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Trichromophoric Pentapeptide: Impact of β-Sheet Conformation on Dual Path to Excimer Emission and Sensing of BSA†

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1. General Experimental Section (Materials and Methods)

All reactions except Boc-protection of primary amine were carried out under nitrogen atmosphere in flame-dried glassware, using a nitrogen filled balloon. Organic extracts were dried over anhydrous sodium sulfate. Solvents were removed in a rotary evaporator under reduced pressure. Silica gel (60- 120 mesh size) was used for the column chromatography. Reactions were monitored by TLC on silica gel 60 F254 (0.25). ^{1}H NMR spectra were recorded either at 400 MHz or at 600 MHz and ^{13}C NMR spectra were recorded either at 100 MHz or at 150 MHz (mentioned accordingly). Coupling constants (J value) were reported in hertz (Hz). The chemical shift were shown in ppm downfield form tetramethylsilane, using residual chloroform (δ = 7.26 in ^{1}H NMR, δ = 77.23 in ^{13}C NMR), DMSO (δ = 2.5 in ^{1}H NMR, δ = 39.5 in ^{13}C NMR), as an internal standard. Mass spectra were recorded with a HR mass spectrometer and data analysed by using built-in software. IR spectra were recorded in KBr on a FT-IR spectrometer.

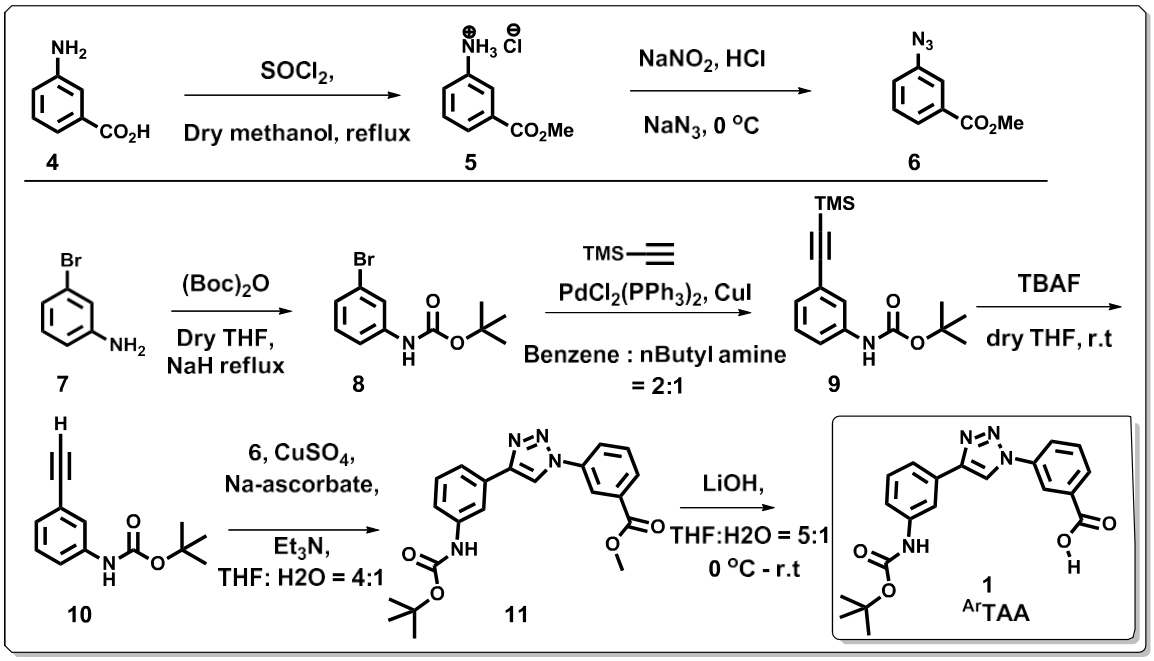
All 2D NMR Experiments were carried out on 600 MHz (Bruker Ascend^{TM} Aeon) spectrometer at room temperature using 7 - 10 mM concentration in d_6-DMSO solvent. Spectra were acquired with 2048 x 256 in both dimension (F2 and F1) and other parameter are given below. Parametre of

TOCSY : Free induction decay (FID) with NS = 16 and DS =32, relaxation delay (D1) 2s, mixing time (D9) 0.08s, acquisition time (AQ) 0.085s, spectral width 12019 Hz.

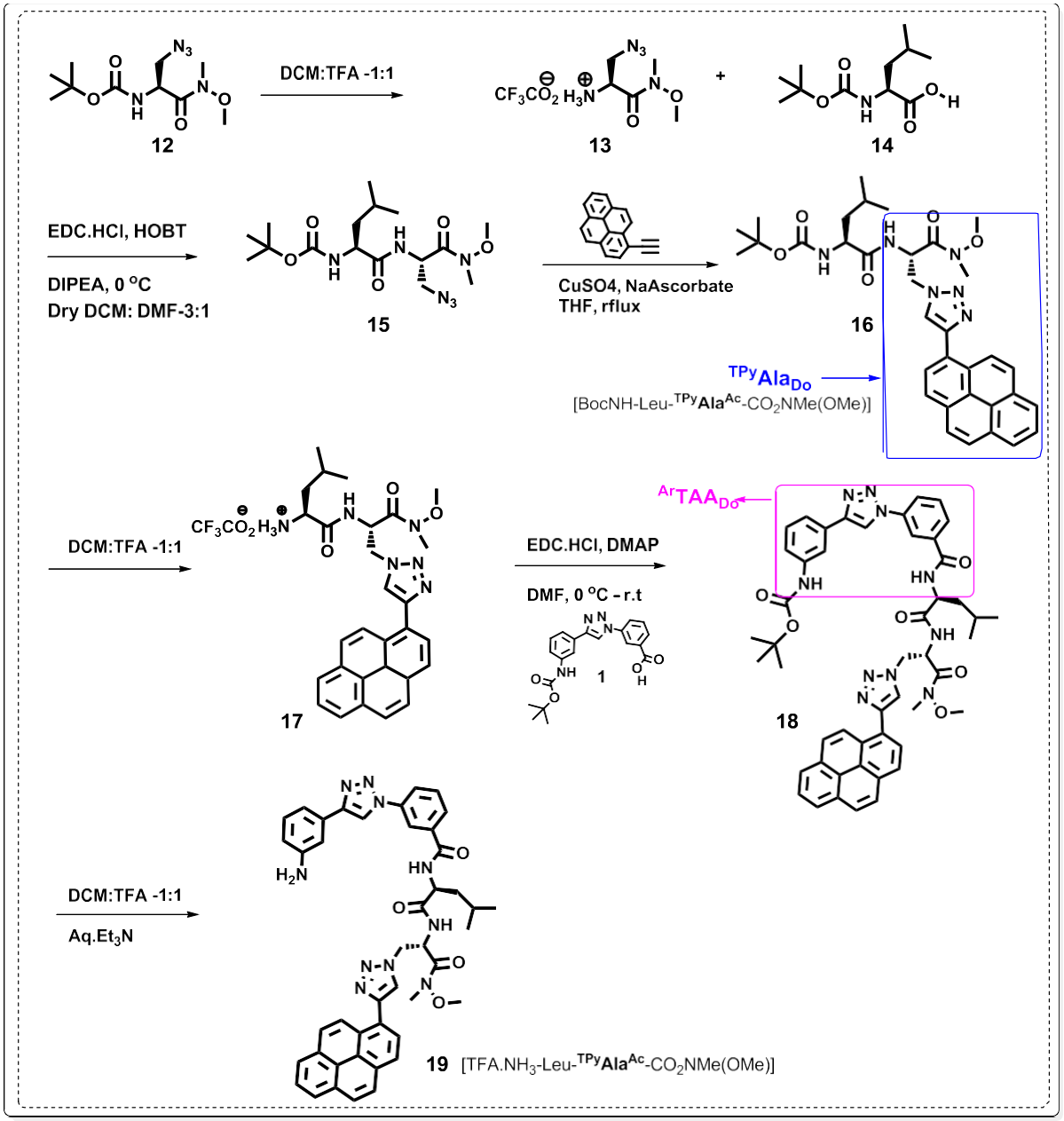
ROESY : Free induction decay (FID) with NS = 16 and DS =16, relaxation delay (D1) 2s, mixing time (P15) 0.02s, acquisition time (AQ) 0.085s, spectral width (SWH) 12019 Hz.

NOESY : Free induction decay (FID) with NS = 8 and DS =16, relaxation delay (D1) 2s, mixing time (D8) 0.6s, acquisition time (AQ) 0.085s, spectral width (SWH) 12019 Hz.

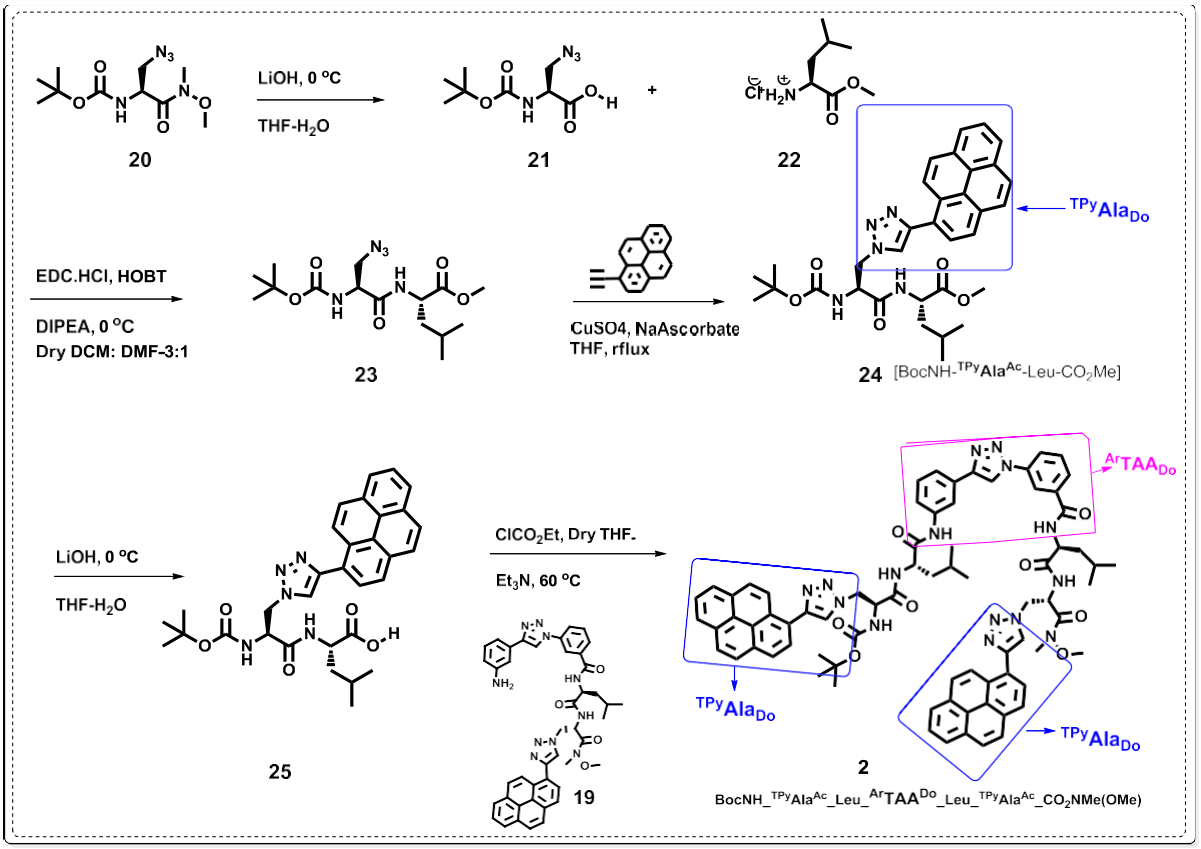
2. Synthetic Schemes



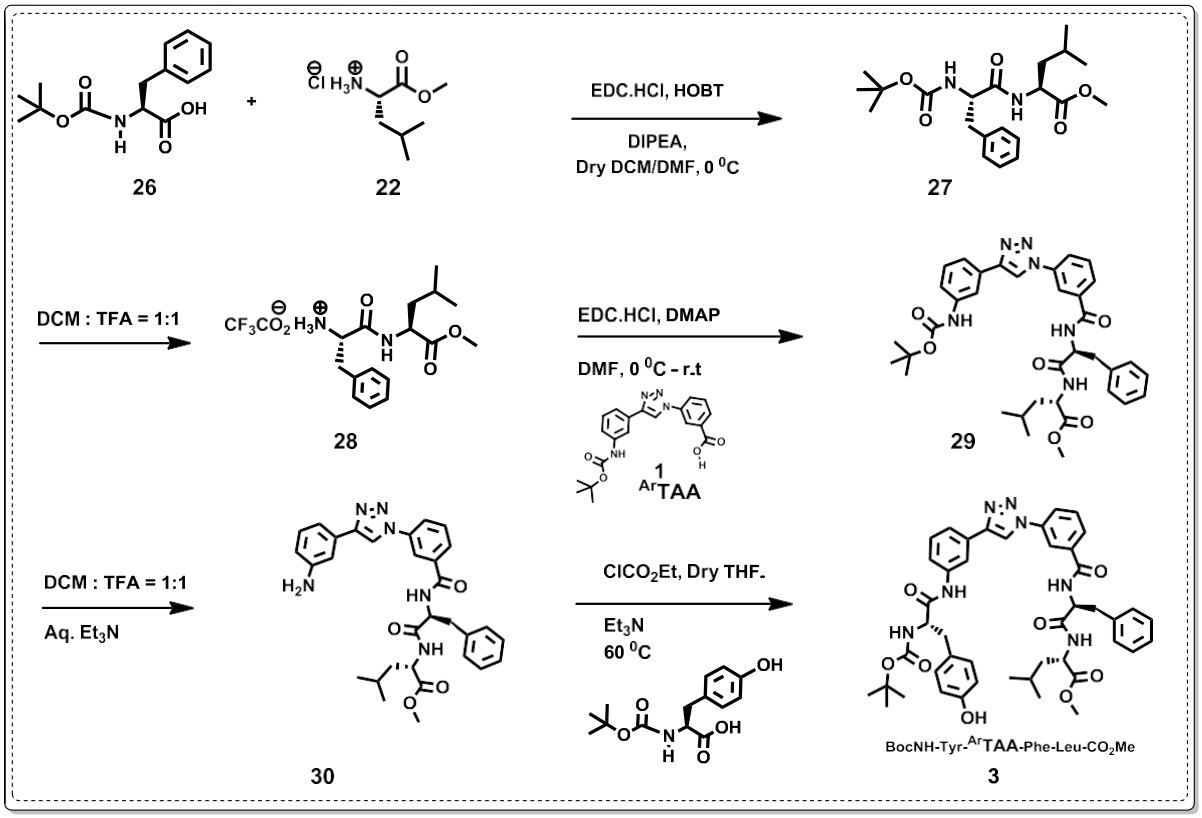
Scheme 1: Synthetic scheme for the triazolyl aromatic amino acid scaffold (**1**,^{Ar}**TAA**).



Scheme 2: Synthetic scheme of triazolyl aromatic amino acid scaffold based unnatural **tripeptide 18**. [BocNH- ^{Ar}**TAA$_{Do}$**_Leu-^{TPy}**Ala$_{Do}$**-CO_2NMe(OMe)].



Scheme 3: Synthetic scheme of aromatic triazolyl amino acid scaffold based unnatural **pentapeptide 2 [**BocNH-^{TPy}**Ala$_{Do}$**-Leu-^{Ar}**TAA$_{Do}$**-Leu-^{TPy}**Ala$_{Do}$**-CO_2NMe(OMe)**].**



Scheme 4: Synthetic scheme of triazolyl aromatic amino acid scaffold based Leu-enkephaline analouge natural **tetrapeptide 3 (**BocNH-Tyr-^{Ar}**TAA-**Phe-Leu-CO_2Me**).**

3. Synthetic Procedure and Characterization Data of Synthesized Peptides

3.1. Some General Procedure for Our Synthetic Scheme of Peptides

3.1.1. ***General procedure for the Peptide coupling*** **:** To a solution N-protected amino acids in 3:1 mixture of dry DCM and DMF, 1-[3-dimethyl amino propyl]-3-ehtylcarbo-diimide hydrochloride (EDC.HCl) (1.2 equiv) and HOBT (1.2 equiv) were added and the reaction mixture was stirred for 1h at 0 ^{0}C. Then the amine salt of wienreb amide or methyl ester protected corresponding amino acids or dipeptide (1.1 equiv) were added followed by diisopropylethylamine (DIPEA) (2.4 equiv). The reaction mixture was stirred for another 18-20 h at 0 ^{0}C to room temperature. Then solvent was dried by rotary evaporator, after which it was partitioned between EtOAc and aqueous $NaHCO_3$ solution (50 ml each). The organic layer was washed with brine solution. Pure product was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 1:1).

3.1.2. ***General procedure of [3+2] Cyclo-addition reaction*** **:** The azido derivative of dipeptide was taken in 5:1 dry THF and water and degassed for 5 min with nitrogen gas. After adding alkyne (1.1 equiv) degassing were continued for the next 5 min. Then, 6 mol % sodium ascorbate and 1 mol% powderd CuSO4 were added. Then 1.2eqv Et_3N was added and reaction mixture was degassed and allowed to proceed for 18-20 h about 65 to 70 ^{o}C. After total consumption of the starting azide, the reaction mixture was evaporated completely and work up was done by EtOAc and NH_4Cl solution. The organic layer was washed with brine, dried over Na_2SO_4.The title trizolyl unnatural dipeptides were separated by column chromatography and characterized.

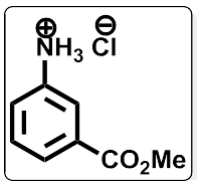
3.1.3. ***General procedure for the deprotection of the methyl ester*** **:** To a solution of the respective methyl ester protected peptide in THF : H_2O = 5 : 1, lithium hydroxide (1.5 equivalent) was added at 0 ^{0}C. The reaction mixture was stirred about 3-4 hour until starting material was consumed. Reaction was monitored by TLC. After completion of the reaction, solvent dried by rotary evaporator. Then water (4-5 ml) was added to the reaction mixture and cooled to 0 ^{o}C. The dilute acetic acid was added to the reaction mixture to adjust pH- 3 to 4. The reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO4. The hydrolysed compound was isolated by column chromatography (Si-gel, $CHCl_3$:MeOH = 10:1). Yield was 90-96%.

3.1.4. ***General procedure for the deprotection of the Boc-group :*** The respective both side protected amino acids or peptides was dissolved in CH_2Cl_2 and cooled to 0 ^{0}C. TFA (equal amount as the solvent) was added and the solution was allowed to warm to room temperature. The stirring was continue at room temperature until the starting material was consumed (TLC monitoring). The reaction mixture was evaporated *in vacuo.* The residual TFA was evaporated by triturating the mixture with dry toluene thrice, evaporated thrice and dried to afford the product in quantitative yield.

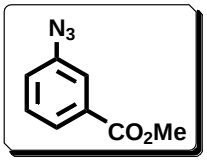
But in some cases to get free amine, water (4-5 ml) was added to the reaction mixture after evaporation of reaction solvent and cooled at 0 ^{0}C temperature. Then diluted aq.Et_3N was added to the reaction mixture to adjust pH- 8. The reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo* to yield the crude product in quantitative yield to use for next step.

3.2. Synthetic route of aromatic triazolyl amino acid scaffold (1,^{Ar}TAA)

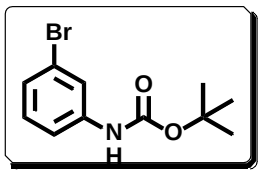
3.2.1. ***Synthesis of 3-(methoxycarbonyl) benzenaminium chloride*** **(5):** *m*-Amino benzoic acid **4** (1500 mg) was taken in a dried RB then it is dissolved by adding dry methanol. Under ice cold condition 1.5 equivalent $SOCl_2$ was added into the reaction mixture. After 15 minute reaction mixture was refluxed for 5-6 hours. After that reaction solvent was dried in rotary evaporator and washed the reaction mixture with toluene 3 times. The title compound **5** was isolated in quantitative yield.



3.2.2. ***Synthesis of methyl 3-azidobenzoate*** **(6) :** Starting material **6** (1500 mg) was taken in a conical then it dissolved by adding water and it was acidified with dil. HCl under ice cool condition. Then drop by drop 1.5 equivalent $NaNO_2$ solution was added to the reaction mixture. Consequently 1.5 eqv. NaN_3 solution was added slowly to the reaction mixture. After few minute to complete the reaction it was work up by ethyl acetate. The combined organic layer was washed by brine solution dried over Na_2SO_4. The title compound **6** was isolated by column chromatography (si-gel, PE) in pure form as colourless oil (1079 mg, Yield 76 %). IR (KBr) 2953, 2844, **2115**, 1727, 1585, 1484, 1443, 1300, 1140, 752 cm^{-1}.

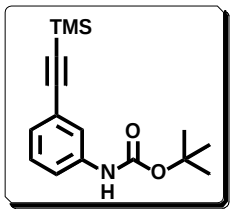


3.2.3.*Synthesis of tert-butyl (3-bromophenyl)carbamate* (8) : In a dry THF, NaH (1.1 eqv) (washed by hexane) and 3-bromo aniline **7** (1 ml, 8.7 mmol) were taken. The reaction



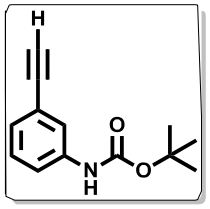
mixture was heated to reflux for one hour then cooled to room temperature. Boc anhydride (1.5 ml, 10.4 mmol) was added and reaction mixture was stirred for 30 minute. A second portion of sodium hydride (same eqv. amount again) was added to the reaction mixture and refluxed overnight. The reaction mixture was cooled to room temperature, carefully quenched by water. The reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4. The Pure product **8** was isolated by column chromatography (Si-gel, PE : EtOAc = 10:1). (2070 mg, 7.6 mmol, Yield 88 %).

3.2.4. *Synthesis of tert-butyl (3-((trimethylsilyl)ethynyl)phenyl)carbamate* (9) : In a dry R.B, compound **8** (800 mg, 2.95 mmol) was taken with dry solvent benzene : n-butyl



amine = 2:1 then catalyst $PdCl_2(PPh_3)_2$ (103.6 mg, 0.147 mmol) was added and degassed by N_2 gas. After fifteen minute of stirring the reaction mixture, TMS acetylene (600 µl, 4.42 mmol) and CuI (11.2 mg, 0.06 mmol) was added and heated to 80 ^{0}C temperature for 12 hour. Then solvent was dried by rotary evaporator, then it was partitioned between EtOAc and aqueous NH_4Cl solution (20 ml each). The organic layer was washed with brine solution. Pure product **9** (665 mg, 2.3 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 10:1). Yield 78 %. ^{1}H NMR ($CDCl_3$; 400 MHz) δ 0.15 (9H, s); 1.42 (9H, s); 6.52 (1H, bs); 7.04 (1H, d, J = 6.8 Hz); 7.1 (1H, t, J = 8.2 Hz); 7.17 (1H, d, J = 8.4 Hz); 7.48 (1H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 0.1, 28.4, 80.8, 94.3, 104.9, 118.8, 121.8, 126.7, 128.9, 130.3, 138.4, 152.7.

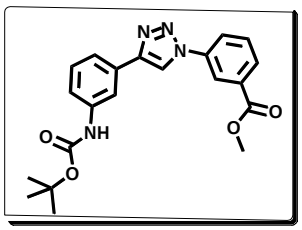
3.2.5. *Synthesis of tert-butyl (3-ethynylphenyl)carbamate* (10) : Compound **9,** 650 mg



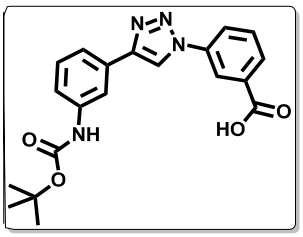
(2.24 mmol) was dissolved in 8 ml THF with 1 ml methanol, 314 mg (5.6 mmol) KOH was added to the solution at room temperature and stirrer overnight. Then solvent was dried by rotary evaporator, then it was partitioned between EtOAc and water. The organic layer was washed with brine solution. Pure product **10** (468 mg, 2.15 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 10:1). Yield 96 %. ^{1}H NMR ($CDCl_3$; 400 MHz) δ 1.5 (9H, s); 3.04 (1H, s); 6.63 (1H, s); 7.14 (1H, d, J = 7.6 Hz); 7.19 (1H, t, J = 8 Hz); 7.43 (1H, d, J = 8 Hz); 7.53 (1H,

s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 28.4, 77.3, 80.9, 83.5, 119.2, 122.1, 122.8, 126.8, 129.1, 138.5, 152.7.

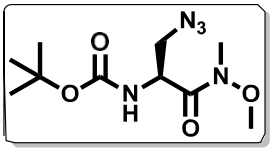
3.2.6. ***Synthesis of methyl 3-(4-(3-((tert-butoxycarbonyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)benzoate*** **(11) :** The ethynyl derivative **10**, 450 mg (2.06 mmol) was taken in a 5:1 dry THF and water system and degassed for 5 min with nitrogen gas. Methyl 3-azidobenzoate **6**, 403 mg (2.3 mmol), 6 mol % sodium ascorbate and 1 mol% powderd CuSO4 were added followed by. Then 1.2 eqv Et_3N was added and reaction mixture was degassed and refluxed for 18-20 hour. After finishing starting material, the reaction mixture was evaporated completely and it was partitioned by EtOAc and NH_4Cl solution. The organic layer was washed with brine, dried over Na_2SO_4. Pure product **11** (670 mg, 1.7 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 2:1). Yield 82 %. IR (KBr) 3328, 3137, 2965, 2919, 1718, 1593, 1448, 1289, 1151, 762 cm^{-1}. ^{1}H NMR (d_6-DMSO; 600 MHz) δ 1.48 (9H, s); 3.91 (3H, s); 7.38-7.34 (2H, m); 7.52 (1H, d, J = 6.6 Hz); 7.78 (1H, t, J = 7.8 Hz); 8.06 (1H, d, J = 7.8 Hz); 8.17 (1H, s), 8.24 (1H, d, J = 7.8 Hz); 8.49 (1H, s); 9.33 (1H, s); 9.45 (1H, s); ^{13}C NMR (d_6-DMSO; 150 MHz) δ 28.5, 52.9, 79.7, 115.4, 118.7, 119.9, 120.1, 120.6, 124.8, 129.5, 129.8, 130.7, 131.1, 131.7, 137.1, 140.4, 147.9, 153.3, 165.8. HRMS calcd for $C_{21}H_{23}N_4O_4$ ($[M + H]^+$) 395.1712, found 395.1757.

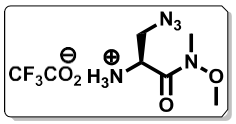


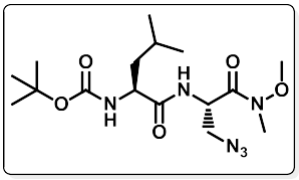
3.2.7. Synthesis of 3-(4-(3-((tert-butoxycarbonyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)benzoic acid **(1):** Using the general procedure of methyl ester hydrolysis, starting from 500 mg (1.26 mmol) of **22** , 463 mg (1.2 mmol) of the title compound **1** (445 mg, 1.17 mmol) was isolated as a white solid material in pure form by column chromatography (Si-gel, PE : EtOAc = 1:2). Yield 93%. ^{1}H NMR (d_6-DMSO; 400 MHz) δ 1.47 (9H, s); 7.36 (2H, s); 7.51 (1H, s); 7.75 (1H, t, J = 7.6 Hz); 8.04 (1H, d, J = 6.8 Hz), 8.16 (1H, s); 8.08 (1H, d, J = 8.0 Hz); 8.46 (1H, s); 9.33 (1H, s); 9.47 (1H, s); ^{13}C NMR (d_6-DMSO; 100 MHz) δ 28.4, 79.6, 115.3, 118.5, 119.9, 120.1, 120.6, 120.7, 124.4, 129.5, 130.8, 132.9, 137.1, 140.4, 147.9, 153.2, 166.7. HRMS calcd for $C_{20}H_{19}N_4O_4$ $[M - H]^-$ 379.3892, found 379.3237.



3.3 ***Synthesis of natural di, tri, tatrapeptides and triazolyl donor/acceptor unnatural pentapeptides*** **(2 & 3):** The targeted peptides were synthesized following the general procedure of peptide coupling protocol as was earlier discussed. The pure product was isolated by column chromatography.

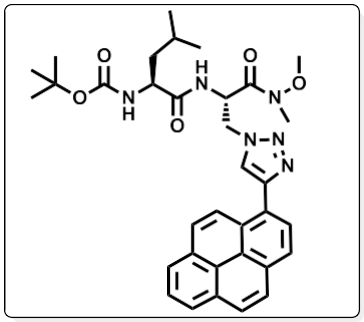
3.3.1. Synthesis of (S)-tert-butyl (3-azido-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate **(12):**  Using our previous method from 1500 mg (7.31 mmol) of N-Boc-protected L-serine, 730 mg (2.68 mmol) of the title compound **12** was preapred. Overall Yield 74 %.

3.3.2. ***Synthesis of Boc deprotected (S)-tert-butyl (3-azido-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate*** **(13):**  Using the general procedure of Boc-deprotection, we have deprotected the Boc-protection of compound **12** (730 mg, 2.68 mmol). The product **13** was obtained in quantitative yield and were used without further purification and characterization.

3.3.3. Synthesis of ***tert-butyl ((S)-1-(((S)-3-azido-1-(methoxy(methyl)amino)-1-oxopropan-2- yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate*** **(15) :** Using general procedure  of peptide coupling, starting from 600 mg (2.59 mmol) of N-Boc-protected leucine **14** and 450 mg (2.59 mmol) of N-deprodected serine azide **13**, 620 mg (1.61 mmol) of the title compound **15** was isolated. Pure product was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 2:1) as colourless oil. Yield 58%. IR (KBr) 3335, 3244, 2966, 2105, 1685, 1643, 1523, 1272, 1170, 928 cm^{-1}. ^{1}H NMR ($CDCl_3$; 400 MHz) δ 0.89 (6H, d, J = 3.2 Hz); 1.4 (9H, s); 1.47-1.43 (1H, m); 1.63 (2H, m); 3.19 (3H, s); 3.55-3.51 (1H, m); 3.63-3.60 (1H, m); 3.72 (3H, s); 4.15 (1H, bs); 5.03 (1H, bs); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 21.8, 23.1, 24.8, 28.3, 32.3, 41.4, 49.6, 51.9, 53.6, 61.8, 80.1, 155.6, 169.1, 172.8. HRMS calcd for $C_{16}H_{31}N_6O_5$ ($[M + H]^+$) 387.2277, found 387.2165.

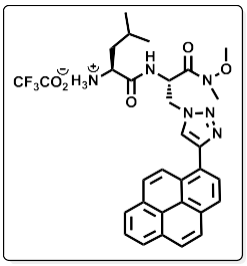
3.3.4. Synthesis of tert-butyl ((S)-1-(((S)-1-(methoxy(methyl)amino)-1-oxo-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate

(16) : Using the general procedure of [3+2] cyclo-addition reaction, starting from 200 mg (0.52 mmol) of azide derivative of dipeptide **15** and 140 mg (0.62 mmol) of 1-



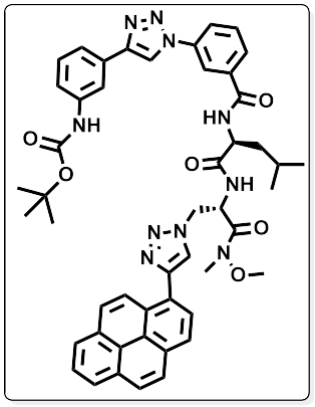
ethynyl pyrene, 247 mg (0.40 mmol) of the title compound **16** was isolated as a light brown solid material (Si-gel, PE : EtOAc = 1:1). Yield 78%; IR (KBr) 3450, 2958, 2928, 2102, 1653, 1509, 1390, 1167, 1049, 848 cm^{-1}. ^{1}H NMR ($CDCl_3$; 600 MHz); δ 0.89 (6H, d, J = 5.4 Hz); 1.38 (9H, s); 1.52-1.48 (1H, m); 1.67-1.59 (2H, m); 3.29 (3H, s); 3.7 (3H, s); 4.11 (1H, bs); 4.89 (1H, dd, J = 4.2 Hz, 9.6 Hz); 4.95 (1H, d, J = 6.6 Hz); 5.05 (1H, bs); 5.36 (1H, bs); 7.22 (1H, d, J = 5.4 Hz); 7.99 (1H, t, J = 7.2 Hz); 8.06 (2H, d, J = 4.8 Hz); 8.09 (1H, t, J = 9.0 Hz); 8.18-8.15 (3H, m); 8.30 (1H, s); 8.31 (1H, s); 8.79 (1H, d, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$; 150 MHz); δ 21.9, 23.1, 24.9, 28.4, 32.9, 41.1, 50.4, 50.7, 53.9, 62.1, 80.4, 124.8, 124.9, 125.0, 125.2, 125.3, 125.4, 126.1, 127.4, 127.5,127.8, 128.2, 128.7, 131.1, 131.4, 131.5, 147.8, 155.9, 168.2, 173.2. HRMS calcd for $C_{34}H_{41}N_6O_5$ ($[M + H]^+$) 613.3133, found 613.3132.

3.3.5. Synthesis of Boc deprotected (S)-1-(((S)-1-(methoxy(methyl)amino)-1-oxo-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)amino)-4-methyl-1-oxopentan-2-aminium 2,2,2-trifluoroacetate (17):**



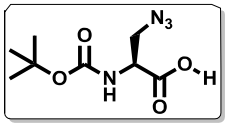
Using the general procedure of Boc-deprotection, compound **16** (240 mg, 0.40 mmol) was deprotected. The product **17** was obtained in quantitative yield and were used without further purification and characterization.

3.3.6. ***Synthesis of tert-butyl (3-(1-(3-(((S)-1-(((S)-1-(methoxy(methyl)amino)-1-oxo-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)carbamate*** **(18):** To a solution of



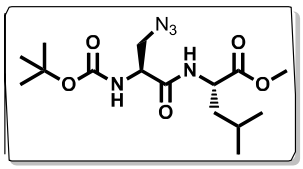
N-protected ^{Ar}**TAA, 1** (130 mg, 0.342 mmol) in dry DMF was cooled to 0 ^{0}C by ice bath. Consequently 1-[3-dimethyl amino propyl]-3-ehtylcarbo-diimide hydrochloride (EDC.HCl) (98 mg, 0.513 mmol), DMAP (125 mg, 1.02 mmol) and amine salt of weinreb amide protected corresponding dipeptide **17** were added and the reaction mixture was stirred for 18 hour at 0 ^{0}C to room temperature. Then solvent was dried by rotary evaporator, after which it was partitioned between EtOAc and water (50 ml each). The organic layer was washed with brine solution. Pure product **18** (230 mg, 0.259 mmol) was isolated in pure form by column chromatography (Si-gel, EtOAc) as solid compound. Yield 76%. IR (KBr) 3452, 3325, 2931, 1726, 1654, 1530, 1385, 1524, 1236, 1160, 1051, 842 cm^{-1}. ^{1}HNMR ($CDCl_3$; 400 MHz) δ 0.87 (6H, d, J = 5.2 Hz); 1.53 (9H, s); 1.74-1.63 (3H, m); 3.30 (3H, s); 3.84 (3H, s); 4.78 (1H, q, J = 6.8 Hz); 4.87 (1H, dd, J = 8.0 Hz, 6.0 Hz); 5.01 (1H, bs); 5.48 (1H, bs); 6.91 (1H, t, J = 8.0 Hz); 7.05 (1H, bs); 7.24 (1H, s); 7.33-7.29 (2H, m); 7.41 (1H, d, J = 8 Hz); 7.51 (1H, d, J = 7.6 Hz); 7.66 (1H, d, J = 6.8 Hz); 7.74 (1H, s); 7.81 (1H, s); 7.90-7.87 (4H, m); 7.99-7.94 (4H, m); 8.08 (2H, t, J = 7.6 Hz); 8.20 (1H, s); 8.51 (1H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 21.7, 22.9, 24.8, 28.3, 32.5, 36.4, 40.4, 50.5, 52.8, 61.9, 80.4, 115.7, 117.9, 118.3, 118.5, 120.1, 122.4, 124.4, 124.6, 124.7, 124.8, 124.9, 125.2, 125.9, 127.1, 127.2, 127.6, 127.9, 128.2, 129.2, 129.4, 130.3, 130.6, 131.0, 131.1, 134.5, 136.2, 139.3, 147.2, 147.8, 153.2, 162.7, 168.3, 173.1. HRMS calcd for $C_{49}H_{51}N_{10}O_6$ ([M + H]$^+$) 875.3988, found 875.3993.

3.3.7. ***Synthesis of (S)-3-azido-2-((tert-butoxycarbonyl)amino)propanoic acid*** **(21):** Using



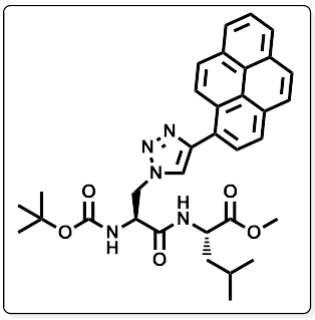
the general procedure of methyl ester hydrolysis , starting from 500 mg (1.83 mmol) of **20** , 387 mg (1.68 mmol) of the title compound **21** was isolated as a yellowish brown material and used for the next step without characterization. Yield 94%.

3.3.8. Synthesis of *(S)-methyl 2-((S)-3-azido-2-((tert-butoxycarbonyl)amino)propanamido)-4-methylpentanoate* (23): Using general procedure of peptide coupling, starting from



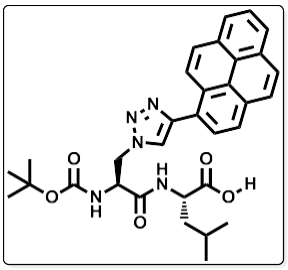
380 mg (1.6 mmol) of N-protected serine azide **21** and 297 mg (1.98 mmol) of N-deprodected amine salt of methyl ester -L-leucine **22**, 306 mg (0.85 mmol) of the title compound **23** was isolated. Pure product was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 2:1) as light yellow oil. Yield 52%. IR (KBr) 3326, 2960, 2873, 2105, 1745, 1666, 1524, 1368, 1250, 1023, 858, 780 cm^{-1}. ^1H NMR ($CDCl_3$; 400 MHz) δ 0.88 (6H, d, J = 5.6 Hz); 1.41 (9H, s); 1.63-1.51 (3H, m); 3.51 (1H, dd, J = 4.8 Hz, 6.8 Hz); 3.68 (3H, s); 3.74-3.70 (1H, m); 4.30 (1H, bs); 4.56 (1H, bs); 5.51 (1H, d, J = 6.4 Hz); 6.97 (1H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 21.9, 22.8, 24.8, 28.3, 41.3, 51.0, 52.2, 52.4, 53.7, 80.8, 155.5, 169.5, 173.1. HRMS calcd for $C_{15}H_{27}N_5O_5Na$ ($[M + Na]^+$) 380.1908, found 380.1909.

