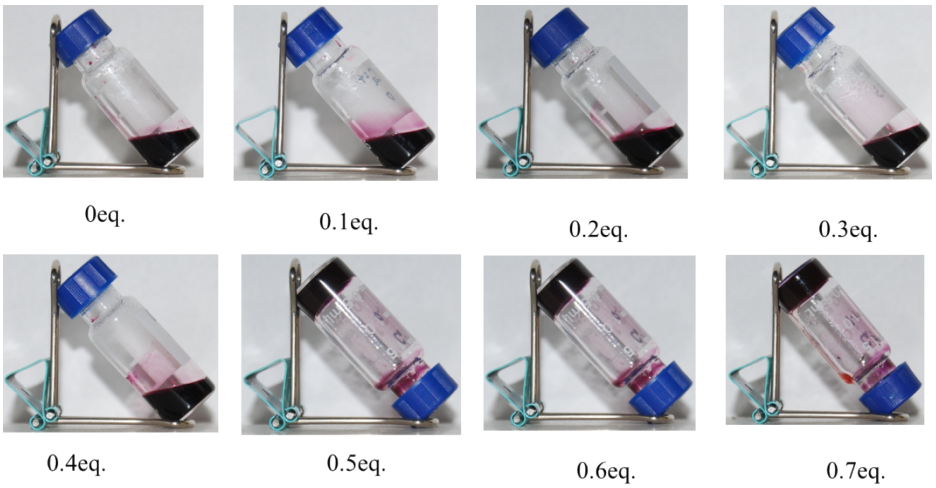



**Figure S14**. Gelation status of **MC*I*-RGD** aqueous solution at different ratio of [$Ca^{2+}$]/[COOH].

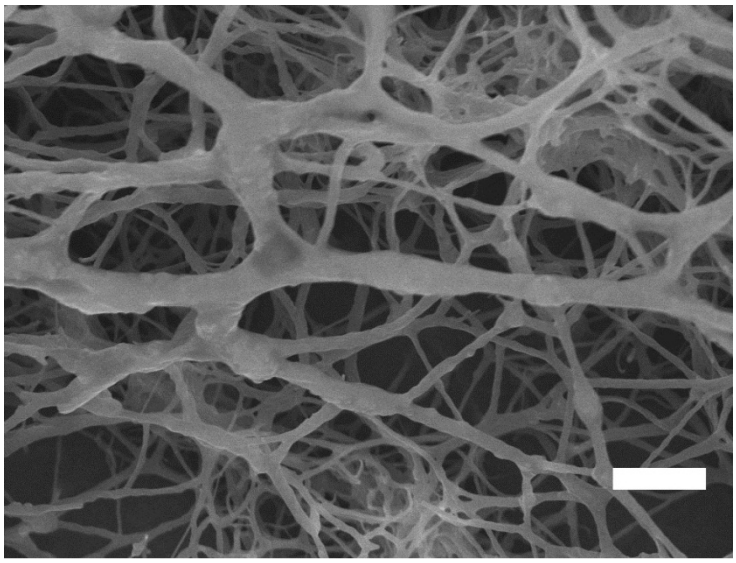


**Figure S15.** The scanning electron micrographs of the hydrogels of **MC*I*-RGD**(10.0 mg/mL, pH 7.4) / $Ca^{2+}$ ([$CaCl_2$]:0.68 mg/mL, pH 7.4). Scale bar = 1 μm.

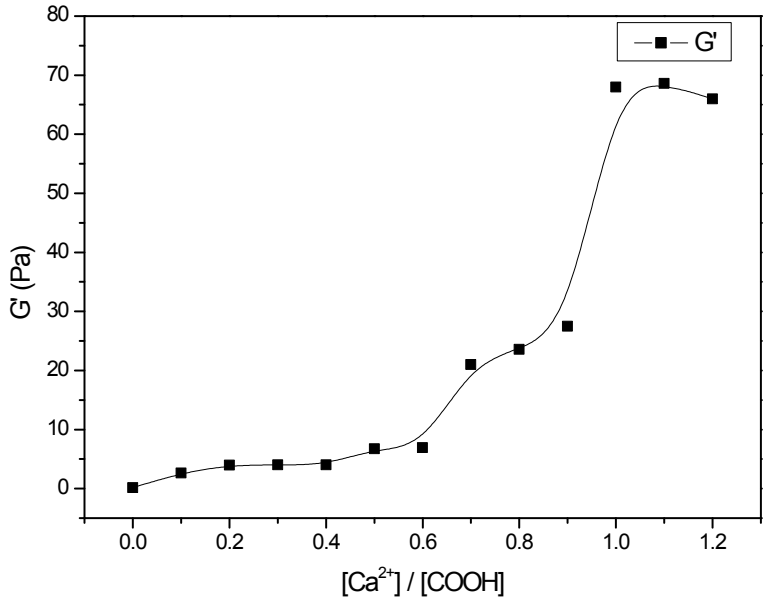



**Figure S16.** Storage moduli of **MC*I***-RGD/$Ca^{2+}$ hydrogel versus [Ca2+]/[COOH].

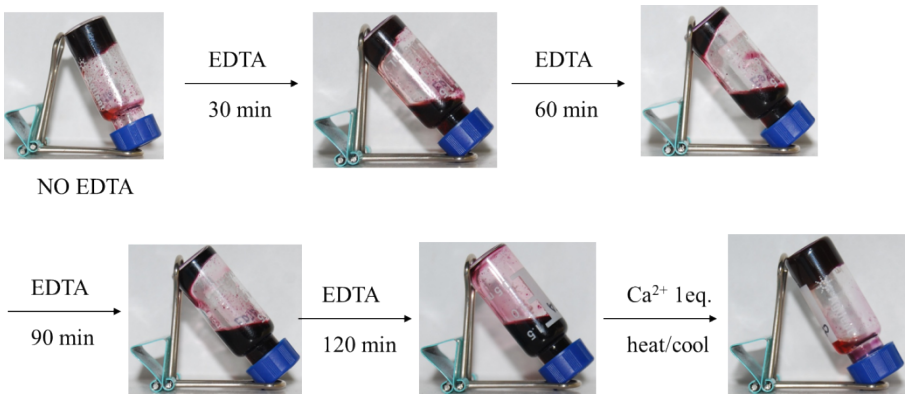
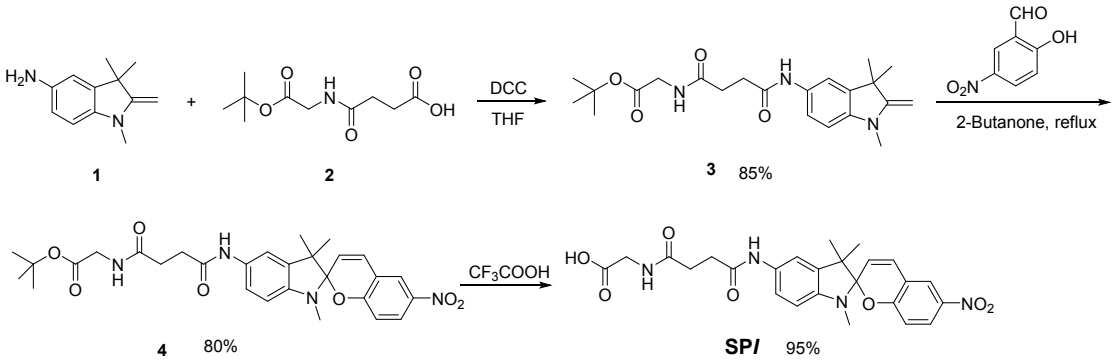



**Figure S17.** Optical images of reversible phase transformation process of the **MC*I***-RGD/$Ca^{2+}$ hydrogel.

## Synthesis and Characterizations

### Synthesis of compound **SP*I***




The starting compounds **1**[1] was prepared form reported methods.

Synthesis of compound **3**

Compound **1** (1.41 g, 7.5 mmol) was dissolved in 20 mL of dry THF, and a solution of compound **2** (2.31 g, 10 mmol) in 20 mL of dry THF was added slowly under $N_2$ atmosphere. Then DCC (2.06 g, 10 mmol) was added and the mixture was stirred over night at room temperature. The reaction mixture was filtrated to remove the insoluble DCU. After evaporation of the solvent, the crude product was re-dissolved in dichloromethane, washed with saturated $NaHCO_3$ aqueous solution, water and saturated NaCl aqueous solution and dried over $Na_2SO_4$. The solvent was removed in vacuum and the product was purified by flash column chromatography on silica gel to afford compound **3** (2.56 g, 85%) as light yellow solid. Compound **3** was very sensitive to air, and slowly turned to purple solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.09 (s,1H), 7.31 (s, 1H), 7.16 (d, $J$ = 7.8 Hz, 1H), 6.48 (t, $J$ = 4.9 Hz, 1H), 6.42 (d, $J$ = 7.8 Hz, 1H), 3.92 (d, $J$ = 4.9 Hz, 2H), 3.81 (s, 2H), 3.00 (s, 3H), 2.68 (s, 4H), 1.46 (s, 9H), 1.31 (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 172.9, 170.4, 169.0, 162.9, 143.1, 137.8, 129.9, 120.1, 115.6, 104.4, 82.1, 73.0, 44.3, 42.2, 32.3, 31.3, 29.9, 28.8, 28.0; MS (ESI+) *m/z*: 402 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{22}H_{32}N_3O_4$ 402.2393; found 402.2391

Synthesis of compound **4**

To a solution of compound **3** (2.41 g, 6.0 mmol) in 40 mL of 2-butanone, 5-nitro-salicylaldehyde (1.20 g, 7.2 mmol) was added. And the mixture was heated at reflux for 3 h. After cooling to room temperature, the solvent was removed in vacuum. The product was purified by flash column chromatography on silica gel to afford compound **4** (2.64 g, 80%). $^{1}$H NMR (300 MHz, $CDCl_3$): *δ* 8.14 (s, 1H), 8.03 – 7.99 (m, 2H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.22 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.92 (d, *J* = 10.4 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.46 (d, *J* = 8.3 Hz, 1H), 6.39 (s, 1H), 5.84 (d, *J* = 10.4 Hz, 1H), 3.94 (d, *J* = 5.1 Hz, 2H), 2.70 (s, 4H), 2.69 (s, 3H), 1.47 (s, 9H), 1.27 (s, 3H)1.18 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): *δ* 172.8, 170.4, 169.0, 159.8, 144.5, 140.9, 136.6, 131.0, 128.3, 125.9, 122.7, 121.5, 120.2, 118.7, 115.4, 115.3, 106.9, 106.7, 82.3, 52.4, 42.2, 32.3, 31.3, 29.0, 28.0, 25.7, 19.8; MS (ESI+) *m/z*: 551 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{29}H_{34}N_4O_7Na$ 573.2325; found 573.2320.

Synthesis of compound **SP*I***

Compound **4** (2.20 g, 4.0 mmol) was dissolved in 100 mL of DCM and 20 mL of trifluoroacetic acid was added. The reaction mixture was stirred for 4 hours at room temperature. Solvent was removed in vacuum when full conversion of compound **4** was accomplished. Cold ether was added to the residue, and compound **SP*I*** (1.88 g, 95%) was obtained after filtration. $^{1}$H NMR (300 MHz, MeOD-$d_4$): *δ* 8.10 (d, *J* = 2.8 Hz, 1H), 8.03 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.32 (d, *J* = 1.9 Hz, 1H), 7.23 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.09 (d, *J* = 10.5 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 6.52 (d, *J* = 8.0 Hz, 1H), 5.96 (d, *J* = 10.5 Hz, 1H), 3.88 (s, 2H), 2.72 (s, 3H), 2.67 – 2.64 (m, 4H), 1.27 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): *δ* 172.1, 170.1, 159.9, 143.8, 140.9, 136.4, 132.6, 128.8, 126.2, 123.3, 121.8, 120.1, 119.4, 119.3, 115.8, 114.6, 107.3, 106.9, 52.4, 31.9, 30.8, 29.1, 26.1, 20.1; MS (ESI-) *m/z*: 493 $[M-H]^-$; HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{25}H_{25}N_4O_7$ 493.1729; found 493.1733.

## Synthesis of SP*I* / SP*III*-linked peptides

The **SP*I*** and **SP*III*-**linked peptides were prepared from the standard solid-phase peptide synthesis and purified with preparative HPLC. The purity of the modified peptides was over 95% as determined by HPLC analysis.

**SP*I*-**Ala-Ala (Mixture of two diastereoisomers): yellow powder; $^{1}$H NMR (300 MHz, MeOD-*d*$_4$): *δ* 8.11 (d, *J* = 2.5 Hz, 1H), 8.04 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.38 (br s, 1H), 7.24 (d, *J* = 6.9 Hz, 1H), 7.13 (d, *J* = 10.2 Hz, 1H), 6.81 (d, *J* = 9.0 Hz, 1H), 6.61 (br s, 1H), 6.04 (br s, 1H), 4.43 (q, *J* = 7.1 Hz, 1H), 4.31 (q, *J* = 7.1 Hz, 1H), 3.90 (d, *J* = 17.0 Hz, 1H), 3.85 (d, *J* = 17.0 Hz, 1H), 2.79 – 2.73 (m, 5H), 2.58 (t, *J* = 6.2 Hz, 2H), 1.37 (d, *J* = 7.1 Hz, 6H), 1.26 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-*d*$_4$): *δ* 174.8, 174.7, 174.4, 174.3, 173.2, 172.0, 171.2, 170.3, 163.5, 159.8, 146.2, 144.8, 144.1, 1410, 137.2, 131.9, 129.3, 128.9, 125.8, 125.6, 123.0, 120.5, 120.1, 119.4, 115.2, 115.0, 107.4, 52.2, 48.8, 48.0, 42.5, 33.7, 31.2, 30.6, 28.6, 25.2, 21.5, 16.7, 16.2; MS (ESI-) *m/z*: 635 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{37}N_6O_9$ 637.2622; found 637.2640.

**SP*I*-**Ala-Phe (Mixture of two diastereoisomers): yellow powder; $^{1}$H NMR (300 MHz, MeOD-*d*$_4$): *δ* 8.10 (d, *J* = 2.9 Hz, 1H), 8.02 (dd, *J* = 8.9, 2.9 Hz, 1H), 7.36 (br s, 1H), 7.24 – 7.15 (m, 6H), 7.10 (d, *J* = 10.5 Hz, 1H), 6.77 (d, *J* = 8.9 Hz, 1H), 6.54 (d, *J* = 7.7 Hz, 1H), 5.98 (d, *J* = 10.5 Hz, 1H), 4.60 (dd, *J* = 8.1, 5.7 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 1H), 3.86 (s, 2H), 3.15 (dd, *J* = 14.0, 5.7 Hz, 1H), 2.98 (dd, *J* = 14.0, 8.1 Hz, 1H), 2.74 (m, 5H), 2.57 (t, *J* = 6.6 Hz, 2H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.22 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-*d*$_4$): *δ* 174.8, 174.7, 174.4, 174.3, 173.2, 171.2, 170.3, 159.8, 146.2, 144.8, 137.2, 132.0, 129.5, 128.9, 125.8, 125.6, 123.0, 120.5, 120.1, 119.4, 115.2, 115.0, 107.4, 52.2, 48.8, 48.0, 42.5, 31.2, 30.6, 28.6, 25.2, 16.7, 16.2; MS (ESI-) *m/z*: 711 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{41}N_6O_9$ 713.2935; found 713.2946.

**SP*I***-Phe-Phe (Mixture of two diastereoisomers): yellow powder; $^{1}$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.06 (d, $J$ = 2.5 Hz, 1H), 7.97 (dd, $J$ = 8.3, 2.5 Hz, 1H), 7.33 (br s, 1H), 7.23 – 7.14 (m, 11H), 7.04 (d, $J$ = 10.4 Hz, 1H), 6.67 – 6.58 (m, 1H), 6.48 (d, $J$ = 8.3 Hz, 1H), 5.91 (d, $J$ = 10.4 Hz, 1H), 4.66 – 4.57 (m, 2H), 3.80 (d, $J$ = 17.1 Hz, 1H), 3.72 (d, $J$ = 17.1 Hz, 1H), 3.17 (dd, $J$ = 13.9, 5.6 Hz, 1H), 3.07 (dd, $J$ = 14.0, 4.6 Hz, 1H), 3.02 – 2.72 (m, 4H), 2.68 (s, 3H), 2.57 – 2.52 (m, 2H), 1.13 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 174.6, 173.3, 172.9, 171.2, 170.3, 159.7, 146.2, 144.7, 144.4, 141.0, 136.9, 129.0, 128.0, 126.4, 125.5, 122.7, 120.8, 120.1, 119.2, 115.1, 107.0, 153.7, 52.1, 49.0, 42.5, 37.0, 31.3, 30.7, 28.2, 25.2, 16.6; MS (ESI-) *m/z*: 787 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{45}N_6O_9$ 789.3248; found 789.3262.

**SP*I***-RGD, yellow powder; MS (ESI-): *m/z*: 821 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{47}N_{10}O_{12}$ 823.3375; found 823.3378.

**SP*I***-VPP, yellow powder; MS (ESI-): *m/z*: 786 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{40}H_{50}N_7O_{10}$ 788.3619; found 788.3625.

**SP*I***-YSV, yellow powder; MS (ESI-): *m/z*: 842 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{50}N_7O_{12}$ 844.3517; found 844.3516.

**SP*I***-SDKP, yellow powder; MS (ESI-): *m/z*: 920 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{56}N_9O_{14}$ 922.3947; found 922.3964.

**SP*I***-AGAS, yellow powder; MS (ESI-): *m/z*: 779 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{36}H_{45}N_8O_{12}$ 781.3157; found 781.3159.

**SP*I***-VVPQ, yellow powder; MS (ESI-): *m/z*: 916 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{60}N_9O_{12}$ 918.4361; found 918.4370.

**SP*I***-VYGGG, yellow powder; MS (ESI-): *m/z*: 926 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{54}N_9O_{13}$ 928.3841; found 928.3841.

**SP*I***-IKVAV, yellow powder; MS (ESI+): *m/z*: 1005 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{50}H_{73}N_{10}O_{12}$ 1005.5409; found 1005.5440.

**SP*I*-**TIGYG, yellow powder; MS (ESI-): *m/z*: 984 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{48}H_{60}N_9O_{14}$ 986.4260; found 986.4259.

**SP*I*-**YIGSR, yellow powder; MS (ESI-): *m/z*: 1069 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{51}H_{67}N_{12}O_{14}$ 1071.4900; found 1071.4920.

**SP*I*-**LGAGGAG, yellow powder; MS (ESI+): *m/z*: 978 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{60}N_{11}O_{14}$ 978.4321; found 978.4331.