3.3.9. *Synthesis of (S)-methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propanamido)-4-methylpentanoate* (24) : Using general procedure



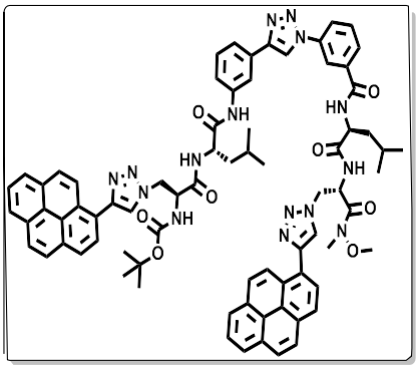
of [3+2] cyclo-addition reaction, starting from 250 mg (0.70 mmol) of azide derivative of dipeptide **23** and 190 mg (0.85 mmol) of 1-ethynyl pyrene, 245 mg (0.68 mmol) of the title compound **24** was isolated as a light brown solid material (Si-gel, PE : EtOAc = 1:1). Yield 60%; IR (KBr) 3336, 2961, 1669, 1515, 1258, 1165, 847, 724 cm^{-1}. ^1H NMR ($CDCl_3$; 600 MHz); δ 0.71 (6H, d, J = 6.0 Hz); 1.47 (9H, s); 1.56-1.49 (3H, m); 3.67 (3H, s); 4.59-4.56 (1H, m); 4.90 (2H, bs); 5.06 (1H, bs); 6.05 (1H, d, J = 5.4 Hz); 7.18 (1H, d, J = 4.8 Hz); 8.03-7.97 (2H, m); 8.06 (2H, q, J = 4.8 Hz); 8.10 (1H, s); 8.17-8.13 (3H, m); 8.20 (1H, d, J = 7.8 Hz); 8.66 (1H, d, J = 9.6 Hz); ^{13}C NMR ($CDCl_3$; 150 MHz); δ 21.7, 22.8, 24.9, 28.4, 41.3, 43.5, 51.1, 52.5, 54.6, 81.3, 124.8, 124.9, 125.0, 125.1, 125.2, 125.3, 125.5, 126.2, 127.2, 127.4, 128.0, 128.3, 128.6, 131.0, 131.3, 131.5, 131.6 147.5, 155.7, 169.0, 172.8. HRMS calcd for $C_{33}H_{38}N_5O_5$ ($[M + H]^+$) 584.2867, found 584.2874.

3.3.10. Synthesis of (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propanamido)-4-methylpentanoic acid (25):



Using the general procedure of methyl ester hydrolysis, starting from 215 mg (0.368 mmol) of **24**, 194 mg (0.34 mmol) of the title compound **25** was isolated as a yellowish brown gummy material (Si-gel, CH_3Cl:MeOH = 10:1). Yield 93%; ^{1}H NMR ($CDCl_3$; d_6-DMSO; 400 MHz); δ 0.82 (6H, m); 1.30 (9H, s); 1.37-1.30 (1H, m); 1.65-1.56 (2H, m); 4.37 (1H, bs); 4.73 (2H, d, J = 8.4 Hz); 5.51-4.90 (1H, m); 6.57 (1H, d, J = 7.6 Hz); 7.89 (1H, s); 8.00-7.94 (1H, m); 8.03 (2H, bs); 8.06 (1H, bs); 8.13 (1H, t, J = 4.0 Hz); 8.15 (1H, s); 8.17 (2H, d, J = 2.8 Hz); 8.34 (1H, s); 8.64 (1H, d, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$; d_6-DMSO; 100 MHz); δ 21.3, 22.8, 24.2, 28.1, 35.9, 40.2, 50.8, 54.1, 79.1, 124.1, 124.4, 124.7, 124.8, 124.9, 125.1, 126.1, 126.8, 127.1, 127.2, 127.4, 127.6, 128.1, 130.3, 130.5, 130.8, 146.1, 155.1, 162.1, 168.6, 174.3. HRMS calcd for $C_{32}H_{34}N_5O_5$ ([M - H]$^-$) 568.2565, found 568.5032.

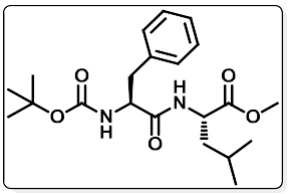
3.3.11. ***Synthesis of tert-butyl ((S)-1-(((S)-1-((3-(1-(3-(((S)-1-(((S)-1-(methoxy(methyl)amino)-1-oxo-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-(4-(pyren-1-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)carbamate*** **(2):**



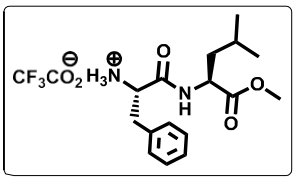
Starting material **25,** 160 mg (0.281 mmol) was taken in a dry 50 ml RB. With creating nitrogen atmosphere and maintaining 0 ^{0}C temperature by ice bath we are added dry THF solvent, ethyl chloroformate and tri-ethylamine followed by. Then free amine of tripeptide **19**, 218 mg (0.281 mmol) was added to the reaction mixture and allowed to stirrer about half an hour. After removing ice bath the reaction mixture was refluxed approximate 4 to 5 hour and monitored by TLC. The pure product **2**, 156 mg (0.12 mmol) was isolated as a solid material (CH_3Cl:MeOH = 10:1). Yield 42%. IR (KBr) 3440, 3323, 2953, 2914, 2849, 1716, 1653, 1586, 1539, 1497, 1388, 1367, 1156, 1052, 849, 757 cm^{-1}. ^{1}H NMR (d_6-DMSO; 600 MHz) δ 0.83 (6H, bs); 0.92 (6H, dd, J = 6.6 Hz, 3 Hz); 1.31 (9H, s); 1.49 (1H, bs); 1.59 (1H, bs); 1.65-1.73 (4H, m);

3.18 (3H, s); 3.79 (3H, s); 4.56 (1H, bs); 4.62 (1H, bs); 4.77-4.70 (3H, m); 4.95 (2H, dd, *J* = 10.2 Hz, 12.6 Hz); 5.39 (1H, bs); 7.26 (1H, d, *J* = 7.8 Hz), 7.36 (2H, dt, *J* = 25.2 Hz, 7.8 Hz); 7.52 (1H, d, *J* = 7.8 Hz); 7.64 (1H, d, *J* = 7.8 Hz); 7.78 (1H, d, *J* = 7.2 Hz); 7.85 (1H, d, *J* = 7.8 Hz); 8.03 (2H, dt, *J* = 22.2 Hz, 7.2 Hz); 8.10 (1H, d, *J* = 9.0 Hz); 8.20-8.17 (5H, m); 8.22 (1H, t, *J* = 6.6 Hz); 8.31-8.24 (8H, m); 8.34 (1H, s); 8.48 (1H, d, *J* = 7.2 Hz); 8.65 (1H, s); 8.68 (1H, s); 8.72 (1H, d, *J* = 7.2 Hz); 8.75 (2H, d, *J* = 9.6 Hz); 8.81 (1H, d, *J* = 9.6 Hz); 9.1 (1H, s); 10.2 (1H, s); ^{13}C NMR (d_6-DMSO; 150 MHz) *δ* 21.4, 21.5, 23.0, 23.1, 24.2, 24.3, 28.1, 31.5, 49.5, 49.9, 50.7, 52.1, 52.3, 54.3, 61.6, 78.8, 116.2, 118.8, 119.3, 119.6, 120.7, 122.4, 123.8, 123.9, 124.2, 124.3, 124.8, 124.9, 125.0, 125.1, 125.2, 125.3, 125.39, 125.4, 125.5, 126.3, 126.4, 126.9, 127.1, 127.2, 127.3, 127.4, 127.6, 127.8, 127.9, 129.4, 129.5, 130.3, 130.4, 130.5, 130.6, 130.8, 130.9, 135.3, 136.3, 139.4, 145.8, 146.1, 147.2, 155.1, 165.1, 168.9, 171.1, 172.4.; HRMS calcd for $C_{76}H_{76}N_{15}O_8$ ($[M + H]^+$) 1326.5998, found 1326.6014.

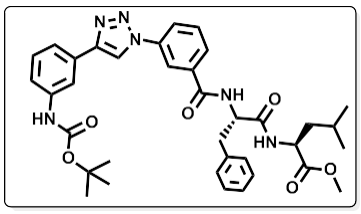
3.3.12. ***Synthesis of ((S)-methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-4-methylpentanoate*** **(27):** Using the general procedure of peptide coupling starting N-protected L-phenylalanine **26**, (1000 mg, 3.77 mmol), and the amine salt of methyl ester -L-leucine **22** (606 mg, 4.15 mmol)**,** the title compound **27 (**950 mg, 2.42 mmol) was isolated as white solid. Yield 64%; IR (KBr) 3391, 3067, 2966, 2937, 1751, 1683, 1648, 1545, 1390, 1200, 703 cm^{-1}. ^{1}H NMR ($CDCl_3$; 400 MHz) *δ* 0.90 (6H, t, *J* = 6 Hz); 1.26 (1H, s); 1.432 (9H, s); 1.66-1.58 (2H, m); 3.10 (2H, ddd, *J* = 6 Hz, 9.2 Hz); 3.694 (3H, s); 4.12 (1H, m); 4.84 (1H, d, *J* = 6.8 Hz); 4.98 (1H, d, *J* = 7.6); 6.66 (1H, d, *J* = 7.2 Hz); 7.10 (2H, d, *J* = 7.6 Hz); 7.29-7.20 (3H, m); ^{13}C NMR ($CDCl_3$; 100 MHz); *δ* 22.1, 22.7, 24.7, 28.4, 31.7, 38.1, 41.3, 52.4, 53.2, 80.1, 127.2, 128.6, 129.4, 135.9, 155.6, 171.8, 172.4. HRMS calcd for $C_{21}H_{32}N_2O_5Na$ ($[M + Na]^+$) 415.2201, found 415.2222.



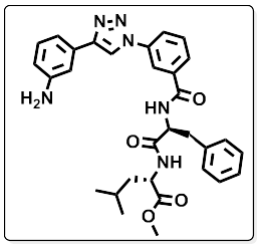
***3.3.13. Synthesis of Boc deprotected ((S)-methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-4-methylpentanoate* (28):** Using the general procedure of Boc-deprotection, compound **27** (212mg, 0.54 mmol) was deprotected. The product **28** was obtained in quantitative yield and were used without further purification and characterization.



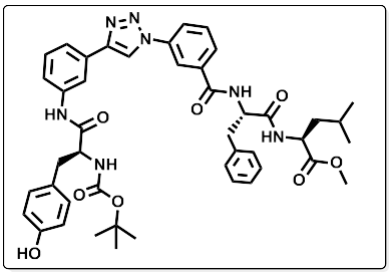
3.3.14. (S)-methyl-2-((S)-2-(3-(4-(3-((tert-butoxycarbonyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)benzamido)-3-phenylpropanamido)-4-methylpentanoate (29): In a dry DMF, N-protected aromatic triazolyl amino acid **^{Ar}TAA, 1** (200 mg, 0.526 mmol) was taken in a dry R.B. Under ice cold condition 1-[3-dimethyl amino propyl]-3-ehtylcarbo-diimide hydrochloride (EDC.HCl) (150.69 mg, 0.789 mmol), DMAP (192.52 mg, 1.578 mmol) were added and stirred for 15 minutes. After that the amine salt of methyl ester protected dipeptide **28** was added and the reaction mixture was stirred for another 18 h at 0 ^{o}C to room temperature. After completion of the reaction, the solvent was dried by rotary evaporator, after which it was partitioned between EtOAc and water (30 ml each). The organic layer was washed with brine solution. Pure product **29 (**250 mg, 0.389 mmol) was separated in pure form by column chromatography (Si-gel, PE: EtOAc = 1:1) as solid compound. Yield 73%; ^{1}H NMR (d_6-DMSO; 400 MHz) δ 0.85 (6H, dd, J = 6 Hz, 16.8 Hz); 1.49 (9H, s); 1.56-1.52 (1H, m); 1.69-1.59 (2H, m); 3.00 (1H, t, J = 12.4 Hz); 3.14 (1H, dd, J = 3.2 Hz, 13.6 Hz); 3.62 (3H, s); 4.32 (1H, m); 4.84 (1H, m); 7.17 (1H, q, J = 8.0 Hz); 7.26 (2H, t, J = 7.6 Hz); 7.37 (3H, s); 7.39 (1H, s); 7.52-7.50 (1H, m); 7.70 (1H, t, J = 8 Hz); 7.94-7.89 (1H, m); 8.10 (1H, dt, J = 1.2 Hz, 8.4 Hz); 8.21 (1H, s); 8.36 (1H, s); 8.58 (1H, d, J = 7.6 Hz); 8.87 (1H, d, J = 8 Hz); 9.25 (1H, s); 9.51 (1H, s); ^{13}C NMR (d_6-DMSO; 100 MHz); δ 21.4, 22.8, 24.3, 28.2, 37.1, 40.1, 50.5, 51.9, 54.7, 79.2, 115.1, 118.3, 119.1, 119.5, 119.7, 122.8, 126,3, 127.5, 128.1, 129.2, 129.3, 129.4, 130.0, 130.6, 135.7, 136.6, 138.2, 140.2, 147.6, 152.9, 165.2, 171.7. 172.9. HRMS calcd for $C_{36}H_{43}N_6O_6$ ($[M + H]^+$) 655.3239, found 655.3231.



3.3.15. ***Synthesis of (S)-methyl 2-((S)-2-(3-(4-(3-aminophenyl)-1H-1,2,3-triazol-1-yl)benzamido)-3-phenylpropanamido)-4-methylpentanoate*** **(30):** Using the general procedure of Boc-deprotection, we are getting the free amine derivative of compound **29** (212mg, 0.54 mmol). The product **30** was obtained in quantitative yield and were used without further purification and characterization.



3.3.16. (S)-methyl-2-((S)-2-(3-(4-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-(4 hydroxyphenyl)propanamido)phenyl)-1H-1,2,3-triazol-1-yl)benzamido)-3-phenylpropanamido)-4-methylpentanoate (3): In a dry THF at 0 ^{0}C temperature N-Boc tyrosine (60.93 mg, 0.213 mmol), ethyl chloroformate (24µl , 0.256 mmol) and trimethyl amine (35 µl, 0.256 mmol) were added. After five minutes free amine and C-terminal protected tripeptide **30** (106 mg, 0.192 mmol) was added and stirred at half an hour at cold condition. Then reaction mixture was refluxed about 4 to 5 hour and reaction monitor by TLC. After completion of the reaction, the solvent was dried by rotary evaporator, after which it was partitioned between EtOAc and water (50 ml each). The organic layer was washed with brine solution. Pure product **3 (**80 mg, 0.098 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 1:1) as solid compound. Yield 46%; %; IR (KBr) 3441, 3331, 2953, 2923, 1744, 1670, 1519, 1439, 1371, 1236, 1164, 1045, 789, 688 cm^{-1}. ^{1}H NMR (d$_6$-DMSO; 600 MHz) δ 0.89 (6H, dd, J = 5.4 Hz, 12.6 Hz); 1.33 (9H, s); 1.52 (1H, bs); 1.65 (2H, bs); 2.74 (1H, d, J = 12.4 Hz); 2.89 (1H, bs); 3.01 (2H, ddd, J = 5.4 Hz, 16.8 Hz); 3.57 (3H, s); 4.26 (1H, bs); 4.48 (1H, q, J = 5.4 Hz); 4.62 (1H, bs); 6.65 (2H, d, J = 7.2 Hz); 7.05 (1H, d, J = 7.8 Hz); 7.12 (2H, d, J = 7.8 Hz); 7.15 (1H, s); 7.21 (4H, s); 7.44 (1H, t, J = 7.8 Hz); 7.61 (2H, dd, J = 7.2 Hz, 15.6 Hz); 7.74 (1H, t, J = 7.8 Hz); 8.01 (1H, d, J = 7.8 Hz); 8.13 (1H, d, J = 7.2 Hz); 8.27 (1H, s); 8.43 (1H, s); 8.45 (1H, s); 8.67 (1H, d, J = 7.8 Hz); 9.19 (1H, s); 9.32 (1H, s); 10.17 (1H, s); ^{13}C NMR (d$_6$-DMSO; CDCl$_3$; 150 MHz); δ 22.2, 22.8, 24.9, 28.3, 37.7, 40.9, 52.4, 52.5, 53.5, 54.0, 57.4, 80.3, 115.6, 116.9,



117.9, 118.0, 119.7, 121.2, 123.4, 127.0, 128.0, 128.1, 128.6, 129.0, 130.0, 130.2, 130.3, 130.7, 134.7, 136.1, 136.7, 147.6, 155.6, 156.5, 166.2, 171.9, 173.5 HRMS calcd for $C_{45}H_{52}N_7O_8$ ($[M + H]^+$) 818.3872, found 818.3871.

4. Spectroscopic Evidences of Various Interactions and β-sheet like Structures in the Peptides 2 and 3

4.1. Study of Circular Dichroism Spectroscopy

Circular dichroism spectra were recorded using a CD spectropolarimeter with a cell path length of 1 mm at in different solvent at room temperature and variable temperature. All the samples were prepared in spectroscopic grade solvent with 100 μM concentration.

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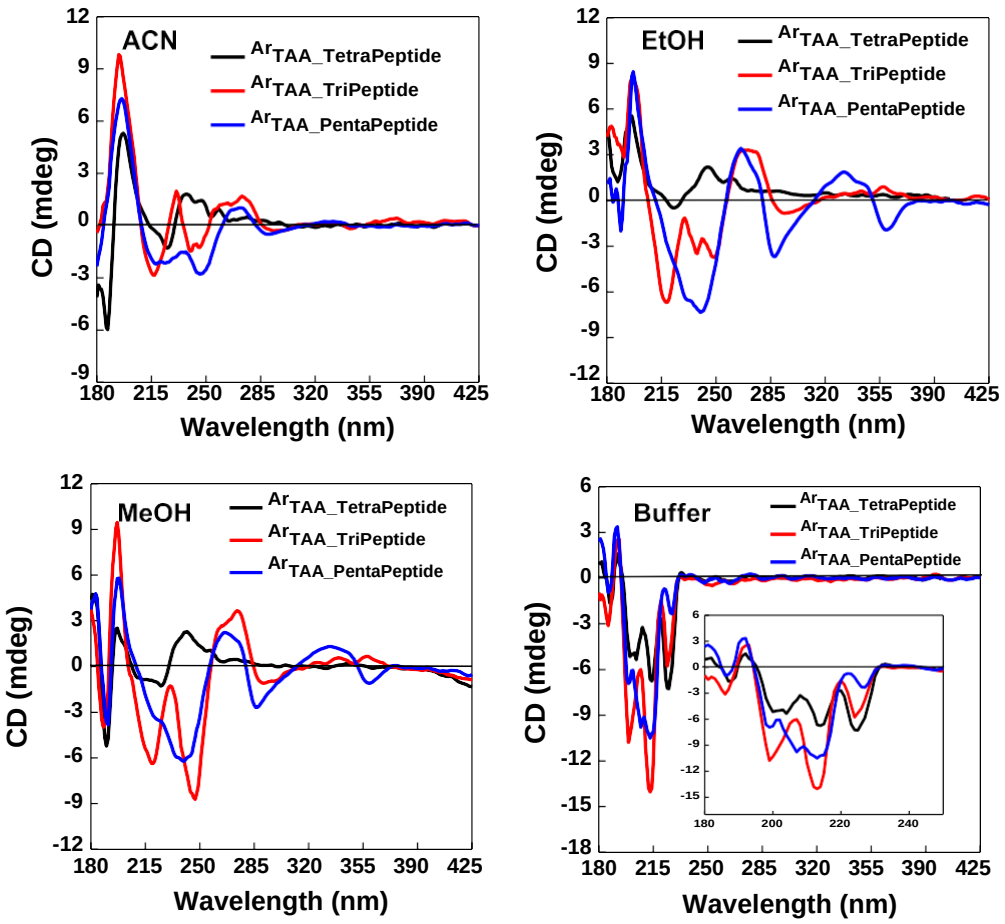


Figure S1. CD spectra of synthesized peptides **2, 3** and **18** in different solvent (100 μM concentration; r.t.).

The fluorescent Pentapeptide **2** showed a strong positive band at around 194 nm and a broad negative band at around 215-239 nm indicating sheet conformation. Moreover, the signature of negative band at 287 nm due to aromatic scaffold and positive bands at 331 and 360 nm indicated the absorption of the fluorescent triazolyl pyrene with π-π stacking interaction between two terminal triazolyl pyrene moiety, which also supported from variable temperature CD spectra in EtOH solvent (Fig. S3).

The tetrapeptide **3** containing the aromatic scaffold in the backbone showed a positive band at 197 nm and a broad negative band at around 209-225 nm indicating sheet conformation. Moreover, the presence of a positive band at 238 and 276 nm signified the absorption of chiral aromatic triazolyl amino acid scaffold as well of the tyrosine in the tetrapeptide **3**. Overall, the our synthesized peptides **2** and **3** showed β-sheet like structure with turn conformation (Fig. S1).

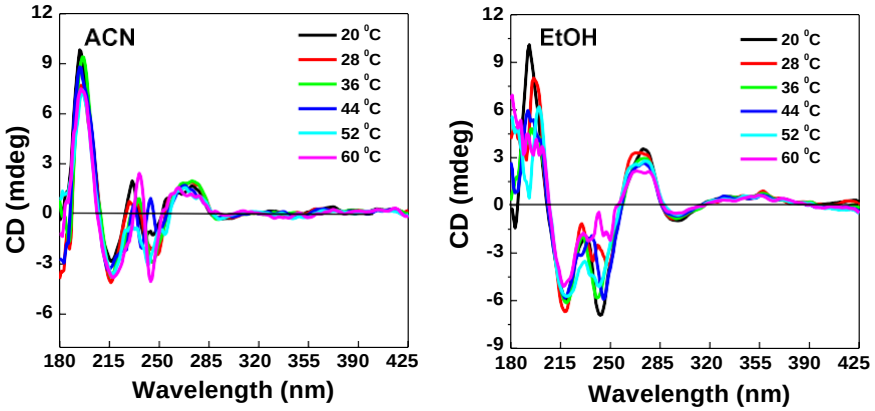


Figure S2. Variable temperature CD spectra of synthesized tripeptides **18** in acetonitrile & ethanol solvent (100 μM concentration).

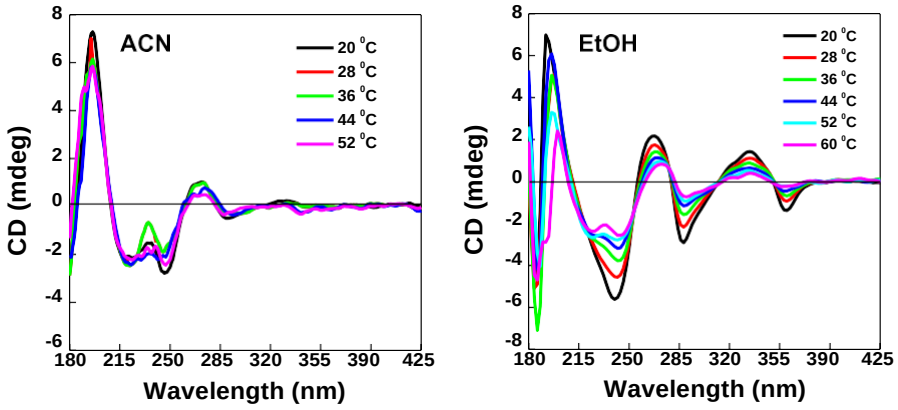


Figure S3. Variable temperature CD spectra of synthesized pentapeptide **2** in acetonitrile & ethanol solvent (100 μM concentration).

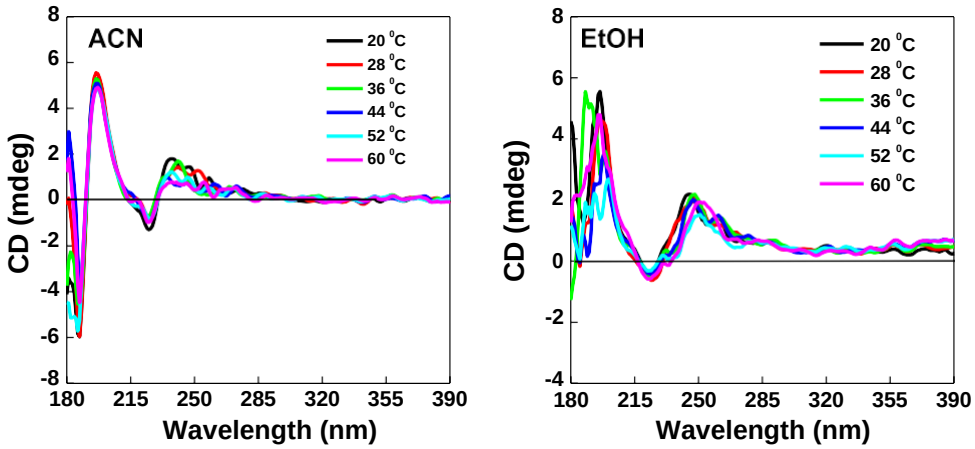


Figure S4. Variable temperature CD spectra of synthesized tetrapeptide **3** in acetonitrile & ethanol solvent (100 μM concentration).

4.2. Infrared Spectroscopy

IR spectra were recorded using dry KBr with solid and dry compound.

Table S1. Summary table of IR spectra

Peptides	Free N-H (cm^{-1})	H bonded N-H (cm^{-1})
Pentapeptide_2	3440	3323
Tetrapeptide_3	3441	3331

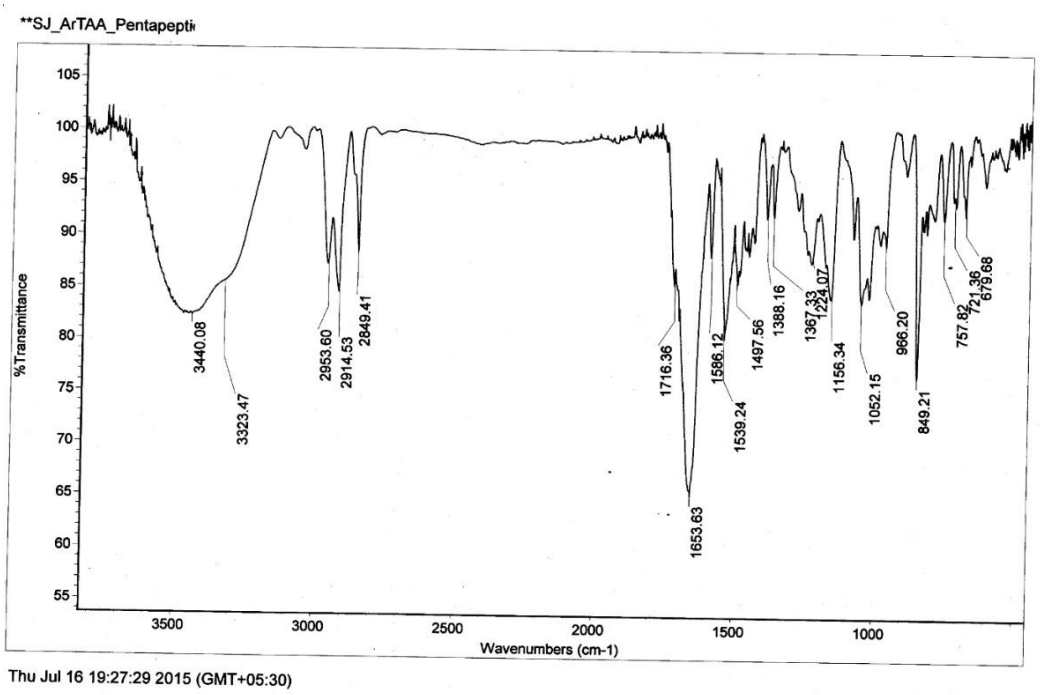


Figure S5. IR spectra of **Pentapeptide 2**

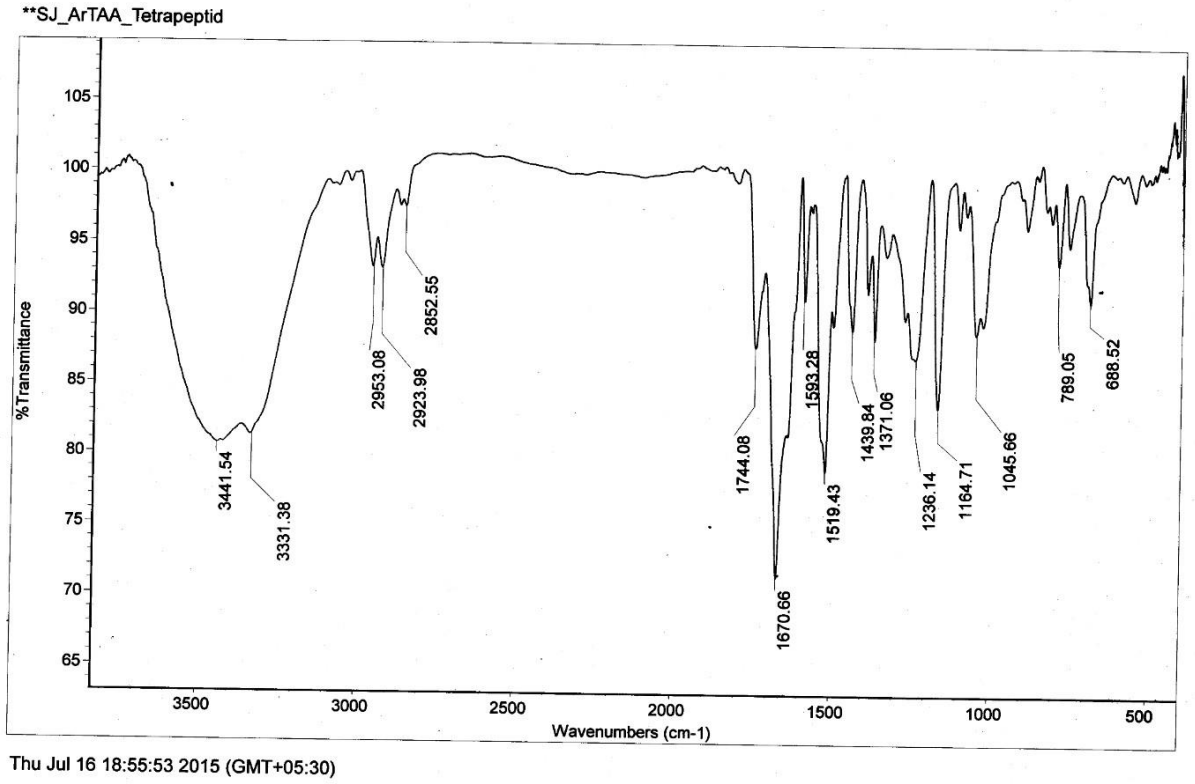


Figure S6. IR spectra of **Tetrapeptide 3**

4.3. Variable Temperature ^{1}H NMR Data of Peptide 2 and 3.

Table S2. Values of temperature coefficients of chemical shifts of amide NH's or triazole-CH's in Pentapeptide **2**.

Kessler limit ($\Delta\delta/\Delta T$) ppb/k	
N_terminal_Ser_NH1	- 4.45 ppb/k
N_terminal_Leu_NH2	- 4.37 ppb/k
N_terminal_^{Py}Tz_CH1	- 1.27 ppb/k
C_ terminal_^{Py}Tz_CH3	-1.79 ppb/k
C_ terminal _Leu_NH4	-4.31 ppb/k
N_ terminal _Ser_NH5	-6.1 ppb/k
^{Ar}TAA_NH3	-4.86 ppb/k
^{Ar}TAA_CH2	-3.13 ppb/k

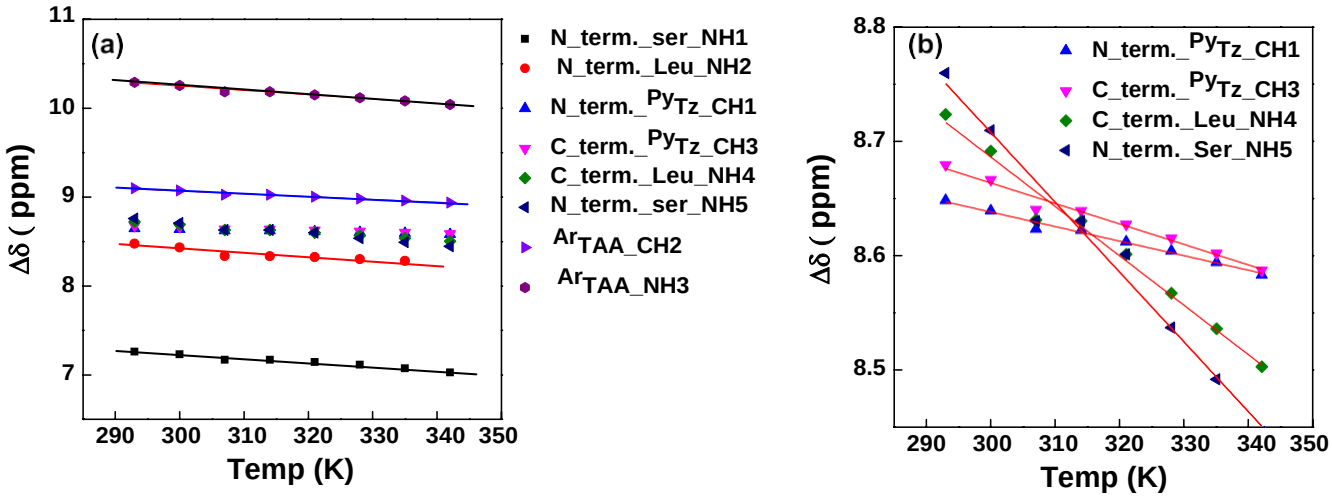


Figure S7. Temperature dependence of amide-NH/triazole-CH chemical shift of Pentapeptide **2.**

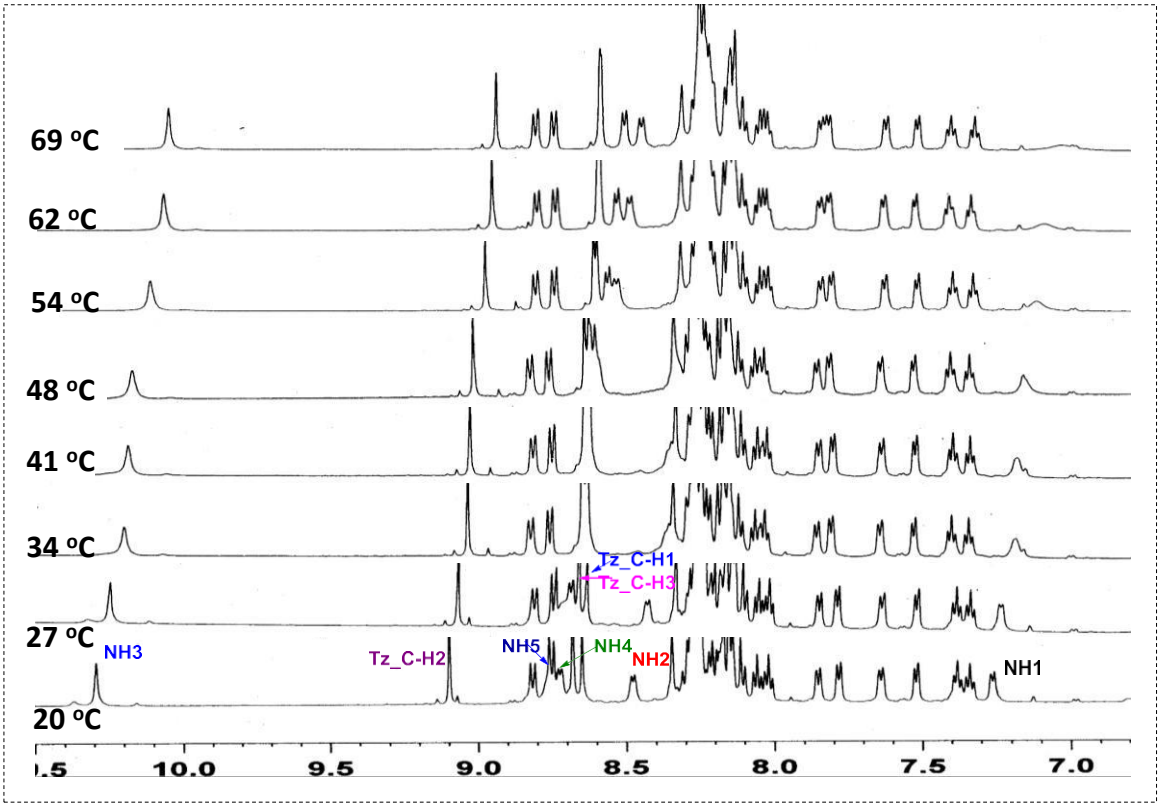


Figure S8. Variable temperature ^{1}H NMR spectra of Pentapeptide **2** in d_6-DMSO showing temperature dependence of amide-NH/triazole-CH chemical shift.

Table S3. Values of temperature coefficients of chemical shifts of amide NH's or triazole-CH in tetrapeptide_**3**.

Kessler limit ($\Delta\delta/\Delta T$) ppb/k	
N_terminal_Tyr_NH1	- 8.68 ppb/k
^{Ar}TAA_NH2	- 6.29 ppb/k
C_terminal_Phe.ala_NH3	-7.2 ppb/k
C_ terminal _ Leu_NH4	-4.51 ppb/k
^{Ar}TAA_triazole_CH1	-3.02 ppb/k
Tyr_OH	-5.56 ppb/k

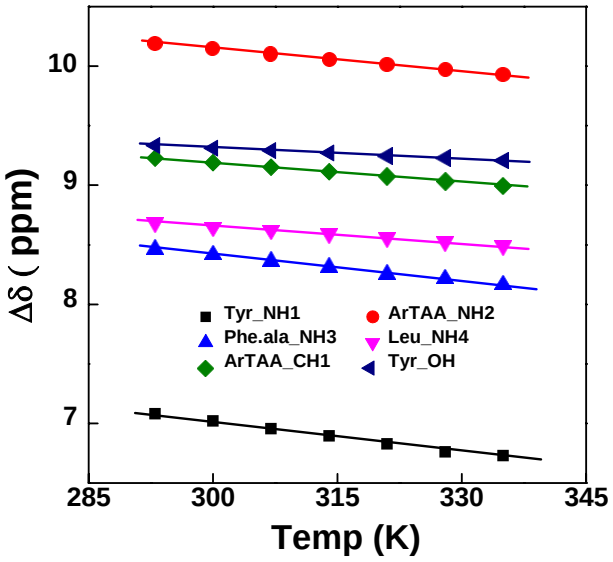


Figure S9. Temperature dependence of amide-NH/triazole-CH chemical shift of tetrapeptide **3.**

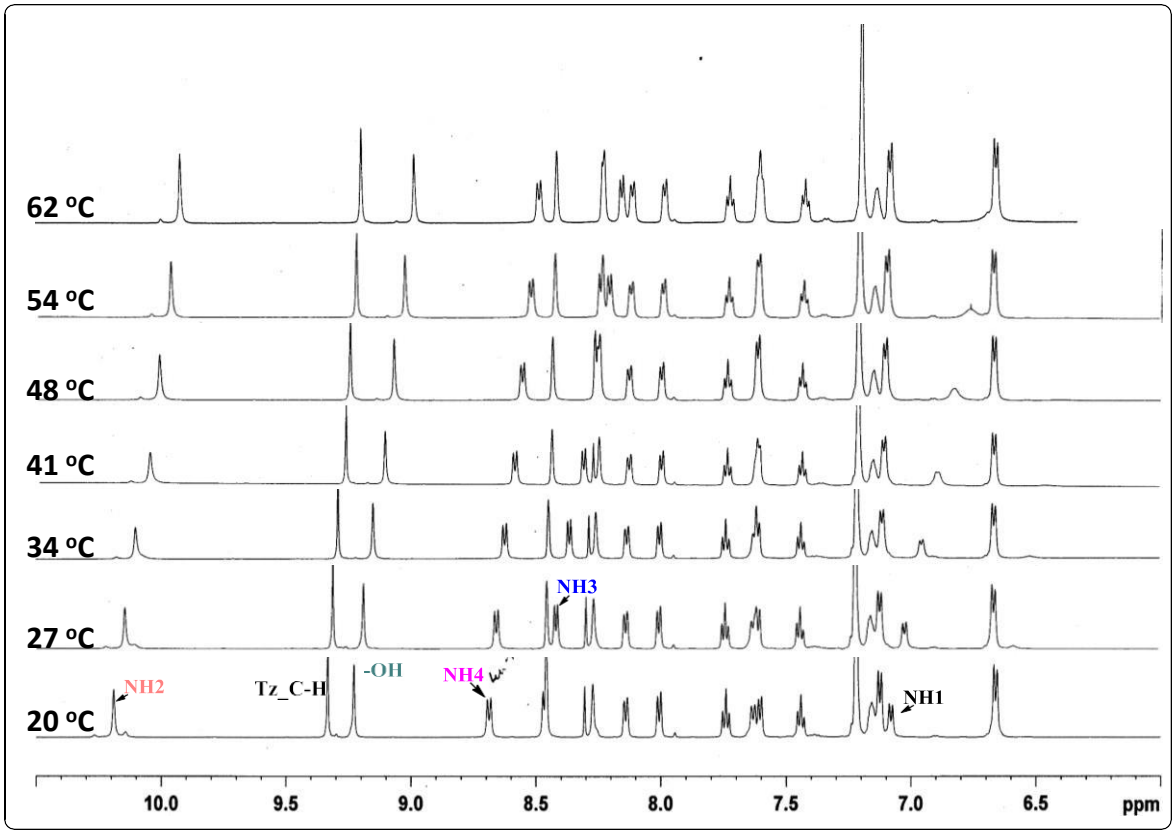


Figure S10. Variable temperature ^{1}H NMR spectra of tetrapeptide **3** in d_6-DMSO showing temperature dependence of amide-NH/triazole-CH chemical shift.

4.4. Conformational Analysis of Peptides 2 and 3 by 2D NMR Spectroscopy.

4.4.1. Conformational Analysis of Pentapeptide 2

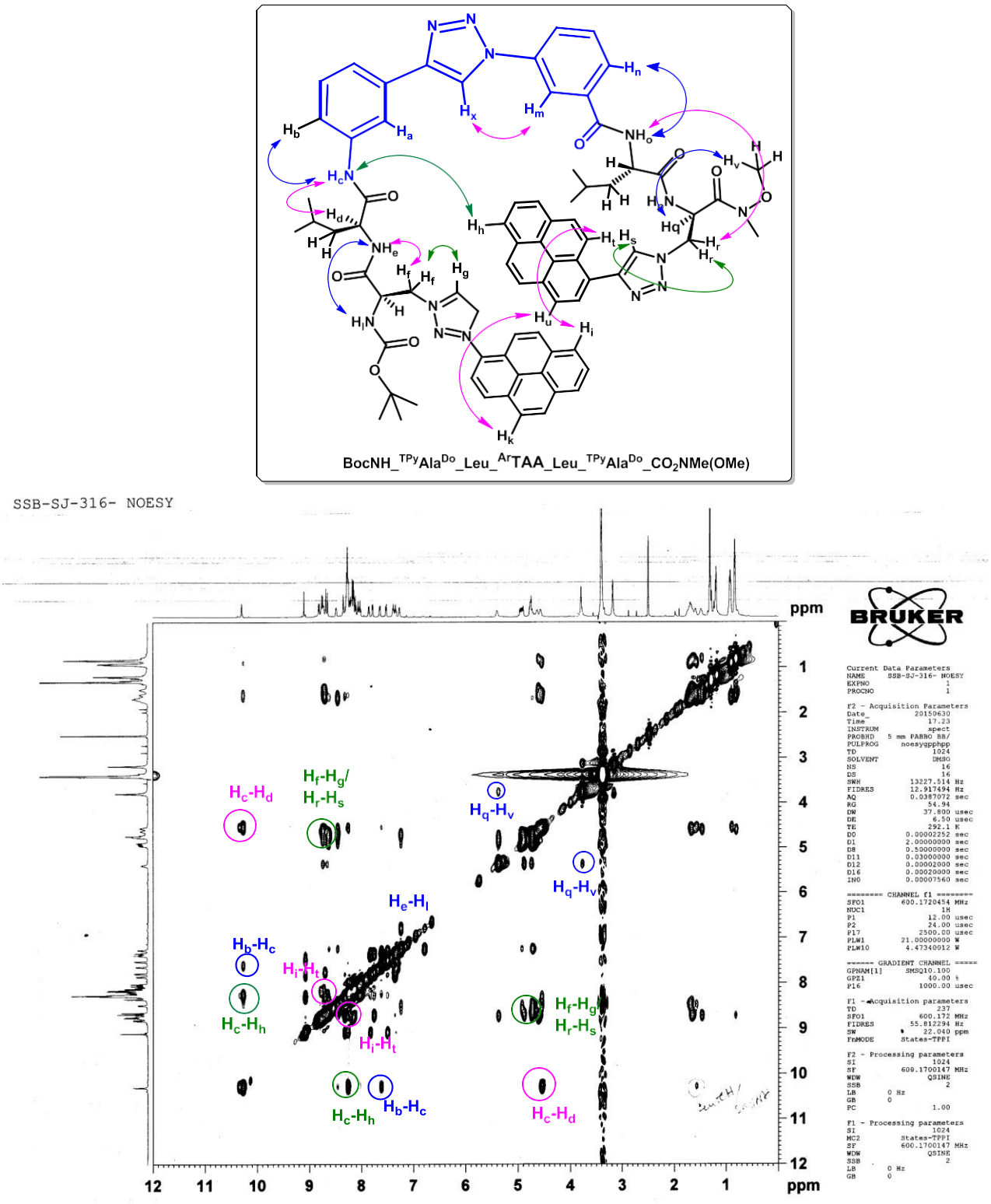


Figure S11. NOESY Spectra of synthesized **Pentapeptide 2.**

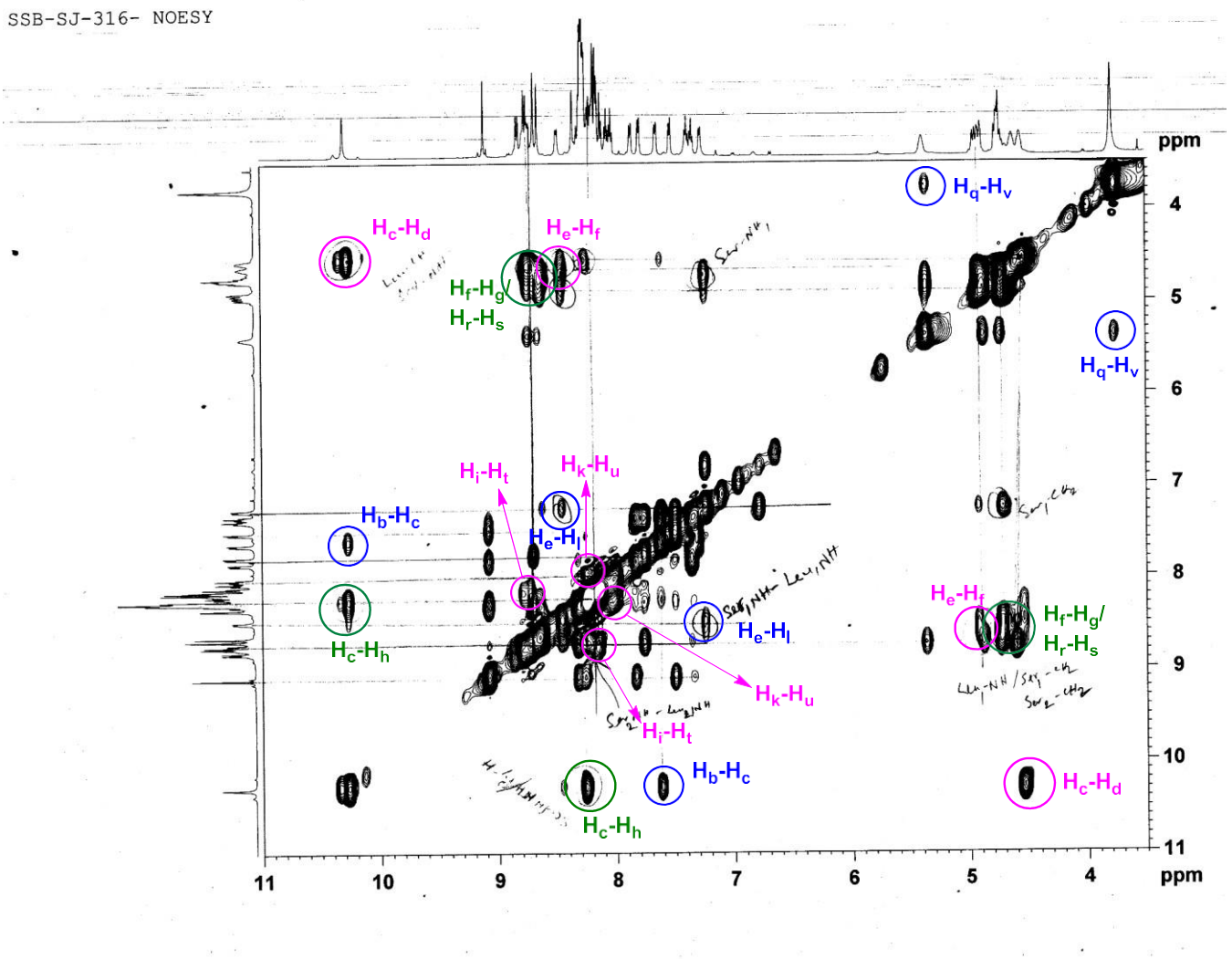


Figure S12. Expanded NOESY Spectra of synthesized **Pentapeptide 2.**

SSB-SJ-316-ROESY

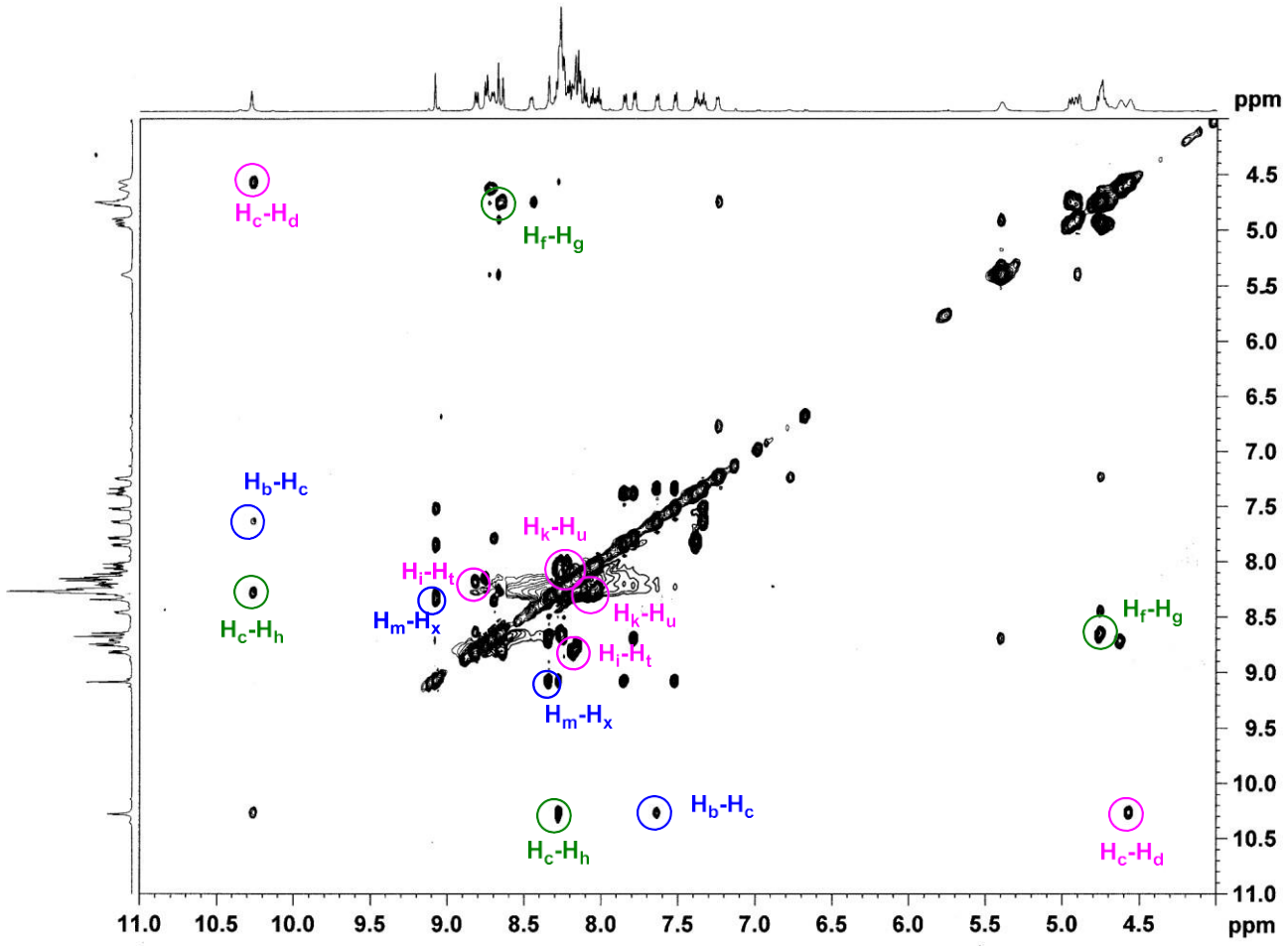


Figure S13. Expanded ROESY Spectra of synthesized **Pentapeptide 2.**

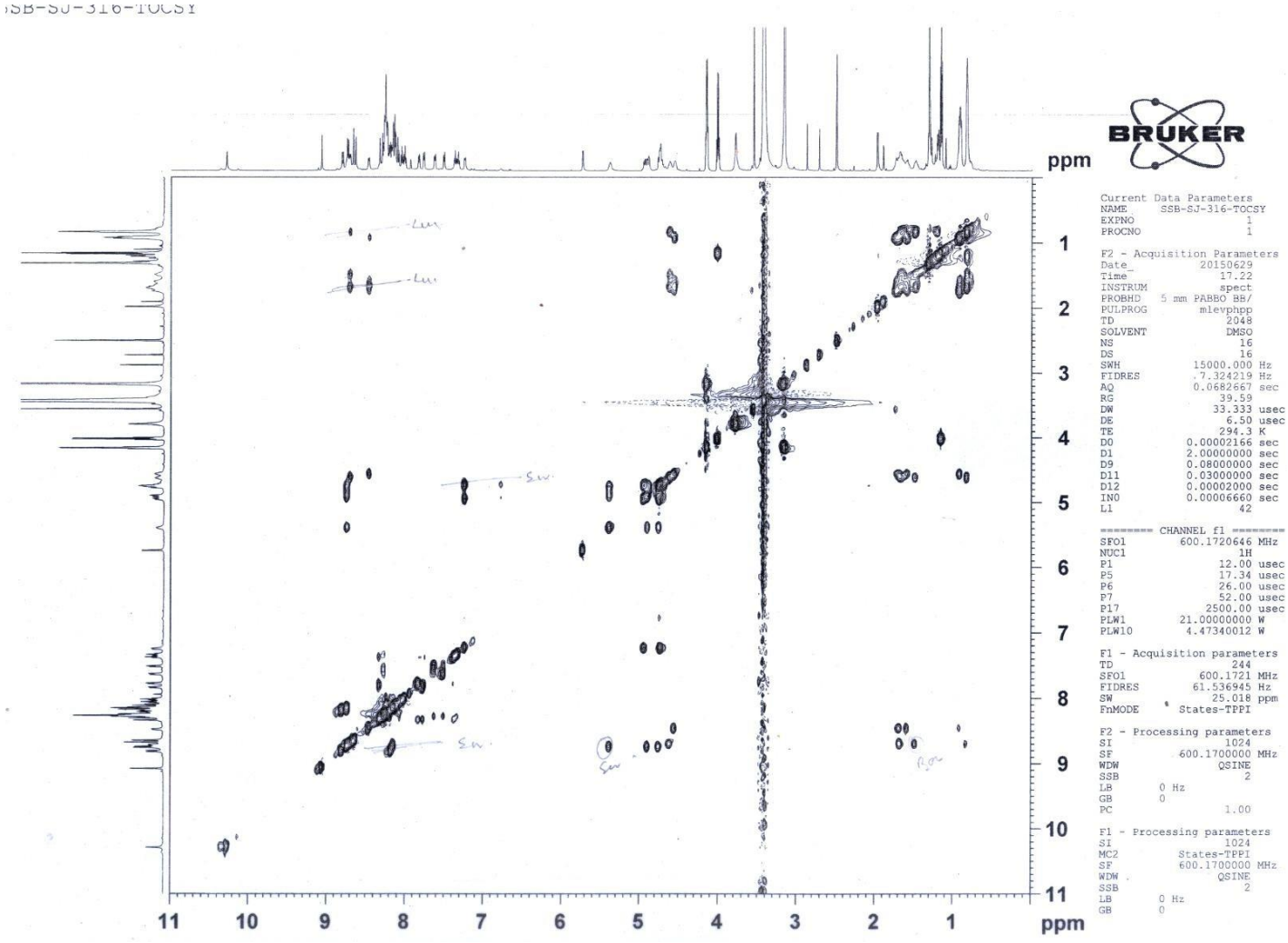
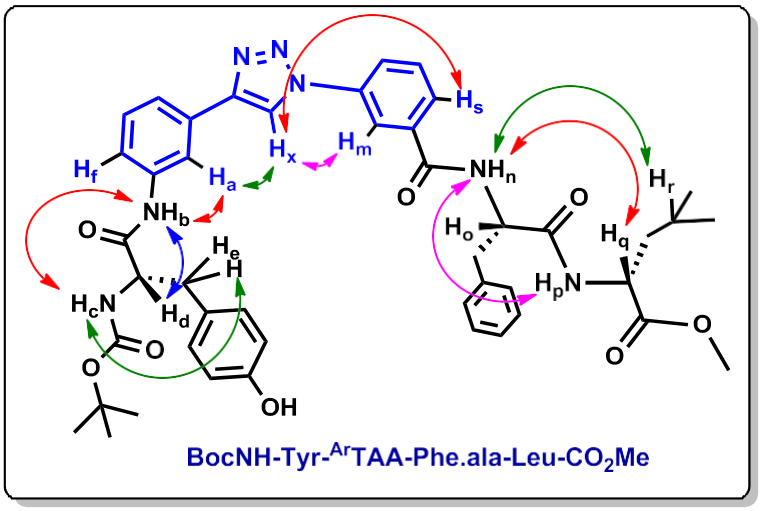


Figure S14. TOCSY Spectra of synthesized **Pentapeptide 2.**

4.4.2. Conformational Analysis of Tetrapeptide 3



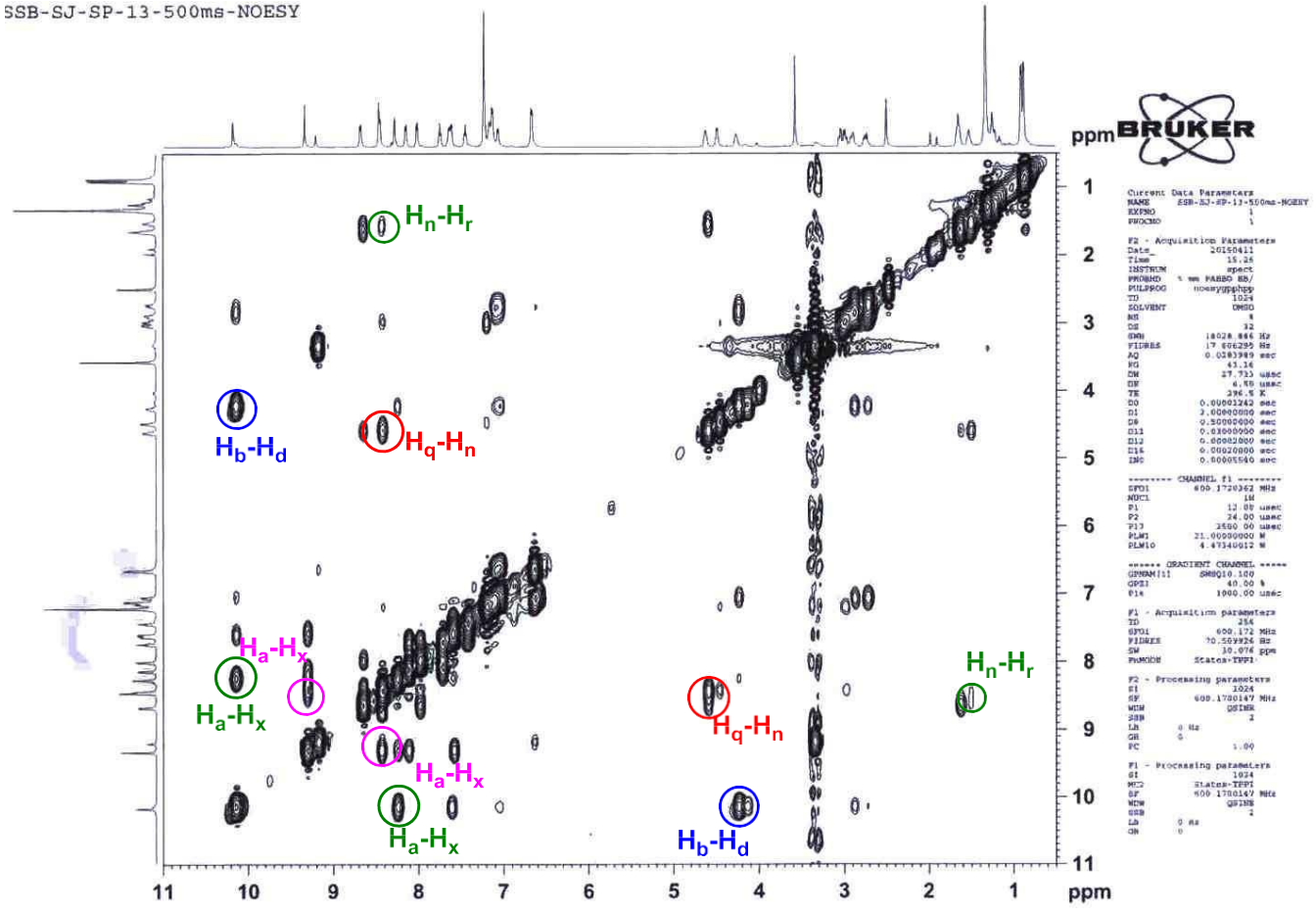


Figure S15. NOESY Spectra of synthesized **tetrapeptide 3.**

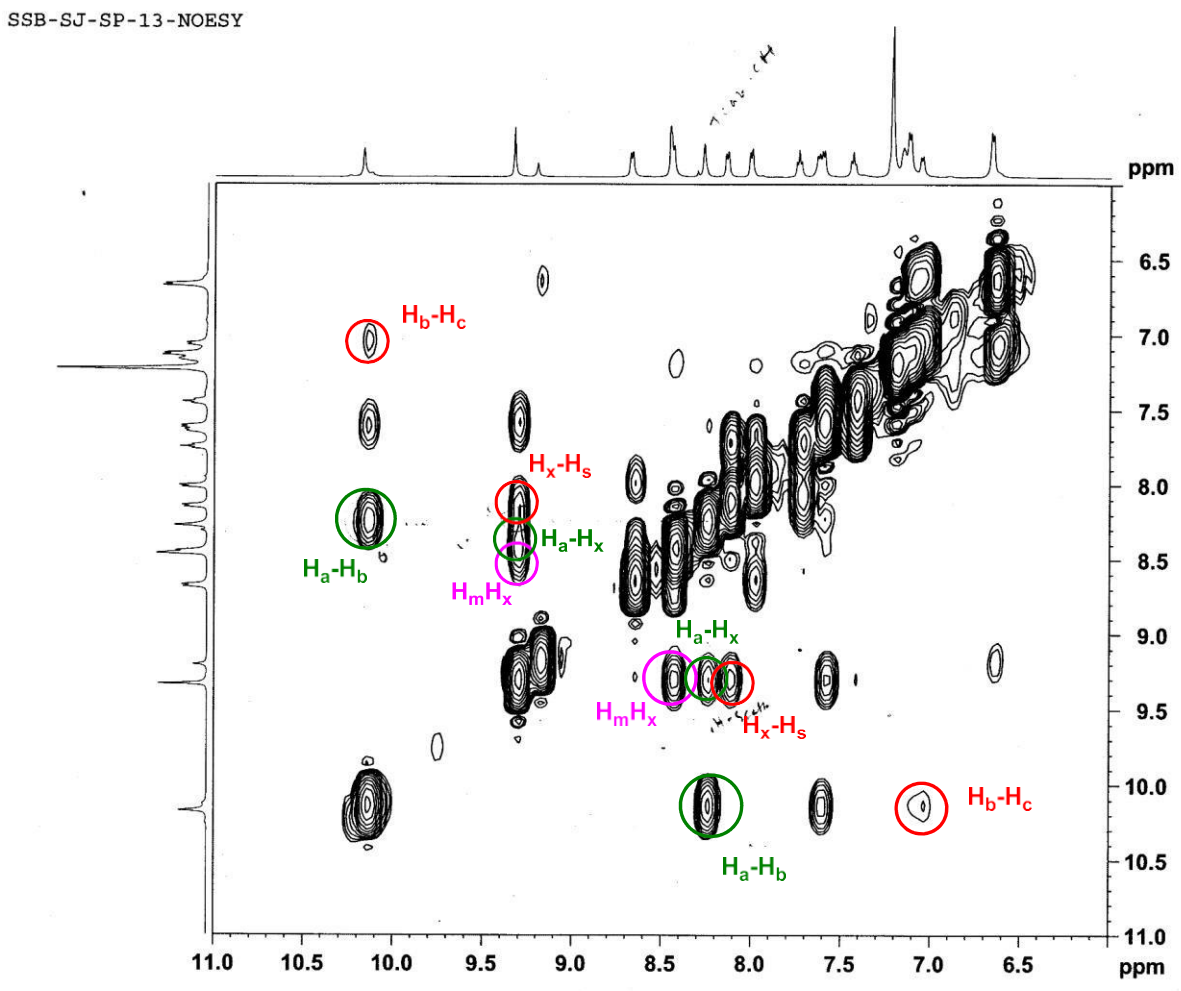


Figure S16. Expanded NOESY Spectra of synthesized **tetrapeptide 3.**

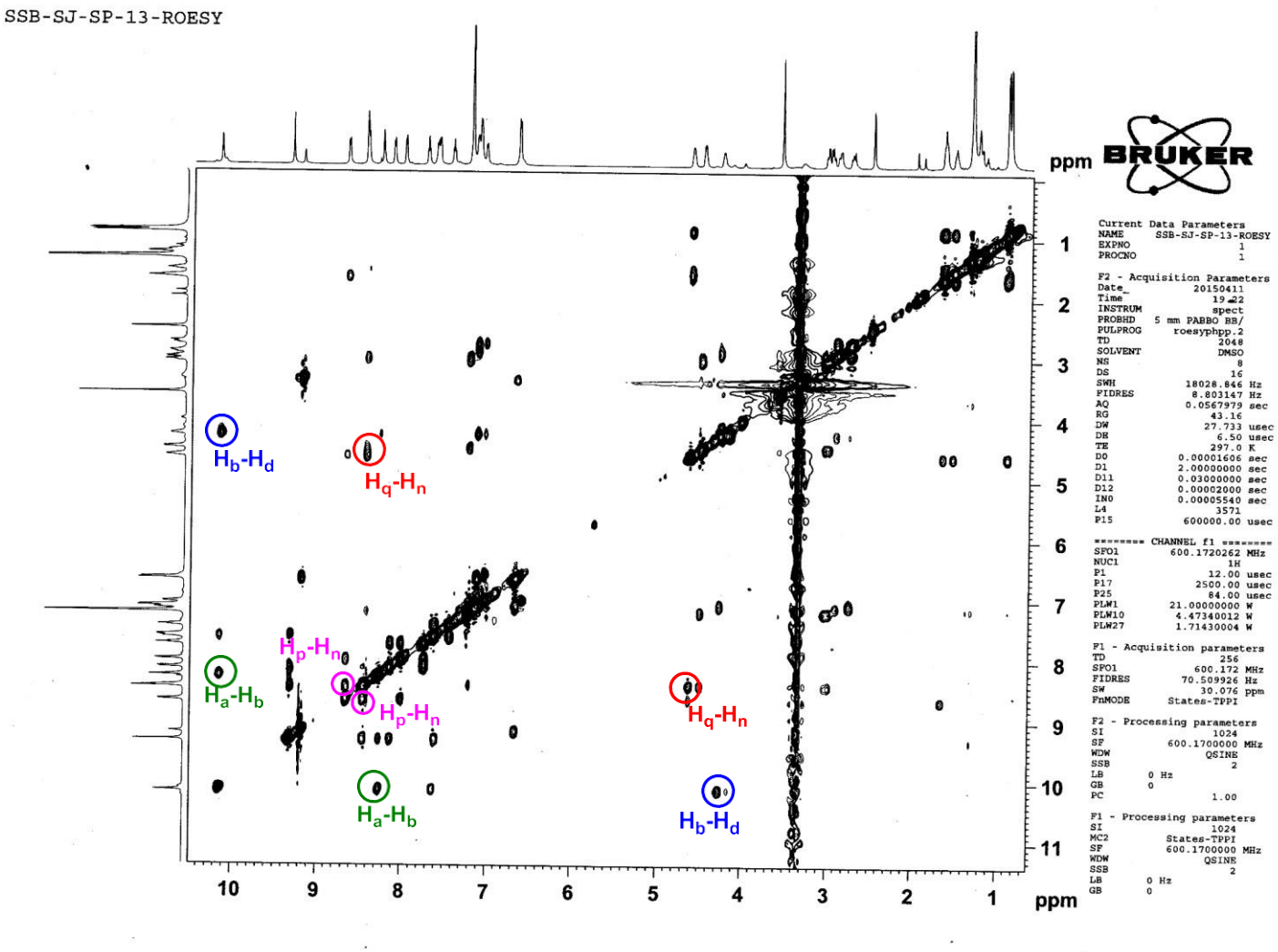


Figure S17. ROESY Spectra of synthesized **tetrapeptide 3.**

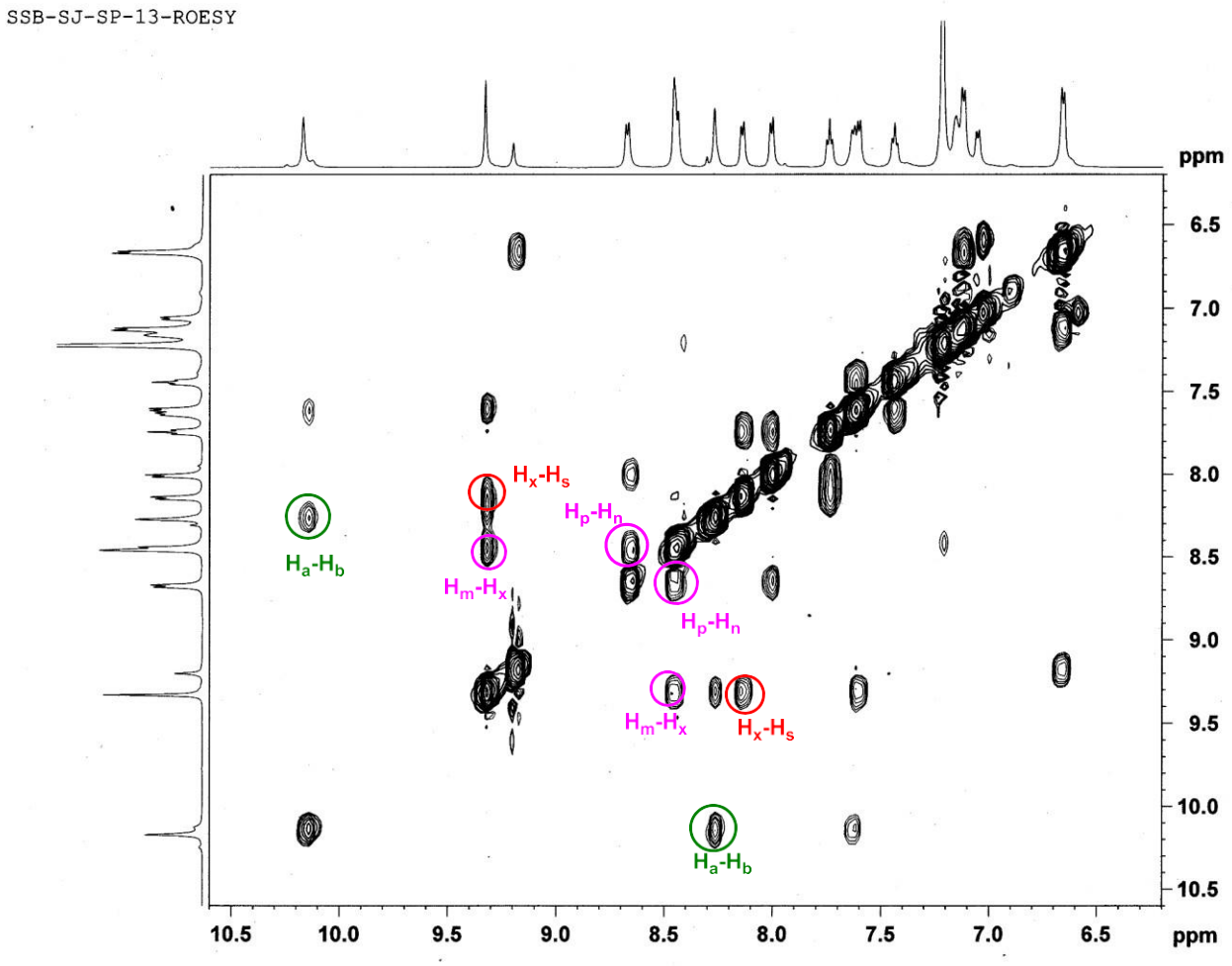


Figure S18. Expanded ROESY Spectra of synthesized **tetrapeptide 3.**

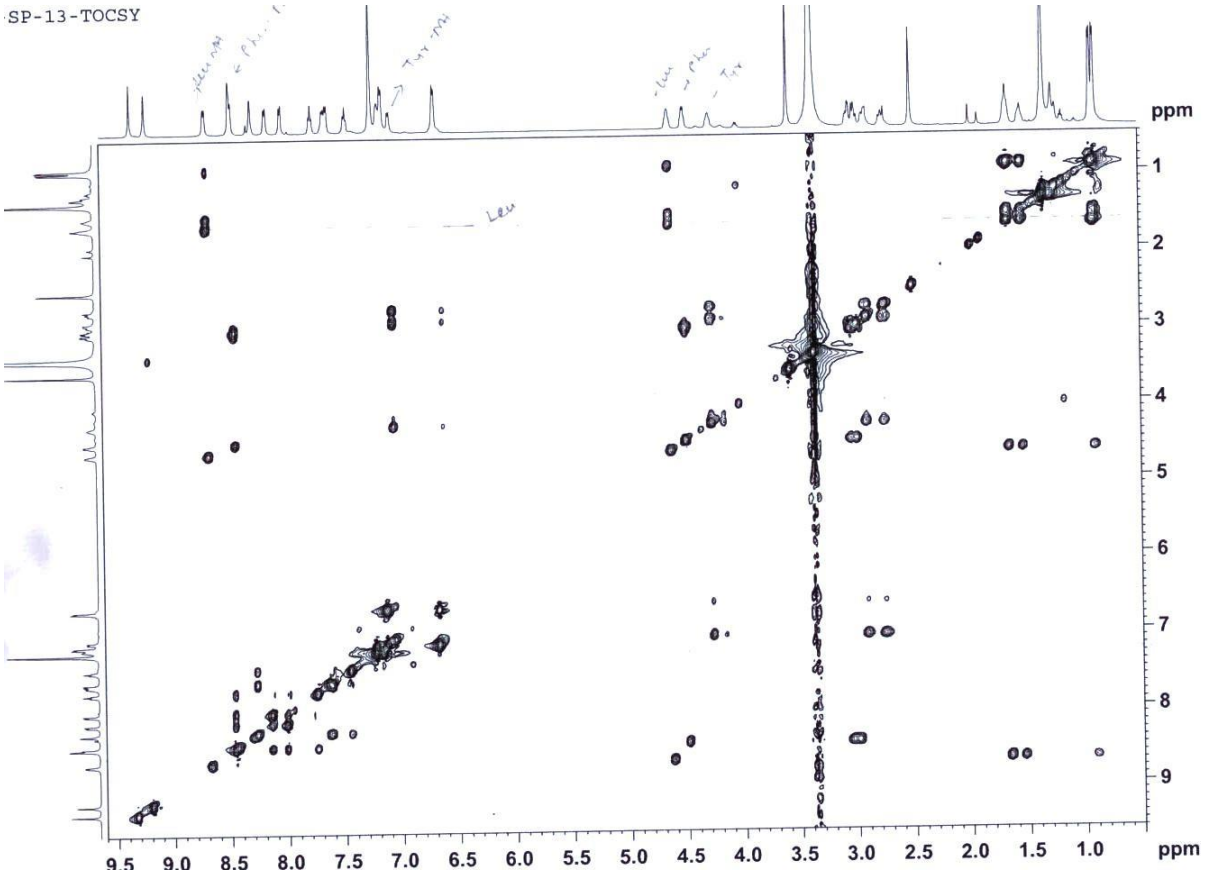


Figure S19. TOCSY Spectra of synthesized **tetrapeptide 3.**

5. Macro Model Study and Molecular Dynamics Simulation for peptide 2 and 3.

5.1. Optimization Structure of Peptides and Conformational Search of Optimized Structures

We are using Schrodinger Macromodel (Maestro vs. 9.1) software with OPLS 2005 force field in water for minimized the structure of our synthesized peptides. A conjugate gradient minimization scheme [PRCG (Polak-Ribiere Conjugate Gradient)] that uses the Polak-Ribiere first derivative method with restarts every 3N iterations was employed for the minimization of the peptides.