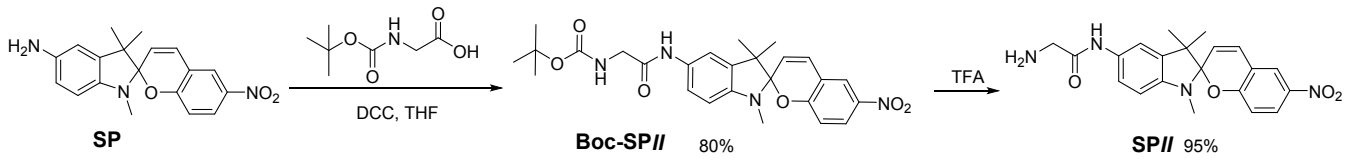
**SP*III*-**Ala-Ala (Mixture of two diastereoisomers): yellow powder; $^1$H NMR (300 MHz, MeOD-*d*$_4$): *δ* 8.09 (d, *J* = 2.7 Hz, 1H), 8.02 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.13 (td, *J* = 7.7, 1.0 Hz, 1H), 7.08 – 7.03 (m, 2H), 6.82 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 9.1 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 5.98 (d, *J* = 10.5 Hz, 1H), 4.32 (penta, *J* = 7.2 Hz, 1H), 3.58 – 3.49 (m, 2H), 2.57 – 2.50 (m, 2H), 1.36 (d, *J* = 6.5 Hz, 3H), 1.27 – 1.25 (m, 6H), 1.15 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-*d*$_4$): *δ* 174.4, 173.3, 172.4, 159.4, 146.6, 141.1, 135.9, 128.2, 128.0, 127.4, 125.3, 122.4, 121.9, 121.3, 119.4, 119.1, 115.2, 106.7, 52.5, 48.8, 47.9, 39.8, 35.0, 24.9, 18.8, 16.8, 16.2; MS (ESI-) *m/z*: 521 $[M-H]^-$; HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{27}H_{31}N_4O_7$ 523.2193; found 523.2232.

**SP*III*-**Phe-Phe (Mixture of two diastereoisomers): yellow powder; $^1$H NMR (300 MHz, MeOD-*d*$_4$): *δ* 8.07 (br s, H), 8.01 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.21 – 7.10 (m, 11H), 7.05 (d, *J* = 7.5 Hz, 1H), 6.98 (d, *J* = 10.6 Hz), 6.94 (d, *J* = 10.6 Hz), 6.82 (t, *J* = 7.4 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 6.61 – 6.57 (m, 1H), 5.86 (d, *J* = 10.6 Hz), 5.84 (d, *J* = 10.6 Hz), 4.63 – 4.58 (m, 2H), 3.47 – 3.39 (m, 1H), 3.27 – 3.13 (m, 2H), 3.06 (dd, *J* = 13.8, 5.1 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.79 – 2.68 (m, 1H), 2.50 – 2.35 (m, 2H), 1.23 (s, 3H), 1.08 (s), 1.06 (s); $^{13}$C NMR (75 MHz, MeOD-*d*$_4$): *δ* 172.8, 172.2, 172.0, 159.4, 146.5, 141.0, 137.0, 136.8, 135.9, 129.0, 128.9, 128.1, 128.0, 127.4, 126.4, 126.3, 125.3, 122.4, 121.8, 121.3, 11904, 119.0, 115.1, 106.6, 54.2, 53.7, 52.4, 39.8, 37.5, 37.0, 34.9, 24.5, 18.7; MS (ESI-) *m/z*: 673 $[M-H]^-$; HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{39}H_{37}N_4O_7$ 673.2668; found 673.2752.

**SP*III***-Ala-Phe (Mixture of two diastereoisomers): yellow powder; $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.07 (d , $J$ = 2.6 Hz, 1H), 8.01 (dd, $J$ = 8.8, 2.6 Hz, 1H), 7.22 – 7.10 (m, 6H), 7.06 (d, $J$ = 7.1 Hz, 1H), 7.03 – 6.96 (m, 1H), 6.82 (t, $J$ = 7.2 Hz, 1H), 6.76 (d, $J$ = 9.0 Hz, 1H), 6.67 (d, $J$ = 7.8 Hz, 1H), 5.94 (d, $J$ = 10.6 Hz), 5.92 (d, $J$ = 10.6 Hz), 4.61 (dd, $J$ = 18.1, 5.3 Hz, 1H), 4.36 – 4.24 (m, 1H), 3.65 – 3.38 (m, 2H), 3.20 – 2.94 (m, 2H), 2.63 – 2.40 (m, 2H), 1.24 (s, 3H), 1.20 (d, $J$ = 7.1 Hz, 3H), 1.13 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 173.3, 172.9, 172.3, 159.4, 146.5, 141.6, 136.8, 135.9, 129.0, 128.0, 127.3, 126.4, 125.3, 122.4, 121.9, 121.9, 121.3, 119.4, 119.0, 115.1, 106.9, 106.6, 55.6, 52.5, 48.7, 39.8, 36.9, 35.0, 34.9, 24.9, 18.7, 16.6; MS (ESI-) *m/z*: 597 $[M-H]^-$; HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{33}H_{33}N_4O_7$ 597.2355; found 597.2425.

**SP*III***-Phe-Ala (Mixture of two diastereoisomers): yellow powder; $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.07 (d, $J$ = 2.8 Hz, 1H), 8.00 (dd, $J$ = 9.0, 2.8 Hz, 1H), 7.18 – 7.10 (m, 6H), 7.07 – 6.98 (m, 2H), 6.82 (td, $J$ = 7.3, 0.6 Hz, 1H), 6.72 (d, $J$ = 9.0 Hz, 1H), 6.61 (d, $J$ = 8.1 Hz, 1H), 5.88 (d, $J$ = 10.7 Hz, 1H), 4.62 (dd, $J$ = 9.3, 5.0 Hz, 1H), 4.33 (q, $J$ = 7.3 Hz, 1H), 3.43 – 3.32 (m, 2H), 3.12 (dd, $J$ = 14.3, 5.0 Hz, 1H), 2.79 (dd, $J$ = 14.3, 9.3 Hz, 1H), 2.47 (br s, 2H), 1.37 – 1.22 (m, 6H), 1.10 (s), 1.06 (s); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 174.3, 172.3, 172.0, 159.4, 146.5, 141.0, 137.0, 135.9, 129.0, 128.1, 128.0, 127.4, 126.3, 125.3, 122.4, 121.8, 121.3, 119.4, 119.0, 115.1, 106.6, 54.2, 52.5, 47.9, 39.8, 37.7, 35.0, 24.8, 18.7, 16.3; MS (ESI-) *m/z*: 597 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{35}N_4O_7$ 599.2506; found 599.2519.

Synthesis of **SP*II***-linked peptides




The starting material **SP** [2] was prepared according to reported method.

Synthesis of compound Boc-**SP*II***

Compound **SP** (337 mg, 1 mmol) was dissolved in 5 mL of dried THF. Then Boc-NH-Gly (175 mg, 1 mmol), DCC (206 mg, 1 mmol) was added and the mixture was stirred over night at room temperature. The reaction mixture was filtrated to remove the insoluble DCU. After evaporation of the solvent, the product was purified by flash column chromatography on silica gel to afford compound Boc-**SP*II*** (396 mg, 80%). $^{1}$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 9.68 (s, 1H), 8.21 (s, 1H), 7.99 (d, $J$ = 9.2 Hz, 1H), 7.35 ~ 7.20 (m, 3H), 7.00 (brs, 1H), 6.89 (d, $J$ = 9.2 Hz, 1H), 6.55 (d, $J$ = 8.1 Hz, 1H), 5.98 (d, $J$ = 10.5 Hz, 1H), 3.67 (s, 2H), 2.63 (s, 3H), 1.38 (s, 9H), 1.18 (s, 3H), 1.11 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 168.0, 159.9, 156.4, 144.1, 140.9, 136.5, 132.1, 128.8, 126.2, 123.3, 121.7, 119.5, 119.4, 115.8, 114.8, 107.4, 106.9, 78.4, 52.4, 44.1, 29.1, 28.7, 26.1, 20.1; MS (ESI+) *m/z*: 495 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{26}H_{31}N_4O_6$ 495.2244; found 495.2244.

Synthesis of **SP*II***

Boc-**SP*II*** (247 mg, 0.5 mmol) was dissolved in 2 mL of 95% trifluoroacetic acid, and the reaction mixture was stirred over night at room temperature. The solvent was removed in vacuum when full conversion of starting material was accomplished. **SP*II***•TFA (242 mg, 95%) was purified with preparative HPLC. $^{1}$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.11 (d, $J$ = 2.6 Hz, 1H), 8.04 (dd, $J$ = 8.8, 2.6 Hz, 1H), 7.36 (d, $J$ = 2.1 Hz, 1H), 7.27 (dd, $J$ = 8.3, 2.1 Hz, 1H), 7.10 (d, $J$ = 10.3 Hz, 1H), 6.79 (d, $J$ = 8.8 Hz, 1H), 6.55 (d, $J$ = 8.3 Hz, 1H), 5.97 (d, $J$ = 10.3 Hz, 1H), 3.83 (s, 2H), 2.73 (s, 3H), 1.28 (s, 3H), 1.20 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 163.6, 159.6, 145.1, 141.1, 136.9, 130.1, 128.3, 125.3, 122.4, 121.1, 119.9, 119.1, 115.0, 114.8, 106.6, 52.0, 40.6, 27.8, 24.7, 18.7; MS (ESI+) *m/z*: 395 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{21}H_{23}N_4O_4$ 395.1719; found 395.1718.

Synthesis of Boc-Gly-**SP*II***

Boc-NH-Gly (88 mg, 0.5 mmol) was dissolved in 10 mL of dry $CH_2Cl_2$. Then DIPEA (173 µL, 1 mmol), HOBt (63 mg, 0.5 mmol) was added, and the mixture was cooled to 0°C. EDC•HCl (96 mg, 0.5 mmol) was added and the reaction mixture was stirred for additional 30 min at 0°C. **SP*II***•TFA (254 mg, 0.5 mmol) was added and stirring was subsequently continued for 30 min at 0°C. The reaction mixture was warmed to room temperature and further stirred overnight. The reaction mixture was diluted with 20 mL of $CH_2Cl_2$, washed with water and saturated NaCl aqueous solution and dried over $Na_2SO_4$. The solvent was removed in vacuum and the product was purified by flash column chromatography on silica gel to afford compound Boc-Gly-**SP*II*** (226 mg, 82%). $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.9 Hz, 1H), 8.03 (dd, $J$ = 9.0, 2.9 Hz, 1H), 7.38 – 7.35 (m, 2H), 7.08 (d, $J$ = 10.3 Hz, 1H), 6.78 (d, $J$ = 9.0 Hz, 1H), 6.53 (d, $J$ = 8.5 Hz, 1H), 5.96 (d, $J$ = 10.3 Hz, 1H), 4.01 (s, 2H), 3.77 (s, 2H), 2.72 (s, 3H), 1.46 (s, 9H), 1.27 (s, 3H), 1.18 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 171.9, 168.0, 159.6, 157.5, 145.0, 141.1, 136.6, 130.3, 128.2, 125.3, 122.4, 121.2, 120.4, 119.1, 115.3, 115.0, 106.7, 106.5, 79.6, 52.0, 43.7, 42.5, 27.8, 27.3, 24.8, 18.8; MS (ESI+) *m/z*: 552 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{28}H_{33}N_5O_7Na$ 574.2278; found 574.2279.

Synthesis of Gly-**SP*II***•TFA

Boc-Gly-**SP*II*** (138 mg, 0.25 mmol) was dissolved in 2 mL of 95% trifluoroacetic acid, and the reaction mixture was stirred over night at room temperature. The solvent was removed in vacuum when full conversion of starting material was accomplished. Gly-**SP*II***•TFA (130 mg, 92%) was purified with preparative HPLC. $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.11 (d, $J$ = 2.7 Hz, 1H), 8.03 (dd, $J$ = 9.0, 2.7 Hz, 1H), 7.33 (d, $J$ = 1.9 Hz, 1H), 7.26 (dd, $J$ = 8.4, 2.1 Hz, 1H), 7.10 (d, $J$ = 10.4 Hz, 1H), 6.79 (d, $J$ = 9.0 Hz, 1H), 6.55 (d, $J$ = 8.4 Hz, 1H), 5.98 (d, $J$ = 10.4 Hz, 1H), 4.08 (s, 2H), 3.78 (s, 2H), 2.73 (s, 3H), 1.24 (s, 6H); $^{13}$C NMR (75 MHz,

MeOD-$d_4$): $\delta$ 167.7, 166.8, 159.7, 144.6, 141.0, 137.0, 130.9, 128.9, 125.5, 122.7, 120.8, 120.3, 119.2, 115.2, 107.0, 52.1, 42.5, 40.2, 28.2, 21.8; MS (ESI+) *m/z*: 452 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{23}H_{26}N_5O_5$ 452.1934; found 452.1933.

Synthesis of Boc-Gly-Gly-**SP*II***

Boc-Gly-Gly-**SP*II*** (247 mg, 81%) was prepared from compound Gly-**SP*II*** as described for compound Boc-Gly-**SP*II***. $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, *J* = 2.7 Hz, 1H), 8.03 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 7.32 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.09 (d, *J* = 10.3 Hz, 1H), 6.79 (d, *J* = 9.1 Hz, 1H), 6.53 (d, *J* = 8.3 Hz, 1H), 5.96 (d, *J* = 10.3 Hz, 1H), 4.01 (s, 2H), 3.94 (s, 2H), 3.77 (s, 2H), 2.72 (s, 3H), 1.43 (s, 9H), 1.28 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 172.3, 170.9, 167.9, 159.6, 145.0, 141.0, 136.6, 130.4, 128.2, 128.1, 125.3, 122.4, 121.2, 120.3, 119.1, 115.3, 115.0, 106.7, 106.5, 79.6, 52.0, 43.6, 42.5, 27.8, 27.3, 24.8, 18.8; MS (ESI+) *m/z*: 609 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{30}H_{36}N_6O_8Na$ 631.2492; found 631.2493.

Synthesis of Gly-Gly-**SP*II***•TFA

Gly-Gly-**SP*II*** (145 mg, 93%) was prepared from compound Boc-Gly-Gly-**SP*II*** as described for compound Gly-**SP*II***•TFA. $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.11 (d, *J* = 2.8 Hz, 1H), 8.03 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.27 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.10 (d, *J* = 10.5 Hz, 1H), 6.79 (d, *J* = 9.0 Hz, 1H), 6.56 (d, *J* = 8.5 Hz, 1H), 5.99 (d, *J* = 10.5 Hz, 1H), 4.03 (s, 4H), 3.77 (s, 2H), 2.74 (s, 3H), 1.24 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 170.5, 168.0, 166.8, 159.7, 144.6, 141.0, 137.0, 130.9, 129.0, 125.4, 122.7, 120.8, 120.4, 119.2, 115.3, 115.0, 107.0, 52.1, 42.5, 42.1, 40.2, 28.2, 25.2; MS (ESI+) *m/z*: 509 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{25}H_{29}N_6O_6$ 509.2149; found 509.2148.

Synthesis of Boc-Ala-Ala-**SP*II***

Boc-Ala-Ala-**SP*II*** (245 mg, 77%) was prepared from compound **SP*II*** as described for compound Boc-**SP*II***. $^{1}$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.8 Hz, 1H), 8.03 (dd, $J$ = 8.9, 2.8 Hz, 1H), 7.44 – 7.35 (m, 2H), 7.09 (d, $J$ = 10.2, 1H), 6.79 (dd, $J$ = 9.0, 2.5 Hz, 1H), 7.09 (d, $J$ = 10.2 Hz, 1H), 6.55 – 6.52 (m, 1H), 5.97 (d, $J$ = 10.2 Hz, 1H), 4.34 – 4.29 (m, 1H), 4.08 – 3.88 (m, 3H), 2.72 (s, 3H), 1.45 – 1.31 (m, 15H), 1.26 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 175.9, 175.0, 174.2, 174.1, 167.9, 159.6, 156.7, 144.9, 141.0, 136.5, 130.5, 130.4, 128.2, 128.1, 125.3, 122.4, 121.2, 120.3, 119.0, 115.2, 115.0, 106.7, 79.5, 78.1, 52.0, 50.6, 50.2, 49.7, 42.7, 27.9, 27.4, 24.8, 18.9, 16.6, 16.1, 16.0, 15.8; MS (ESI+) *m/z*: 637 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{32}H_{40}N_6O_8Na$ 659.2805; found 659.2808.

Synthesis of Ala-Ala-**SP*II***•TFA

Ala-Ala-**SP*II***•TFA (150 mg, 92%) was prepared from compound Boc-Ala-Ala-**SP*II*** as described for compound Gly-**SP*II***•TFA. $^{1}$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.6 Hz, 1H), 8.04 (dd, $J$ = 8.9, 2.6 Hz, 1H), 7.40 (d, $J$ = 2.0 Hz, 1H), 7.28 (dd, $J$ = 8.2, 1.8 Hz, 1H), 7.12 (d, $J$ = 10.4 Hz, 1H), 6.79 (d, $J$ = 8.9 Hz, 1H), 6.60 (d, $J$ = 8.2 Hz, 1H), 6.01 (d, $J$ = 10.4 Hz, 1H), 4.44 (q, $J$ = 7.0 Hz, 1H), 4.18 – 3.96 (m, 3H), 2.76 (s, 3H), 1.54 (d, $J$ = 7.0 Hz, 3H), 1.44 (d, $J$ = 6.3 Hz, 3H), 1.25 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 172.0, 168.1, 166.2, 158.1, 142.1, 139.2, 135.9, 130.1, 128.5, 123.9, 121.5, 118.7, 118.3, 117.8, 113.4, 106.2, 50.6, 47.8, 47.1, 40.9, 27.2, 25.7, 23.5, 20.2, 14.6, 14.3; MS (ESI+) *m/z*: 537 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{27}H_{33}N_6O_6$ 537.2462; found 537.2464.

Synthesis of Boc-Phe-Gly-**SP*II***

Boc-Phe-Gly-**SP*II*** (307 mg, 88%) was prepared from compound **SP*II***•TFA as described for compound Boc-NH-Gly-Gly-**SP*II***. $^{1}$H NMR (300 MHz, MeOD-$d_4$): $^{1}$H

NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.8 Hz, 1H), 8.02 (dd, $J$ = 9.0, 2.8 Hz, 1H), 7.39 – 7.34 (m, 2H), 7.31 – 7.21 (m, 5H), 7.09 (d, $J$ = 10.3 Hz, 1H), 6.78 (d, $J$ = 9.0 Hz, 1H), 6.54 (d, $J$ = 8.3 Hz, 1H), 5.96 (d, $J$ = 10.3 Hz, 1H), 4.25 (t, $J$ = 8.0 Hz, 1H), 4.09 – 3.71 (m, 4H), 3.12 (dd, $J$ = 13.6, 6.6 Hz, 1H), 2.91 (dd, $J$ = 13.6, 8.4 Hz, 1H), 2.72 (s, 3H), 1.35 (s, 9H), 1.28 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 174.4, 170.8, 167.9, 159.6, 156.7, 145.0, 141.0, 137.0, 136.6, 130.4, 129.0, 128.2, 128.1, 126.4, 125.4, 122.4, 121.2, 120.4, 119.0, 115.3, 106.7, 106.5, 79.6, 56.7, 52.0, 42.8, 42.6, 37.2, 27.9, 27.3, 24.8, 18.9; MS (ESI+) *m/z*: 699 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{37}H_{42}N_6O_8Na$ 721.2962; found 721.2967.

Synthesis of Phe-Gly-**SP*II***•TFA

Phe-Gly-**SP*II***•TFA (162 mg, 91%) was prepared from compound Boc-Phe-Gly-**SP*II*** as described for compound Gly-**SP*II***•TFA. $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.8 Hz, 1H), 8.03 (dd, $J$ = 9.0, 2.7 Hz, 1H), 7.38 – 7.29 (m, 6H), 7.25 (dd, $J$ = 8.3, 2.2 Hz, 1H), 7.10 (d, $J$ = 10.3 Hz, 1H), 6.79 (d, $J$ = 9.0 Hz, 1H), 6.55 (d, $J$ = 8.6 Hz, 1H), 5.97 (d, $J$ = 10.3 Hz, 1H), 4.18 – 4.10 (m, 1H), 4.07 (d, $J$ = 16.6 Hz, 1H), 4.03 (s, 2H), 3.87 (d, $J$ = 16.6 Hz, 1H), 3.27 (dd, $J$ = 16.3, 6.5 Hz, 1H), 3.06 (dd, $J$ = 16.3, 8.1 Hz, 1H), 2.73 (s, 3H), 1.23 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 170.3, 169.2, 168.0, 159.7, 144.4, 141.0, 137.2, 134.3, 131.2, 129.4, 129.1, 128.7, 127.5, 125.5, 122.9, 120.5, 119.3, 115.3, 115.1, 107.3, 54.5, 52.2, 42.6, 42.1, 37.1, 28.5, 21.8; MS (ESI+) *m/z*: 599 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{32}H_{34}N_6O_6Na$ 621.2438; found 621.2439.

Synthesis of Boc-Phe-Phe-**SP*II***

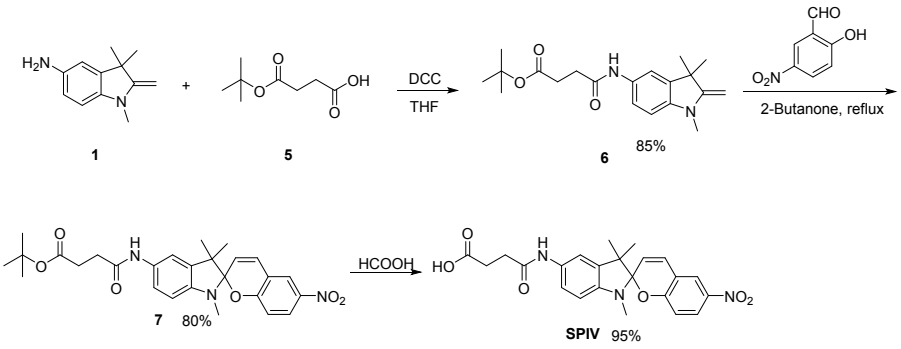
Boc-Phe-Phe-**SP*II*** (335 mg, 85%) was prepared from compound **SP*II***•TFA as described for compound Boc-Gly-**SP*II***. $^1$H NMR (300 MHz, MeOD-$d_4$): $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.6 Hz, 1H), 8.04 – 7.99 (m, 1H), 7.41 – 7.07

(m, 17H), 6.77 – 6.73 (m, 1H), 6.55 – 6.52 (m, 1H), 5.96 (d, *J* = 10.3 Hz, 1H), 4.50 (t, *J* = 7.8 Hz, 1H), 4.29 – 4.22 (m, 1H), 4.09 – 3.74 (m, 2H), 3.26 – 2.78 (m, 4H), 2.72 (s, 3H), 1.33 (s, 9H), 1.27 (s, 3H), 1.18 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 173.9, 173.3, 172.8, 172.5, 167.8, 159.6, 144.9, 141.0, 137.0, 136.6, 130.4, 129.0, 128.9, 128.8, 128.2, 128.1, 126.5, 126.4, 125.4, 122.4, 121.2, 120.4, 119.0, 115.3, 115.0, 106.7, 106.6, 79.5, 56.3, 56.0, 55.5, 52.0, 42.8, 37.6, 37.5, 36.7, 28.0, 27.4, 24.9, 18.9; MS (ESI+) *m/z*: 789 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{44}H_{48}N_6O_8Na$ 811.3431; found 811.3431.

Synthesis of Phe-Phe-**SP*II***•TFA

Phe-Phe-**SP*II***•TFA (193 mg, 96%) was prepared from compound Boc-Phe-Phe-**SP*II*** as described for compound Gly-**SP*II***•TFA. $^1$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.09 (d, *J* = 2.6 Hz, 1H), 8.02 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.41 – 7.04 (m, 16H), 6.78 – 6.74 (m, 1H), 6.58 (d, *J* = 8.3 Hz, 1H), 5.99 (d, *J* = 10.3 Hz, 1H), 4.69 (dd, *J* = 8.0, 6.6 Hz, 1H), 4.13 – 4.85 (m, 3H), 3.25 – 2.80 (m, 4H), 2.75 (s, 3H), 1.23 (s, 6H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 172.5, 172.1, 168.6, 168.6, 167.9, 167.8, 159.8, 144.2, 140.9, 137.4, 136.8, 136.7, 134.1, 133.9, 131.4, 129.7, 129.2, 129.1, 128.9, 128.8, 128.7, 128.3, 128.2, 127.5, 126.7, 126.6, 125.6, 123.1, 120.5, 120.3, 119.4, 115.3, 115.1, 107.6, 55.5, 55.1, 54.2, 54.1, 52.2, 42.8, 42.7, 37.2, 37.1, 37.0, 29.3, 28.7, 21.8; MS (ESI+) *m/z*: 689 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{41}N_6O_6$ 689.3088; found 689.3092.

Synthesis of **SP*IV***




The starting compounds **1**[1] and **5**[3] were prepared form reported methods.

Synthesis of compound **6**

Compound **1** (1.41 g, 7.5 mmol) was dissolved in 20 mL of dry THF, and a solution of compound **5** (1.74 g, 10 mmol) in 20 mL of dry THF was added slowly under $N_2$ atmosphere. Then DCC (2.06 g, 10 mmol) was added and the mixture was stirred over night at room temperature. The reaction mixture was filtrated to remove the insoluble DCU. After evaporation of the solvent, the crude product was re-dissolved in dichloromethane, washed with saturated $NaHCO_3$ aqueous solution, water and saturated NaCl aqueous solution and dried over $Na_2SO_4$. The solvent was removed in vacuum and the product was purified by flash column chromatography on silica gel to afford compound **6** (2.20 g, 85%) as slight yellow solid. **6** was sensitive to air and slowly turned to purple solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.57 (s, 1H), 7.30 (d, $J$ = 2.1 Hz, 1H), 7.14 (dd, $J$ = 8.3, 2.1 Hz, 1H), 6.43 (d, $J$ = 8.3 Hz), 3.82 (s, 2H), 2.69 – 2.65 (m, 2H), 2.60 – 2.55 (m, 2H), 1.46 (s, 9H), 1.32 (s, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 172.6, 169.8, 162.9, 143.3, 138.0, 129.6, 120.0, 115.6, 104.5, 81.0, 73.1, 44.3, 32.1, 31.0, 29.9, 28.9, 28.1; MS (ESI+) *m/z*: 345 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{29}N_2O_3$ 345.2178; found 345.2175.

Synthesis of compound **7**

To a solution of compound **6** (2.07 g, 6.0 mmol) in 40 mL of 2-butanone, 5-nitro-salicylaldehyde (1.20 g, 7.2 mmol) was added. And the mixture was heated at reflux for 1 h. After cooling to room temperature, the solvent was removed in vacuum. The product was purified by flash column chromatography on silica gel to afford compound **7** (2.58 g, 87%). $^{1}$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.10 (d, $J$ = 2.5 Hz, 1H), 8.03 (dd, $J$ = 9.0, 2.5 Hz, 1H), 7.33 (d, $J$ = 2.2 Hz, 1H), 7.22 (dd, $J$ = 8.4, 2.2 Hz, 1H), 7.09 (dd, $J$ = 10.3 Hz, 1H), 6.79 (d, $J$ = 9.0 Hz, 1H), 6.52 (d, $J$ = 8.4 Hz, 1H), 5.96 (d, $J$ = 10.3 Hz, 1H), 3.83 (s, 2H), 2.72 (s, 3H), 2.67 – 2.64 (m, 4H), 1.47 (s, 9H), 1.27 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 172.7, 169.9, 159.8, 144.7, 140.9, 136.8, 130.8, 128.3, 125.9, 122.7, 121.4, 120.0, 118.6, 115.5, 115.3, 107.0, 106.6, 81.1, 52.4, 32.3, 31.0, 29.0, 28.1, 25.8, 19.9; MS (ESI+) *m/z*: 516 $[M+Na]^+$; HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{27}H_{31}N_3O_6Na$ 516.2111; found 516.2114.

Synthesis of compound **SP*IV***

Compound **7**(1.97 g, 4.0 mmol) was dissolved in 10 mL of formic acid, and the reaction mixture was stirred over night at room temperature. The excessive formic acid was removed in vacuum when full conversion of starting material was accomplished. Cold ether was added to the residue, and compound **SP*IV*** (1.66 g, 95%) was obtained after filtration. $^{1}$H NMR (300 MHz, MeOD-$d_4$): $\delta$ 8.10 (d, $J$ = 2.8 Hz, 1H), 8.03 (dd, $J$ = 8.8, 2.8 Hz, 1H), 7.32 (d, $J$ = 1.9 Hz, 1H), 7.23 (dd, $J$ = 8.0, 2.0 Hz, 1H), 7.09 (d, $J$ = 10.5 Hz, 1H), 6.79 (d, $J$ = 8.8 Hz, 1H), 6.52 (d, $J$ = 8.0 Hz, 1H), 5.96 (d, $J$ = 10.5 Hz, 1H), 3.88 (s, 2H), 2.72 (s, 3H), 2.67 – 2.64 (m, 4H), 1.27 (s, 3H), 1.19 (s, 3H); $^{13}$C NMR (75 MHz, MeOD-$d_4$): $\delta$ 172.1, 170.1, 159.9, 143.8, 140.9, 136.4, 132.6, 128.8, 126.2, 123.3, 121.8, 120.1, 119.4, 119.3, 115.8, 114.6, 107.3, 106.9, 52.4, 31.9, 30.8, 29.1, 26.1, 20.1; MS (ESI-) *m/z*: 493 $[M-H]^-$; HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{25}H_{25}N_4O_7$ 493.1729; found 493.1733.

## Synthesis of SP*I* to SP*VIII*-linked RGD peptides

The **SP*IV*** to **SP*VIII***-linked RGD peptides were prepared from the standard solid-phase peptide synthesis and purified with preparative HPLC. The purity of the modified peptides was over 95% as determined by HPLC analysis.

**SP*IV***-RGD, yellow powder; MS (ESI-): *m/z*: 764 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{35}H_{44}N_9O_{11}$ 766.3160; found 766.3152.

**SP*V*-**RGD, yellow powder; MS (ESI-): *m/z*: 849 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{51}N_{10}O_{12}$ 851.3688; found 851.3691.

**SP*VI***-RGD, yellow powder; MS (ESI-): *m/z*: 877 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{55}N_{10}O_{12}$ 879.4001; found 879.4005.

**SP*VII***-RGD, yellow powder; MS (ESI-): *m/z*: 883 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{42}H_{49}N_{10}O_{12}$ 885.3531; found 885.3529.

**SP*VIII***-RGD, yellow powder; MS (ESI-): *m/z*: 897 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{51}N_{10}O_{12}$ 899.3688; found 899.3693.

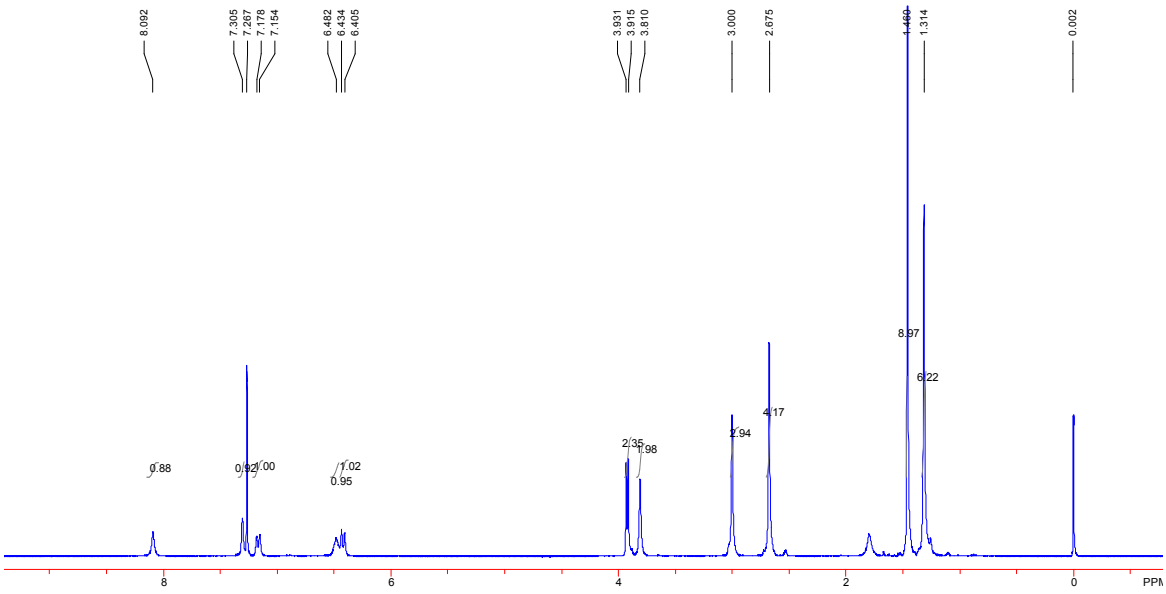
References

[1] D. J. Gale, and J. F. K. Whilshire, *J. Soc. Dyers Colour.*, 1974, **90**, 97-100.

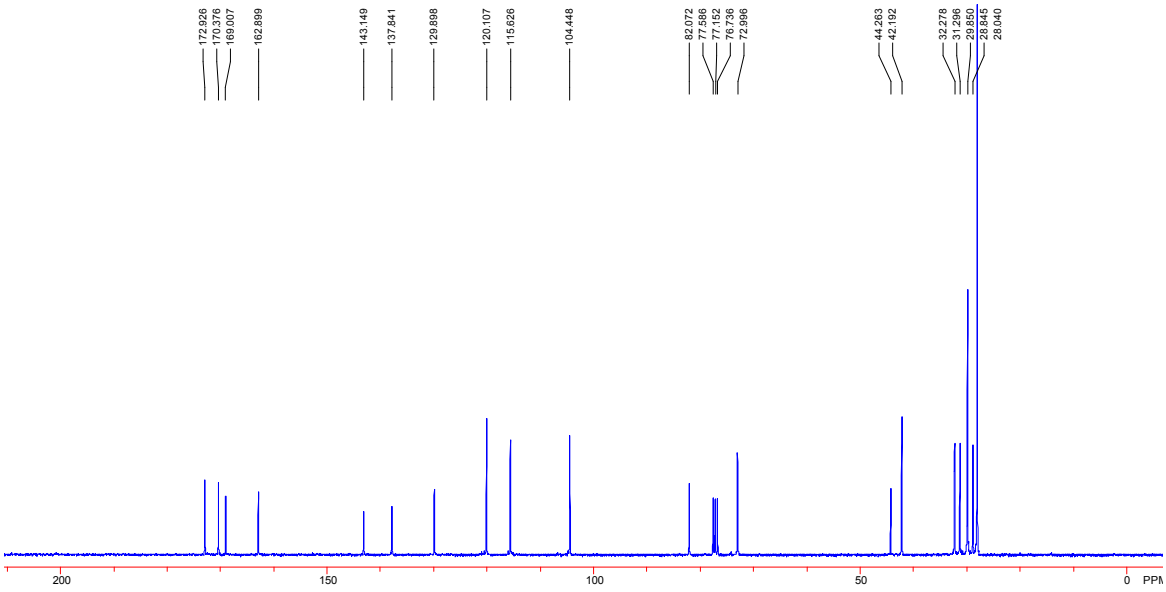
[2] F. P. Shvartsman, and V. A. Krongauz, *J. Phys. Chem.*, 1984, **88**, 6448-6453.

[3] F. Liu, H. Y. Zha, and Z. J. Yao, *J. Org. Chem.*, 2003, **68**, 6679-6684.