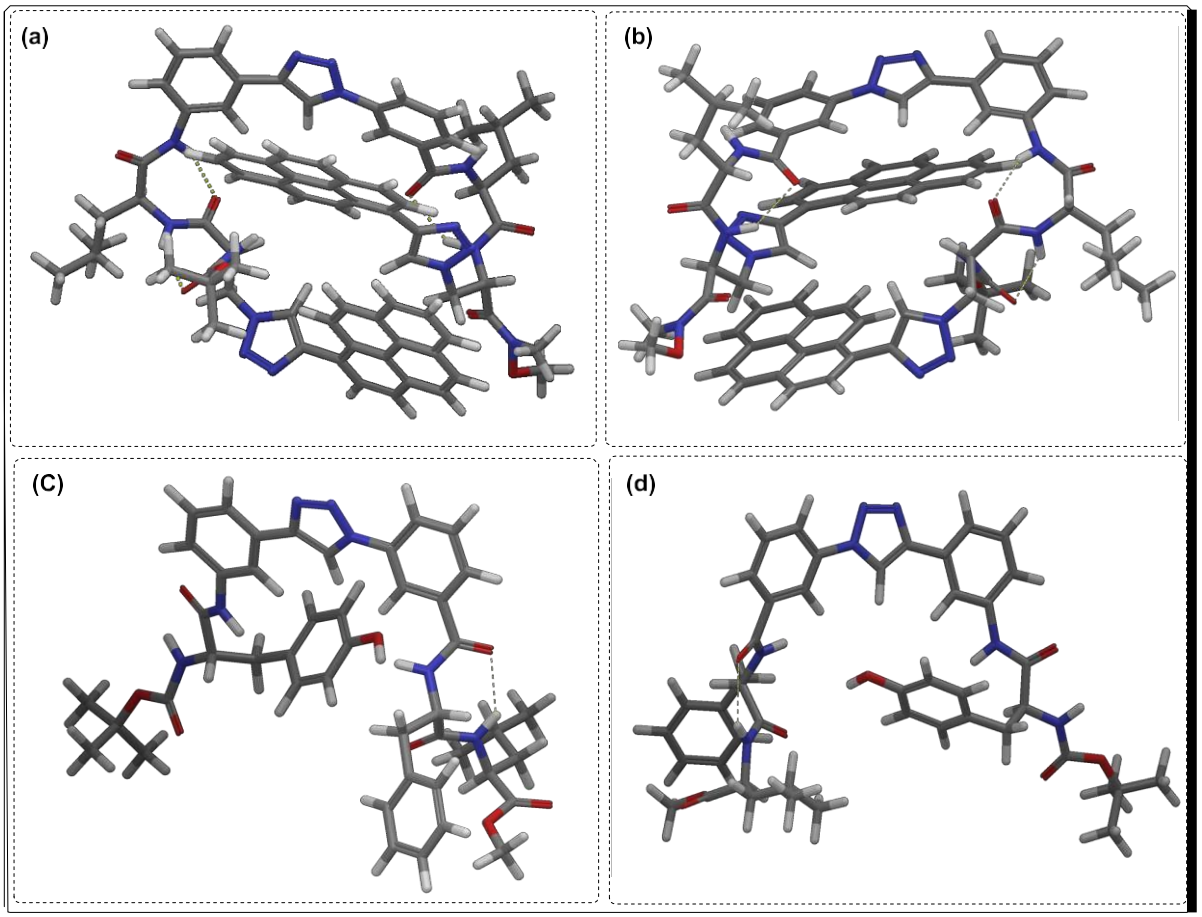


Figure S20. OPLS 2005 force field energy minimized conformations (a) front side view, (b) back side view of Pentapeptide **2** (**BocNH-^{TPy}Ala^{Do}-Leu-^{Ar}TAA-Leu-^{TPy}Ala^{Do}-CONMe(OMe)**). and (c) front side view, (d) back side view of Tetrapeptide **3** (**BocNH-Tyr-^{Ar}TAA-Phe-Leu-CO_2Me**) in water.

Next, we carried out conformational search using OPLS 2005 force field at constant dielectric in water with "large scale low-frequency-mode conformational search" (Mixed torsional/Large scale low-mode sampling = MCMM/LMCS) method using Schrodinger Macromodel (Maestro vs. 9.0) software package. It is a hybrid functional method wherein Low-Mode Conformational Search Methods is hybridized with Monte Carlo Multiple Minimum (MCMM) global searching. This method uses a combination of the random changes in torsion angles and/or molecular position from the MCMM method, together with the low-mode steps from the LLMOD method used in Large scale low-mode.

A total of 500 structures were processed with 500 maximum no. of steps iteration. A global search analysis eliminates redundant conformers using RMS deviation for all compared atoms exceed the threshold Cutoff of 0.5 Å. An optimal minimization method was chosen for minimizing the generating conformers.

A total of 65 (for Pentapeptide **2**) and 116 (for tetrapeptide **3**) minimized and well converged conformers were generate out of which the one conformer appeared 3 times (for Pentapeptide 2), and 5 times (for tetrapeptide 3) which remained within 1.00 k.cal/mole (4.18 kJ/mole) global minimum with a convergence threshold of 0.047 to 0.045 RMSD (threshold cutoff = 0.05). These conformers were taken as the starting structures for MD simulation studies.

5.2. Molecular Dynamics Simulation of Optimized Structure of the Peptides 2 and 3.

The MD simulations were done by Schrodinger Macromodel (Maestro vs. 9.1) software package with OPLS 2005 force field in which the systems were subjected to 100 ps simulations time (with time step of 1.5 fs and equilibrium time 1.0 ps) at constant temperature (300 K) and pressure (1 atm) with shaking all bonds . An optimal minimization method was chosen for minimizing the generated structures (with maximum iteration of 1000) with gradiant convergence threshold of 0.05.

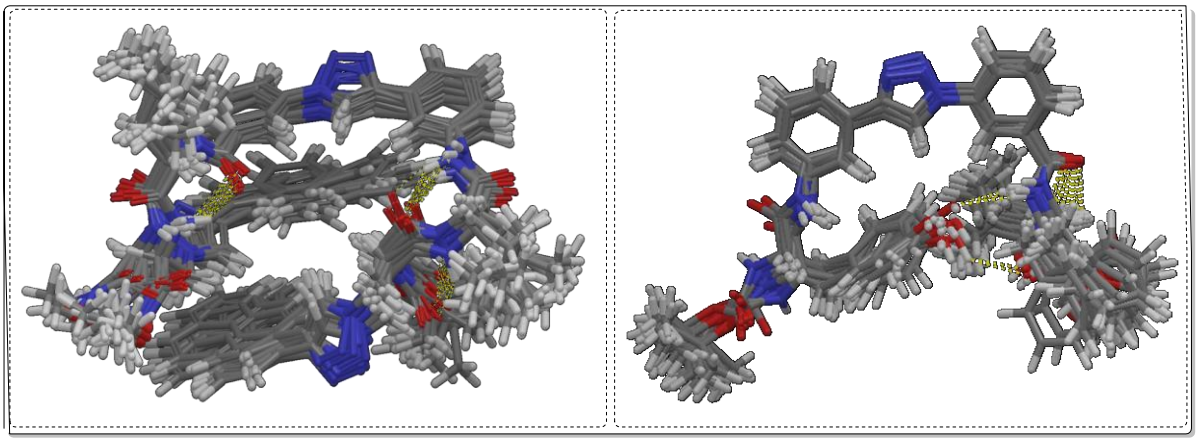


Figure S21. Well Converged structures (within 21 kJ/mole global minima) obtained from molecular dynamics simulation for the peptides (a) Pentapeptide **2** (**BocNH ^{TPy}Ala^{Do}- Leu-^{Ar}TAA-Leu- ^{TPy}Ala^{Do} -CONMe(OMe)**), (b) Tetrapeptide **3** (**BocNH-Tyr-^{Ar}TAA-Phe-Leu-CO_2Me**).

6. Study of Photophysical Property

6.1. UV-visible & fluorescence measurements method

All the UV –visible spectra of the peptides (10 µm) were measured in different solvents using a UV-Visible spectrophotometer using cell of 1 cm path length at 25 ^{0}C. All the sample solutions were prepared before an hour for the experiment.

All the sample solutions with same concentration as described in UV measurement experiments for fluorescence study. Emission spectra were obtained using a fluorescence spectrophotometer at 25 ^{0}C using 1 cm path length cell. The excitation wavelengths for the monomers were set at λ^{abs}_{max}. Time resolved fluorescence decays were measured using time resolved fluorescence spectrophotometer. The fluorescence quantum yields (Φ_f) were determined using quinine sulphate as a reference with the known Φ_f = 0.54 in 0.1 molar solution in sulphuric acid.

6.2. UV-visible and Fluorescence Spectra

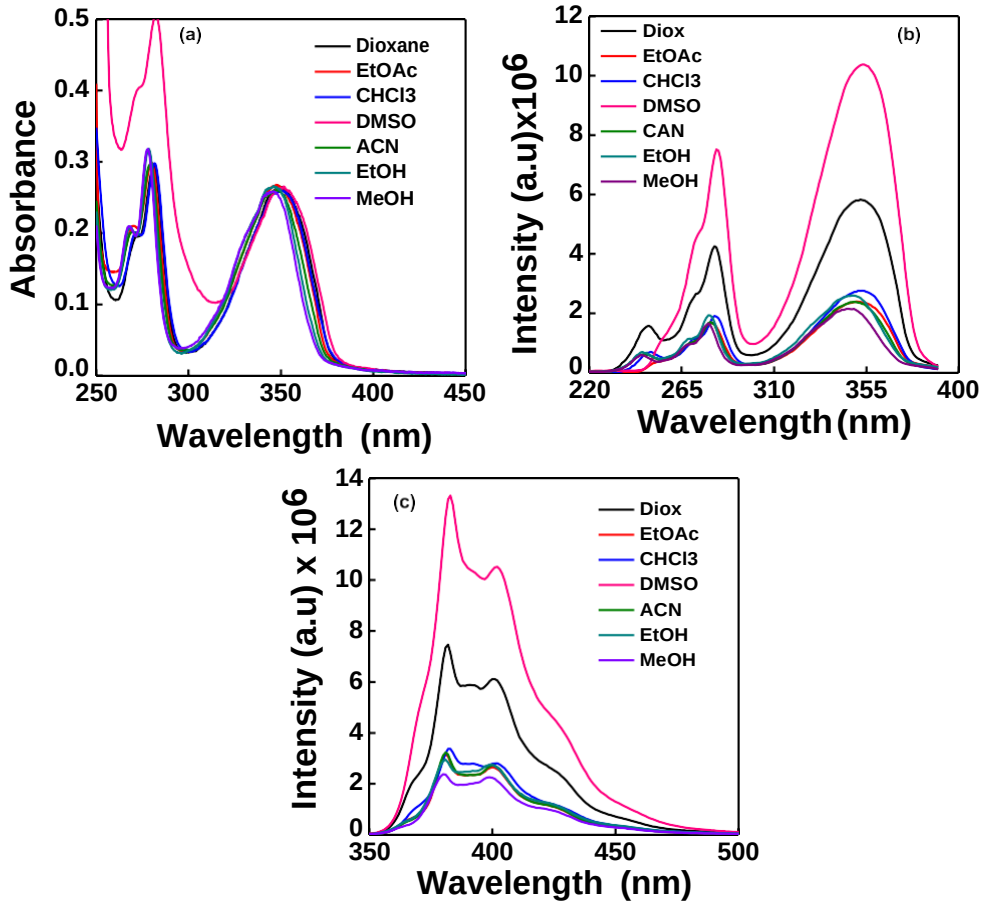


Figure S22. UV-Visible (a), Excitation spectra (b), Fluorescence spectra (c) of **Dipeptide 16** in different solvents (10 µM, r.t.; $\lambda_{ex} = \lambda_{max}$ (345 nm) of each solvent).

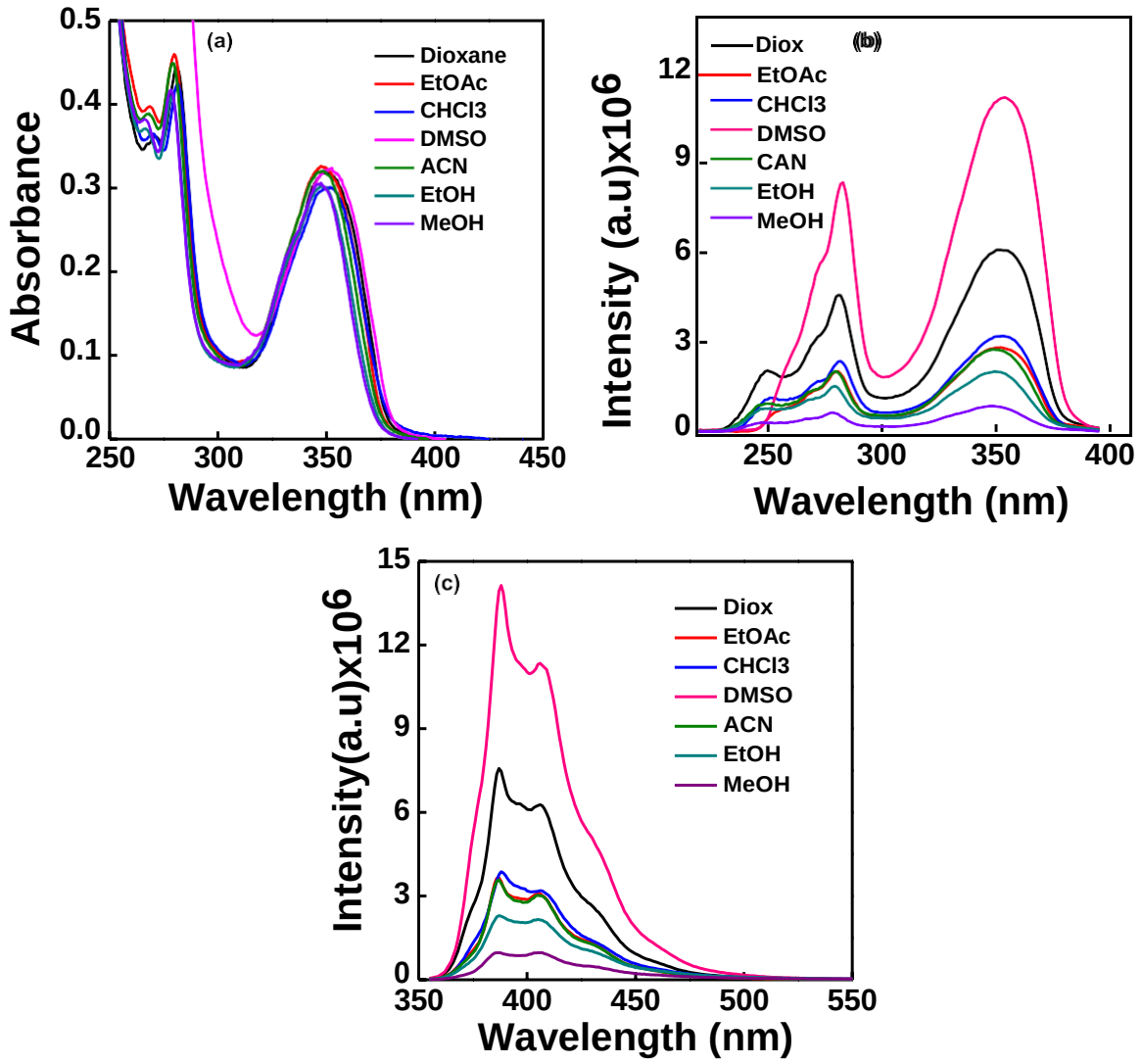


Figure S23. UV-Visible (a), Excitation spectra (b), Fluorescence spectra (c) of **Tripeptide 18** in different solvents (10 µM, r.t.; $\lambda_{ex} = \lambda_{max}$ (345 nm) of each solvent).

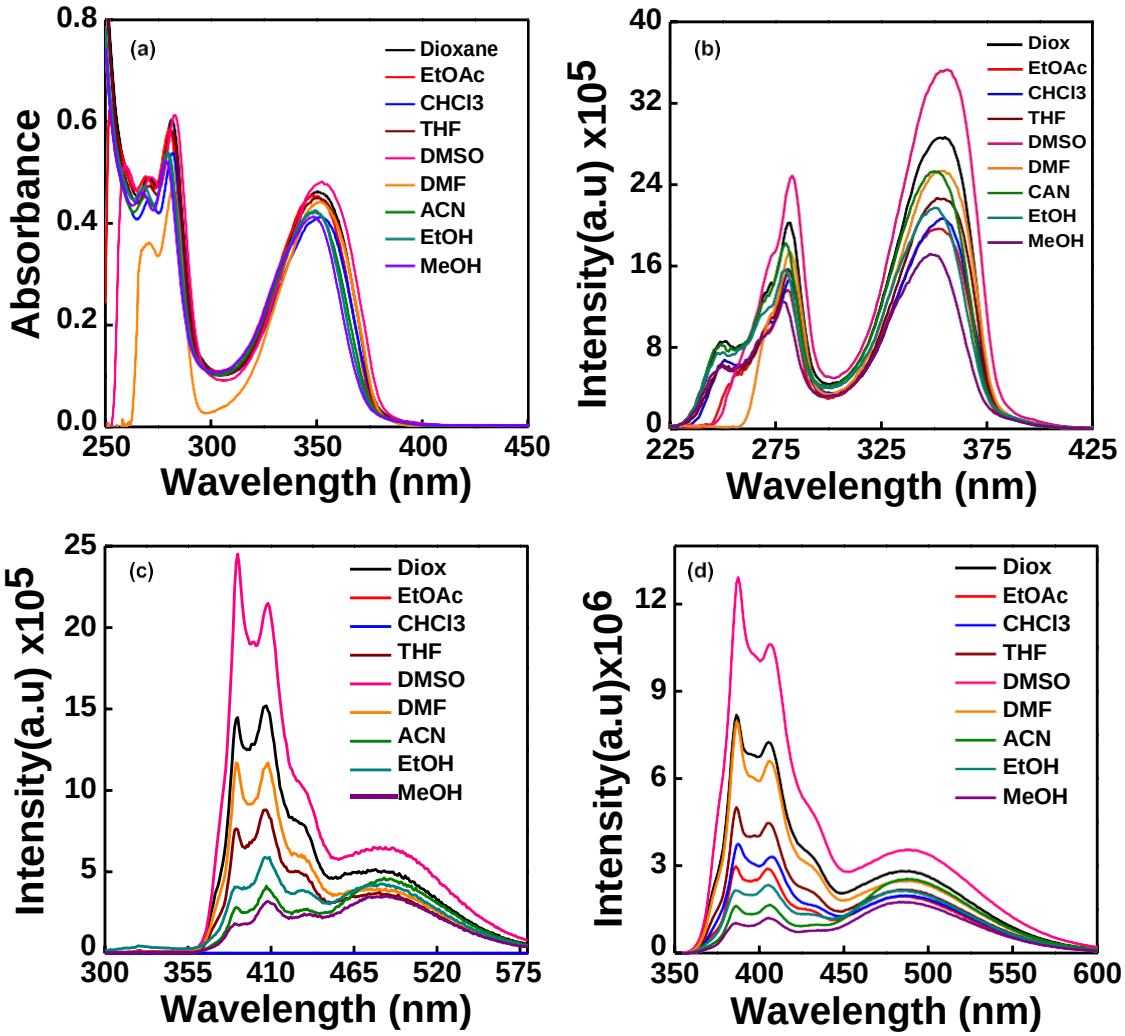


Figure S24. UV-Visible (a), Excitation spectra (b), Fluorescence spectra of **Pentapeptide 2** in different solvents (c) λ_{ex} = 290 nm, (d) λ_{ex} = 345 nm (10 µM, r.t.; of each solvent).

6.3. Photophysical Properties Summary of synthesized peptides 16, 18 & 2

Table S4: Summary table of photophysical properties of the **Dipep. 16**

Entry	Solvents	Δf	UV-Vis & Fluorescence		
			λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
Leu_^{TPy}Ala^{Do}_ Dipep. 16	Dioxane	0.021	281, 349	382, 400, 423	0.326
	$CHCl_3$	0.148	281, 350	382, 401, 422	0.158
	EtOAc	0.201	279, 347	381, 499, 421	0.133
	DMSO	0.265	282, 350	383, 402, 425	0.56
	EtOH	0.290	278, 345	380, 400, 421	0.140
	ACN	0.307	279, 345	380, 400, 422	0.137
	MeOH	0.309	278, 344	380, 399, 418	0.110

Table S5: Summary table of photophysical properties of the **Tripep. 18**

Entry	Solvents	Δf	UV-Vis & Fluorescence		
			λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
^{Ar}TAA _leu_^{TPy}Ala^{Do} _Tripep. 18	Dioxane	0.021	281, 349	387, 406, 429	0.303
	$CHCl_3$	0.148	281, 351	387, 406, 430	0.173
	EtOAc	0.201	280, 348	386, 406, 426	0.139
	DMSO	0.265	280, 350	387, 406, 428	0.57
	EtOH	0.290	279, 348	387, 405, 427	0.114
	ACN	0.307	279, 347	386, 406, 428	0.138
	MeOH	0.309	278, 346	386, 407, 430	0.051

Table S6: Summary table of photophysical properties of the **Pentapep. 2**

Entry	Solvents	Δf	UV-Vis & Fluorescence		
			λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
^{TPy}Ala^{Do}_leu_^{Ar}TAA_leu_^{TPy}Ala^{Do}_Pentapep. 2	Dioxane	0.021	281, 351	387, 406, 426, 488	0.358
	$CHCl_3$	0.148	281, 351	388, 407, 428, 486	0.225
	EtOAc	0.201	280, 349	388, 407, 428, 485	0.183
	THF	0.210			0.246
	DMSO	0.265	282, 352	387, 406, 428, 488	0.498
	DMF	0.275			0.338
	EtOH	0.290	279, 349	324, 386, 406, 426, 485	0.192
	ACN	0.307	279, 349	386, 406, 487	0.191
	MeOH	0.309	278, 348	385, 406, 485	0.140

6.4. Study of Fluorescence Resonance Energy Transfer (FRET) in Pentapeptide 2.

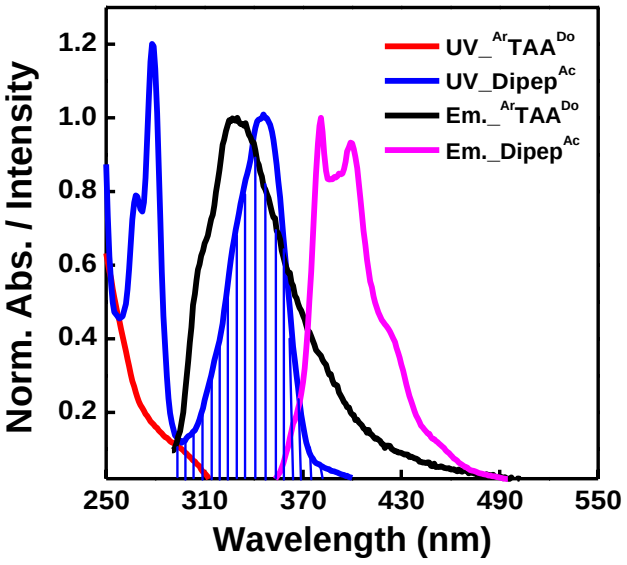


Figure S25. (a) Overlapping of emission spectra of **triazolyl aromatic scaffold ^{Ar}TAA, 1** (act as a FRET donor) and the absorption spectra of **Dipeptide containing fluorescent amino acid ^{TPy}Ala^{Do}, 16** (act as a FRET acceptor) (10 μM each, r.t.; λ_{ex} = 290 nm in EtOH).

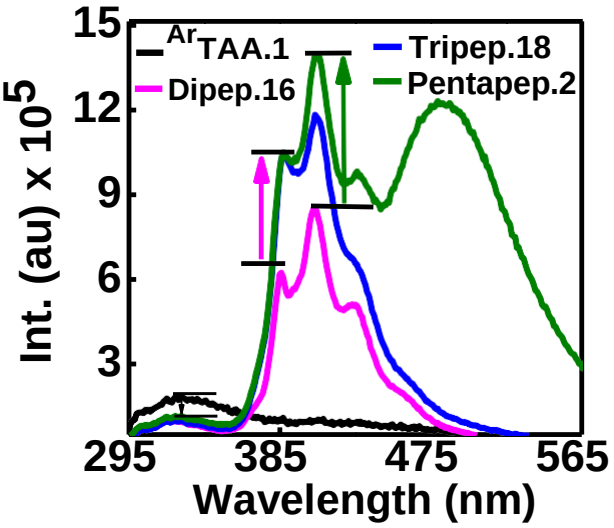


Figure S26. Fluorescence spectra of individual donor (**[Ar]TAA, 1**) and acceptor amino acid **dipeptide 16** and the **Pentapeptide 2** which contain these two moieties. In **Pentapeptide 2**, acceptor emission increased by almost 1.8 times in presence of donor, whereas the donor emission decreases almost 1.6 times of the individual donor emission. This change in fluorescence intensity is visual evidence of FRET (10 μM each, r.t.; λ_{ex} = 290 nm in EtOH).

Calculation of the Forster distance and FRET efficiency

The fluorescence resonance energy transfer (FRET) and the Förster distance for the FRET were calculated using the following three equations. The efficiency of energy transfer, *E*, was calculated using the equation (1)

$$E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{F}{F_0} \quad \text{.....(1)}$$

where F and F_0 are the fluorescence intensity of donor in the presence and absence of acceptor, r is the distance between donor and the acceptor and R_0 is the critical distance when the energyv transfer efficiency is 50% . The Förster distance R_0 (Å) was calculated by the following equation (2)

$$R_0 = [8.79 \times 10^{-5} \kappa^2 n^{-4} \Phi_D J(\lambda)]^{1/6} \quad \textbf{.....(2)}$$

where κ^2 is the orientation, n is the refractive index of the medium, Φ_D is the quantum yield of the donor in the absence of acceptor $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor given by the following equation (3)

$$J = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \quad \textbf{.....(3)}$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$ with the total intensity normalized to unity. $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor as a function of wavelength (λ).

Using the values of $\kappa^2 = 2/3$, $n = 1.36$, $\Phi_D = 0.068$, and the obtained overlap integral, $J(\lambda)$ $=5.9255 \times 10^{16}$

the R_0 and r values were calculated which were found to be $R_0 = 58$ Å and $r = 63$ Å. R_0 is the critical distance when the energy transfer efficiency is 50 % and r is the distance between the donor and acceptor.

Energy Transfer efficiency (E) = 1- F/F_0 = 38 %. F and F_0 are the fluorescence intensity of donor in the presence and absence of acceptor.

Picture of Di, Tri and Pentapeptide under Fluorescence light

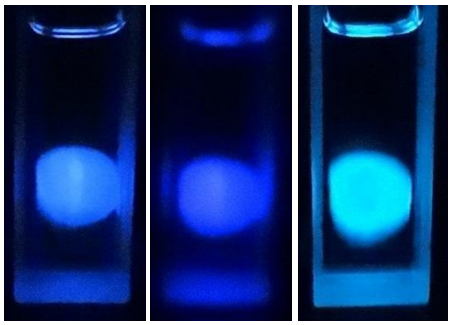


Figure S27. Picture under fluorescence light (λ_{ex} = 280 nm) of **(a)** Dipeptide **16, (b)** Tripeptide **18** and **(c)** Pentapeptide **2** in ethanol solvents.

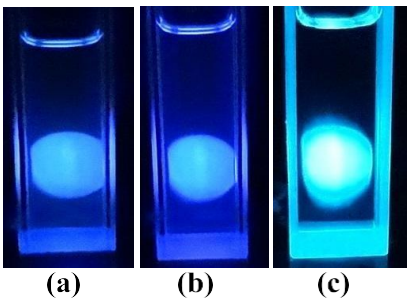


Figure S28. Picture under fluorescence light (λ_{ex} = 350 nm) of **(a)** Dipeptide **16, (b)** Tripeptide **18** and and **(c)** Pentapeptide **2** in ethanol solvents.

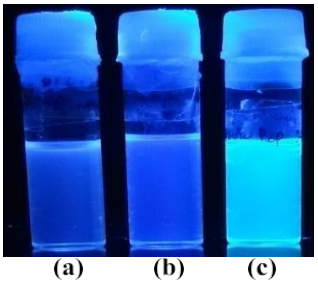


Figure S29. Picture under transilluminator (λ_{ex} = 365 nm) of **(a)** Dipeptide **16, (b)** Tripeptide **18** and and **(c)** Pentapeptide **2 in** ethanol solvent.

6.5. Life time table and Trace

Table S7: Summary table of fluorescence lifetimes of the peptides **1**, **18 & 2 at λ_{ex} = 290 nm**

Entry	Solvents	Φ_f	λ [nm]	τ_1 [ns]	τ_2 [ns]	$<\tau>$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
^{Ar}TAA. 1	**Dioxane**	0.0043	330	4.22 (100%)	------	4.22	0.01	2.36	0.93
	EtOH	0.07	330	3.65 (49%)	8.85 (51%)	6.21	0.112	1.5	1.03
	ACN	0.005	320	1.8 (100%)	------	1.8	0.027	5.53	0.85
	MeOH	0.004	330	3.48 (100%)	------	3.48	0.011	2.86	0.92
^{Ar}TAA_leu_^{TPy}Ala^{Do}_Tripep. 18	**Dioxane**	0.0015	330	2.68 (100%)	------	2.68	0.005	3.73	0.87
	EtOH	0.005	330	2.15 (14%)	6.7 (86%)	6.07	0.008	1.64	1.03
	ACN	0.001	320	1.21 (100%)	------	1.21	0.008	8.26	0.81
	MeOH	0.0011	330	3.36 (100%)	------	3.36	0.003	2.97	0.92
^{TPy}Ala^{Do}_leu_^{Ar}TAA_ leu_^{TPy}Ala^{Do}_ Pentapep. 2	**Dioxane**	0.001	330	3.83 (100%)	----	3.83	0.002	2.61	0.90
	EtOH	0.004	330	2.05 (15%)	6.38 (85%)	5.69	0.007	1.75	0.98
	ACN	0.0006	320	1.8 (100%)	----	1.8	0.003	5.51	0.88
	MeOH	0.0007	330	3.23 (100%)	----	3.23	0.002	3.09	0.92

For lifetimes of the fluorescent amino acids λ_{ex} = 290 nm; Concentration of each fluorescent amino acid = 10 μM; $<\tau>$, k_f, and k_{nr} are weighted means from the biexponential fits: $<\tau> = 1/(\alpha_1/\tau_1 + \alpha_2/\tau_2)$, $k_f = \Phi_f/<\tau>$, and $k_{nr} = (1- \Phi_f)/<\tau>$.

Table S8: Summary table of fluorescence lifetimes of the peptides **16**, **18 & 2 at λ_{ex} = 290 nm**

Entry	Solvents	Φ_f	λ_{em} [nm]	τ_1 [ns]	τ_2 [ns]	$<\tau>$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
Leu_^{TPy}Ala^{Do}_ Dipep. 16	**Dioxane**	0.242	405	33.05 (100%)	----	33.05	0.073	0.229	1.02
	EtOH	0.152	405	20.83 (100%)	----	20.83	0.072	0.407	0.99
	ACN	0.126	405	15.78 (100%)	----	15.78	0.079	0.554	0.97
	MeOH	0.113	405	17.40 (100%)	----	17.40	0.065	0.51	0.95
^{Ar}TAA^{Do} _leu_^{TPy}Ala^{Ac}_ Tripep**. 24**	**Dioxane**	0.197	405	33.25 (100%)	----	33.25	0.058	0.389	1.03
	EtOH	0.116	405	14.01 (100%)	----	14.01	0.046	0.748	0.92
	ACN	0.108	405	16.53 (100%)	----	16.53	0.047	1.30	0.98
	MeOH	0.055	405	7.1 (100%)	----	7.1	0.037	1.22	1.01
^{TPy}Ala^{Do}_leu_^{Ar} TAA_ leu_^{TPy}Ala^{Do}_ Pentapep. 2	**Dioxane**	0.130	405	4.8 (5%)	23.3 (95%)	22.34	0.058	0.038	0.94
	EtOH	0.059	405	4.69 (18%)	14.34 (82%)	12.58	0.046	0.074	0.91
	ACN	0.035	405	4.34 (50%)	10.65 (50%)	7.42	0.047	1.30	0.85
	MeOH	0.03	405	3.7 (39%)	10.7 (61%)	7.95	0.037	1.22	0.91
^{TPy}Ala^{Do}_leu_^{Ar} TAA_ leu_^{TPy}Ala^{Do}_ Pentapep. 2	**Dioxane**	0.147	485	24.53 (35%)	32.77 (65%)	42.64	0.034	0.20	1.05
	EtOH	0.119	485	25.9 (100%)	----	25.9	0.045	0.340	1.05
	ACN	0.115	485	17.36 (100%)	----	17.36	0.066	0.510	0.92
	MeOH	0.095	485	19.03 (100%)	----	19.03	0.049	0.476	0.99

For lifetimes of the fluorescent amino acids λ_{ex} = 290 nm; Concentration of each fluorescent amino acid = 10 μM; $<\tau>$, k_f, and k_{nr} are weighted means from the biexponential fits: $<\tau> = 1/(\alpha_1/\tau_1 + \alpha_2/\tau_2)$, $k_f = \Phi_f/<\tau>$, and $k_{nr} = (1- \Phi_f)/<\tau>$.

Table S9: Summary table of fluorescence lifetimes of the peptides **16**, **18 & 2 at λ_{ex} = 336 nm**

Entry	Solvents	Φ_f	λ [nm]	τ_1 [ns]	τ_2 [ns]	$<\tau>$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
Leu_^{TPy}Ala^{Do}_ Dipep. 16	**Dioxane**	0.326	405	33.65 (100%)	----	33.65	0.096	0.200	1.02
	EtOH	0.140	405	21.39 (100%)	----	21.39	0.065	0.402	1.03
	ACN	0.137	405	15.89 (100%)	----	15.89	0.086	0.543	0.92
	MeOH	0.115	405	18.59 (100%)	----	18.59	0.059	0.479	1.00
^{Ar}TAA_leu_^{TPy}Ala^{Do}_Tripep. 18	**Dioxane**	0.303	405	33.14 (100%)	----	33.14	0.091	0.210	0.99
	EtOH	0.114	405	14.39 (100%)	----	14.39	0.079	0.616	0.96
	ACN	0.138	405	16.51 (100%)	----	16.51	0.083	0.522	0.97
	MeOH	0.051	405	5.1 (50%)	10.45 (50%)	7.72	0.066	0.123	0.95
^{TPy}Ala^{Do} _leu_^{Ar}TAA_ leu_^{TPy}Ala^{Do}_ Pentapep. 2	**Dioxane**	0.171	405	7.56 (6%)	23.68 (94%)	22.77	0.075	0.346	0.99
	EtOH	0.057	405	5.2 (20%)	14.28 (80%)	12.45	0.045	0.757	0.93
	ACN	0.038	405	4.22 (50%)	10.31 (50%)	7.32	0.051	0.131	0.94
	MeOH	0.03	405	4.44 (48%)	12.15 (52%)	8.38	0.003	0.116	0.92
^{TPy}Ala^{Do}_leu_^{A} ^{r}TAA_ leu_^{TPy}Ala^{Do}_ Pentapep. 2	**Dioxane**	0.192	485	23.45 (30%)	31.52 3(7%)	37.43	0.051	0.216	1.05
	EtOH	0.136	485	1.54 (4%)	23.19 (96%)	22.42	0.060	0.385	1.01
	ACN	0.150	485	16.82 (100%)	----	16.82	0.089	0.505	0.96
	MeOH	0.111	485	18.53 (100%)	----	18.53	0.059	0.48	0.94

For lifetimes of the fluorescent amino acids λ_{ex} = 336 nm; Concentration of each fluorescent amino acid = 10 μM; $<\tau>$, k_f, and k_{nr} are weighted means from the biexponential fits: $<\tau> = 1/(\alpha_1/\tau_1 + \alpha_2/\tau_2)$, $k_f = \Phi_f/<\tau>$, and $k_{nr} = (1- \Phi_f)/<\tau>$.

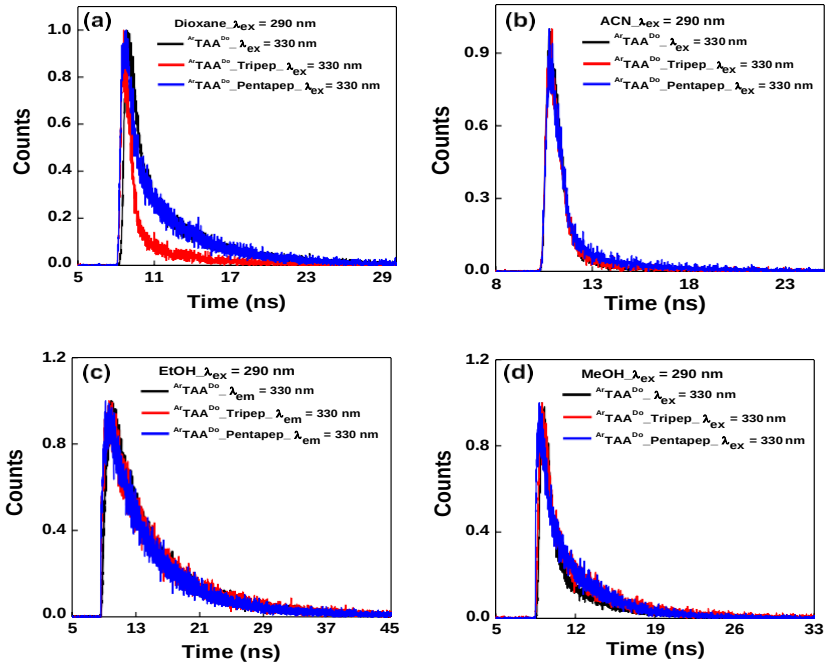


Figure S30. Time resolved fluorescence spectra of ^{Ar}**TAA , Tripeptide 18** and **pentapeptide 2** using 290 LED in (a) Dioxane (λ_{ex} = 290 nm, λ_{em} = 330 nm); (b) ACN (λ_{ex} = 290 nm, λ_{em} = 330 nm); (c) ethanol (λ_{ex} = 290 nm, λ_{em} = 330 nm); (d) methanol (λ_{ex} = 290 nm, λ_{em} = 330 nm);

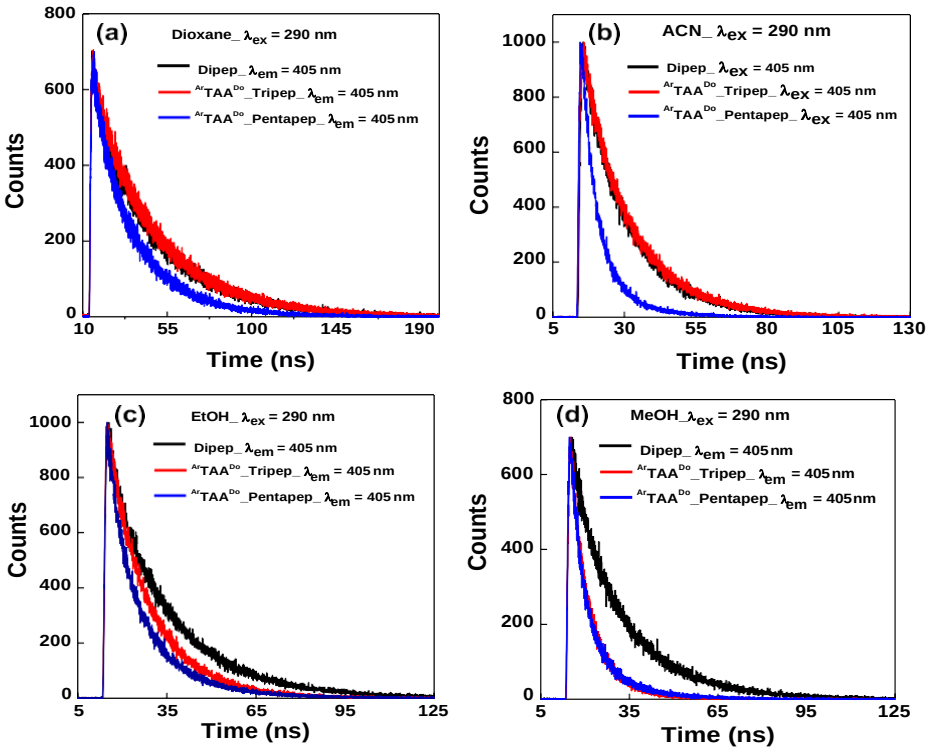


Figure S31. Time resolved fluorescence spectra of **Dipeptide 16 , Tripeptide 18** and **pentapeptide 3** using 290 LED in (a) Dioxane (λ_{ex} = 290 nm, λ_{em} = 405 nm); (b) ACN (λ_{ex} = 290 nm, λ_{em} = 405 nm); (c) ethanol (λ_{ex} = 290 nm, λ_{em} = 405 nm); (d) methanol (λ_{ex} = 290 nm, λ_{em} = 405 nm).