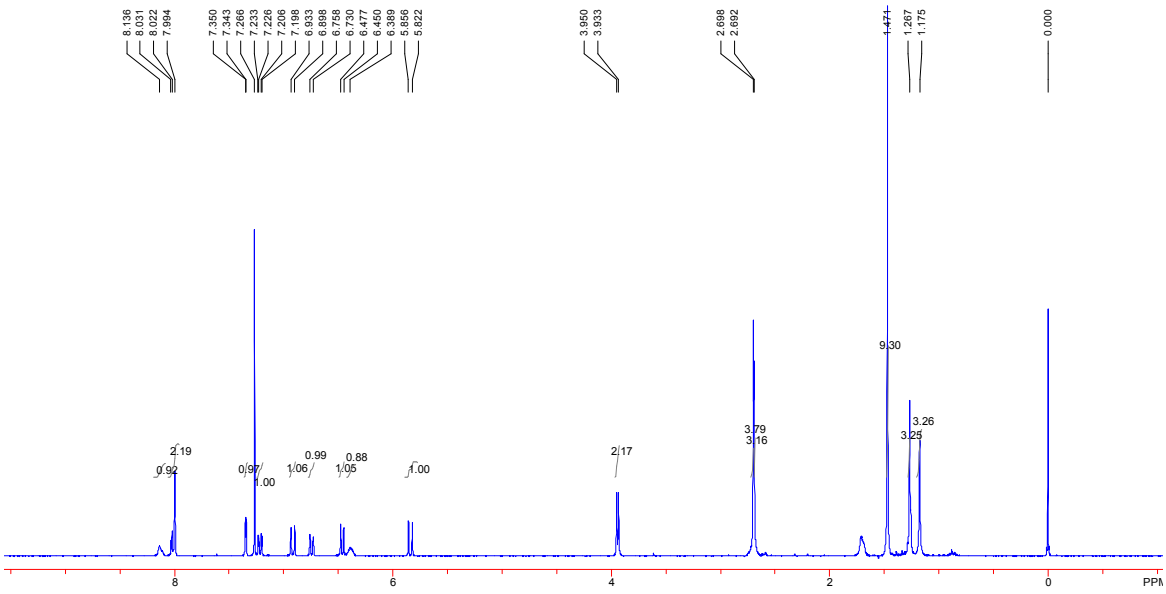
## $^{1}$H NMR of compound **3**



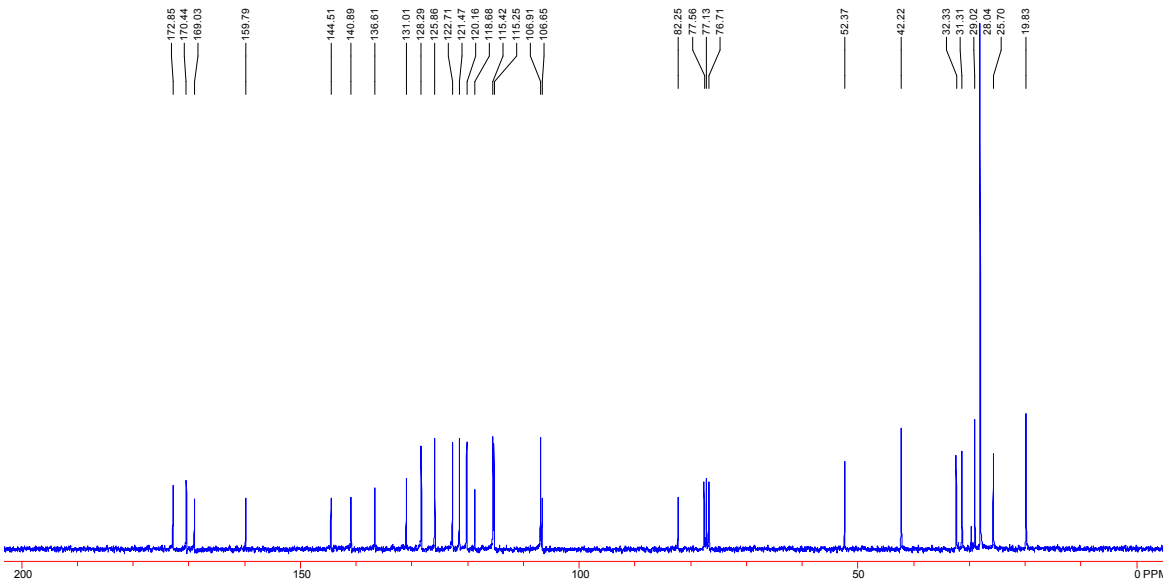

## $^{13}$C NMR of compound **3**



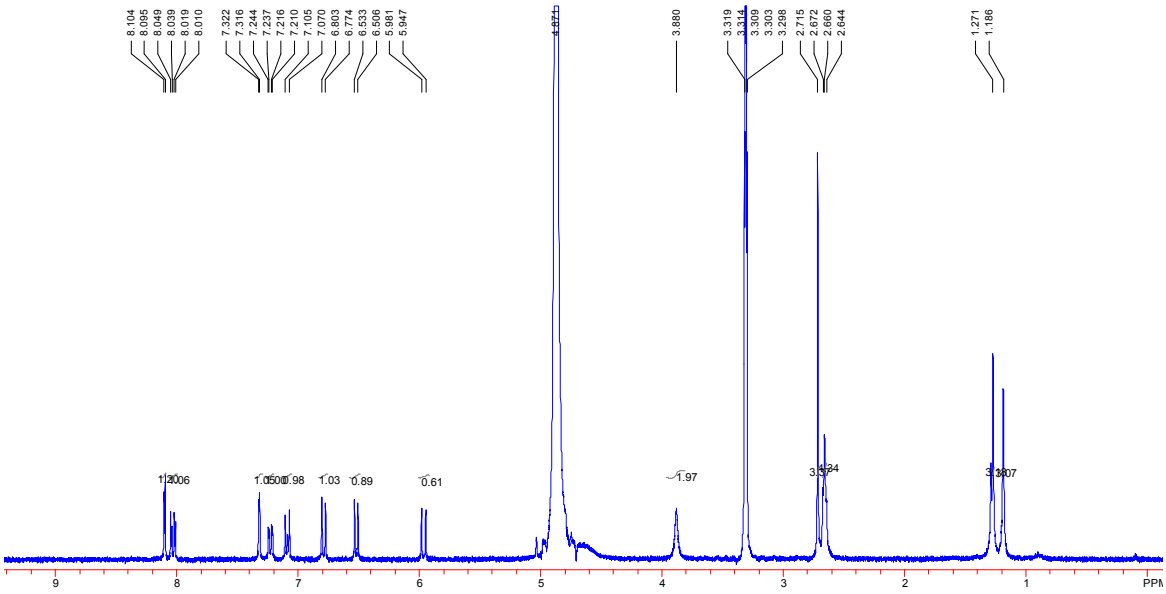
## $^{1}$H NMR of compound **4**



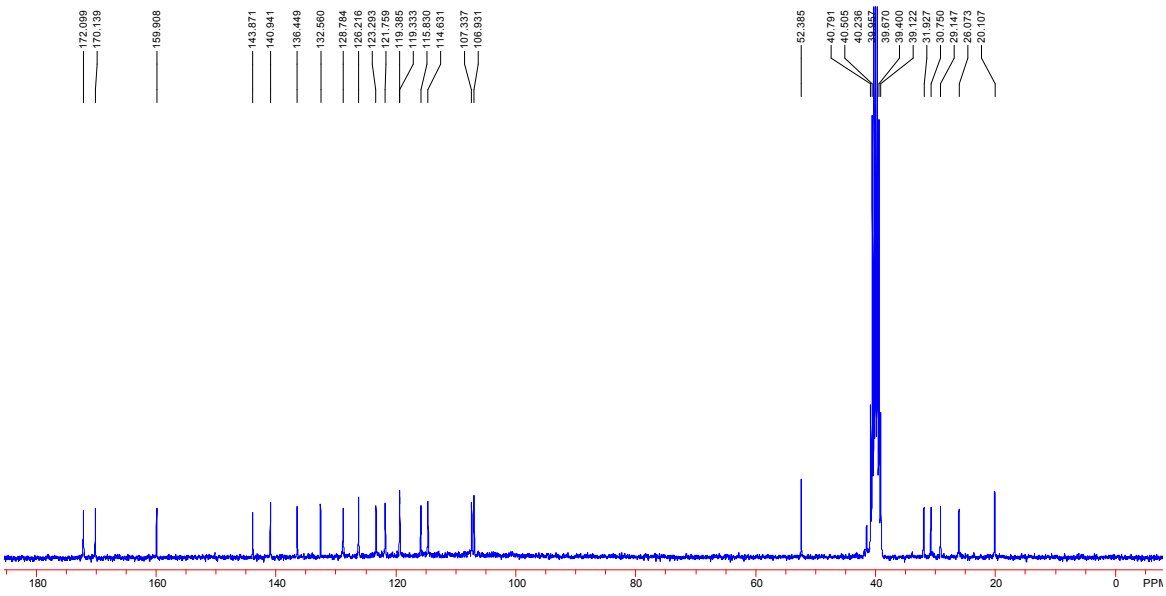

## $^{13}$C NMR of compound **4**



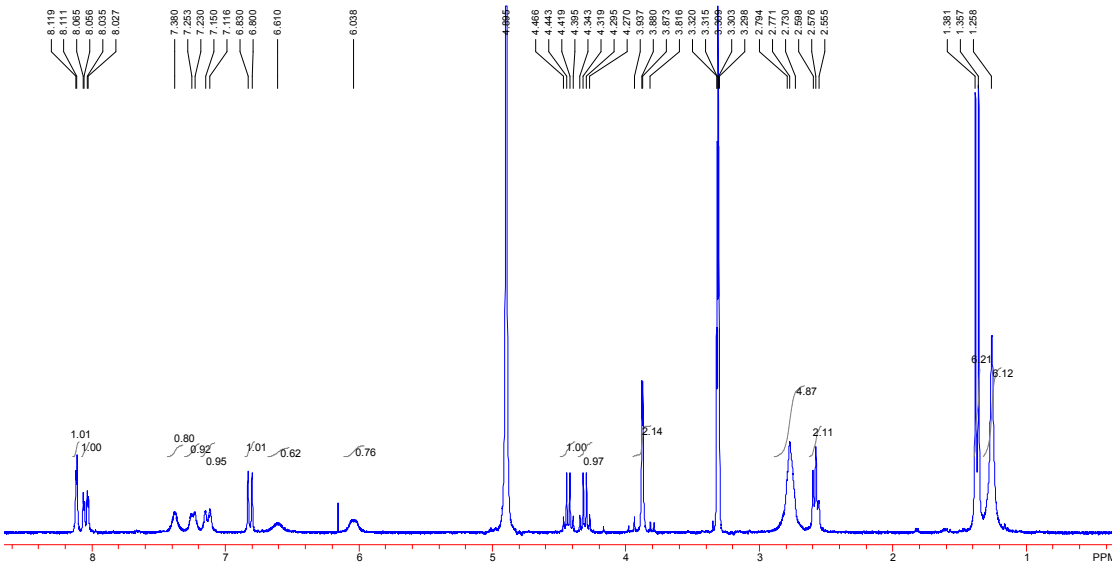
$^{1}$H NMR of compound ***SPI***



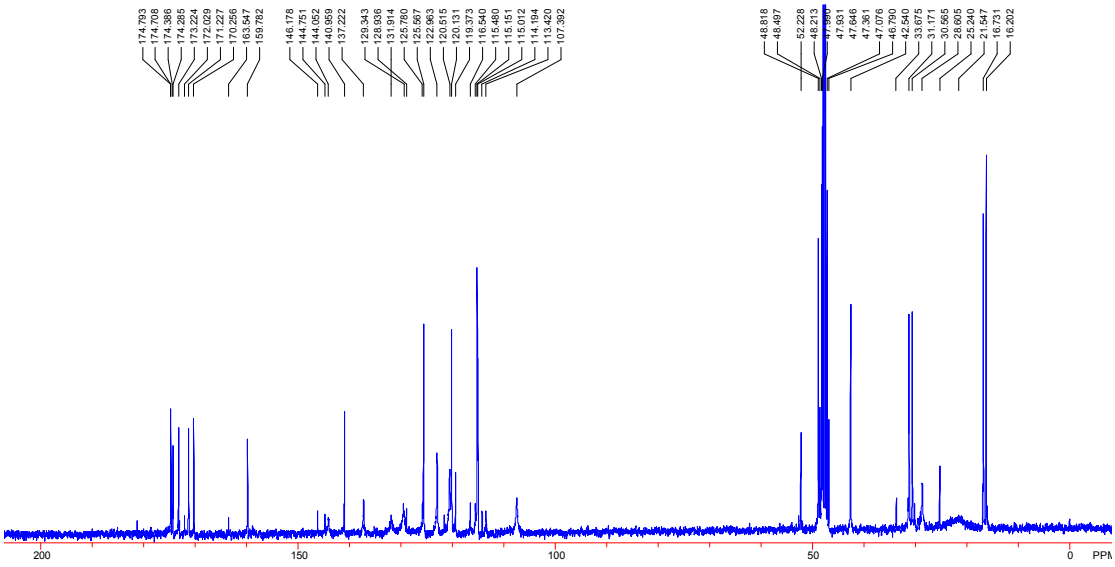

$^{13}$C NMR of compound ***SPI***



$^{1}$H NMR of compound **SP*I*-Ala-Ala**



$^{13}$C NMR of compound **SP*I*-Ala-Ala**



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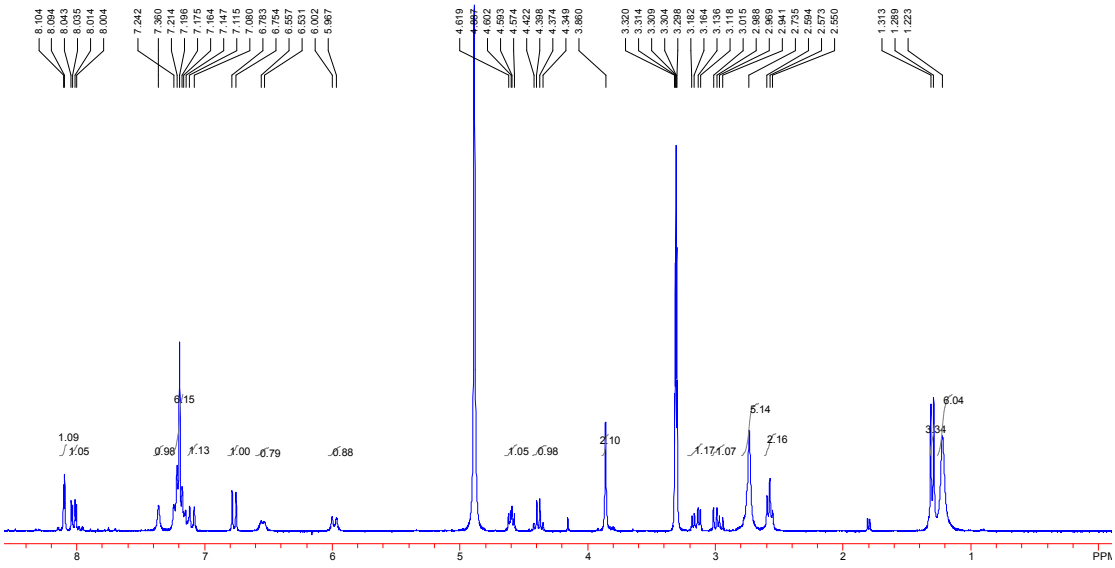


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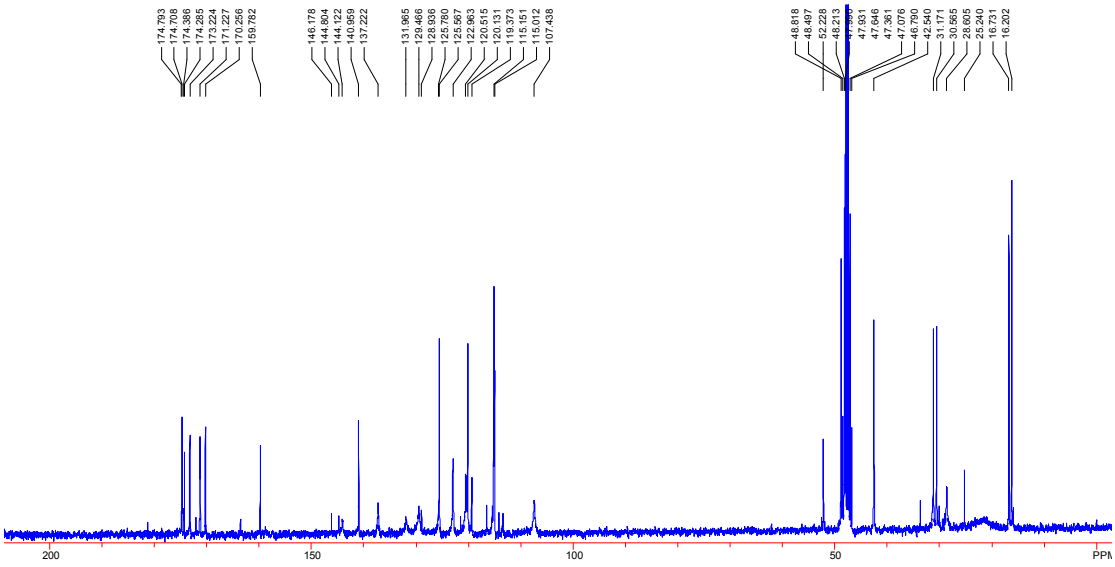

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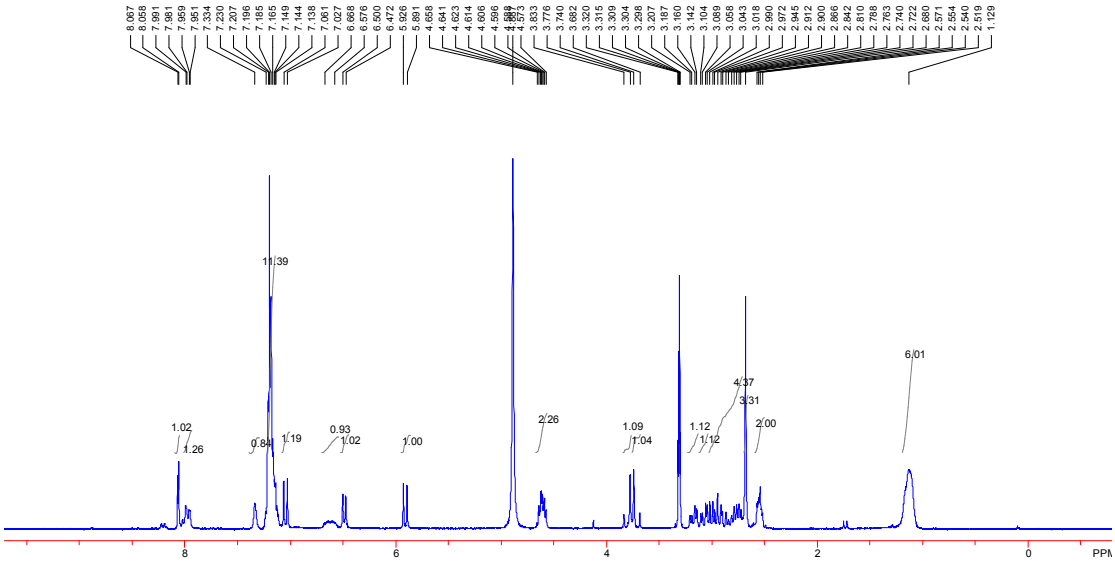
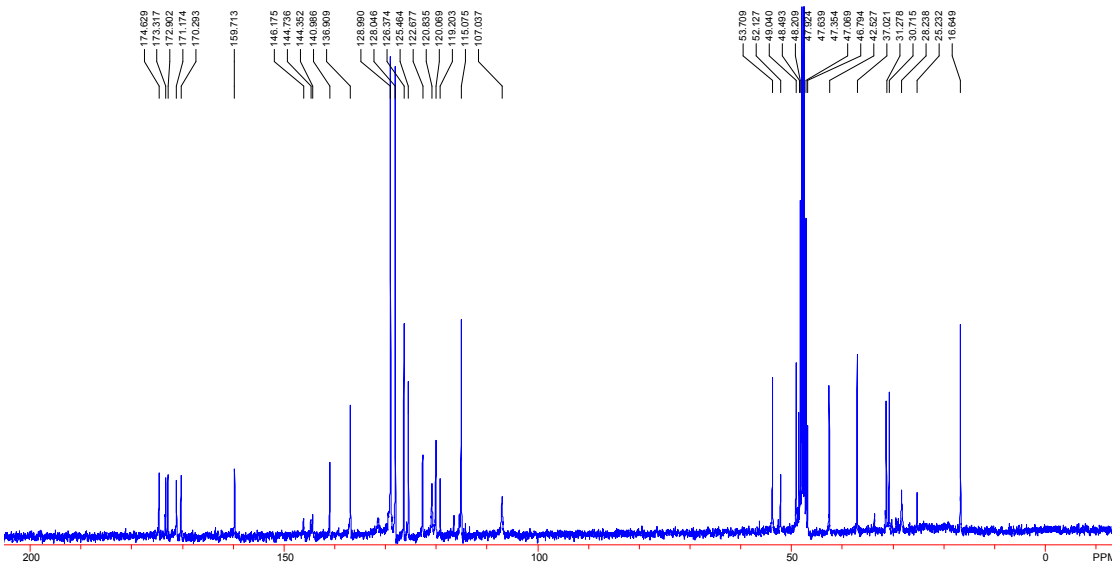
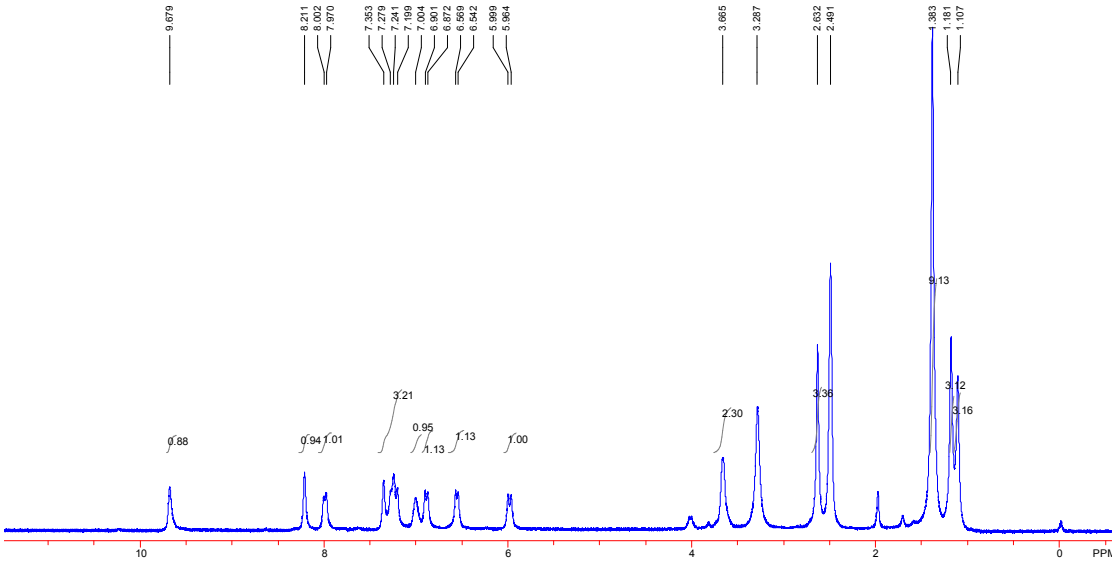