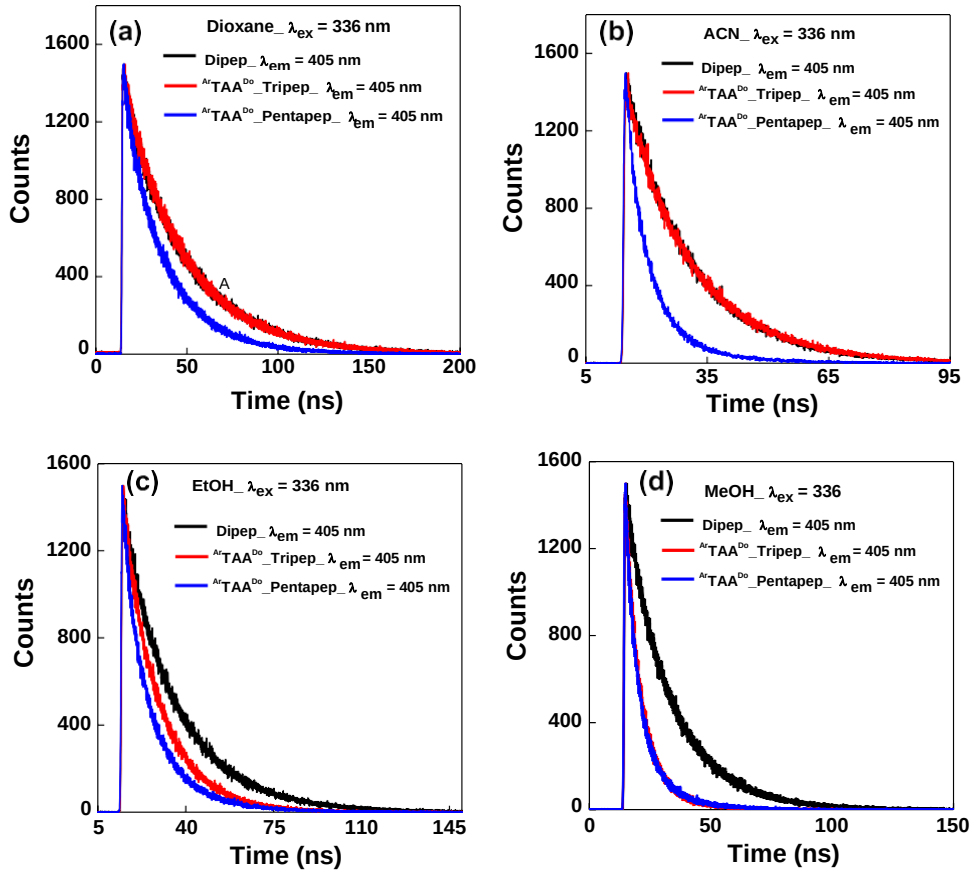


Figure S32. Time resolved fluorescence spectra of **Dipeptide 16 , Tripeptide 18** and **pentapeptide 2** using 336 LED in (a) Dioxane (λ_{ex} = 336 nm, λ_{em} = 405 nm); (b) ACN (λ_{ex} = 336 nm, λ_{em} = 405 nm); (c) ethanol (λ_{ex} = 336 nm, λ_{em} = 405 nm); (d) methanol (λ_{ex} = 336 nm, λ_{em} = 405 nm).

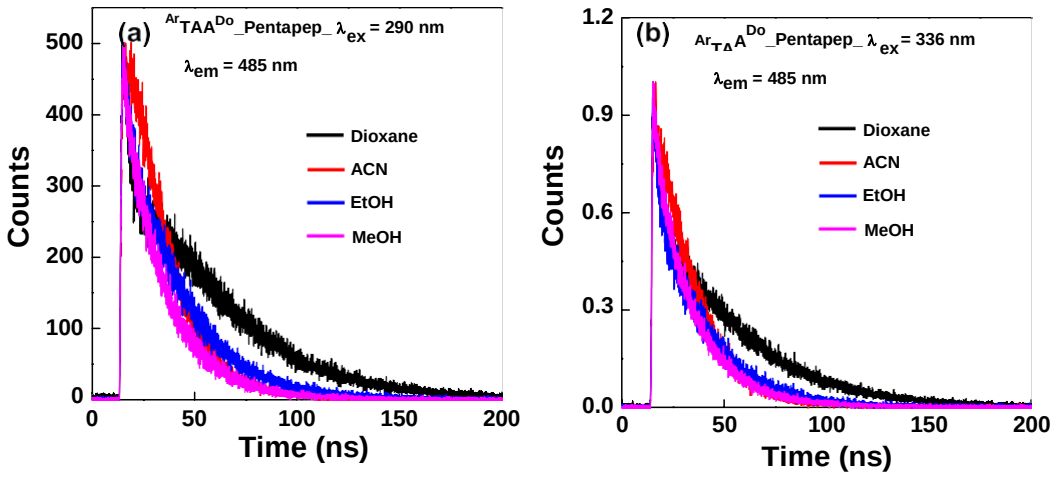


Figure S33. Time resolved fluorescence spectra of **Pentapeptide 2** using (a) 290 nm (λ_{ex} = 290 nm, λ_{em} = 485 nm); (b) 336 nm LED in different solvent (λ_{ex} = 336 nm, λ_{em} = 485 nm).

7. Studies on the interaction of Pentapeptide 2 with BSA

7.1 General experimental

7.1.1 Materials

BSA, Na_2HPO4 and $NaH_2PO_4.H_2O$ (for preparation of phosophate buffer) were purchased from Merck, India and used without further purification. Water was taken from a Milli-Q purification system. All solutions were prepared before 1 hour of experiments done. The probe molecules (Pentapeptide **2**) was synthesized and purified according to the procedure described.

7.1.2 Preparation of BSA Solution

Phosphate buffer of pH 7.0 was used to prepare the solution of BSA (Merck). A 250 μM of stock BSA solution was prepared by dissolving 0.0222 gm of BSA in 1.28 mL phosphate buffer (20 mM) of pH 7.0. From that stock solution sub stock of 1000 μM BSA was prepared. The compound stock solution was prepared in DMF because of the poor solubility in water.
0.6 mg of Pentapeptide was dissolved in 1 mL DMF to make a stock probe solution of concentration 1092.4 μM.

7.1.3 General experimental on interaction study of BSA by photophysical study:

All the spectral measurements were carried out at room temperature. To study the interaction of compound with BSA, an aqueous solution of peptide (4 μM for peptide) was titrated with different concentrations of BSA (ranging from 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28 μM). The total volume of the final solution for each sample was 3 mL. The % of DMF content did not exceed 3%. The presence of 2% DMF does not induce structural changes to biomolecules. Each sample solution was mixed well before spectral measurements.

7.1.4 UV-Visible Study

The UV–Visible absorbance measurements were performed using Shimadzu UV- 2550 UV Visible spectrophotometer with a cell of 1 cm path length at 298 K. All the UV-Visible studies were carried out in 20 mM phosphate buffer of pH 7.02 containing solution at 298 K. 2 % DMF was used to solubilize the probe. The measurements were taken in absorbance mode and the absorbance values of the sample solutions were measured in the wavelength regime of 200– 700 nm. All the experiments were carried out with freshly prepared sample solutions.

7.1.5 Fluorescence Study

All fluorescence and steady state anisotropy experiments were performed using a Fluoromax 4 spectrophotometer with a cell of 1 cm path length at 298 K. The excitation wavelength for probe (Pentapeptide **2**) was set at 280 nm, 350 nm and emission spectra were measured in the wavelength regime of 290–650 nm. Steady state anisotropy of the solutions was measured using Fluoromax 4 spectrophotometer. Time resolved fluorescence anisotropy decay were measured using Life Space-II (Edinburgh Instruments) time resolved fluorescence spectrophotometer. The fluorescence quantum yields (Φ) were determined using quinine sulphate as a reference with the known Φ (0.54) in 0.1 molar solution in sulphuric acid.

7.2. Study of UV-visible and fluorescence photophysical properties of probe 2 (Pentapeptide) in presence BSA

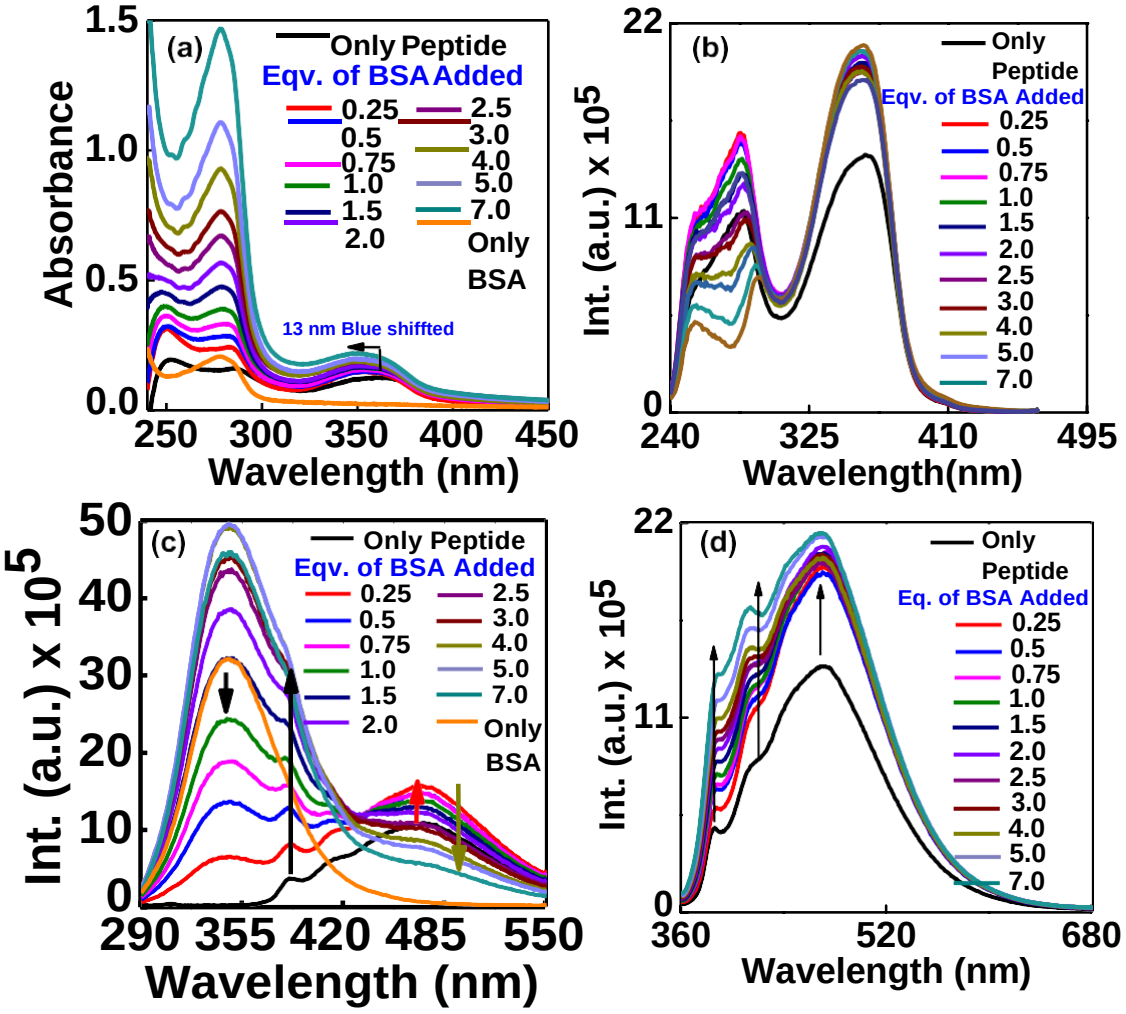


Figure S34: (a) UV-visible and (b) excitation spectra (λ_{ex} = 470 nm), (c) emission spectra (λ_{ex} = 280 nm) and (d) emission spectra (λ_{ex} = 350 nm) of Pentapeptide **2** in presence of increasing BSA concentration at 298K. [Pentapeptide] = 4 μM and [BSA] = 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28 μM

Table 10: Summary table of photophysical properties of the peptide **2** in BSA

Entry	Solvents	UV-Vis & Fluorescence		
		λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
Only BSA	Buffer (pH=7.0)	278	346	0.058
Only Penta peptide	Buffer (pH=7.0)	251, 284, 364	385, 411, 472	0.073
1 eqv.Peptide +0.25 eqv.BSA	Buffer (pH=7.0)	283, 354	342, 385, 414, 472	0.083
1 eqv.Peptide +0.50 eqv.BSA	Buffer (pH=7.0)	282, 353	344, 384, 412, 471	0.083
1 eqv.Peptide +0.75 eqv.BSA	Buffer (pH=7.0)	280, 353	345, 484, 411, 472	0.084
1 eqv.Peptide +1.00 eqv.BSA	Buffer (pH=7.0)	281, 352	345, 383, 410, 473	0.078
1 eqv.Peptide +1.50 eqv.BSA	Buffer (pH=7.0)	280, 350	346, 382, 410, 472	0.079
1 eqv.Peptide +2.0 eqv.BSA	Buffer (pH=7.0)	279, 350	346, 381, 409, 411	0.079

7.3. Benesi-Hildebrand plot to Evaluation Binding Constant

The association constant (K) of the fluorophore with BSA was determined by a Benesi-Hildebrand plot using the following equation 1,

$$\frac{1}{(I-I_0)} = \frac{1}{(I_\infty - I_0)} + \frac{1}{(I_\infty - I_0)K[BSA]} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Where I_0, I and I_α are the emission intensities of pentapeptide in the absence of BSA, in the presence of an inetermediate and at infinite concentration of BSA respectively. From the slop of the $1/(I - I_0)$ *vs.*$1/[BSA]$ plot of equation 1, binding constant K was determined and its value is $1.8 \times 10^5\ M^{-1}$. ΔG = -7.16 Kcal.

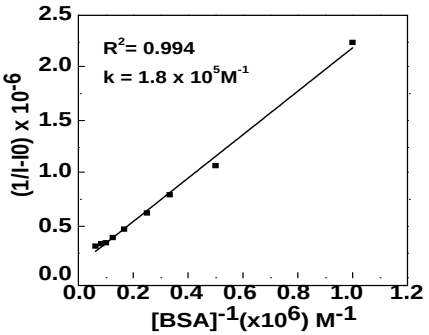


Figure S35: Benesi- Hildebrand plot of Pentapeptide **2** fluorophore in presence of increasing BSA concentration. . [pentapeptide] = 4 µM and [BSA] = 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28 µM.

Isothermal titration calorimetry measurement

ITC tritation of BSA with pentapeptide **2** was performed at 298 K in phosphate bufffer pH 7.02. The protien solution (1 µm) was taken in a sample cell and titrant solution of pentaptide **2** (15 µm) was fitted in the syringe. The resulting data was fitted with two site model. The binding constant (K), binding stoichiomatry (N), enthalpy change (DH), entropy change (DS) and change in free energy were obtained from the fitted data (**Table 11**).

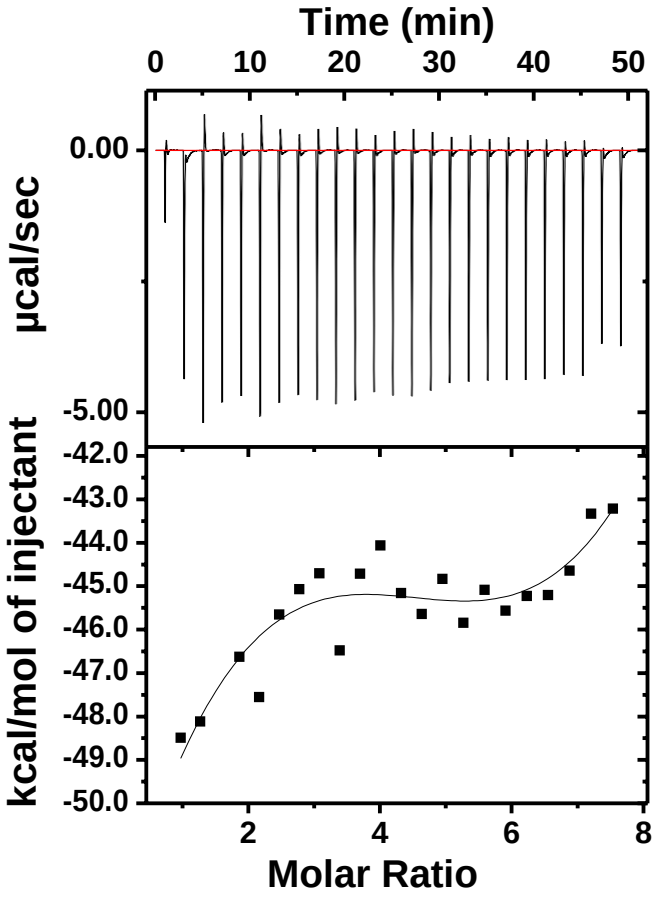


Figure 36: Plot of isothermal titration calorimetry.

Table 11: Thermodynamical parameter of **BSA-peptide** system from ITC measurement

Sequential Binding				
K_1 (M^{-1})	N_1	ΔH_1 (cal/mol)	ΔS_1 (cal/mol/deg)	ΔG_1 (Kcal)
9.81 x 10^3	---	-4.694 x 10^5	-1.56 x 10^3	- 5.44
K_2 (M^{-1})	N_2	ΔH_2 (cal/mol)	ΔS_2 (cal/mol/deg)	ΔG_2 (Kcal)
2.33 x 10^3	---	-2.924 x 10^6	-9.79 x 10^3	- 4.59
Two sites binding				
K_1 (M^{-1})	N_1	ΔH_1 (cal/mol)	ΔS_1 (cal/mol/deg)	ΔG_1 (Kcal)
1.78 x 10^4	0.939	4.106 x 10^6	1.38 x 10^4	-5.8
K_2 (M^{-1})	N_2	ΔH_2 (cal/mol)	ΔS_2 (cal/mol/deg)	ΔG_2 (Kcal)
1.43 x 10^4	0.946	-5.349 x 10^6	-1.79 x 10^4	- 5.6

7.4. FRET study between Pentapeptide_2 and BSA Protein

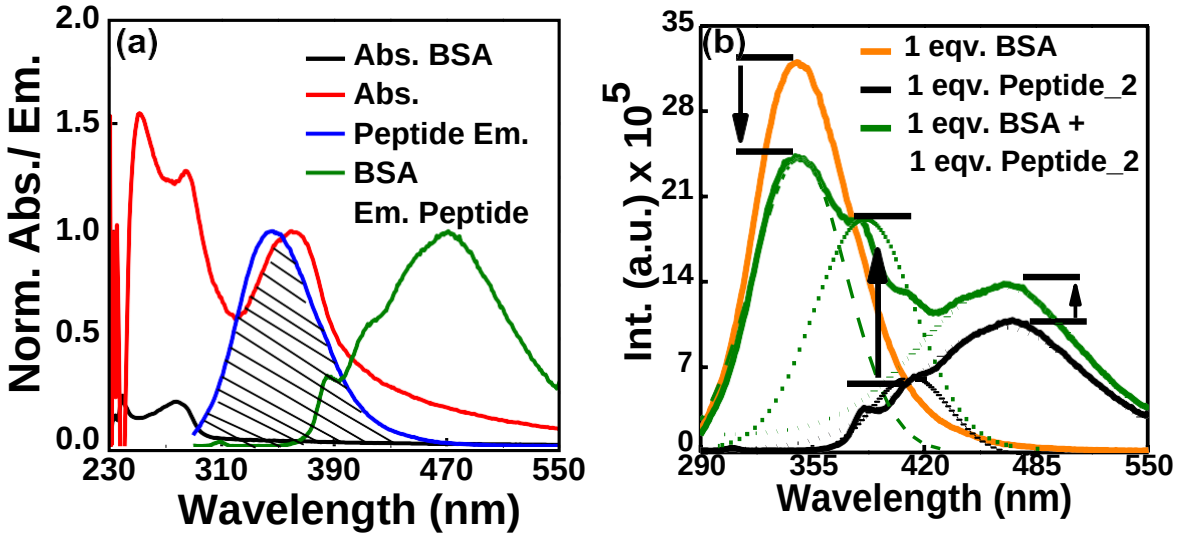


Figure S37. (a) Overlaping of emission spectra of **BSA** (act as a FRET donor) and the absorption spectra of **Pentapeptide 2** (act as a FRET acceptor) (4 μM each, r.t.; λ_{ex} = 280 nm in Buffer). (b) Fluorescence spectra of individual donor (**BSA**) and acceptor **Pentapeptide 2** and 1 eqv. **BSA-Pentapep. 2**. In **Pentapeptide 2**, acceptor emission increased by almost 2.2 times in presence of donor, whereas the donor emission decreases almost 1.3 times of the individual donor emission. This change in fluorescence intensity is visual evidence of FRET (4 μM each, r.t.; λ_{ex} = 280 nm in buffer).

Calculation of the Forster distance and FRET efficiency

The fluorescence resonance energy transfer (FRET) and the Förster distance for the FRET were calculated using the following three equations. The efficiency of energy transfer, *E*, was calculated using the equation (1)

$$E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{F}{F_0} \quad \text{.....(1)}$$

where *F* and F_0 are the fluorescence intensity of donor in the presence and absence of acceptor, *r* is the distance between donor and the acceptor and R_0 is the critical distance when the energyv transfer efficiency is 50% . The Förster distance R_0 (Å) was calculated by the following equation (2)

$$R_0 = [8.79 \times 10^{-5} \kappa^2 n^{-4} \Phi_D J(\lambda)]^{1/6} \quad \textbf{.....(2)}$$

where κ^2 is the orientation, n is the refractive index of the medium, Φ_D is the quantum yield of the donor in the absence of acceptor $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor given by the following equation (3)

$$J = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \quad \text{.....(3)}$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$ with the total intensity normalized to unity. $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor as a function of wavelength (λ).

Using the values of $\kappa^2 = 2/3$, $n = 1.33$, $\Phi_D = 0.058$, and the obtained overlap integral, $J(\lambda) = 4.33113 \times 10^{17}$

the R_0 and r values were calculated which were found to be $R_0 = 82.59$ Å and $r = 99$ Å. R_0 is the critical distance when the energy transfer efficiency is 50 % and r is the distance between the donor and acceptor.

Energy Transfer efficiency (E) = 1- F/F_0 = 25 %. F and F_0 are the fluorescence intensity of donor in the presence and absence of acceptor.

Picture of Pentapeptide 2 and Pentapeptide 2 - BSA under Fluorescence light

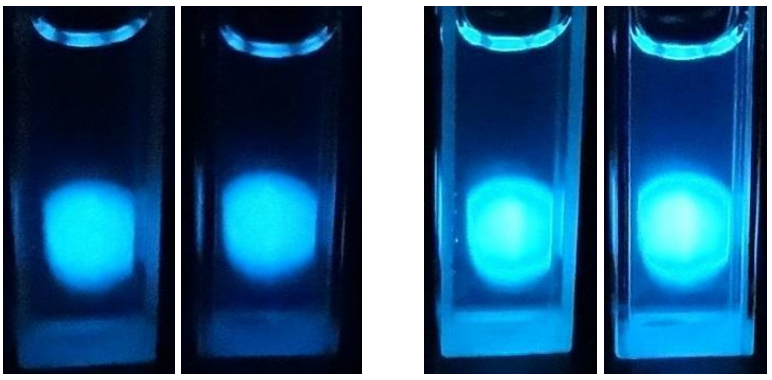
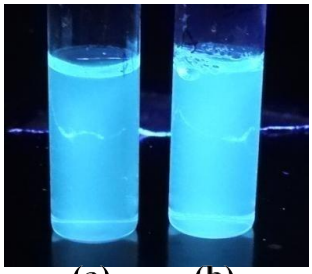


Figure S38. Picture under fluorescence light (λ_{ex} = 280 nm) of **(a)** Pentapeptide **2, (b)** Pentapeptide **2 –BSA** and λ_{ex} = 350 nm **(c)** Pentapeptide **2 (d)** Pentapeptide **2 –BSA** in phosphate buffer.



(a) **(b)**

Figure S39. Picture under transilluminator (λ_{ex} = 365 nm) of **(a)** Pentapeptide **2, (b)** Pentapeptide **2 –BSA** in phosphate buffer.

7.5. Life time table and Trace

Table S12: Summary table of fluorescence lifetimes of the peptides **2** with increasing BSA concentation **at λ_{ex} = 290 nm**

Entry	Solvents	Φ_f	λ [nm]	τ_1 [ns]	τ_2 [ns]	$<\tau>$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
Only BSA	**Buffer P^H = 7**	0.058	350	4.03 (35%)	7.00 (65%)	6.51	0.097	1.57	1.01
1 eqv peptide+ 0. 5 eqv BSA	**Buffer P^H = 7**	0.048	350	2.32 (24%)	5.57 (76%)	4.84	0.010	1.96	1.06
1 eqv peptide+ 1.00 eqv BSA	**Buffer P^H = 7**	0.049	350	2.82 (17%)	6.42 (83%)	5.79	0.077	1.64	1.01
1 eqv peptide+ 2.00 eqv BSA	**Buffer P^H = 7**	0.045	350	2.99 (17%)	6.49 (83%)	5.89	0.066	1.63	0.98
Only Peptide 2	**Buffer P^H = 7**	0.030	410	2.85 (31%)	25.19 (68%)	19.74	0.020	0.486	1.06
1 eqv peptide+ 0.50 eqv BSA	**Buffer P^H = 7**	0.039	410	5.09 (42%)	27.58 (58%)	18.29	0.026	0.519	1.03
1 eqv peptide+ 1.00 eqv BSA	**Buffer P^H = 7**	0.036	410	5.97 (54%)	32.79 (45%)	18.17	0.024	0.525	1.05
1 eqv peptide+ 2.00 eqv BSA	**Buffer P^H = 7**	0.032	410	6.0 (63%)	26.90 (36%)	13.58	0.028	0.707	1.01
Only Peptide 2	**Buffer P^H = 7**	0.041	470	2.92 (33%)	13.79 (66%)	10.13	0.03	0.94	1.04
1 eqv peptide+ 0.5 eqv BSA	**Buffer P^H = 7**	0.046	470	3.25 (36%)	14.61 (63%)	10.48	0.046	0.906	1.04
1 eqv peptide+ 1 eqv BSA	**Buffer P^H = 7**	0.044	470	3.41 (34%)	14.49 (65%)	10.65	0.042	0.896	1.03
1 eqv peptide+ 2.00 eqv BSA	**Buffer P^H = 7**	0.044	470	3.40 (33%)	14.56 (67%)	10.84	0.035	0.886	1.03

For lifetimes of the fluorescent amino acids λ_{ex} = 290 nm; Concentration of the peptide = 10 μM; $<\tau>$, k_f, and k_{nr} are weighted means from the biexponential fits: $<\tau> = 1/(\alpha_1/\tau_1 + \alpha_2/\tau_2)$, $k_f = \Phi_f/<\tau>$, and $k_{nr} = (1- \Phi_f)/<\tau>$.

Table S13: Summary table of fluorescence lifetimes of the peptides **2** with increasing BSA concentation **at λ_{ex} = 375 nm**

Entry	Solvents	Φ_f	λ [nm]	τ_1 [ns]	τ_2 [ns]	$<\tau>$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
Only Peptide 3	**Buffer P^H = 7**	0.043	410	2.19 (38%)	9.8 (62%)	6.90	0.105	1.34	0.99
1 eqv peptide+ 0.50 eqv BSA	**Buffer P^H = 7**	0.049	410	2.53 (34%)	21.16 (66%)	14.74	0.056	0.622	1.09
1 eqv peptide+ 1.00 eqv BSA	**Buffer P^H = 7**	0.051	410	2.62 (33%)	21.66 (67%)	15.36	0.05	0.60	1.09
1 eqv peptide+ 2.00 eqv BSA	**Buffer P^H = 7**	0.052	410	2.92 (35%)	21.27 (65%)	14.74	0.053	0.624	1.07
Only Peptide 2	**Buffer P^H = 7**	0.048	470	3.86 (37%)	16.62 (62.7 %)	11.86	0.061	0.781	1.07
1 eqv peptide+ 0.50 eqv BSA	**Buffer P^H = 7**	0.055	470	3.79 (37%)	16.63 (63%)	11.85	0.070	0.773	1.04
1 eqv peptide+ 1.00 eqv BSA	**Buffer P^H = 7**	0.055	470	3.9 (38%)	17.34 (61%)	12.19	0.063	0.567	1.08
1 eqv peptide+ 2.00 eqv BSA	**Buffer P^H = 7**	0.056	470	3.88 (37%)	16.92 (62%)	12.09	0.065	0.761	1.08

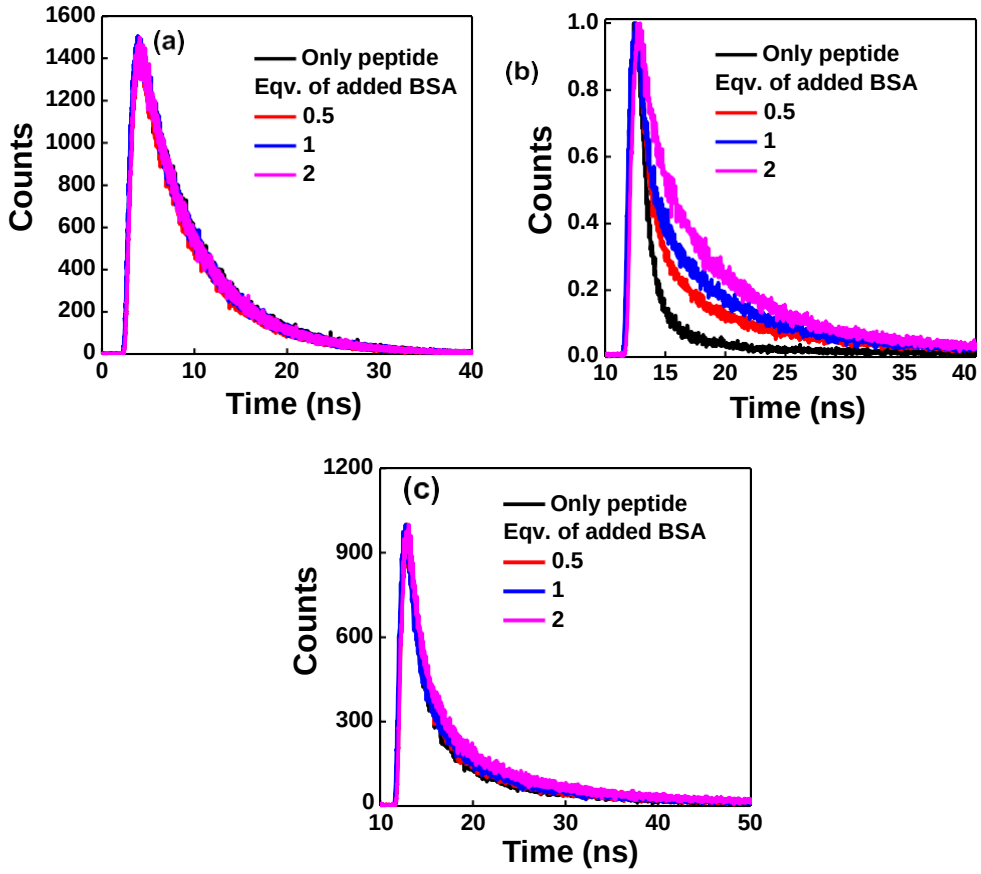


Figure S40. Time resolved fluorescence spectra of pentapeptide **2** tritated with increasing concentration of BSA using 290 LED in (a) λ_{ex} **= 290 nm,** λ_{em} **= 350 nm**; (b**)** λ_{ex} **= 290 nm,** λ_{em} **= 410 nm**. (c**)** λ_{ex} **= 290 nm,** λ_{em} **= 470 nm**. [peptide] = 10 µM and [BSA] = 5, 10, 15 and 20 µM.

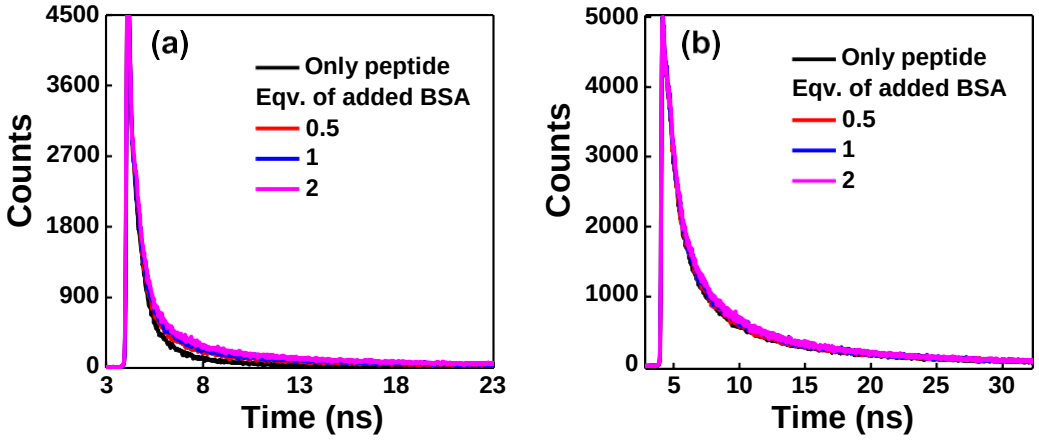


Figure S41. Time resolved fluorescence spectra of pentapeptide **2** tritated with increasing concentration of BSA using 375 LESER in (a) λ_{ex} **= 375 nm,** λ_{em} **= 410 nm**; (b) λ_{ex} **= 375 nm,** λ_{em} **= 470 nm**. [peptide] = 10 µM and [BSA] = 5, 10, 15 and 20 µM.

7.6. Steady State Anisotropy and Polarisation study

Table S14: Summary table of steady state anisotropy and polarisation of the peptides **2** with increasing BSA concentation at **λ_{ex} = 350 nm, λ_{em} = 385 nm and 472 nm**.

Entry	Solvents	Anisotropy		Polarisation	
		λ_{em} = 385 nm	λ_{em} = 472 nm	λ_{em} = 385 nm	λ_{em} = 472 nm
Only Penta peptide	Buffer (pH=7.0)	0.025	0.0153	0.038	0.02278
1 eqv.Peptide +0.25 eqv.BSA	Buffer (pH=7.0)	0.08887	0.02081	0.12764	0.03089
1 eqv.Peptide +0.50 eqv.BSA	Buffer (pH=7.0)	0.10623	0.01931	0.15131	0.02869
1 eqv.Peptide +0.75 eqv.BSA	Buffer (pH=7.0)	0.11312	0.02053	0.1606	0.03049
1 eqv.Peptide +1.00 eqv.BSA	Buffer (pH=7.0)	0.11492	0.02225	0.16302	0.033
1 eqv.Peptide +1.50 eqv.BSA	Buffer (pH=7.0)	0.12768	0.02412	0.18003	0.03575
1 eqv.Peptide +2.0 eqv.BSA	Buffer (pH=7.0)	0.13569	0.02882	0.19061	0.04261
1 eqv.Peptide +2.5 eqv.BSA	Buffer (pH=7.0)	0.13217	0.03047	0.18596	0.04502
1 eqv.Peptide +3.0 eqv.BSA	Buffer (pH=7.0)	0.14056	0.03123	0.19699	0.04612
1 eqv.Peptide +4.0 eqv.BSA	Buffer (pH=7.0)	0.13998	0.0337	0.19624	0.04971
1 eqv.Peptide +5.0 eqv.BSA	Buffer (pH=7.0)	0.14484	0.03464	0.20259	0.05108
1 eqv.Peptide +7.0 eqv.BSA	Buffer (pH=7.0)	0.15	0.04307	0.2093	0.06325

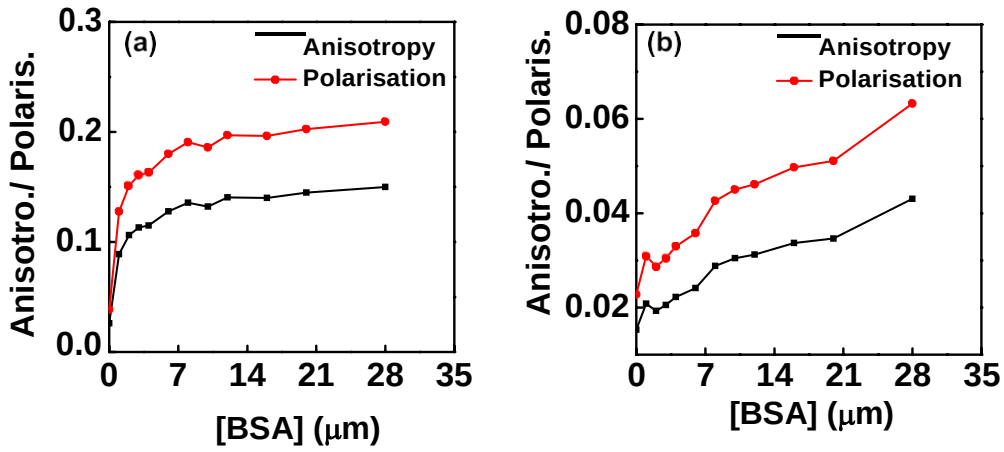


Figure S42: Steady-state anisotropy and polarisation plot (a) λ_{em} = 385 nm (b) λ_{em} = 472 nm of Pentapeptide fluorophore in presence of increasing BSA concentration. [Pentapeptide] = 4 μM and [BSA] = 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28 μM.

7.7. Study of Circular Dichroism Spectroscopy

Circular dichroism spectra were recorded using a CD spectropolarimeter with a cell path length of 1 mm at 25 ^{o}C. Samples concentration 10 μM .

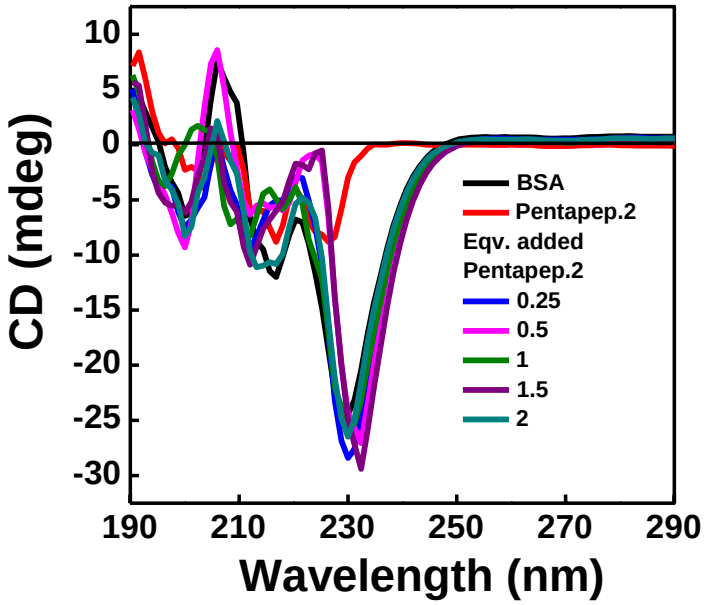


Figure S43. CD spectra of BSA in presence of increasing concentration of fluorophoric pentapeptide **2** in phosphate buffer (2% DMF). [BSA] = 10 μM and [Pentapeptide] = 2.5, 5, 10, 15, 20, μM.

7.8. Molecular docking calculation

Docking calculations were carried out using Autodock4 (*Bikadi, Hazai, 2009*). The amino acid sequence of BSA protein was observed from the NCBI website, http://www.ncbi.nlm.nih.gov/protein/CAA76847.1. The 3D model of the BSA protein

was built using the 3D structure 1AO6 chain 'A' as template using the ESyPred3D11 web

server. This template shares 72.4% identities with the BSA sequence. Following is the

BSA sequence which was used to generate the 3D model. >gi|3336842|emb|CAA76847.1| bovine serum albumin [Bos taurus]

http://www.rcsb.org/pdb/results/results.do?qrid=D081659C&tabtoshow=Current

MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYL

QQCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYG

DMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKFWGKYL

YEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLTSS

ARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCH

GDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLP

PLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATL

EECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRY

TRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEK

TPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQ

IKKQTALVELLKHKPKATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKL

VVSTQTALA

Among the various conformation of Peptide **2** with BSA from docking result, the docking pose (rank-2) was shown in **Figure S44**. This conformer showed that the peptide binds in hydrophobic pocket of subdomain IB in site-I. From the result it was also observed that the C-terminal pyrene triazole of peptide 2 is surrounded by the hydrophobic amino acid side chain. Thus it is conclude that the interaction between Peptide 2 and BSA is strongly hydrophobic in nature. It was also observed that the close proximity of tryptophan (Trp-134) with triazolyl pyrene also support the possibilities of energy transfer (FRET) process in the peptide 2 - BSA protein sysrem. The free energy of binding (ΔG) of Pentapeptide 2-BSA complex obtained from the docking calculation is -7.10 kcal mol^{-1} which is close to our experimental value -7.16 kcal mol^{-1} calculated from the Benesi–Hildebrand plot.

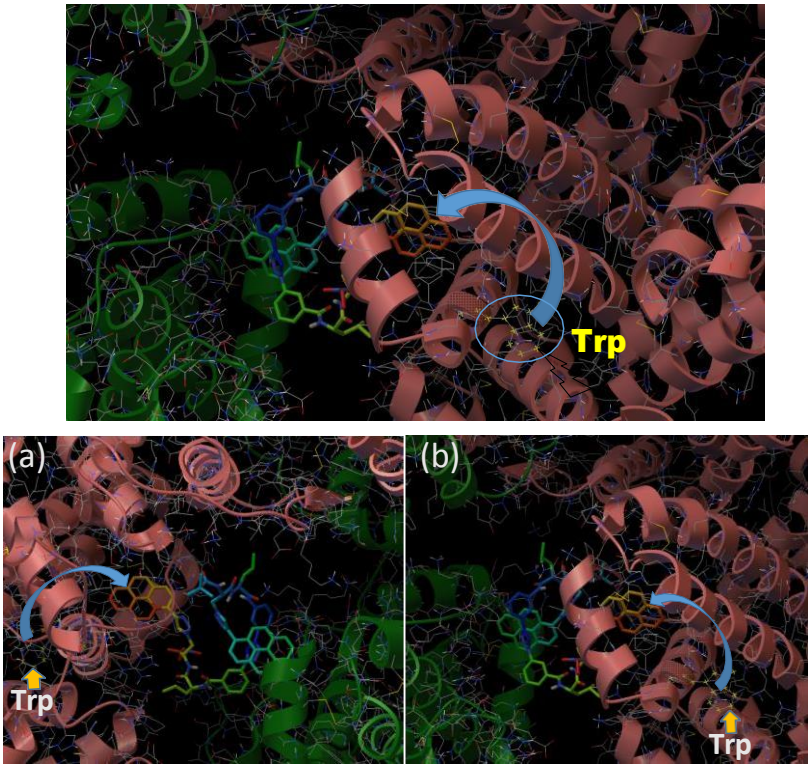


Figure S44: Docking pose of **pentapeptide 2 - BSA** (a) front side view (b) back side view.

Table S15: Energy of Pentapeptide **2-BSA** complex obtained from Autodock4

Interaction Energy	kcal/mol
Estimated Free Energy of Binding	-7.10
Final Intermolecular Energy	-14.55
vdW + Hbond + desolv Energy	-13.45
Electrostatic Energy	-1.11
Final Total Internal Energy	-8.67
Torsional Free Energy	+7.46
Unbound System's Energy	-8.67

7.9. Determination of the Detection Limit of BSA protein

We have determined quantitatively the BSA protein by our synthesized pentapeptide **2.** To evaluate the detection limit of BSA, we have plotted $(I_{min} - I)/(I_{min} - I_{max})$ *vs* log of added concentration of BSA taking increased **TPy** monomer emission wavelength at 385 nm when excited at 280 nm (absorption of the scaffold, ^{Ar}**TAA** and BSA). The linear fit was drawn by taking the nine points (conc. of BSA 1 µM to 28 µM) of the linear region. The extended line where it crossed the coordinate axes is the detection limit of BSA which was found to be 0.054 µg/ml.

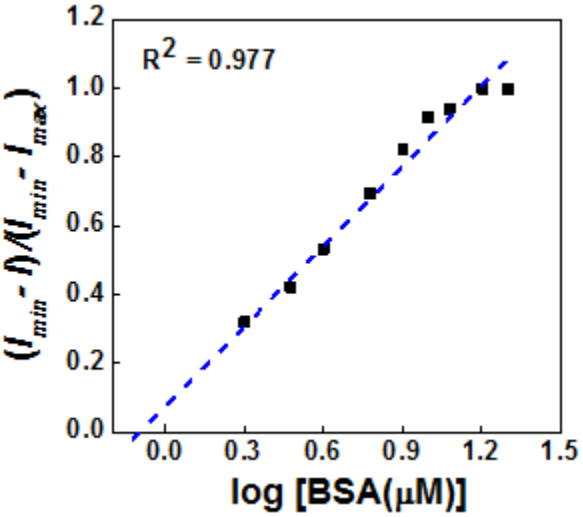


Figure S45. Plot of $(I_{min} - I)/(I_{min} - I_{max})$ *vs* log (Conc.BSA) of pentapeptide 2 tritated with BSA protein.

8. Studies on the interaction of Pentapeptide 2 with α-Amylase

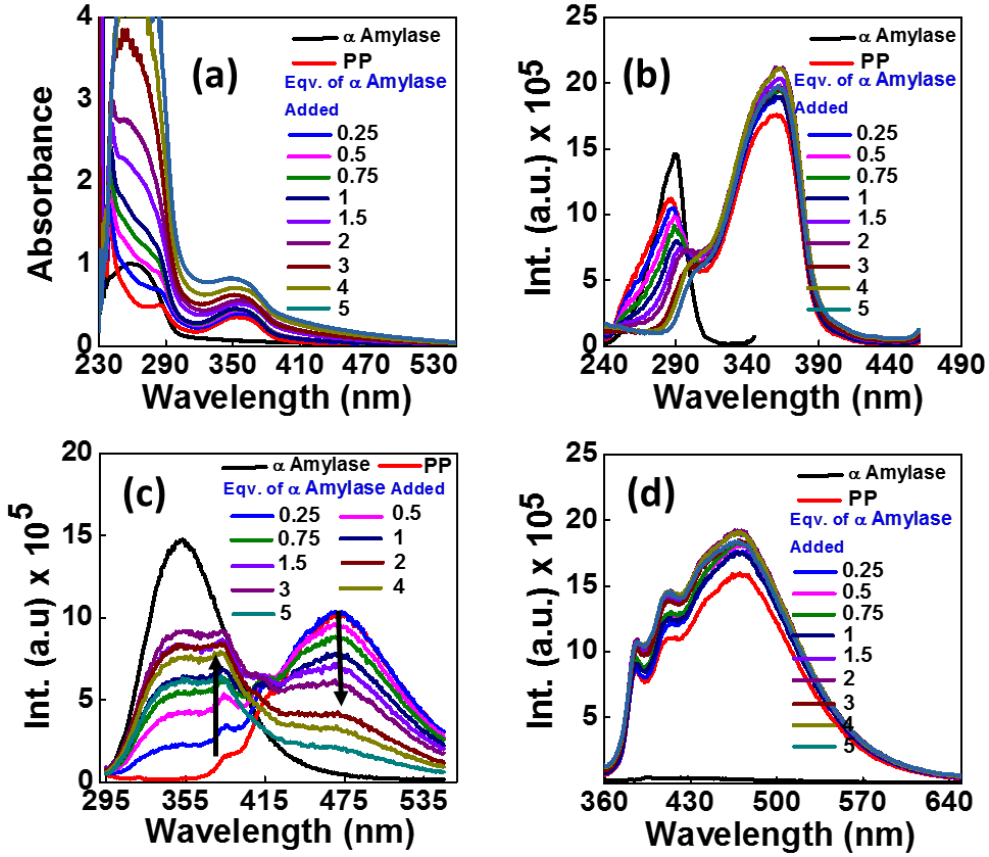


Figure S46: (a) UV-visible and (b) excitation spectra (λ_{ex} = 470 nm), (c) emission spectra (λ_{ex} = 280 nm) and (d) emission spectra (λ_{ex} = 350 nm) of Pentapeptide **2** in presence of increasing α-amylase concentration at 298K. [Pentapeptide] = 10 μM and [α-amylase] = 0, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50 μM

8.1. Relative change of florescence intensity of pentapeptide 2 with BSA and α-Amylase

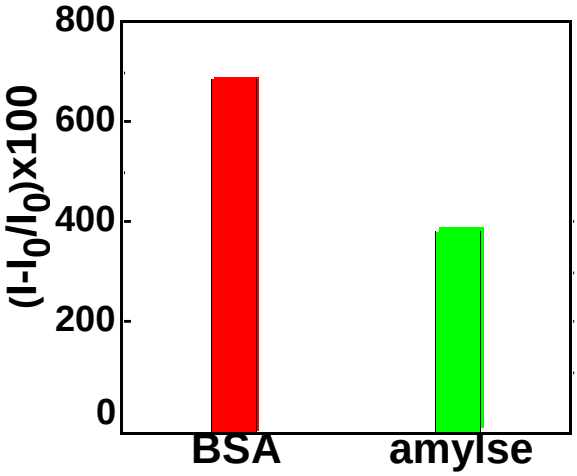


Figure S47: Plot of relative change of the fluorescence intensity of pentapeptide **2** with BSA and α-Amylase protein.