$^{13}$C NMR of compound **SP*I*-Phe-Phe**



$^{1}$H NMR of compound Boc-**SP*II***

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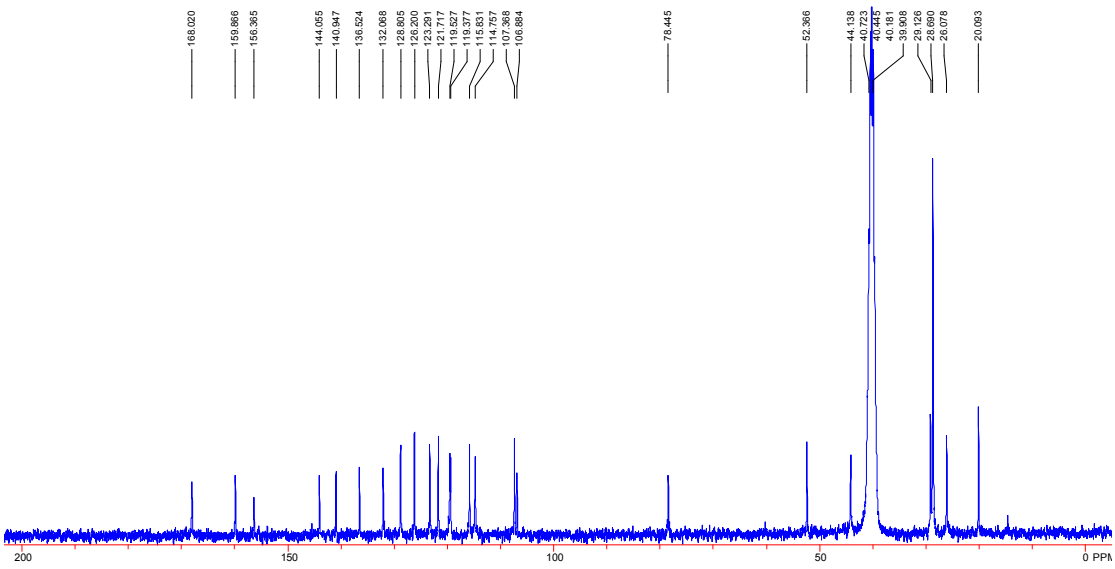
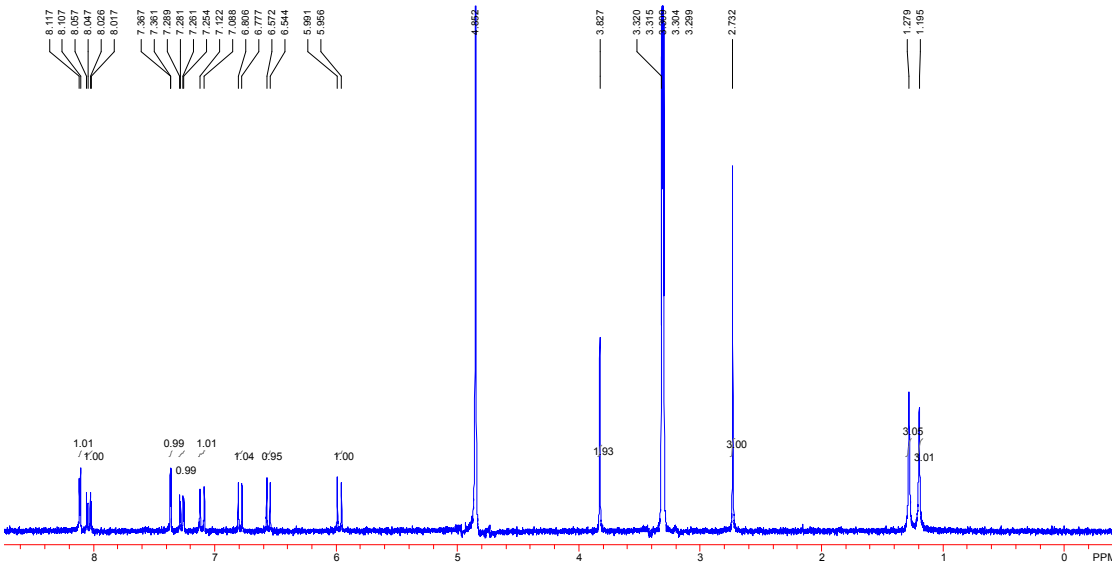
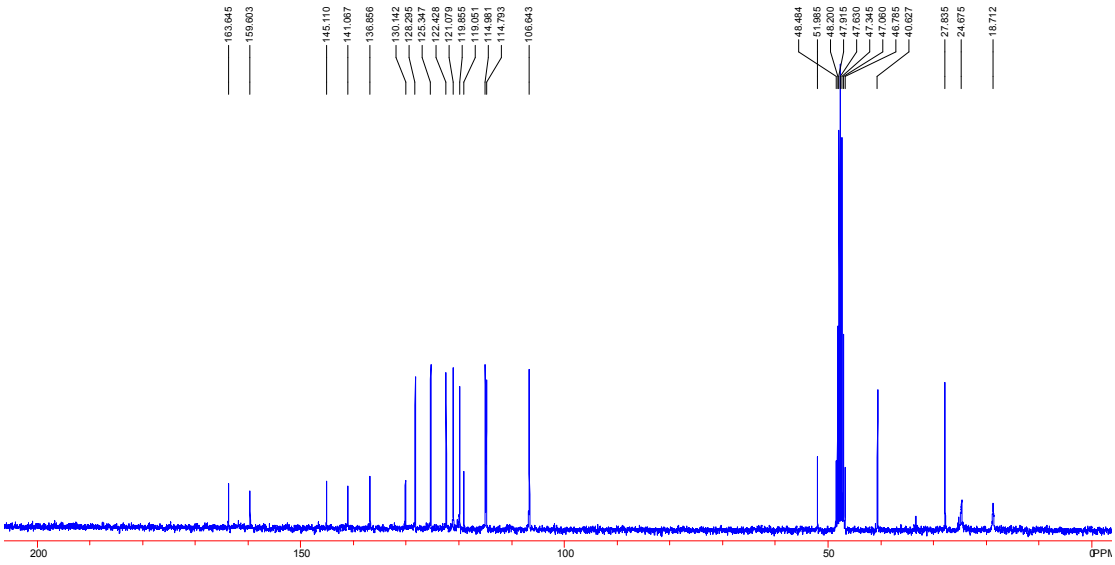


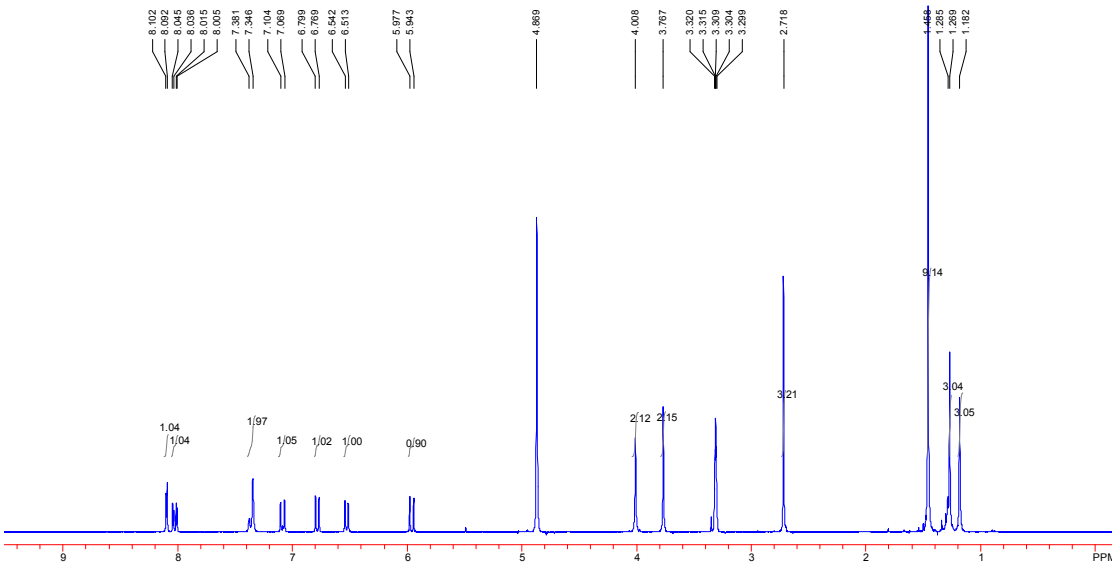
$^{1}$H NMR of compound **SP*II*•**TFA



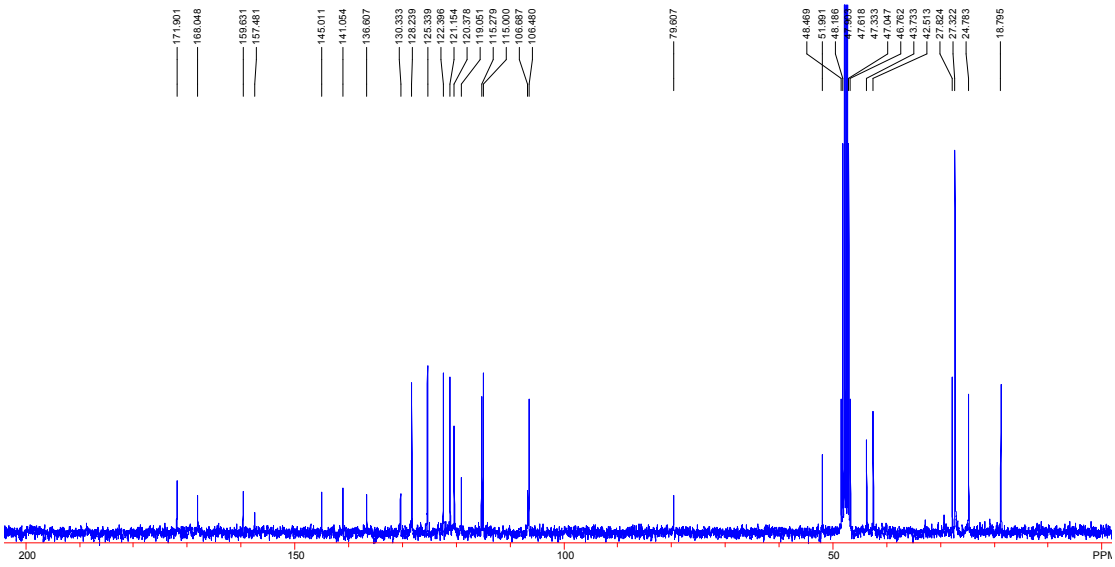

$^{13}$C NMR of compound **SP*II*•**TFA



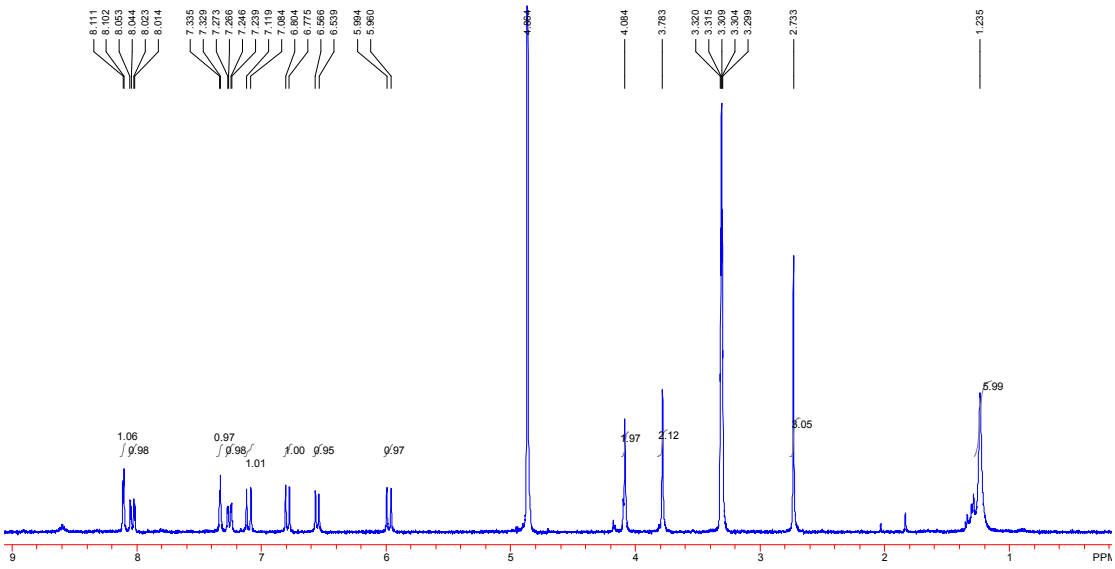
## $^{1}$H NMR of compound Boc-Gly-***SPII***




## $^{13}$C NMR of compound Boc-Gly-***SPII***



$^{1}$H NMR of compound Gly-**SP*II***•TFA

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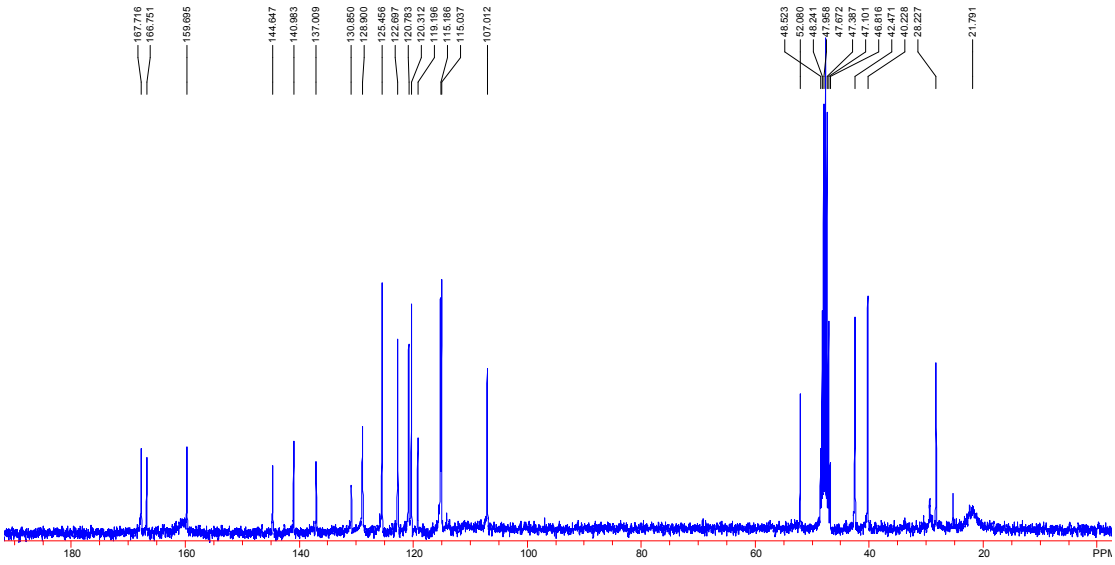
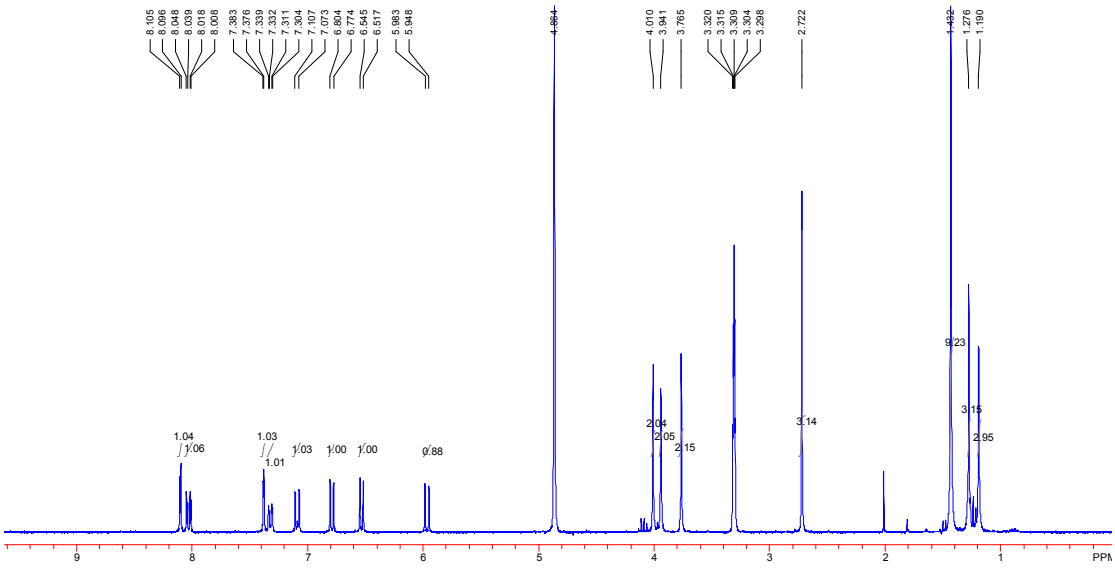