9. ^{1}H and ^{13}C NMR spectra of synthesized compound

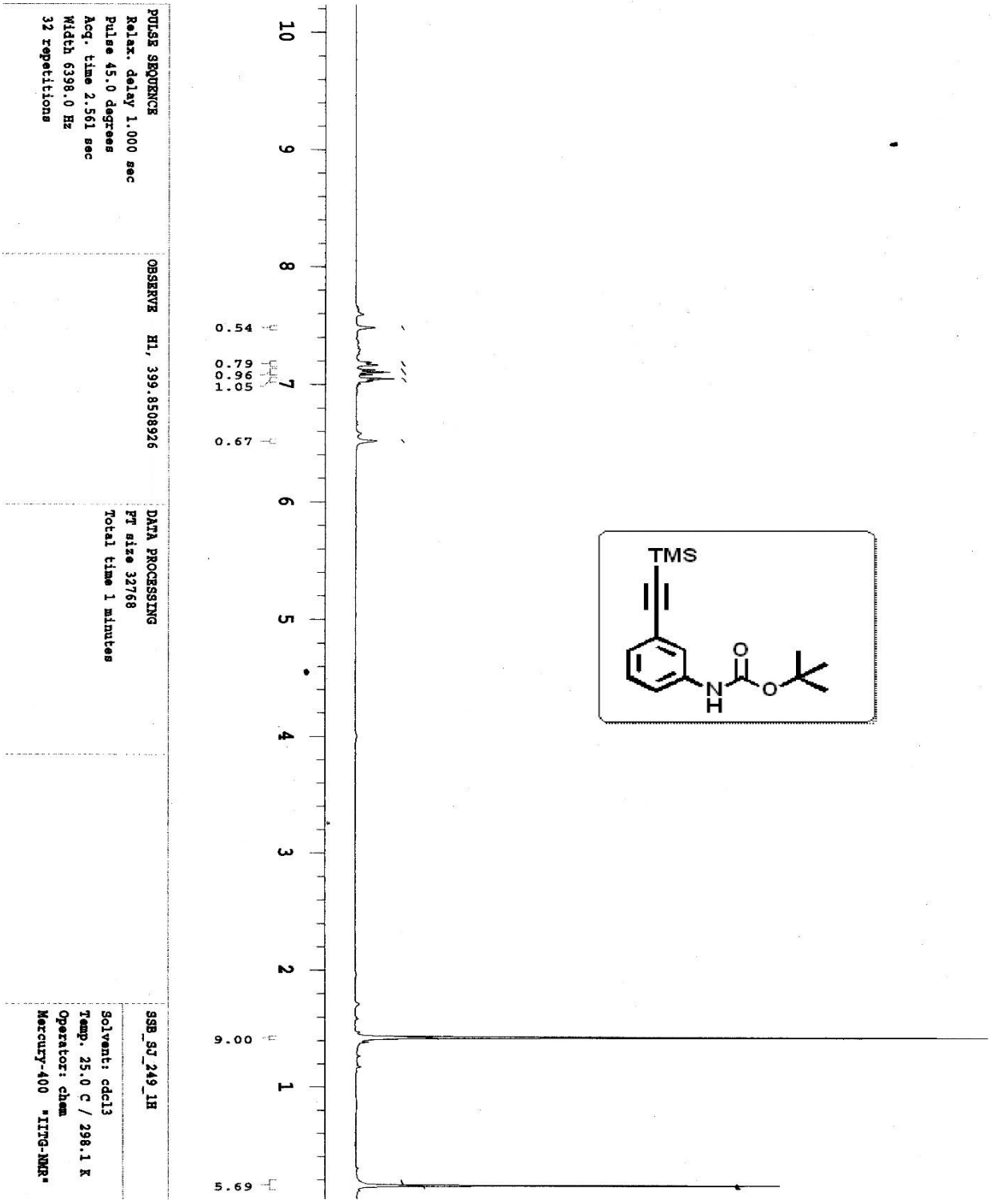


Figure S48. ^{1}H Spectra of synthesized compound **9.**

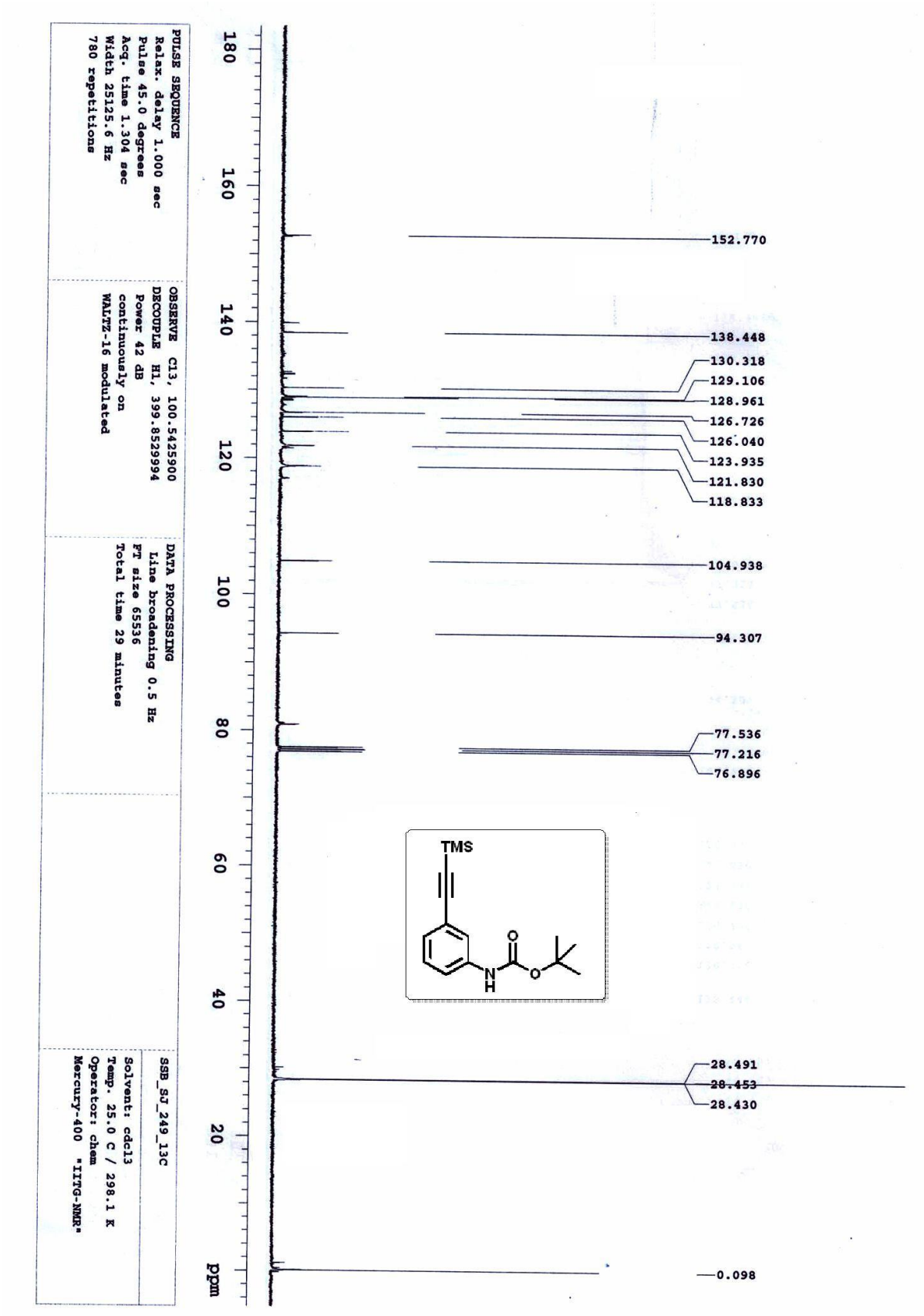


Figure S49. ^{13}C Spectra of synthesized compound **9.**

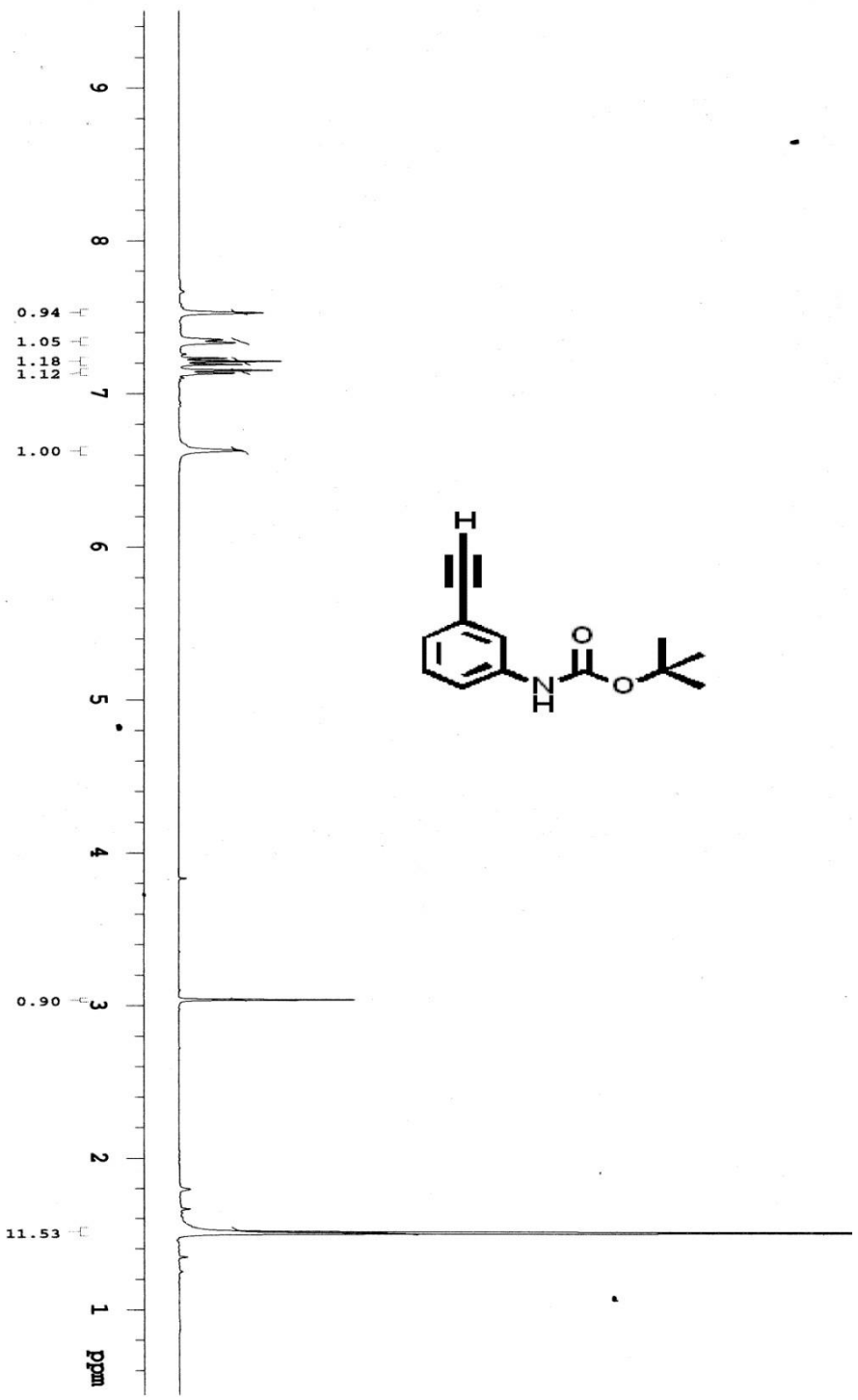


Figure S50. ^{1}H Spectra of synthesized compound **10.**

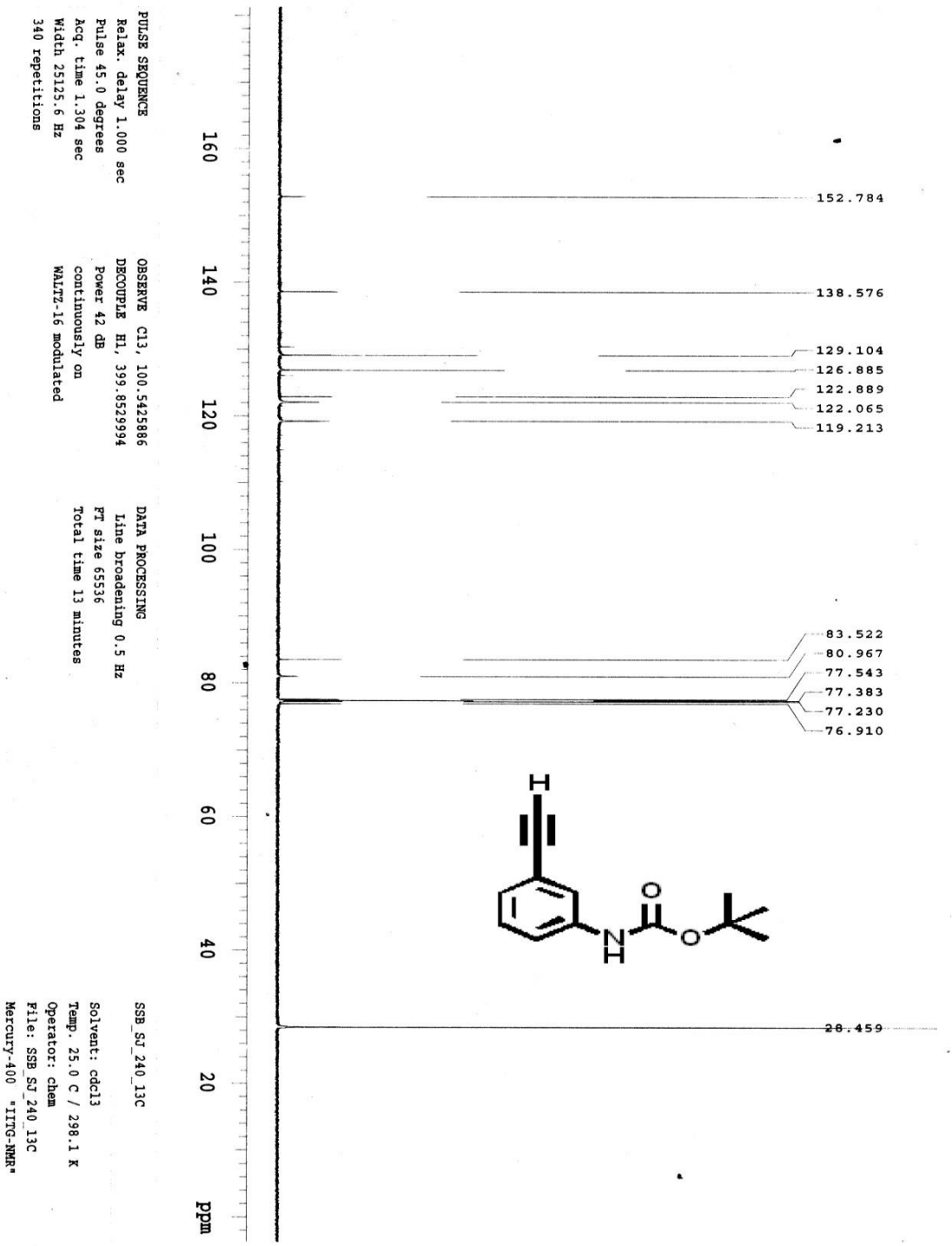


Figure S51. ^{13}C Spectra of synthesized compound **10.**

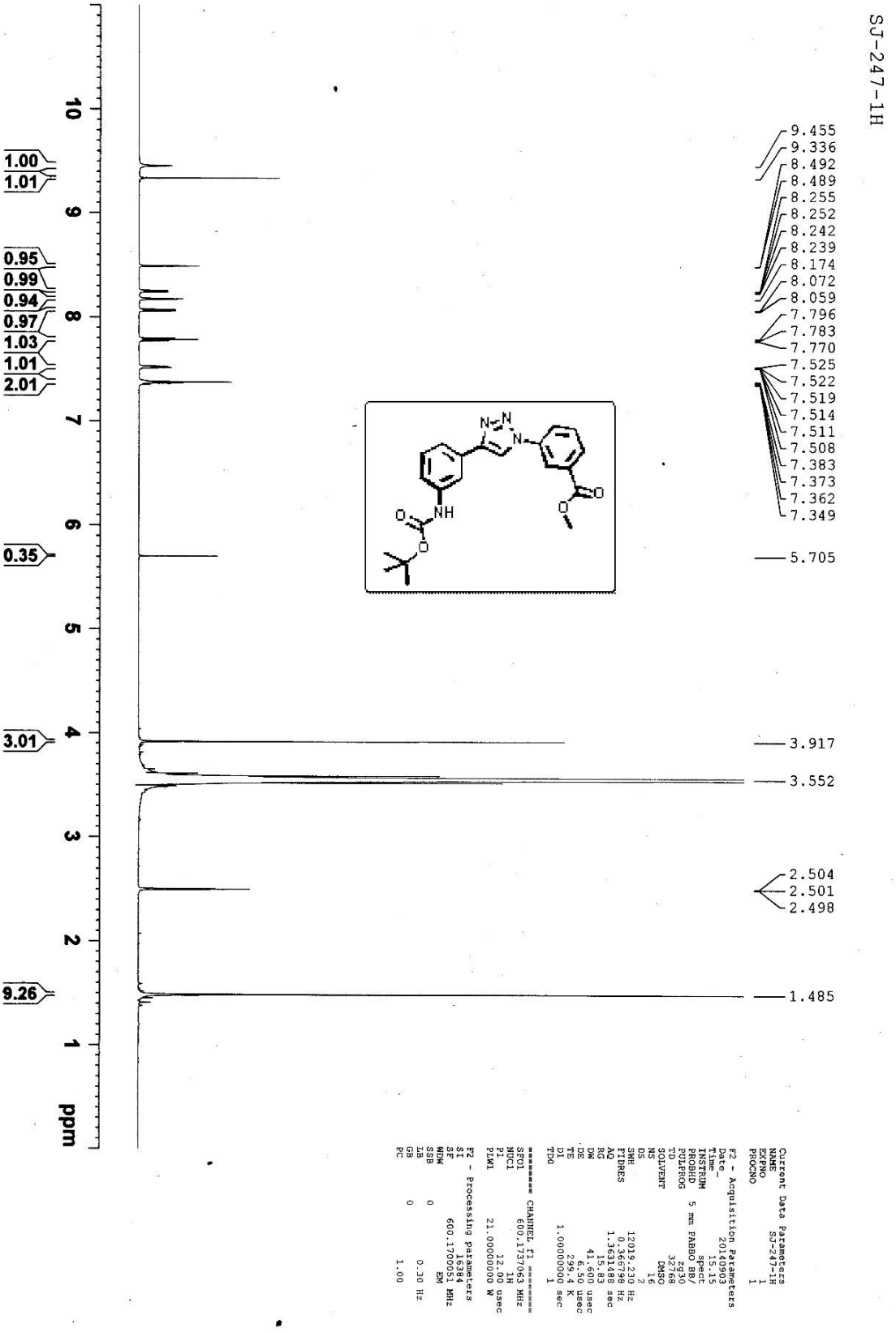


Figure S52. ^{1}H Spectra of synthesized compound **11.**

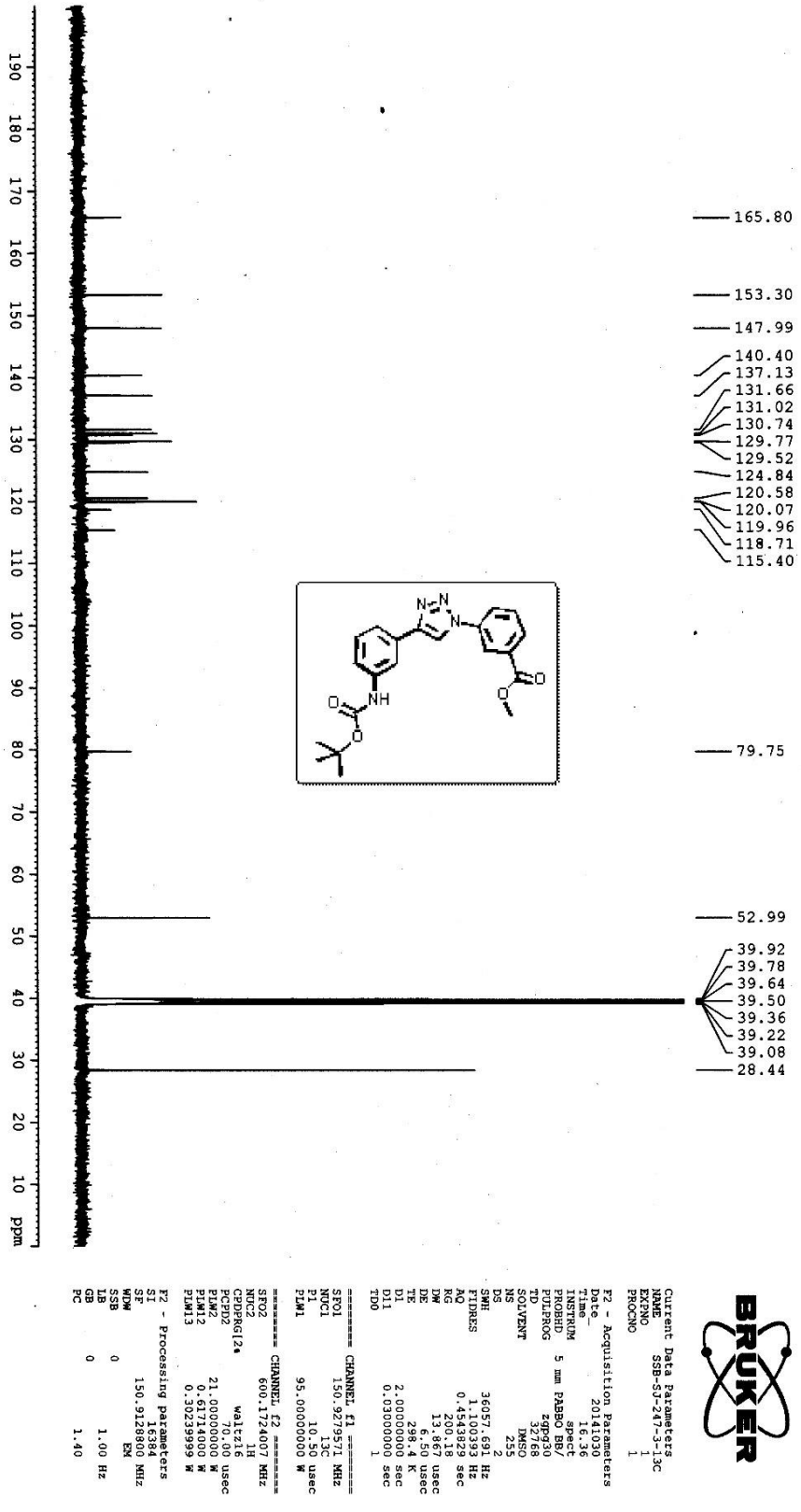


Figure S53. ^{13}C Spectra of synthesized compound **11.**

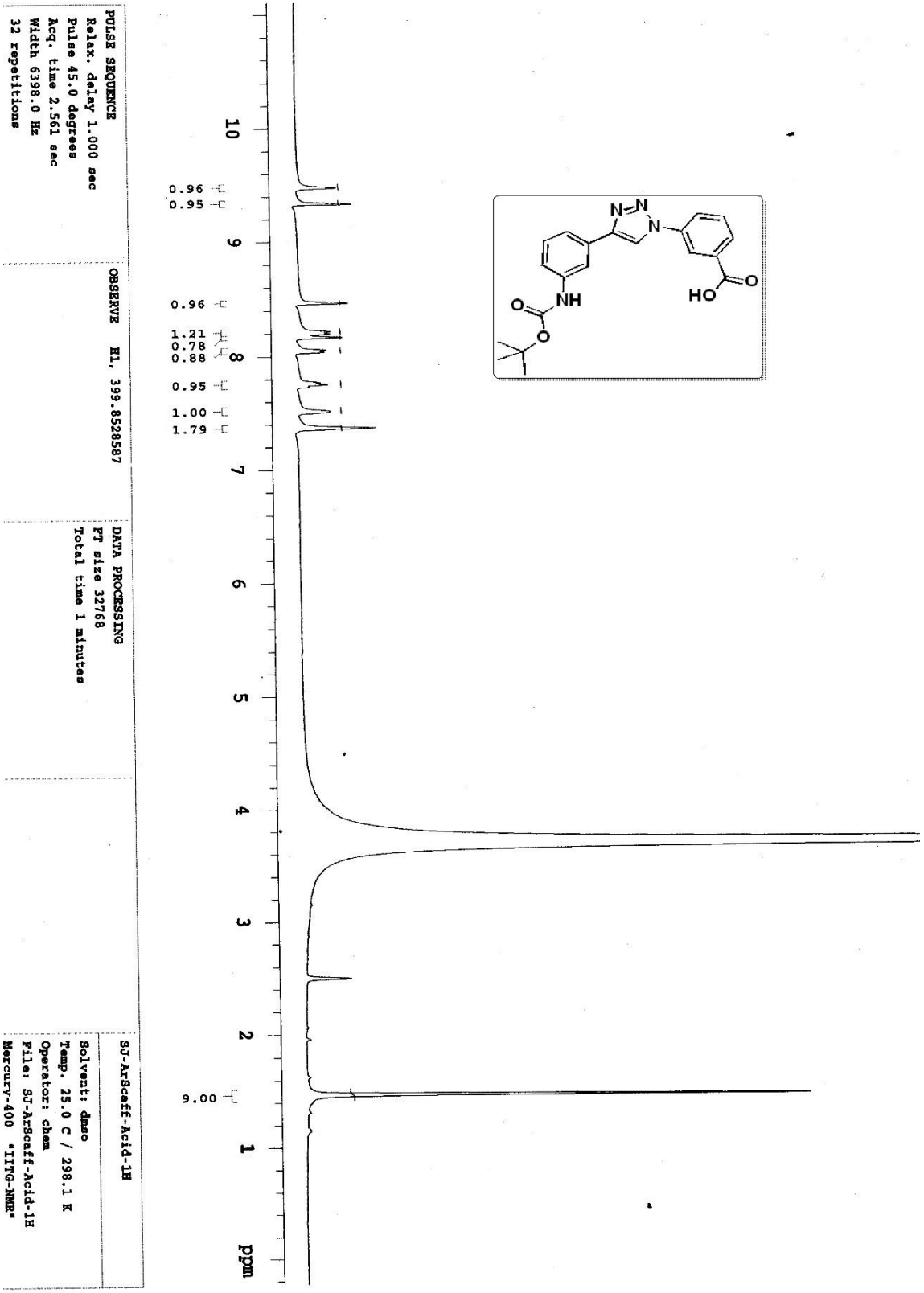


Figure S54. ^{1}H Spectra of synthesized compound **1.**

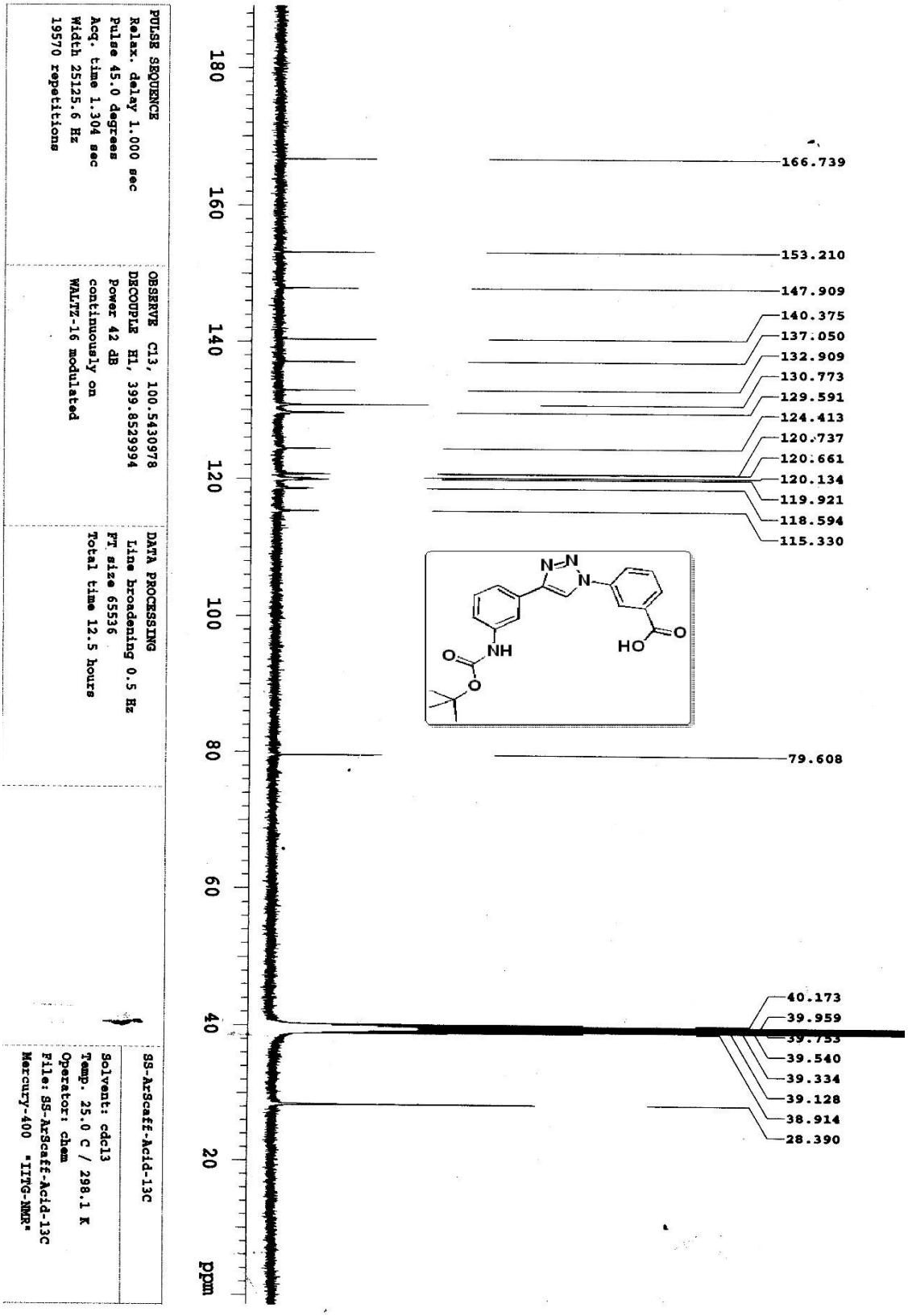


Figure S55. ^{13}C Spectra of synthesized compound **1.**

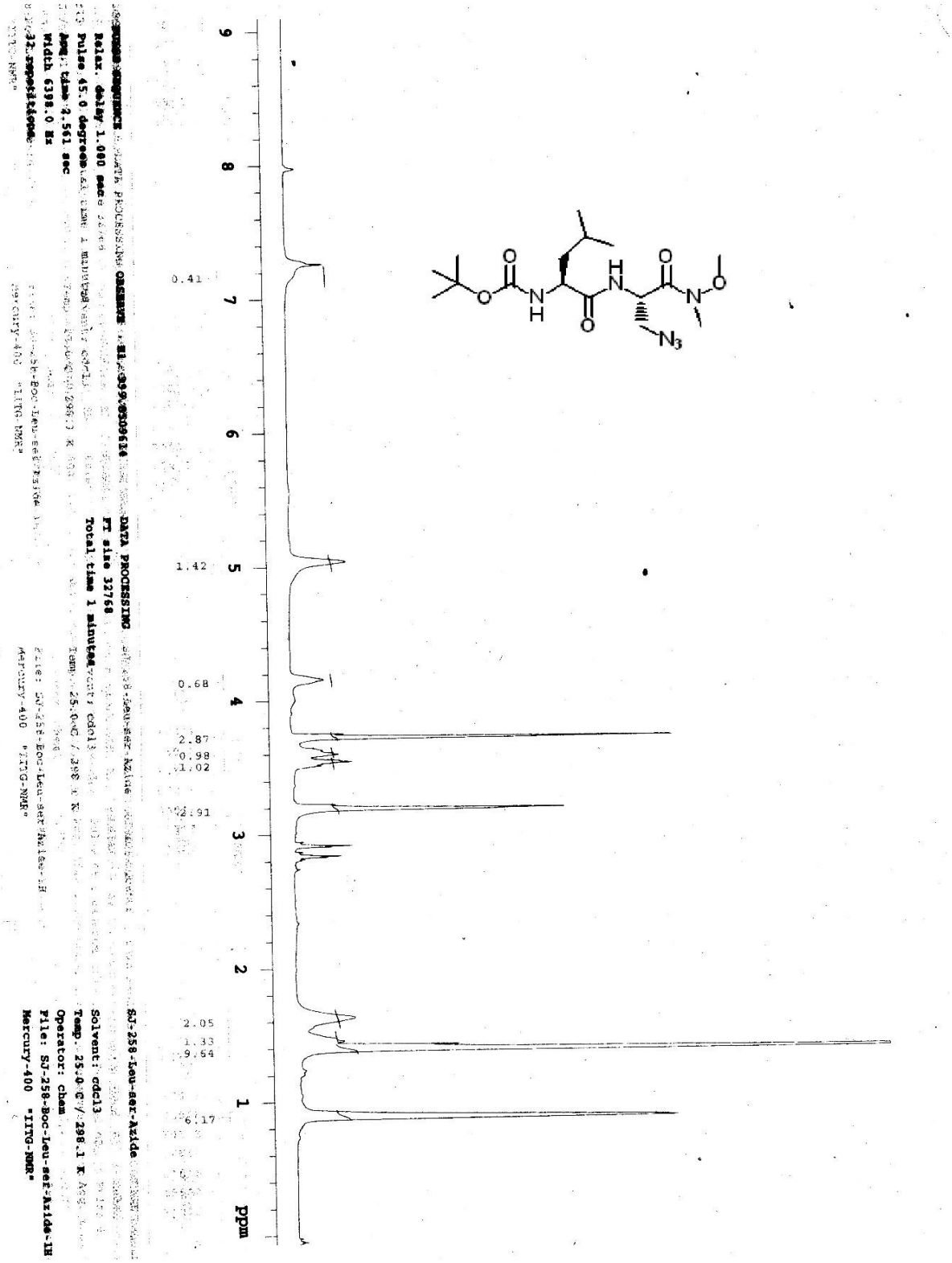


Figure S56. ^{1}H Spectra of synthesized compound **15.**

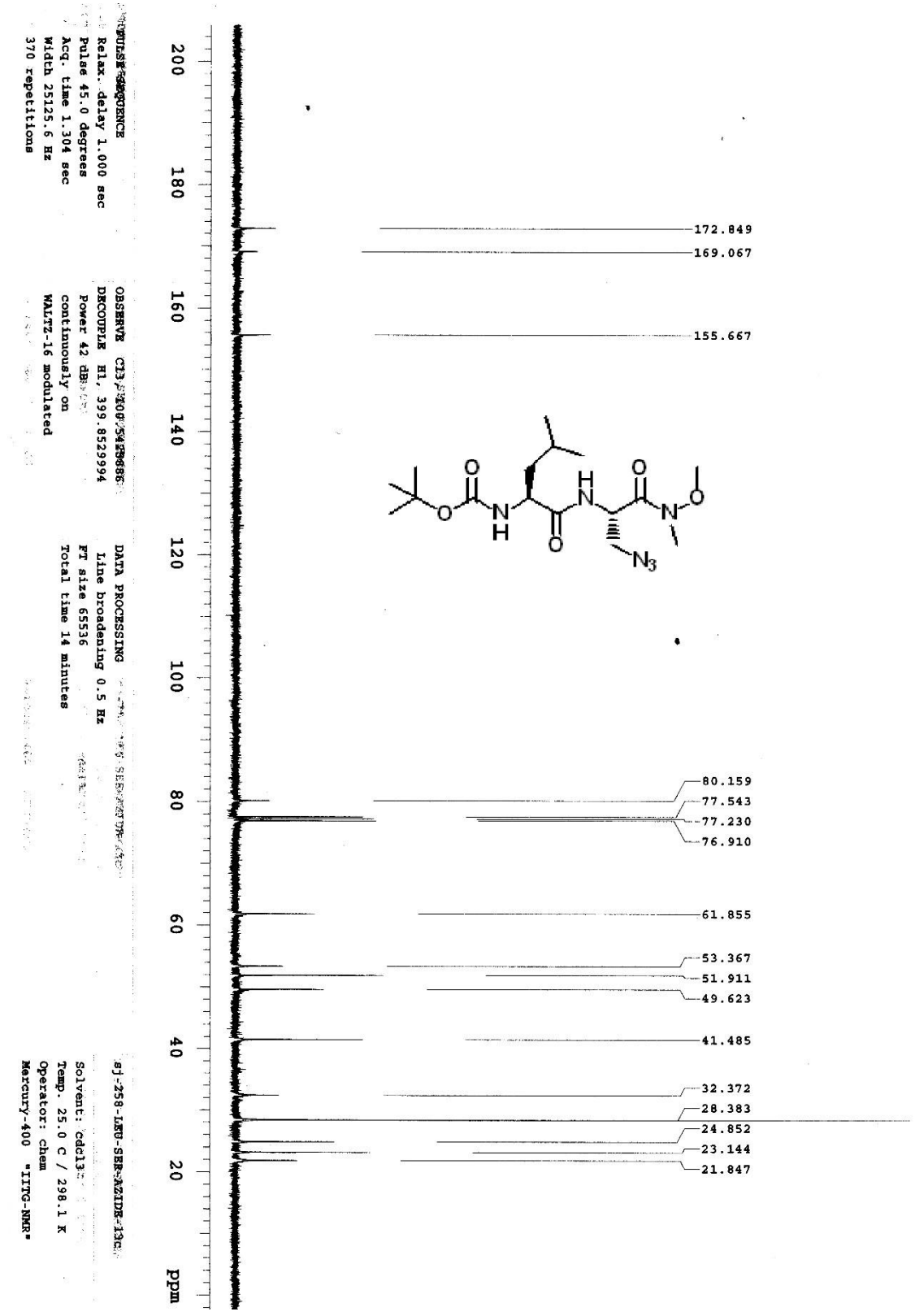


Figure S57. ^{13}C Spectra of synthesized compound **15.**

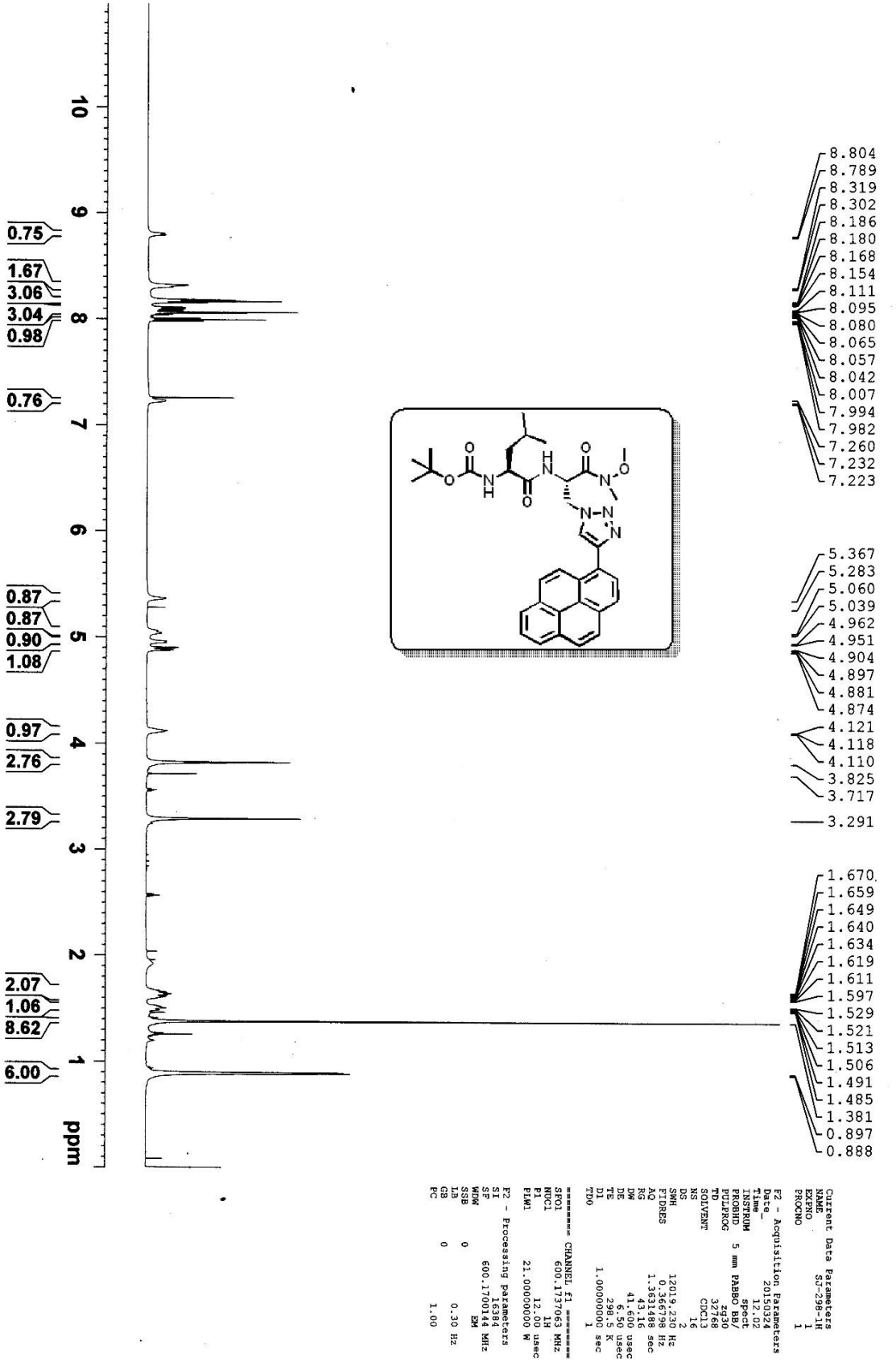


Figure S58. ^{1}H Spectra of synthesized compound **16.**

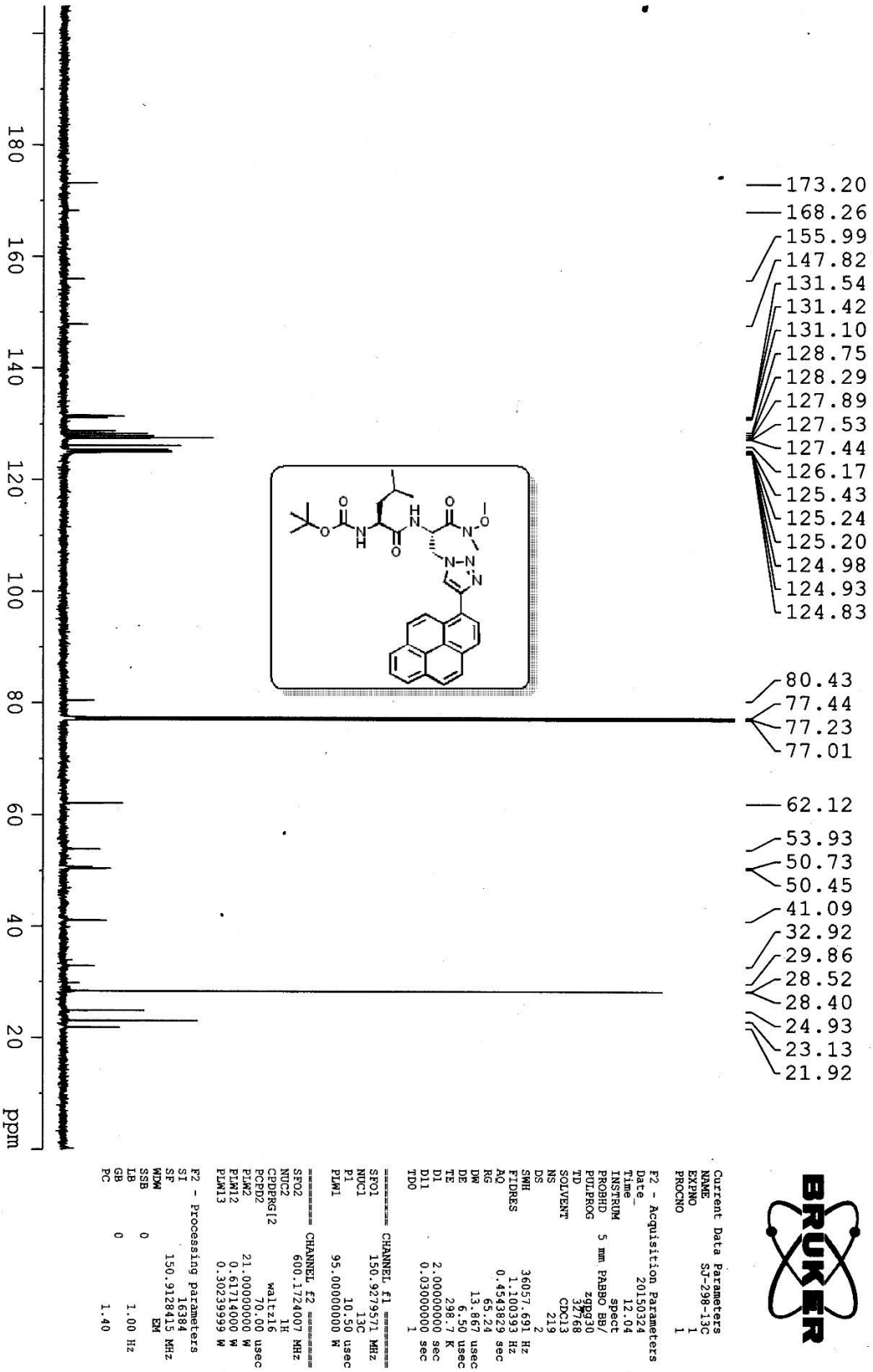


Figure S59. ^{13}C Spectra of synthesized compound **16.**

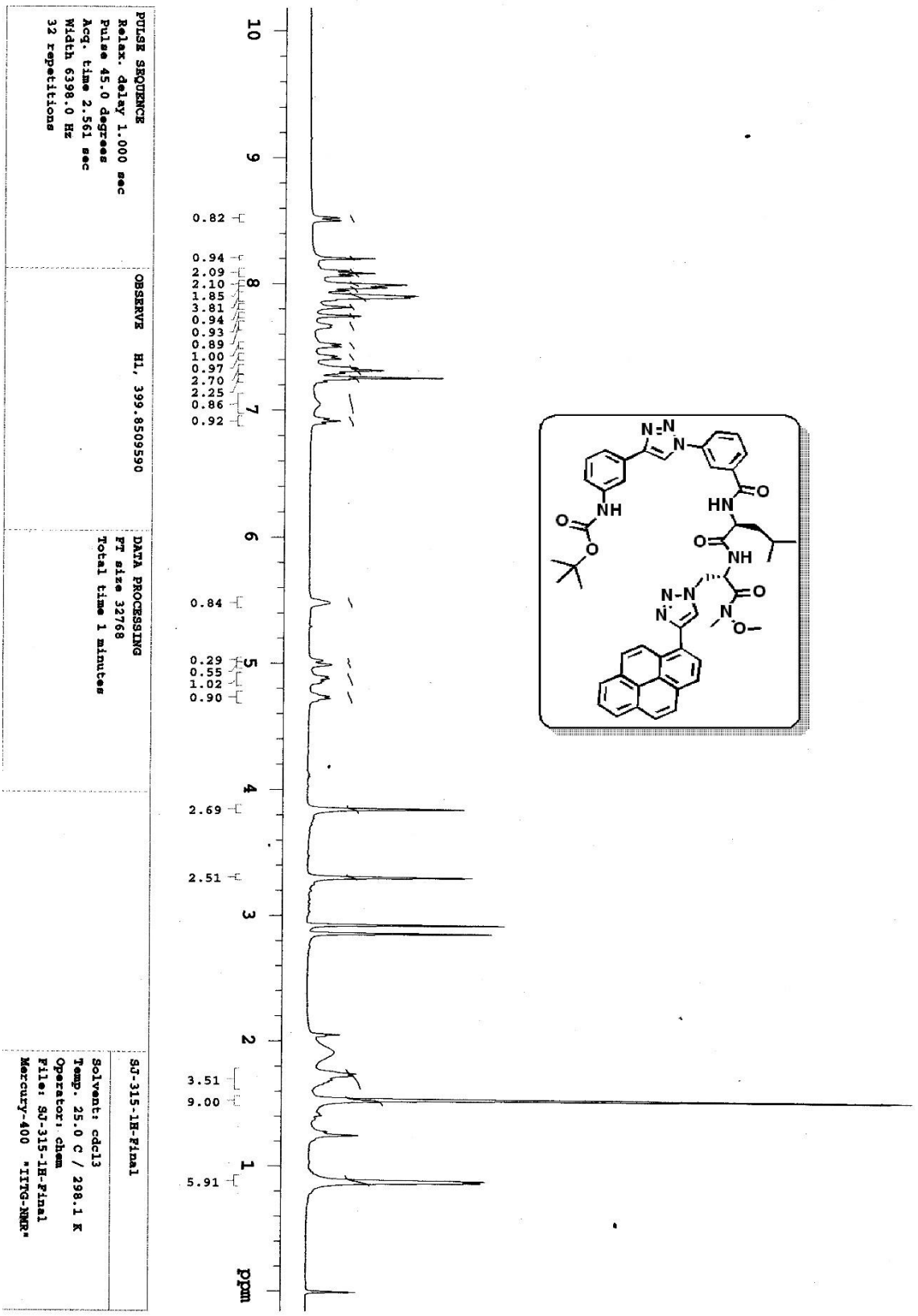


Figure S60. ^{1}H Spectra of synthesized compound **18.**

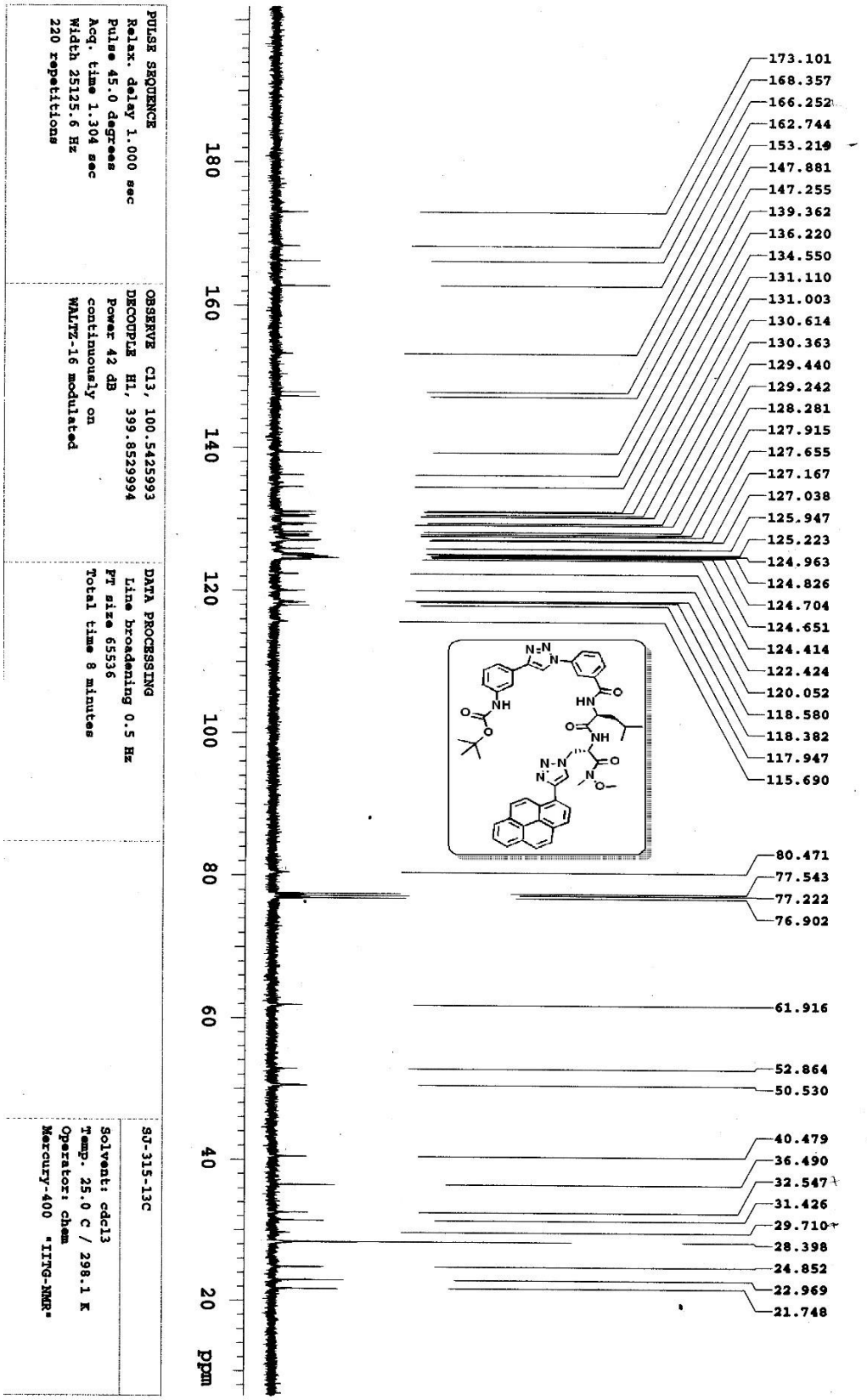