$^{1}$H NMR of compound Boc-Gly-Gly-***SPII***

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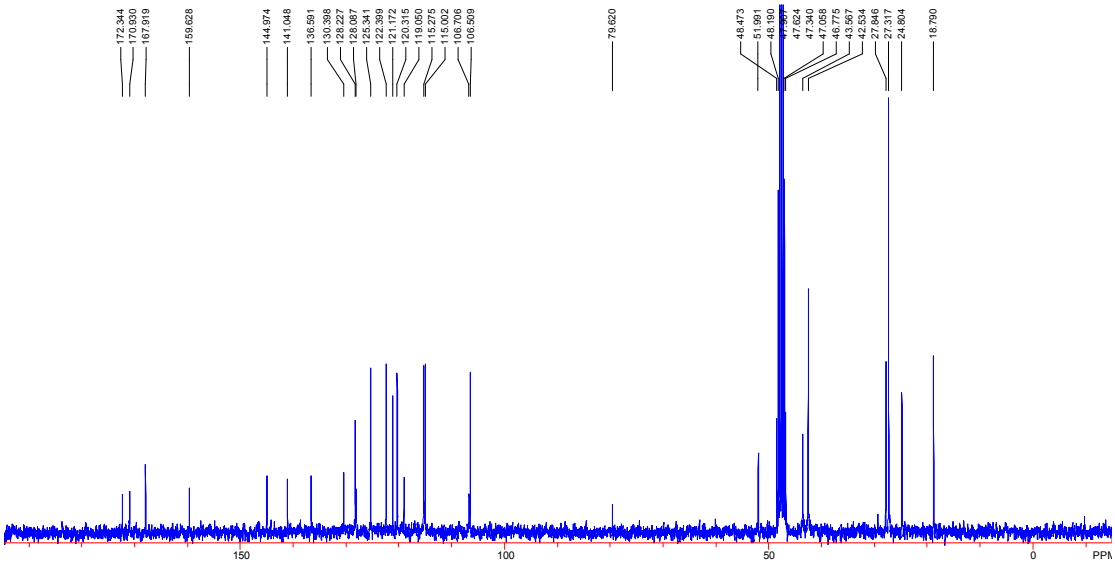
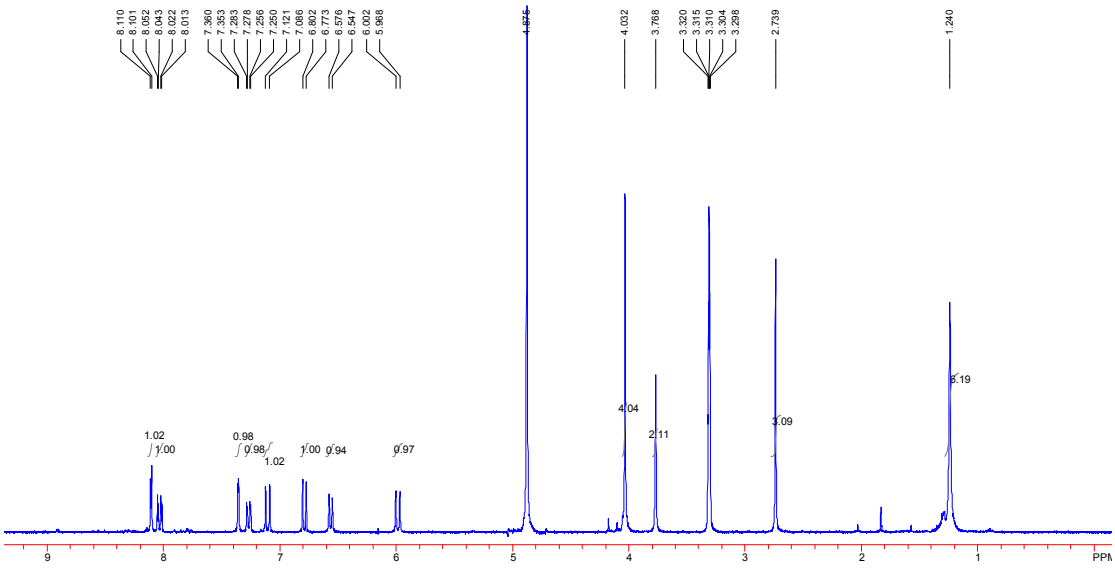
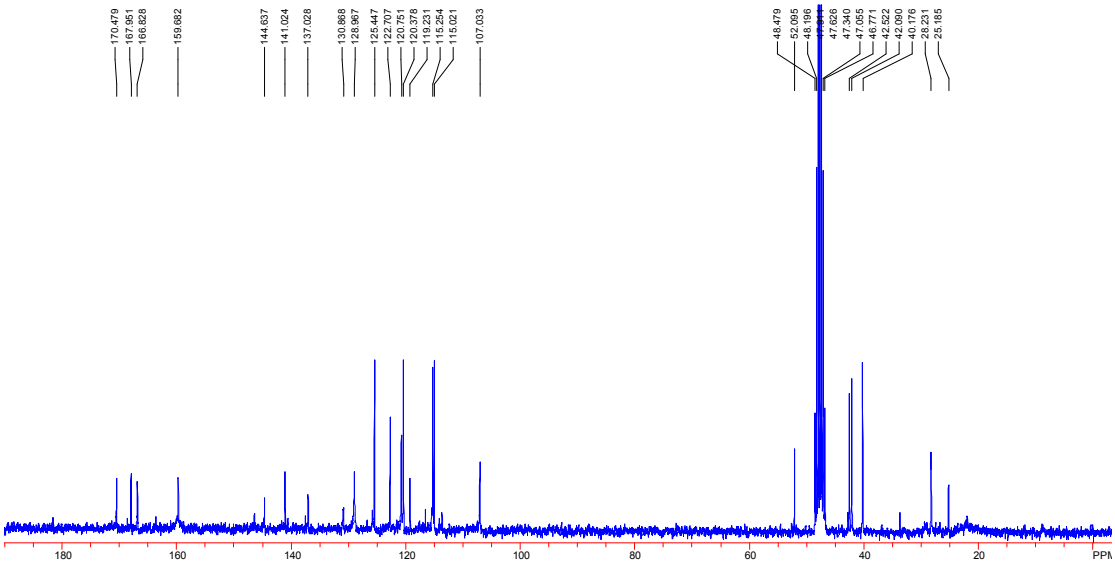


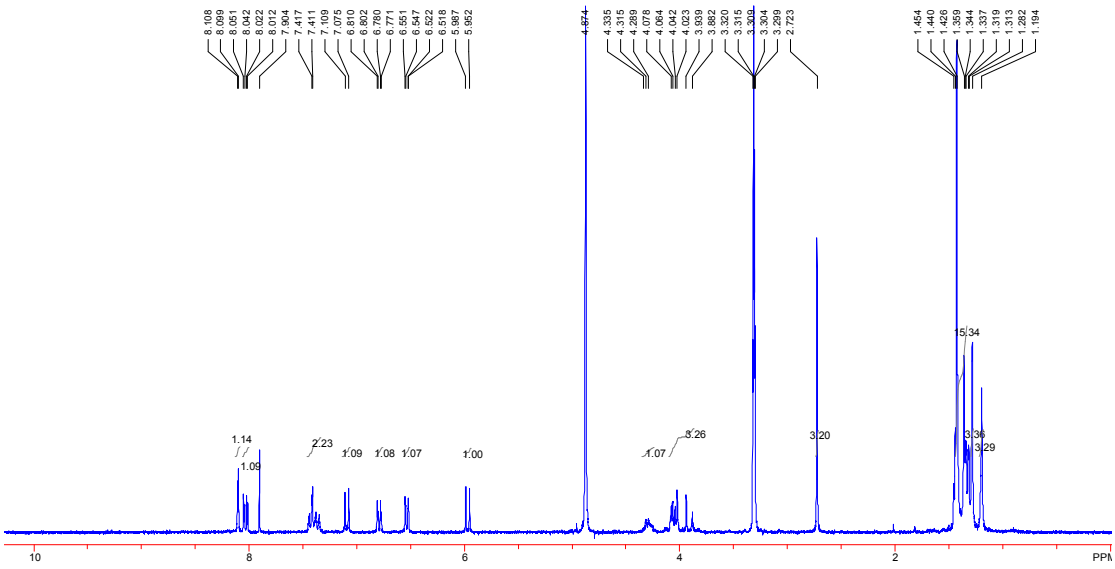
## $^{1}$H NMR of compound Gly-Gly-**SP*II*•**TFA




## $^{13}$C NMR of compound Gly-Gly-**SP*II*•**TFA



$^{1}$H NMR of compound Boc-Ala-Ala-***SPII***

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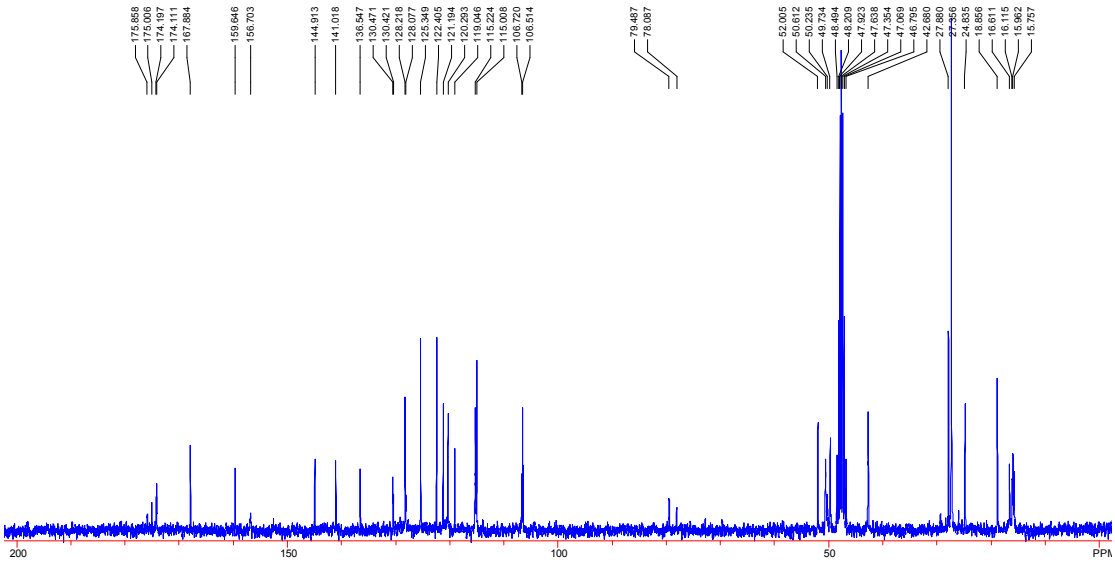

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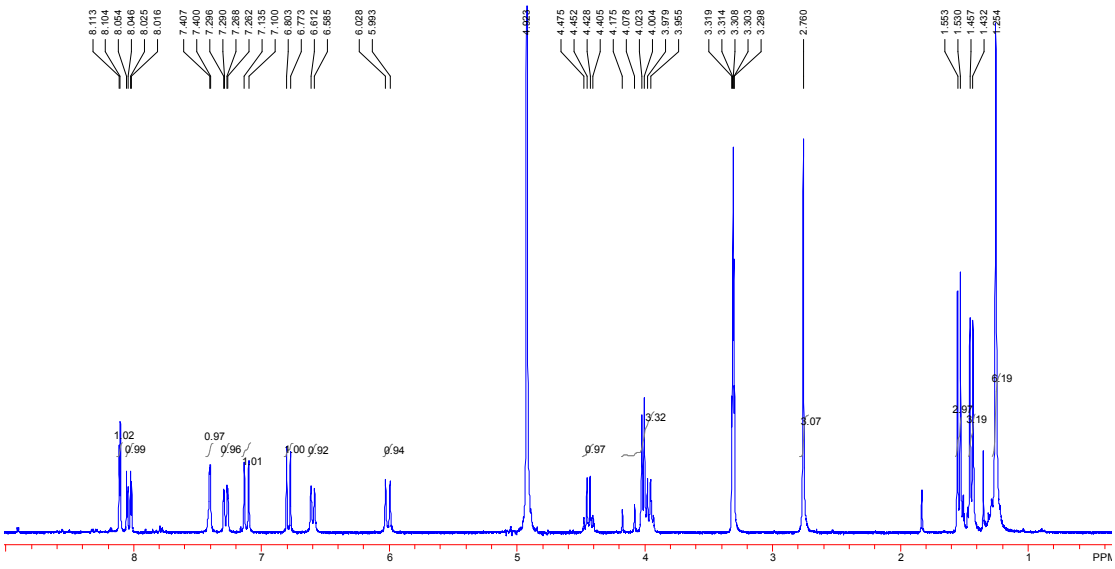


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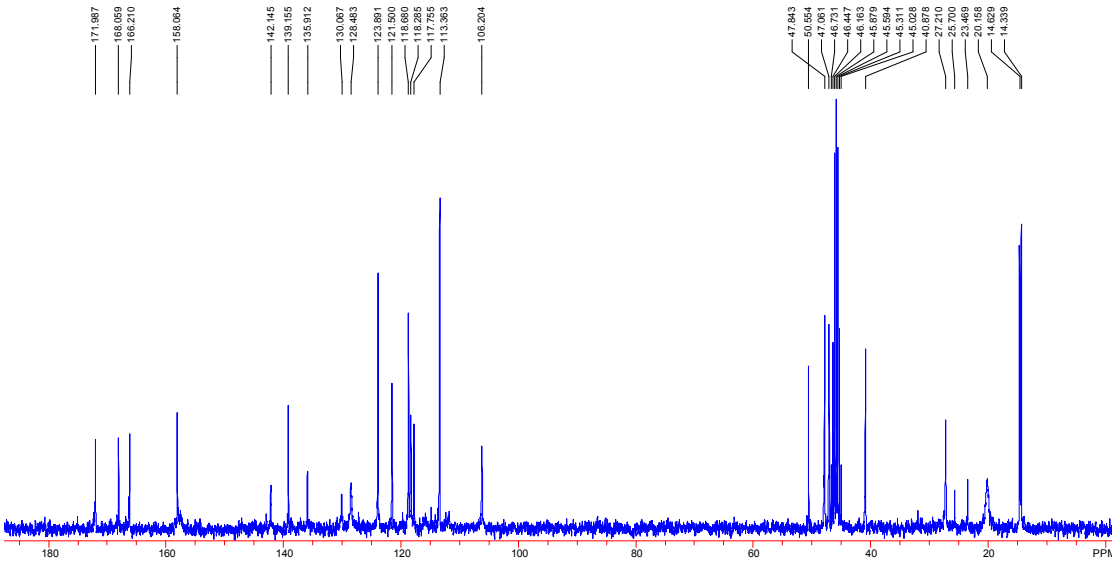
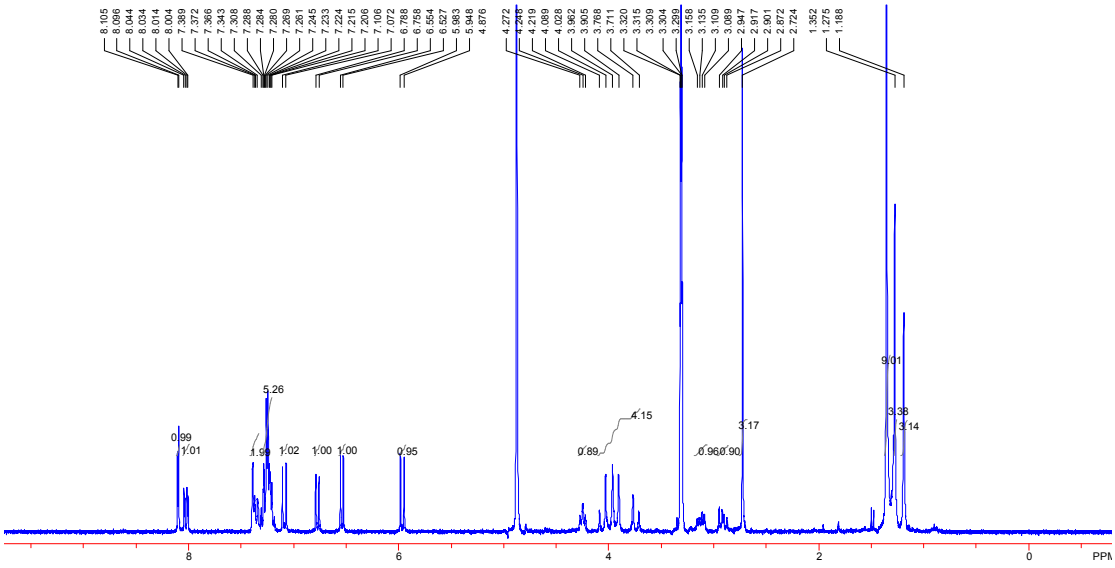
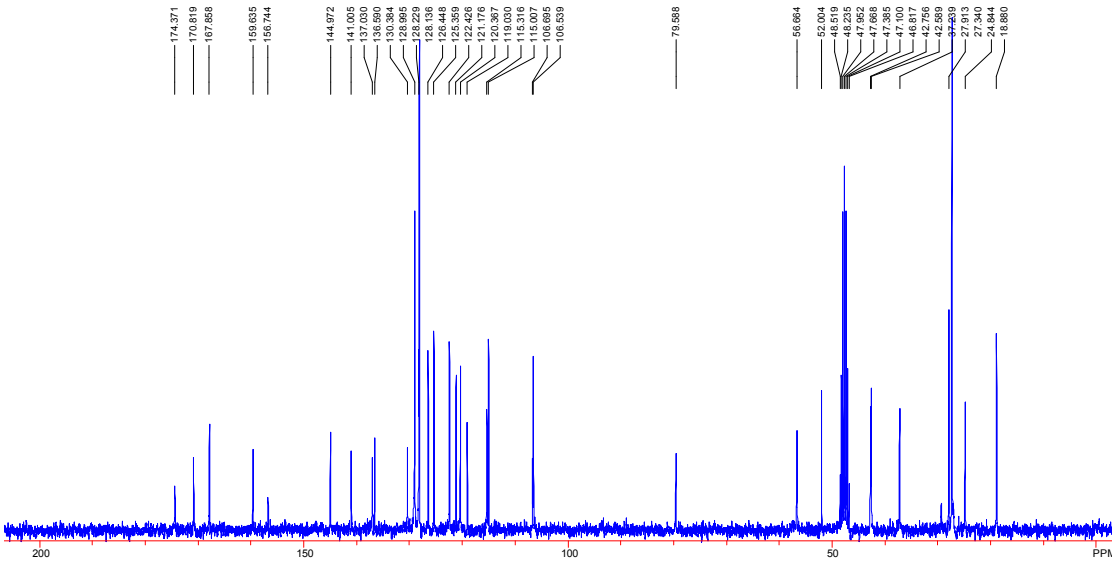


$^{1}$H NMR of compound Boc-Phe-Gly-***SPII***




$^{13}$C NMR of compound Boc-Phe-Gly-***SPII***



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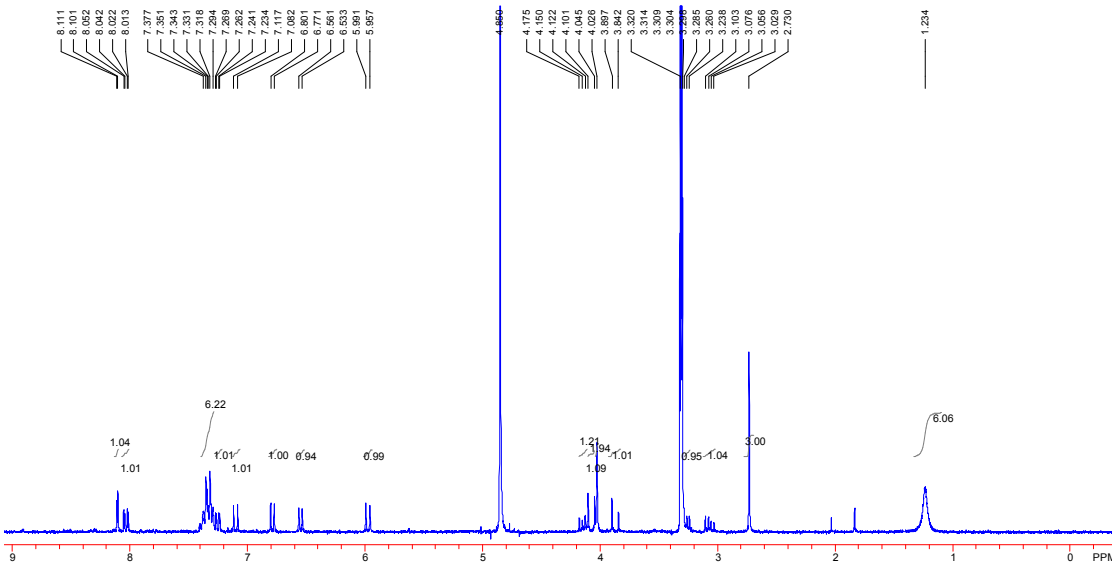