Figure S61. ^{13}C Spectra of synthesized compound **18.**

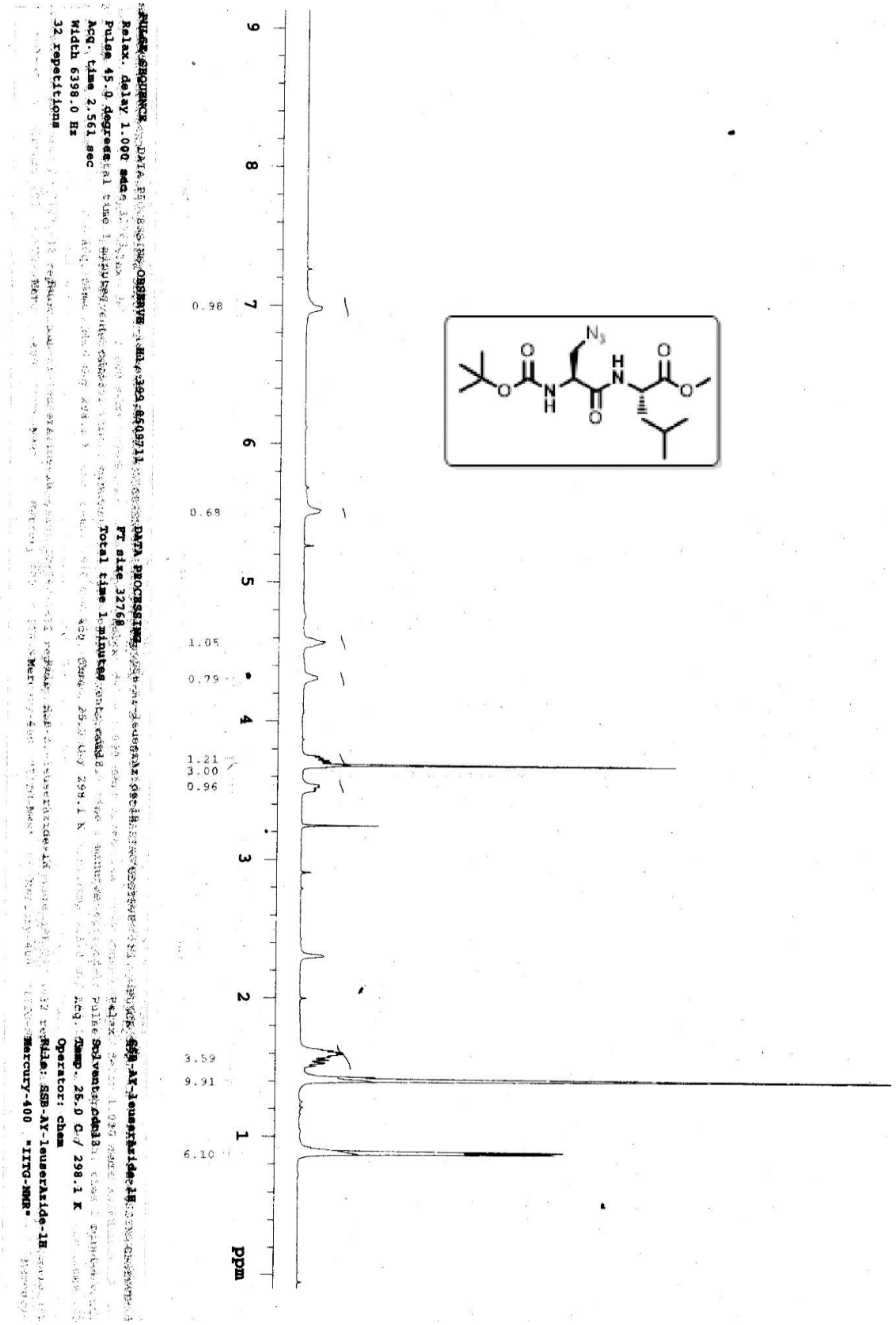


Figure S62. ^{1}H Spectra of synthesized compound **23**

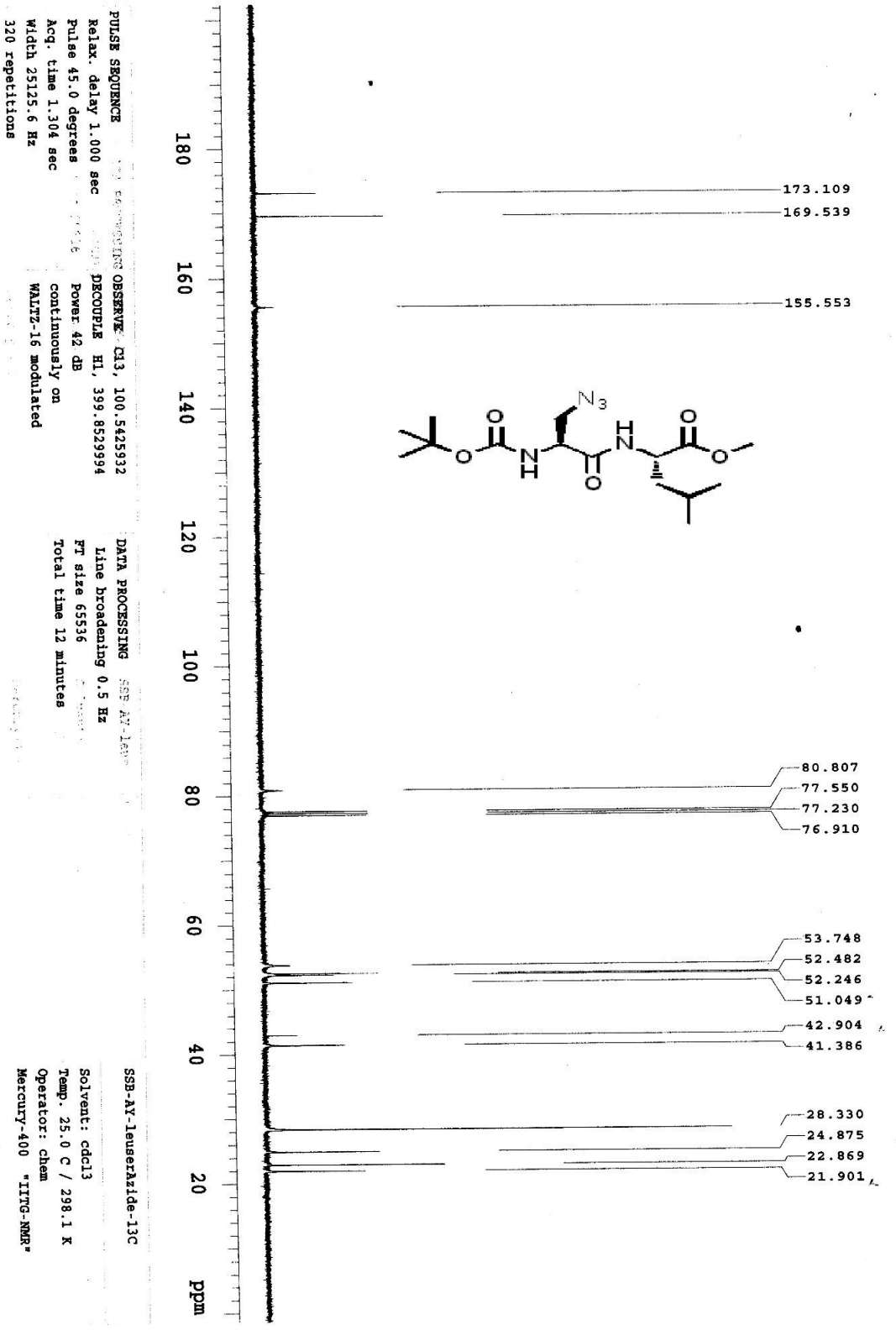


Figure S63. ^{13}C Spectra of synthesized compound **23.**

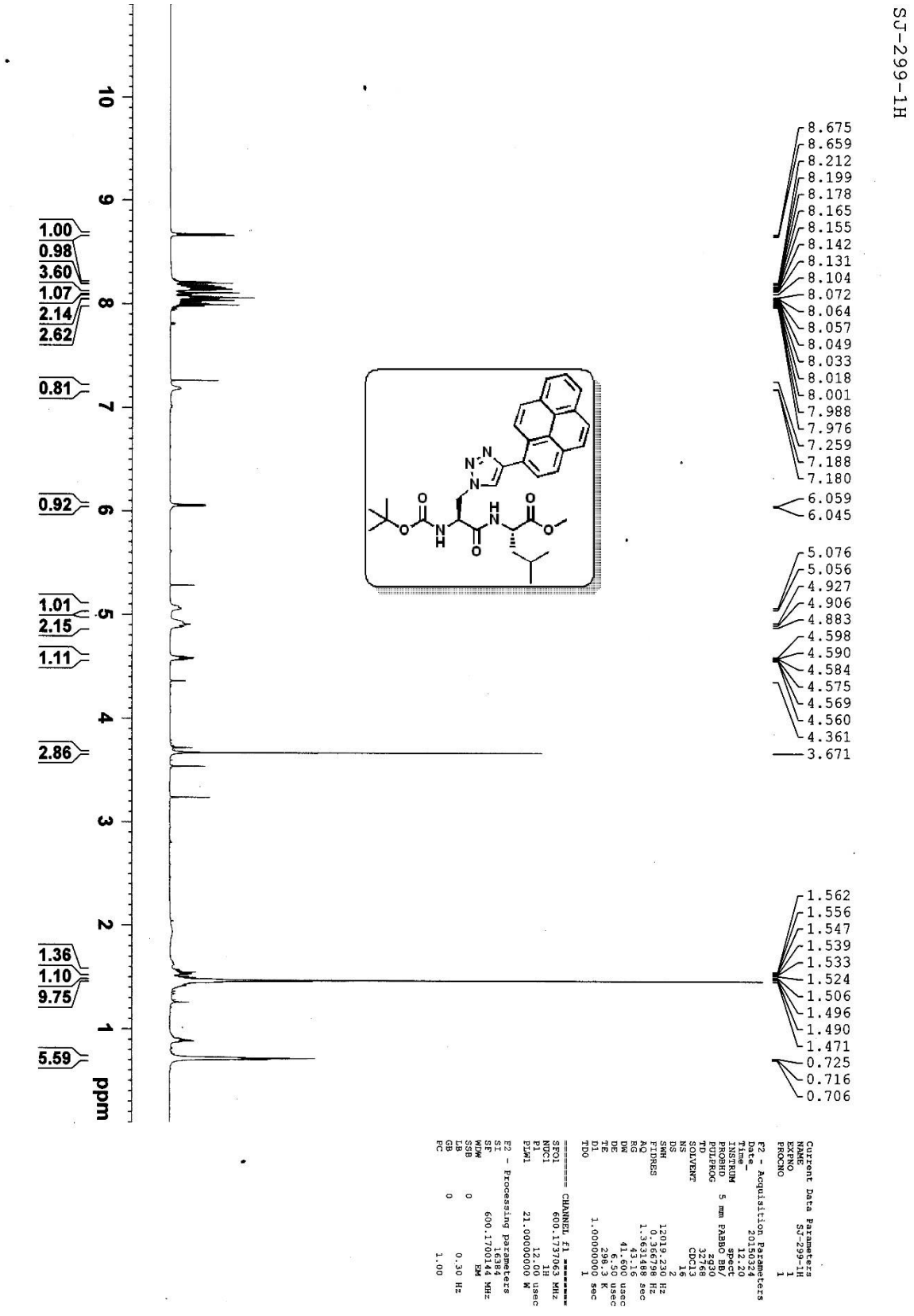


Figure S64. ^{1}H Spectra of synthesized compound **24.**

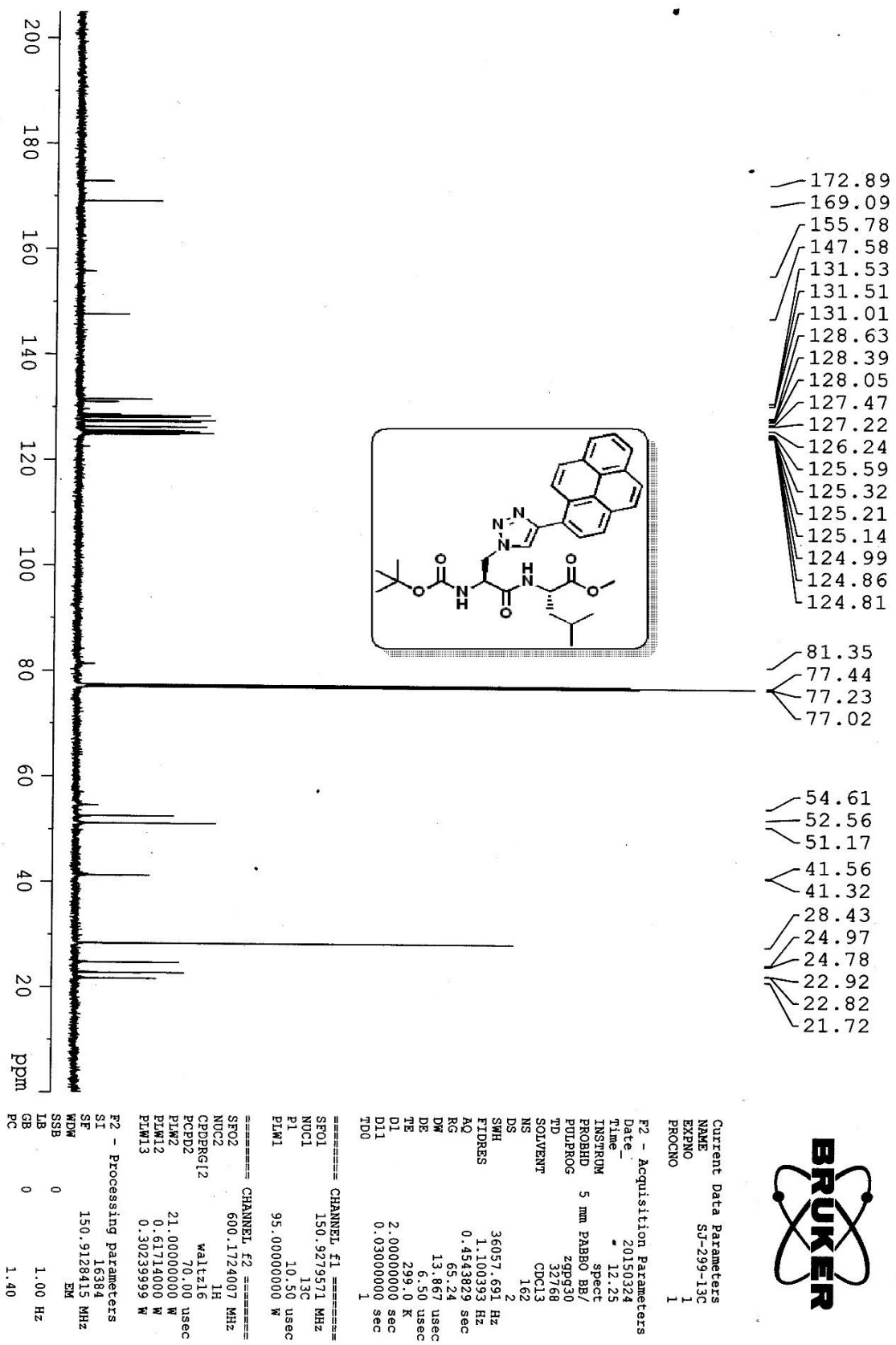


Figure S65. ^{13}C Spectra of synthesized compound **24.**

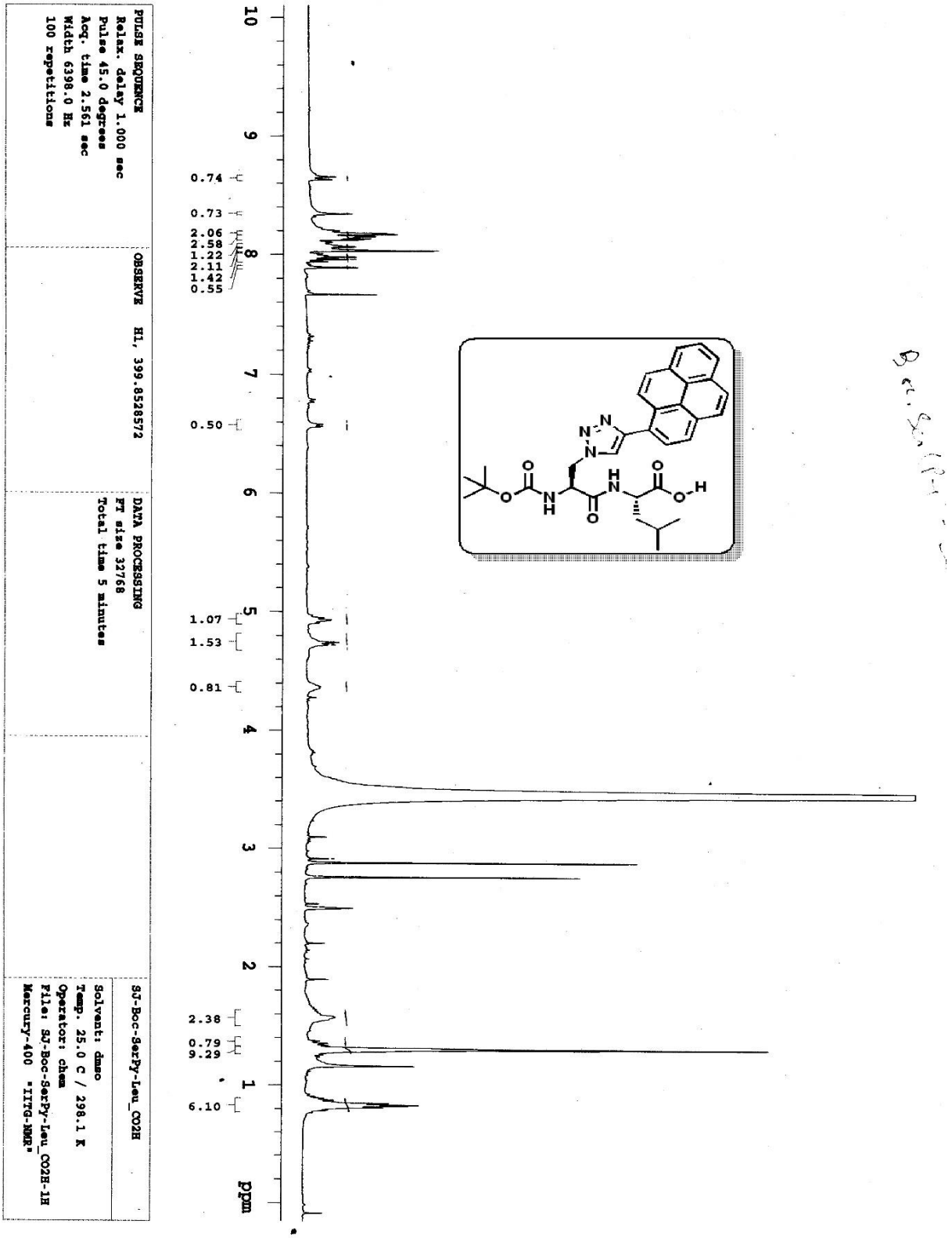


Figure S66. ^{1}H Spectra of synthesized compound **25.**

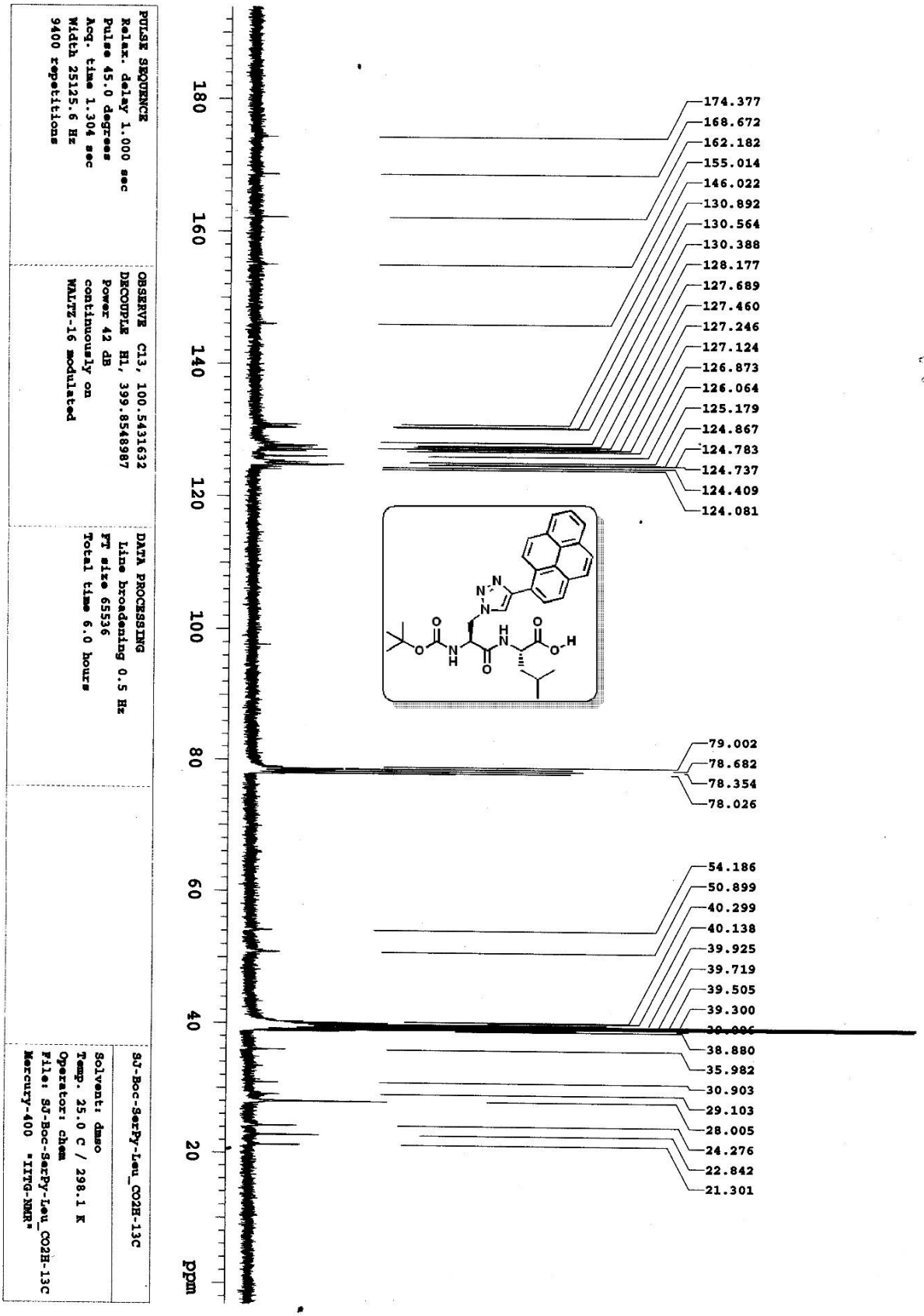


Figure S67. ^{13}C Spectra of synthesized compound **25.**

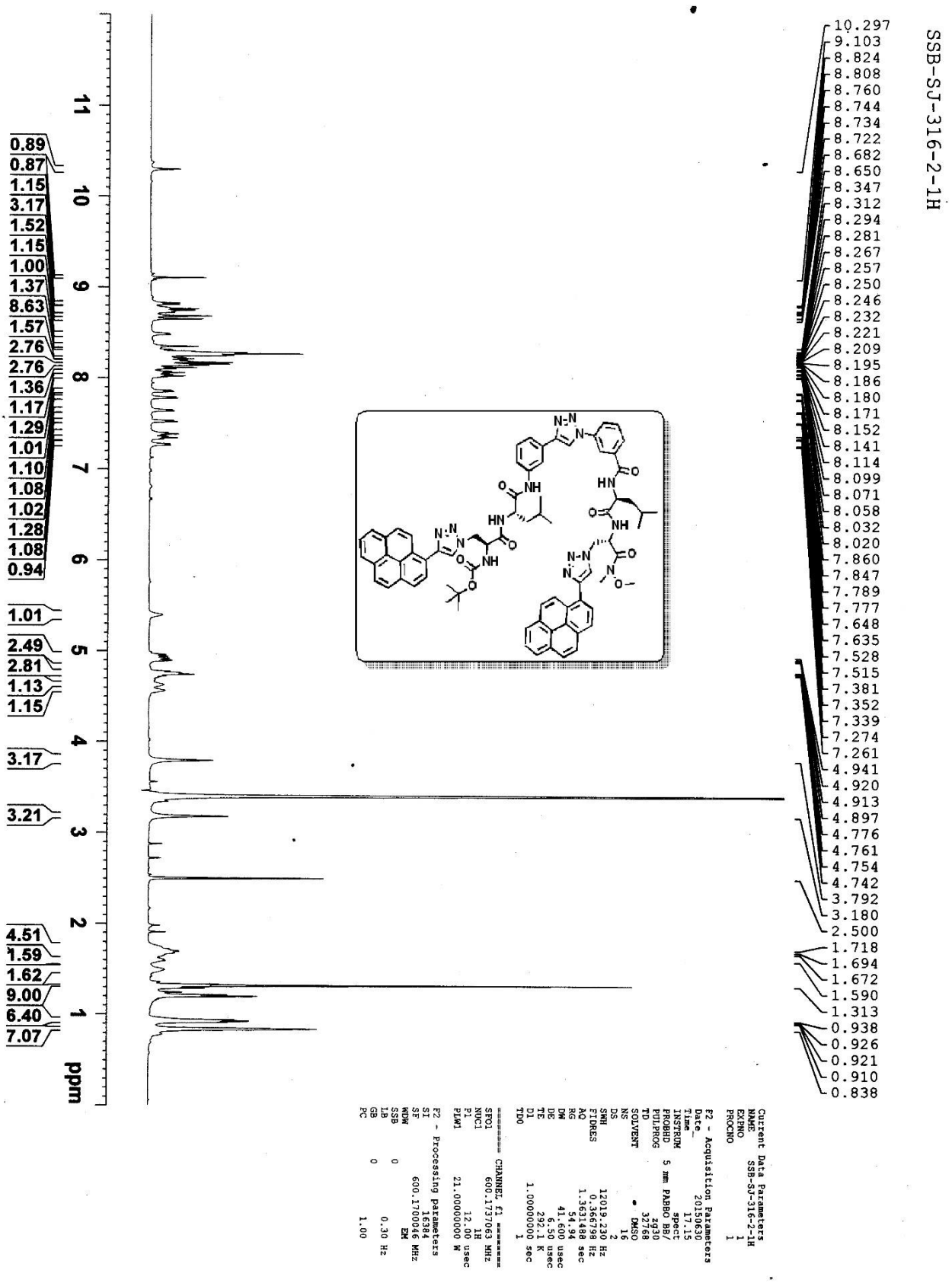


Figure S68. ^{1}H Spectra of synthesized compound **2.**

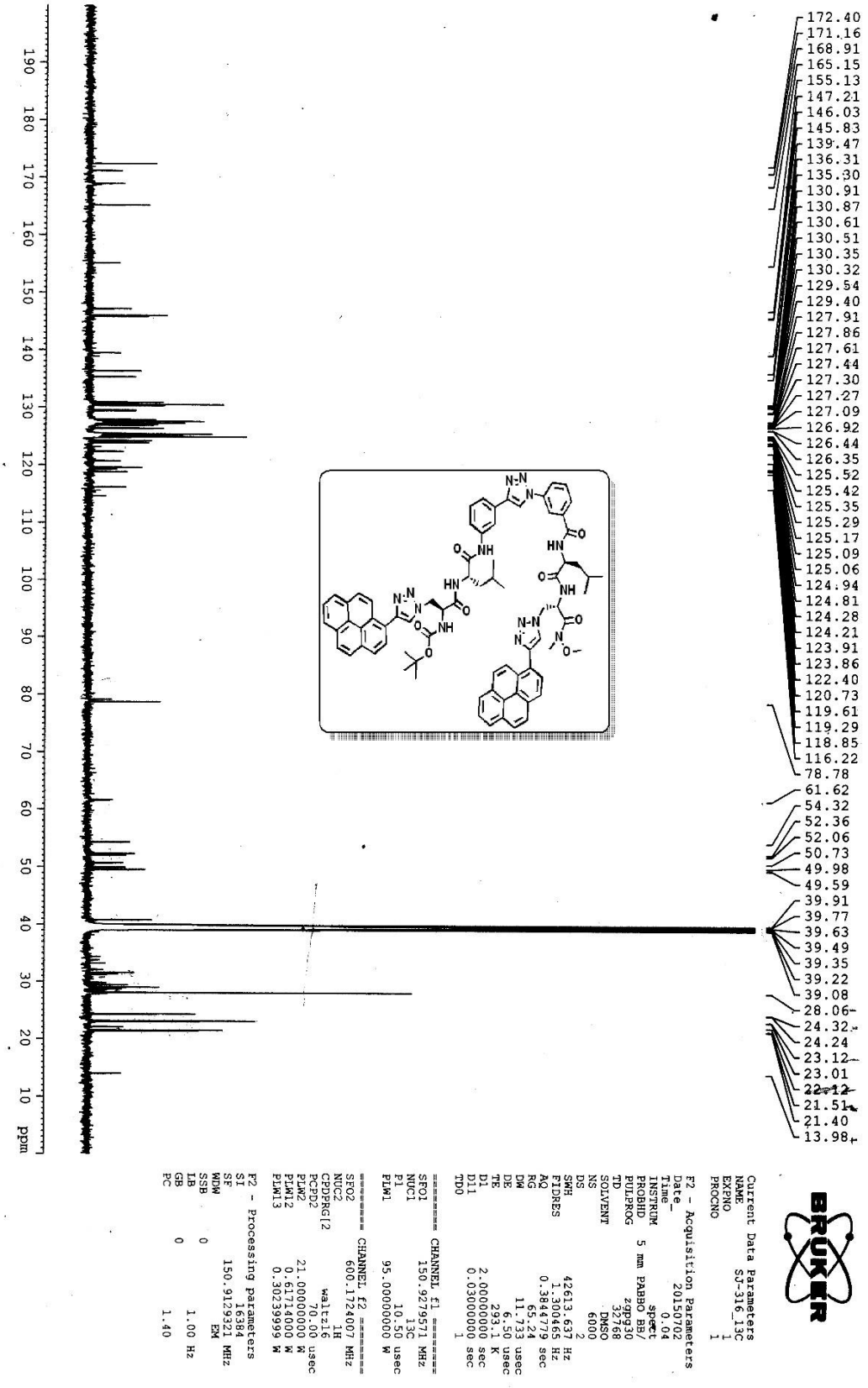


Figure S69. ^{13}C Spectra of synthesized compound **2.**

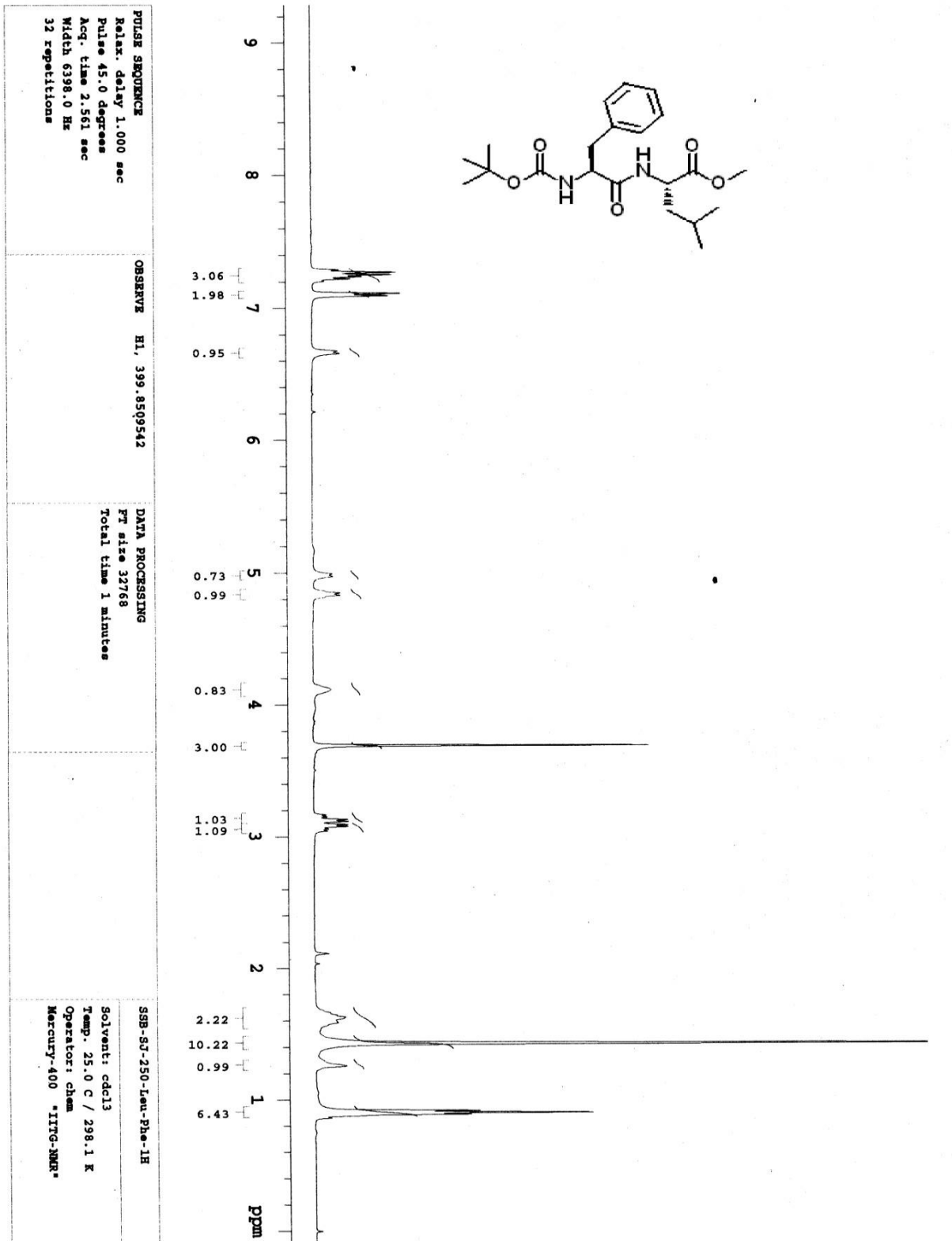


Figure S70. ^{1}H Spectra of synthesized compound **27.**

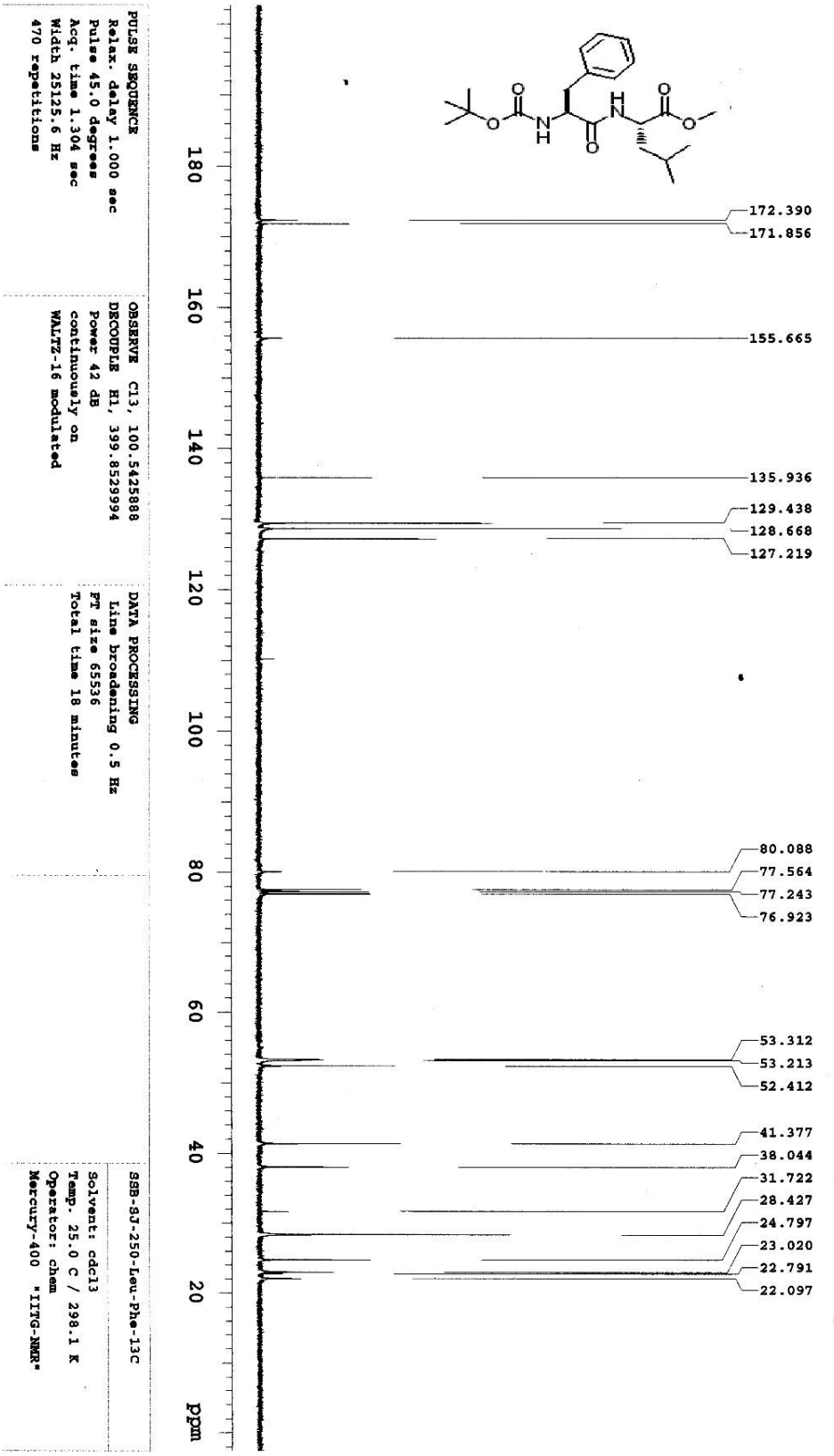


Figure S71. ^{13}C Spectra of synthesized compound **27.**

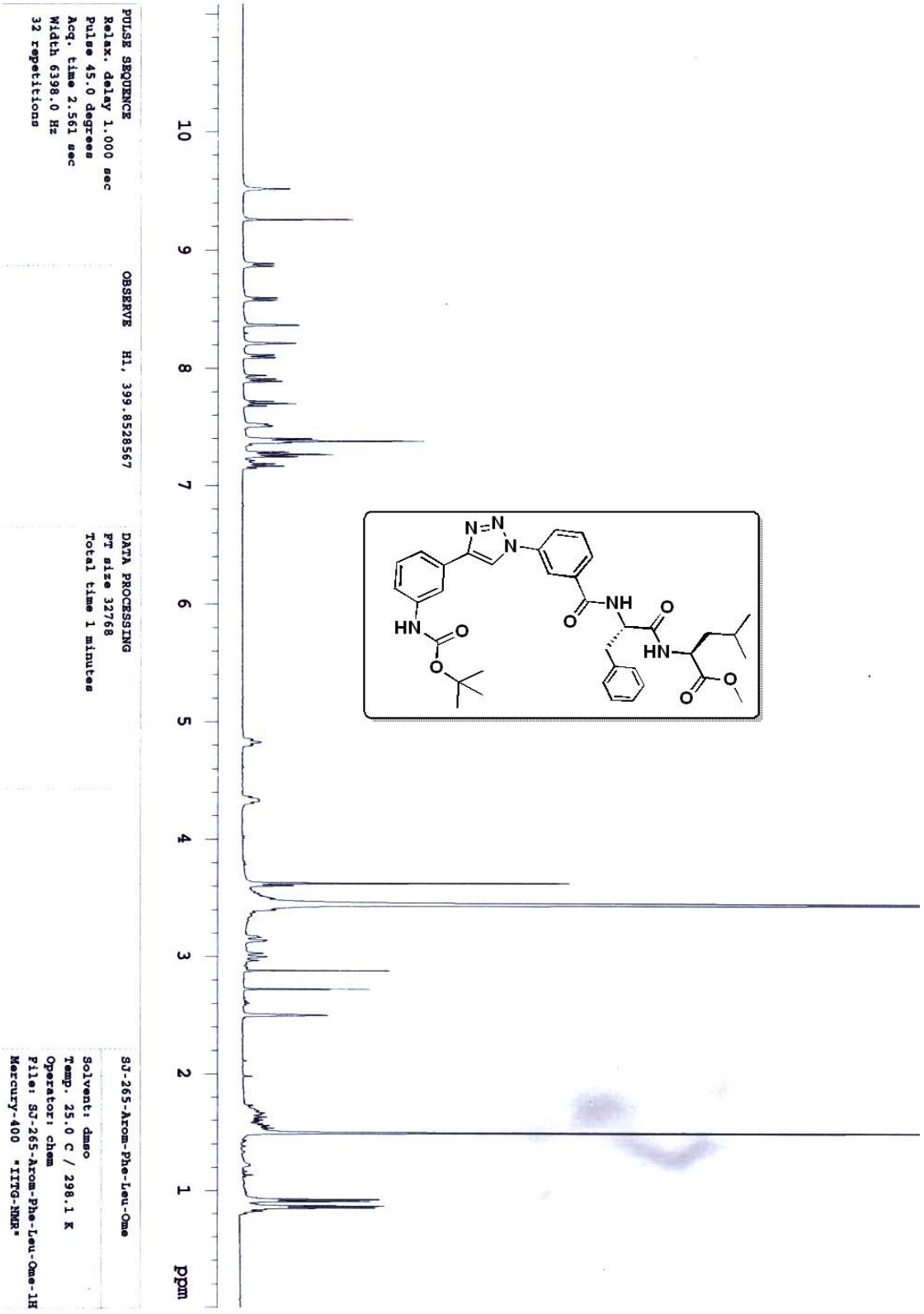


Figure S72. ^{1}H Spectra of synthesized compound **29.**

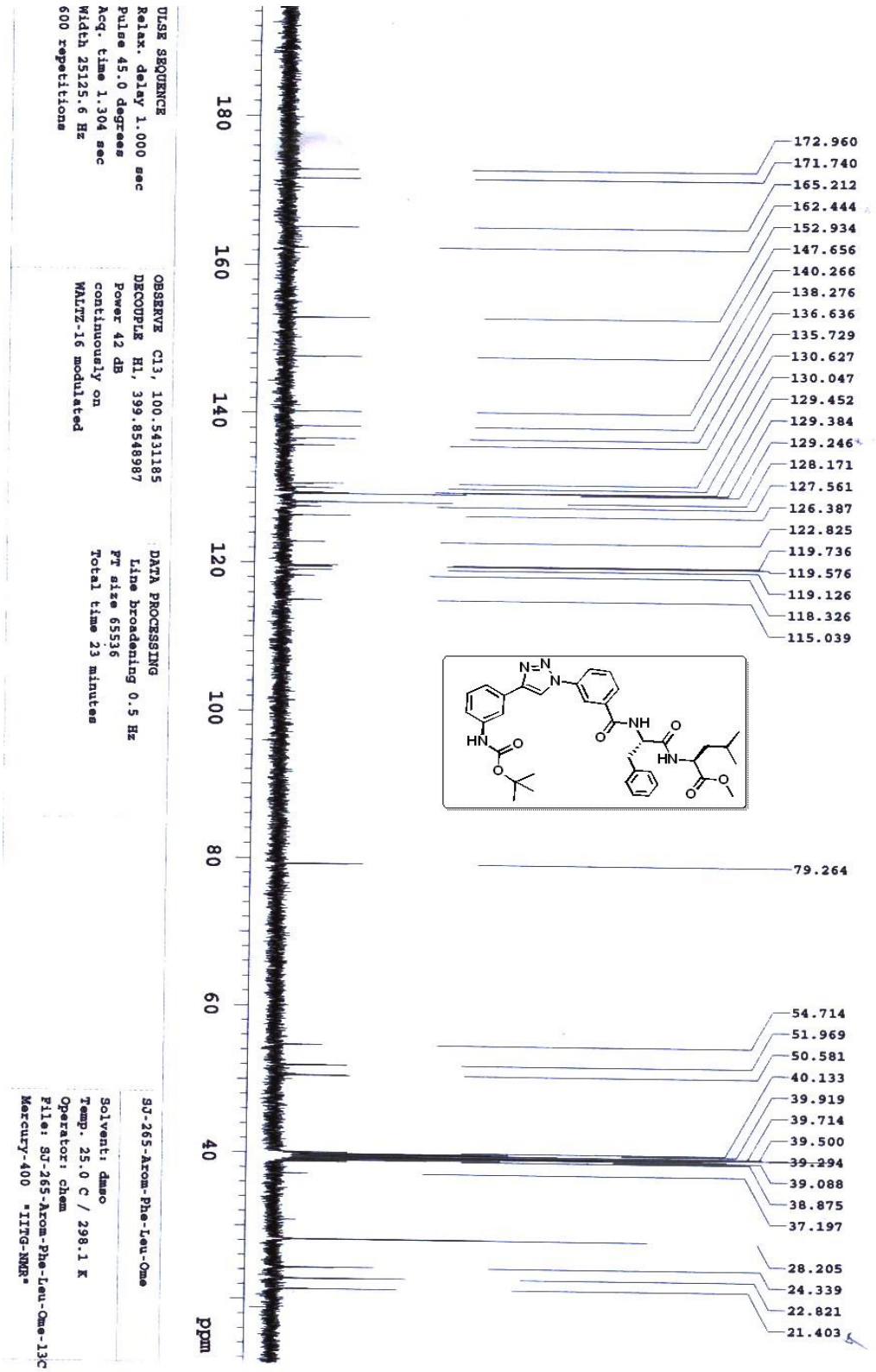


Figure S73. ^{13}C Spectra of synthesized compound **29.**

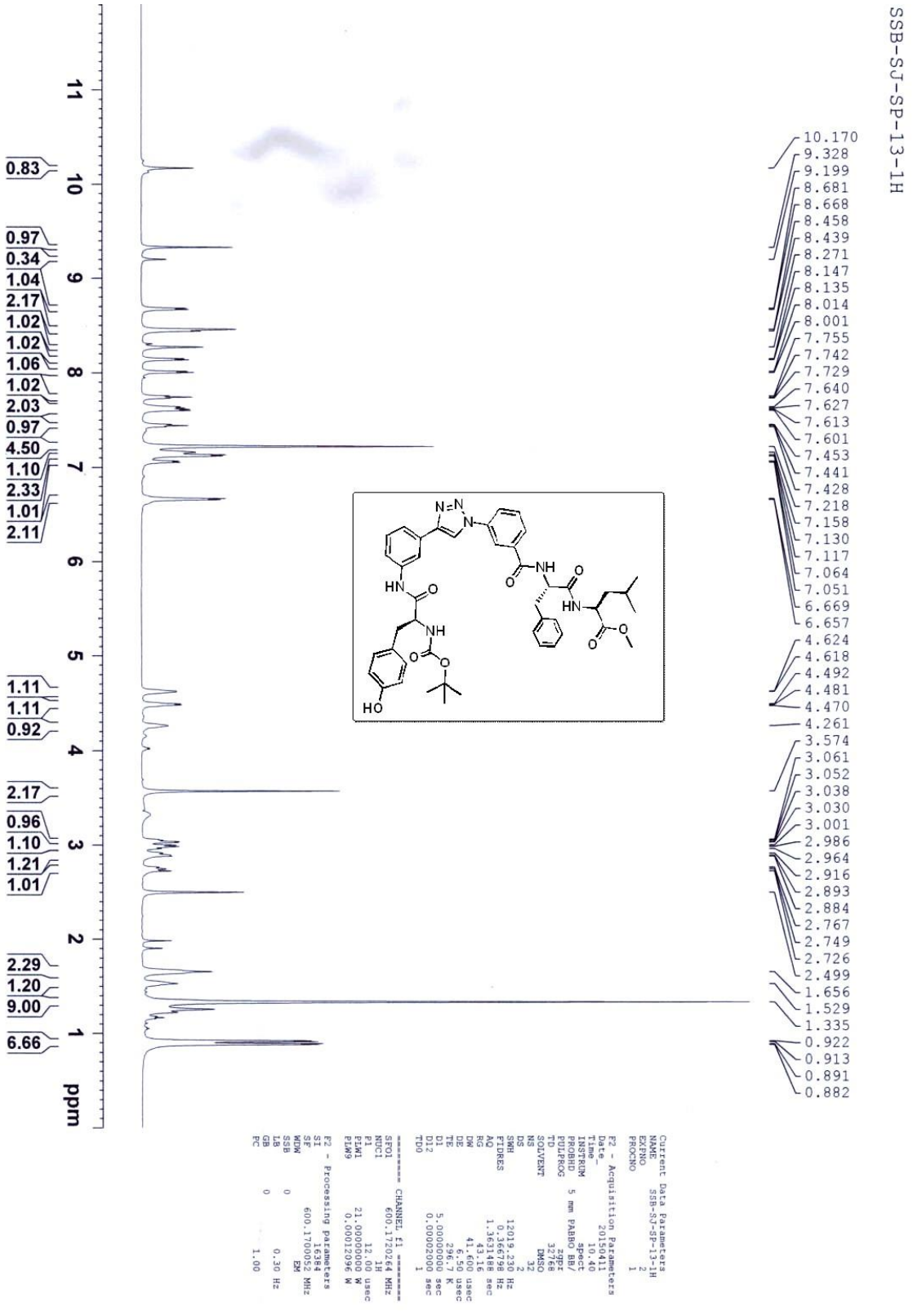


Figure S74. ^{1}H Spectra of synthesized compound **3.**

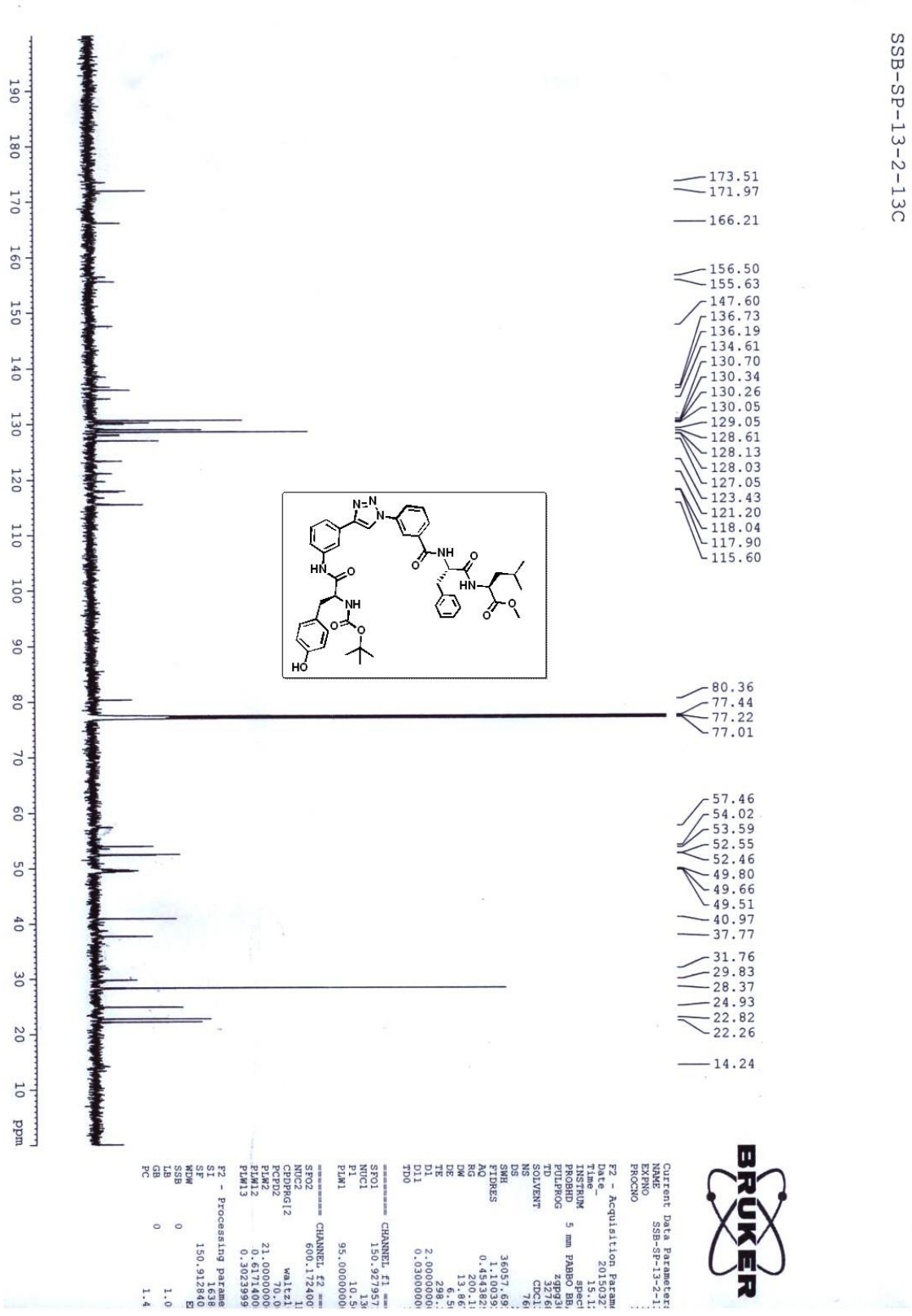


Figure S75. ^{13}C Spectra of synthesized compound **3.**