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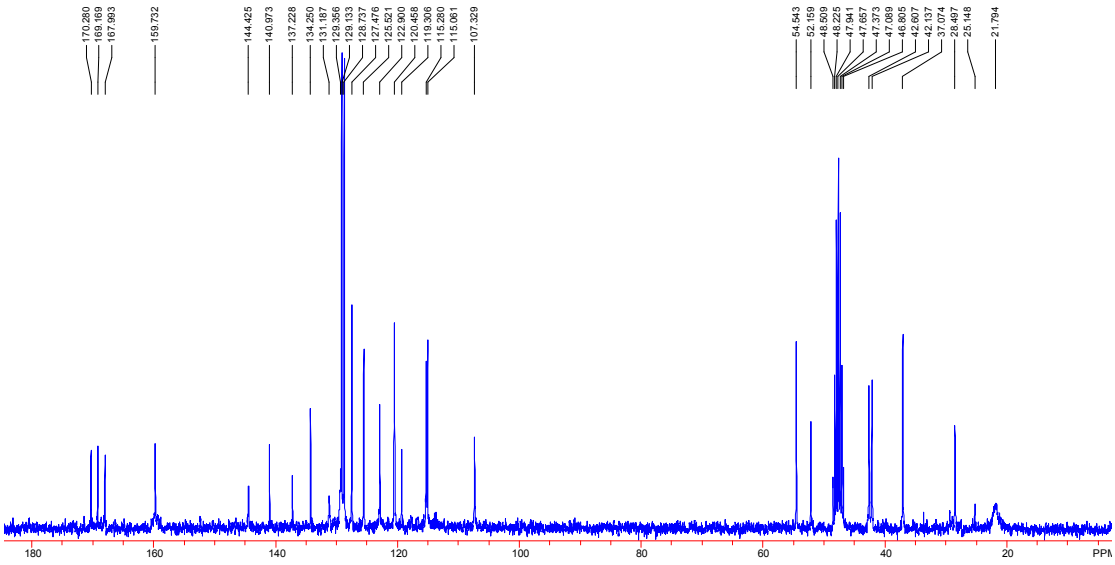
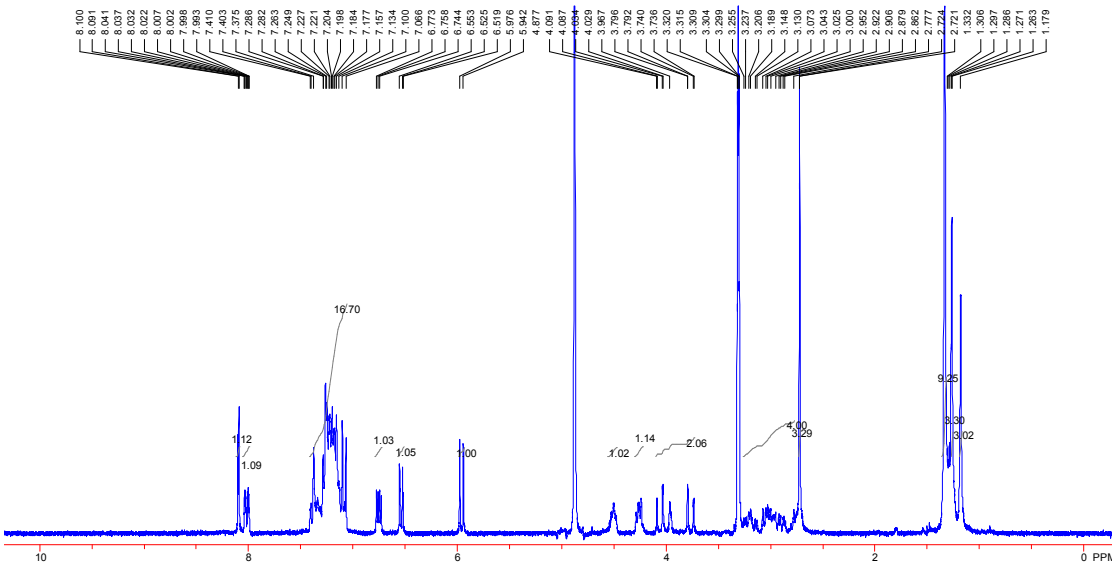
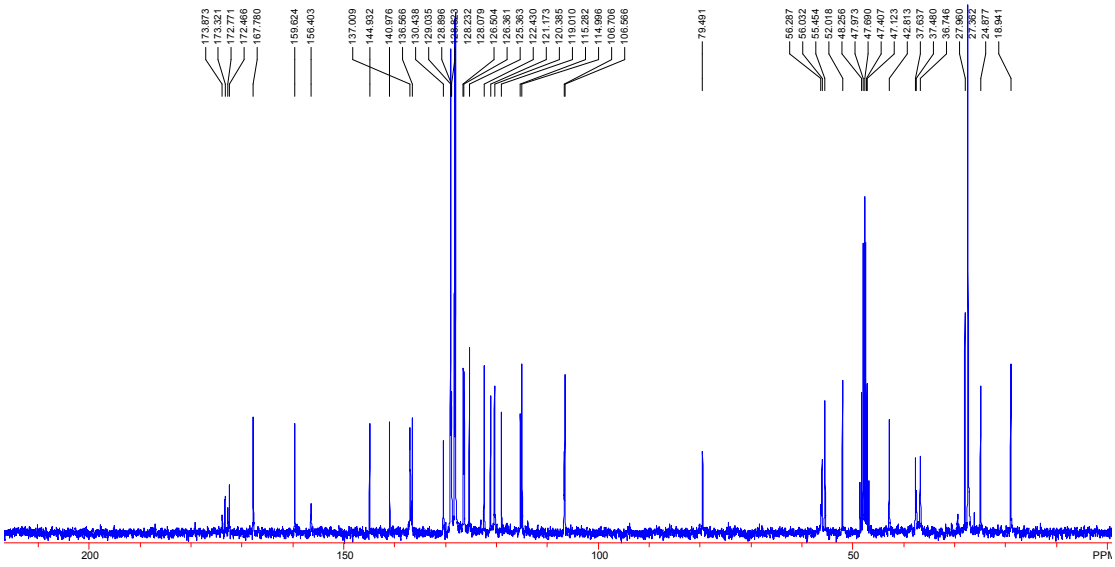


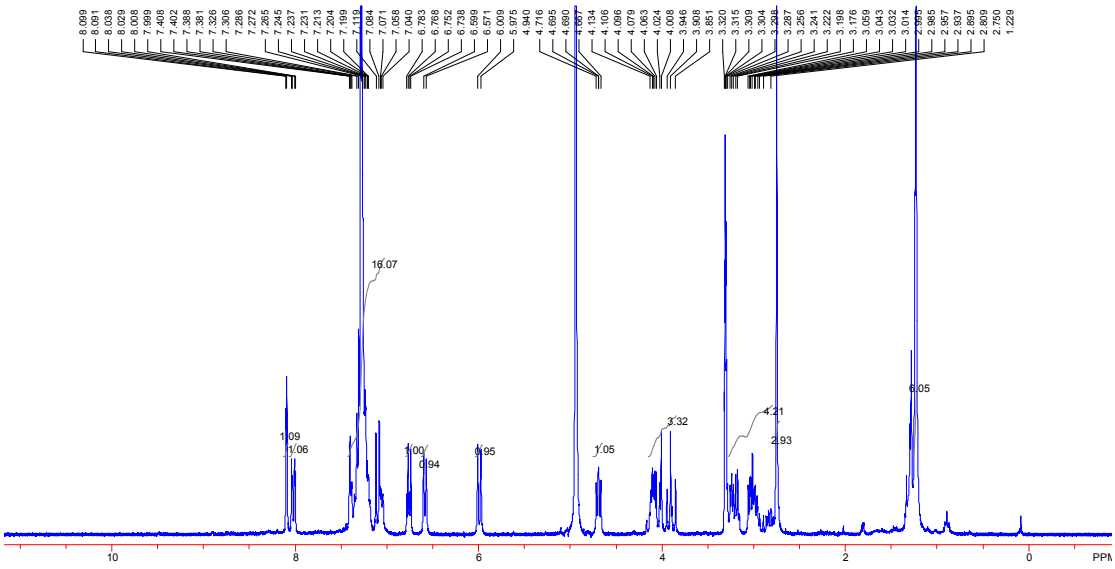
$^{1}$H NMR of compound Boc-Phe-Phe-***SPII***



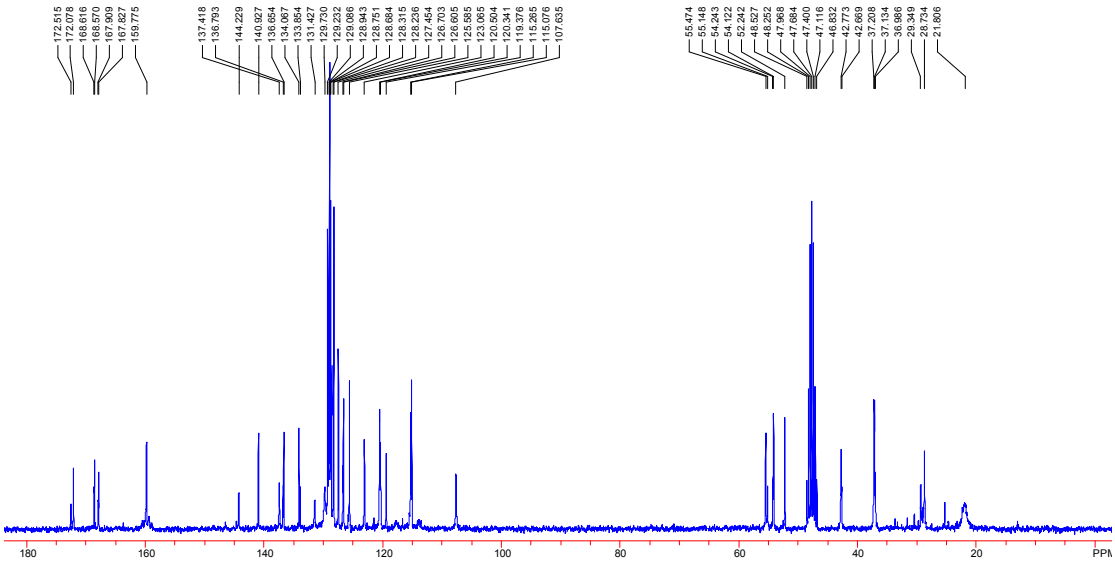

$^{13}$C NMR of compound Boc-Phe-Phe-***SPII***



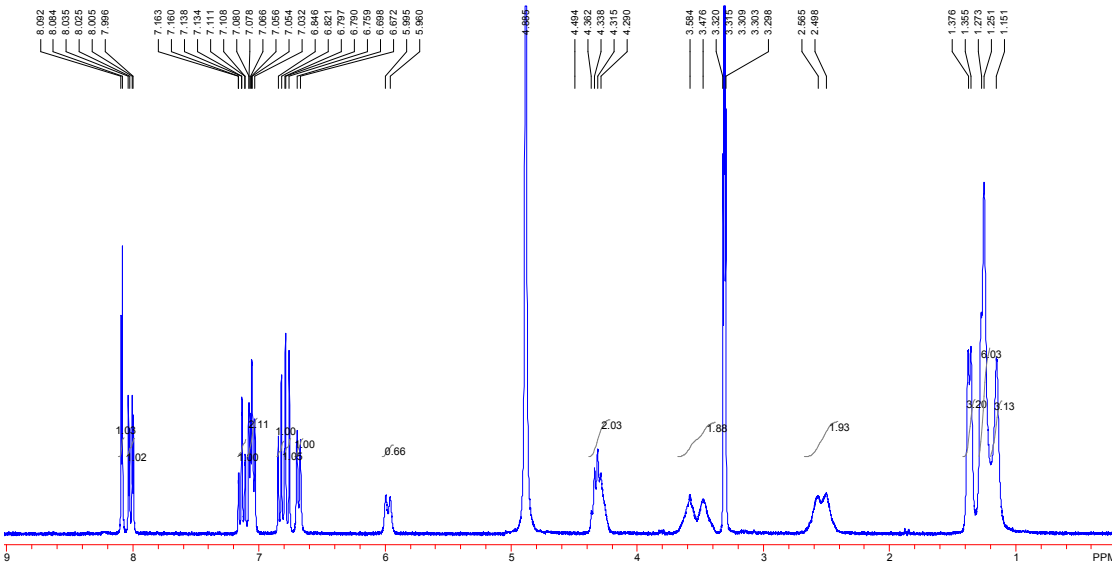
$^{1}$H NMR of compound Phe-Phe-**SP*II*•**TFA



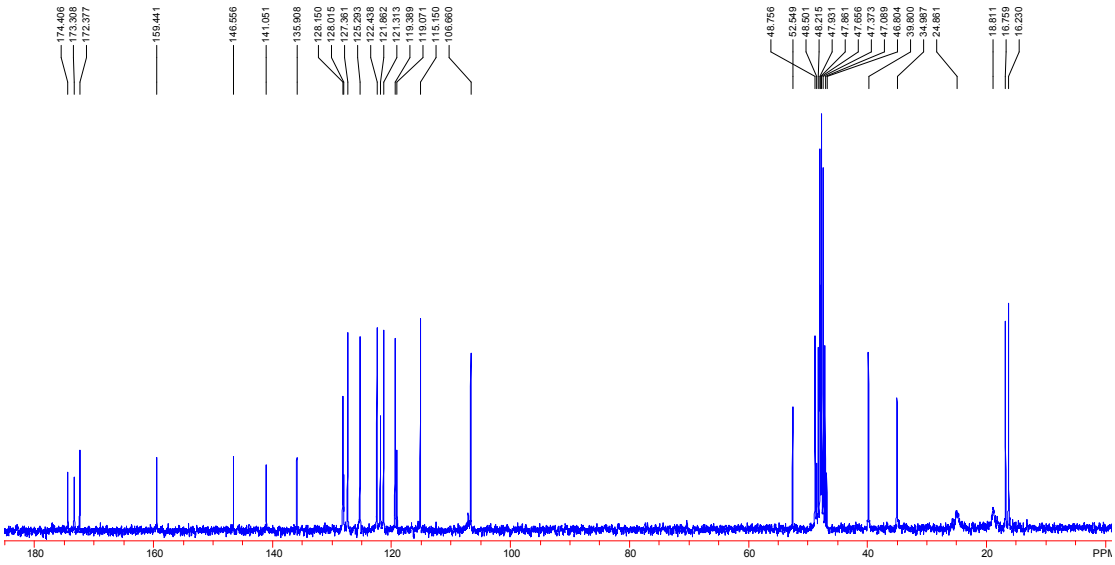

$^{13}$C NMR of compound Phe-Phe-**SP*II*•**TFA



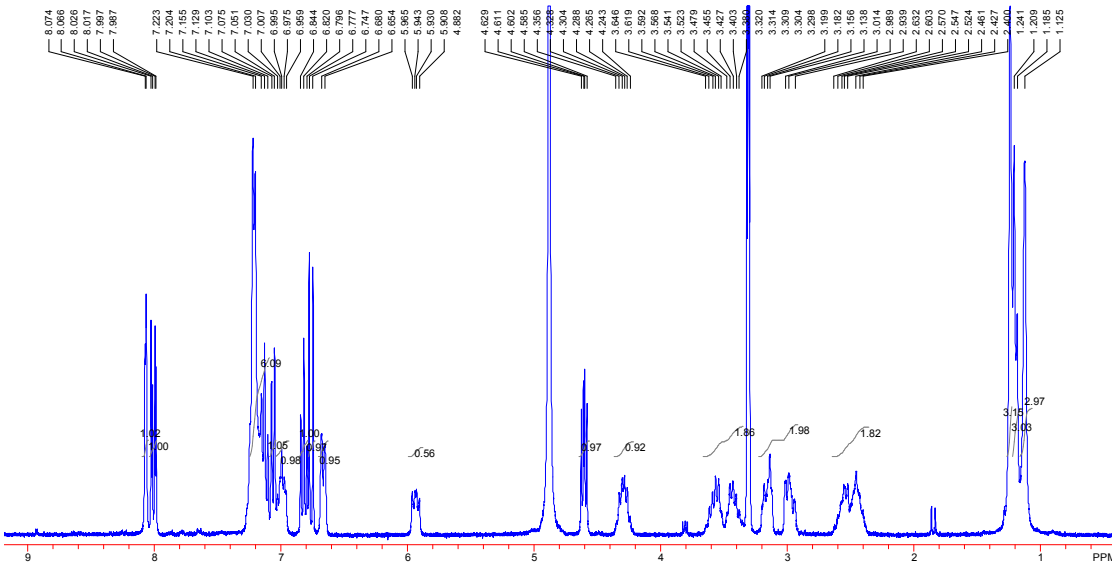
$^{1}$H NMR of compound **SP*III***-Ala-Ala



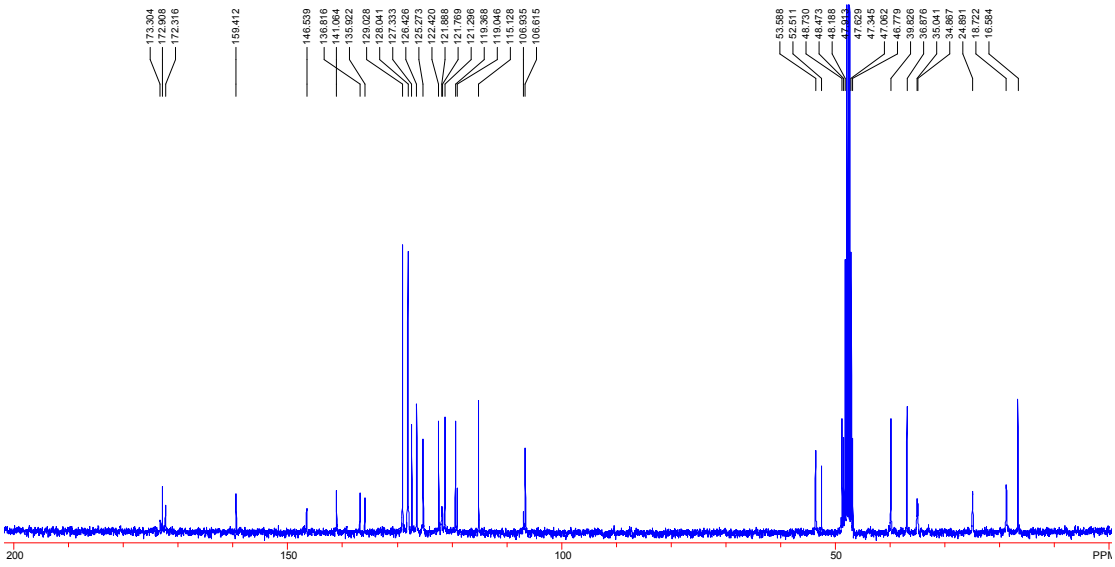

$^{13}$C NMR of compound **SP*III***-Ala-Ala



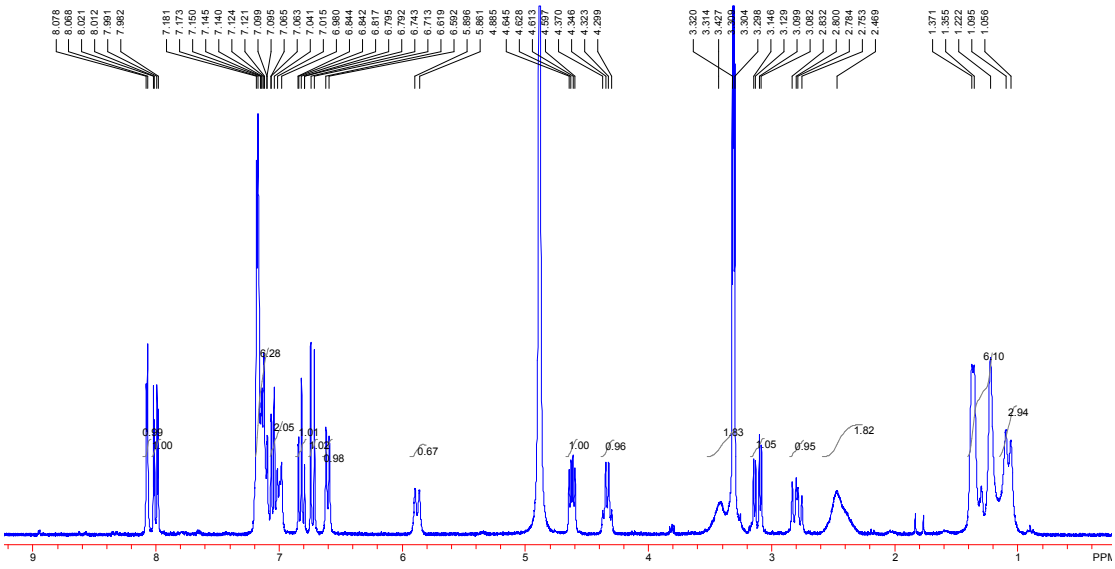
$^{1}$H NMR of compound **SP*III*-Ala-Phe**



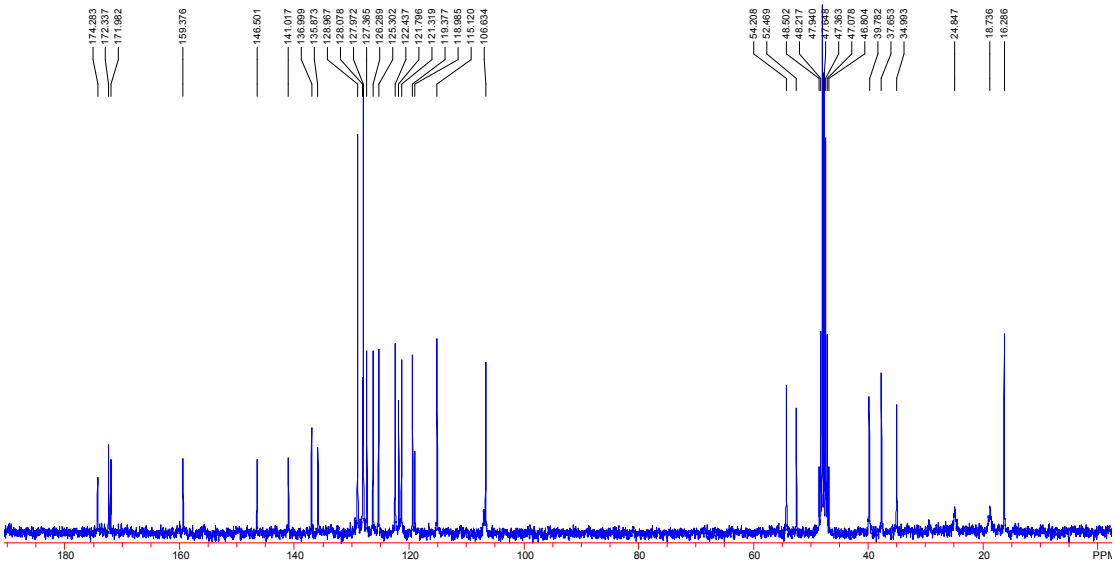
$^{13}$C NMR of compound **SP*III*-Ala-Phe**



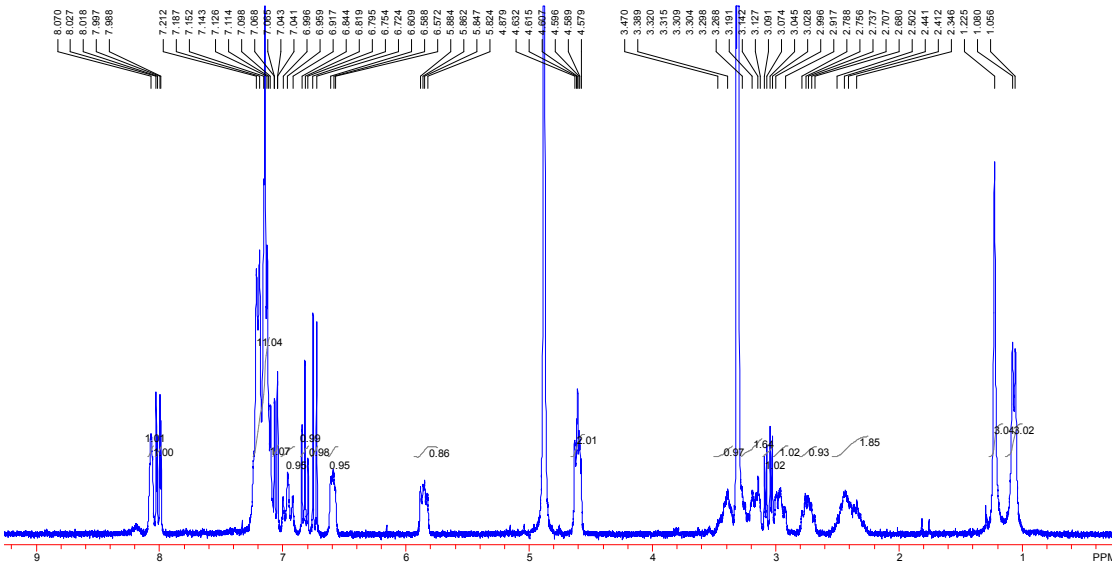
$^{1}$H NMR of compound **SP*III***-Phe-Ala



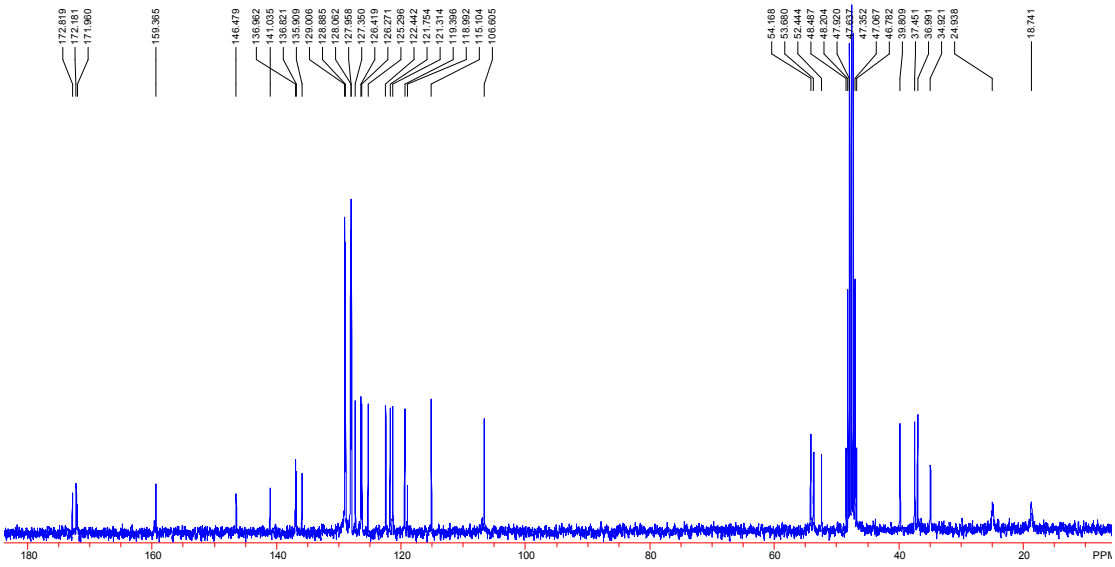
$^{13}$C NMR of compound **SP*III***-Phe-Ala



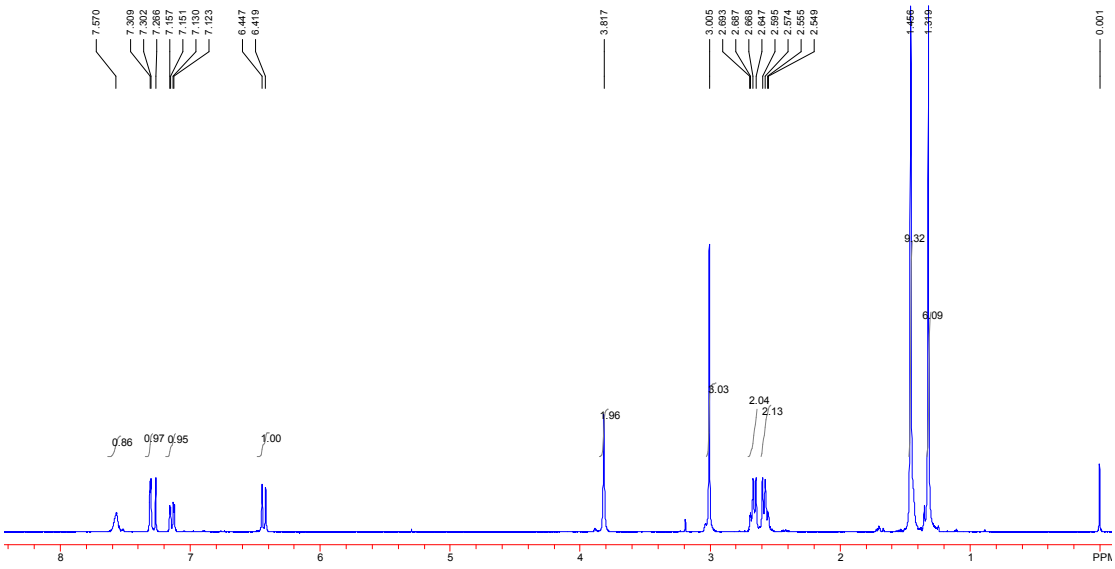
$^{1}$H NMR of compound **SP*III***-Phe-Phe



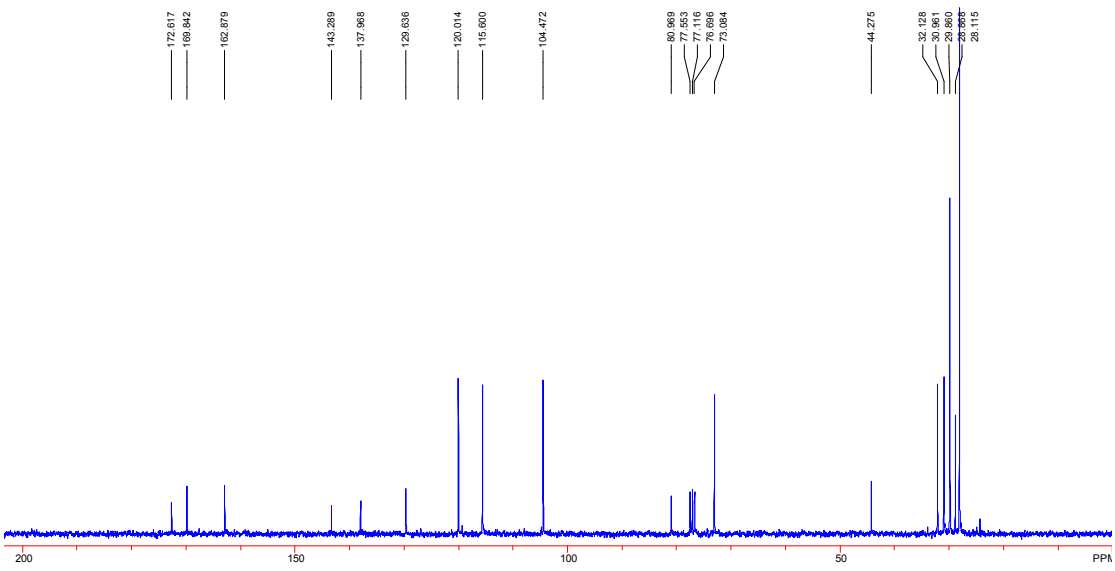
$^{13}$C NMR of compound **SP*III***-Phe-Phe



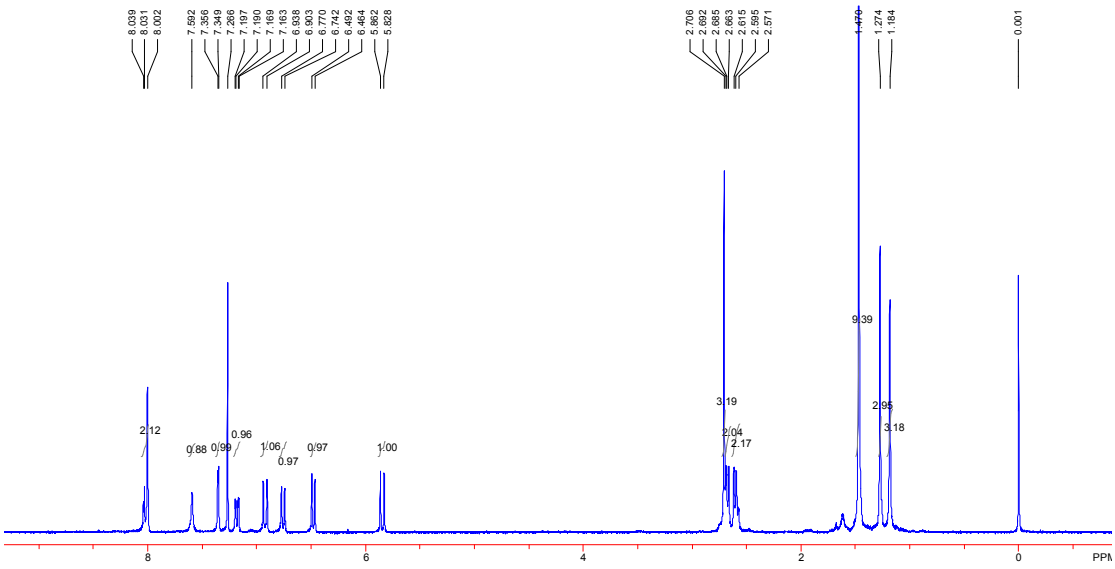
## $^{1}$H NMR of compound **6**



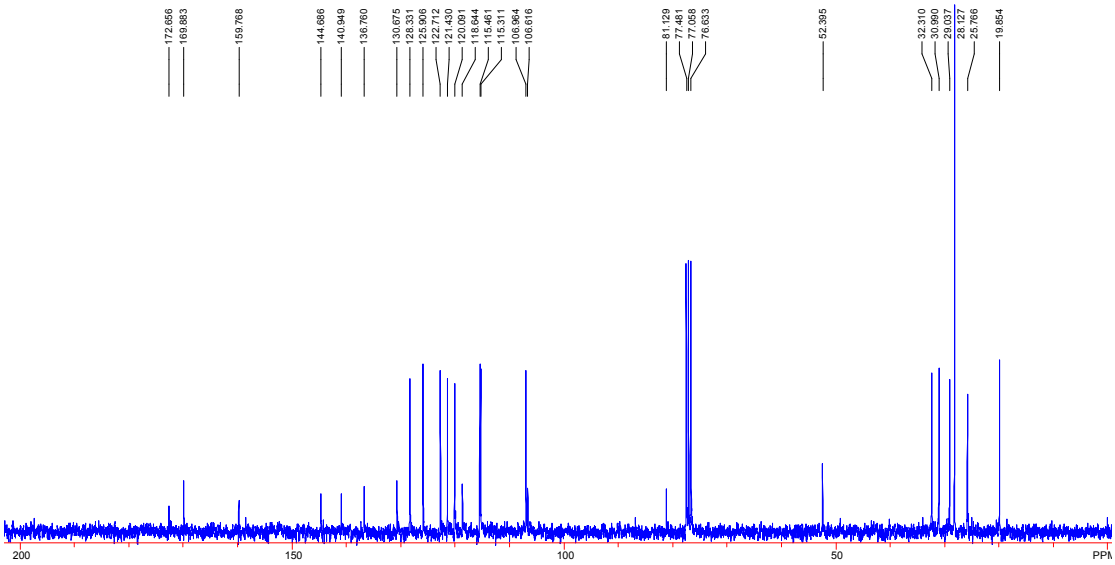

## $^{13}$C NMR of compound **6**



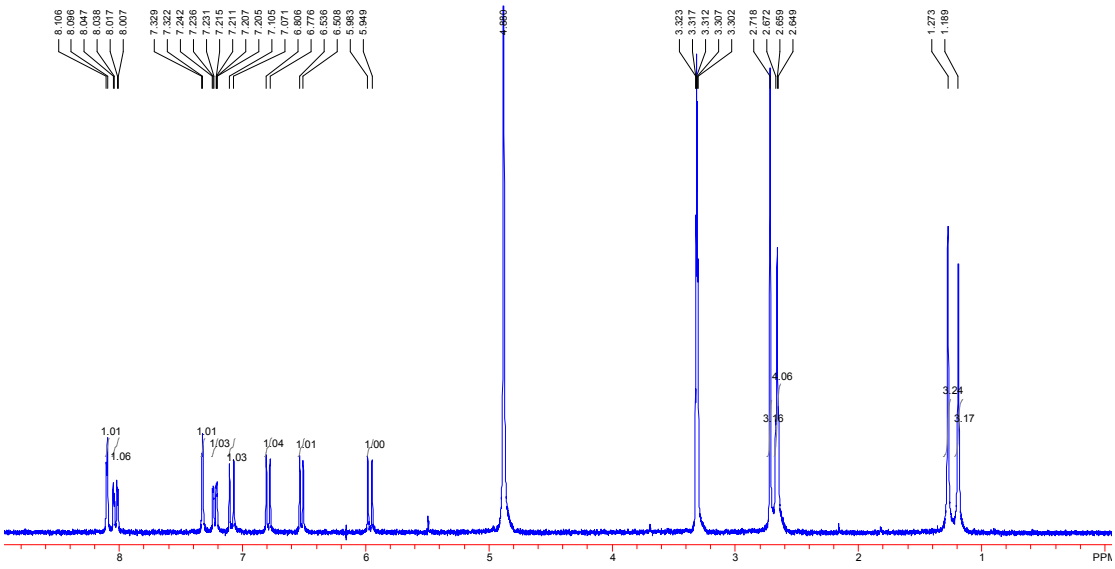
## $^{1}$H NMR of compound **7**




## $^{13}$C NMR of compound **7**



## $^{1}$H NMR of compound **SP*IV***




## $^{13}$C NMR of compound **SP*IV***