Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2018

Relay FRET Event in a Designed Trichromophoric Pentapeptide Containing *o-, m*-Aromatic-Amino Acid Scaffold

Subhendu Sekhar Bag* and Afsana Yashmeen

Bioorganic Chemistry Laboratory, Department of Chemistry, Indian Institute of Technology Guwahati,North Guwhati-781039, Assam, India. Fax: +91-361-258-2349; Tel: +91-361-258-2324.E-mail:ssbag75@iitg.ernet.in

1. Experimental Section

1.1. Materials and Methods

All reactions were carried out under inert atmosphere using flame-dried glassware. Combined organic layers were dried over anhydrous sodium sulfate. After work up solvents were removed in a rotary evaporator under reduced pressure. For column chromatography Silica gel (60-120 mesh) was used. Reactions were monitored by TLC on silica gel 60 F254 (0.25). BSA, Na_2HPO_4 and $NaH_2PO_4.H_2O$ (for preparation of phosophate buffer) were purchased from Merck, India and used without further purification. Milli-Q Water was taken for solution preparation. All solutions were prepared freshly before doing the experiments. The probe molecules (pentapeptide **3**) were synthesized and purified according to the procedure described.

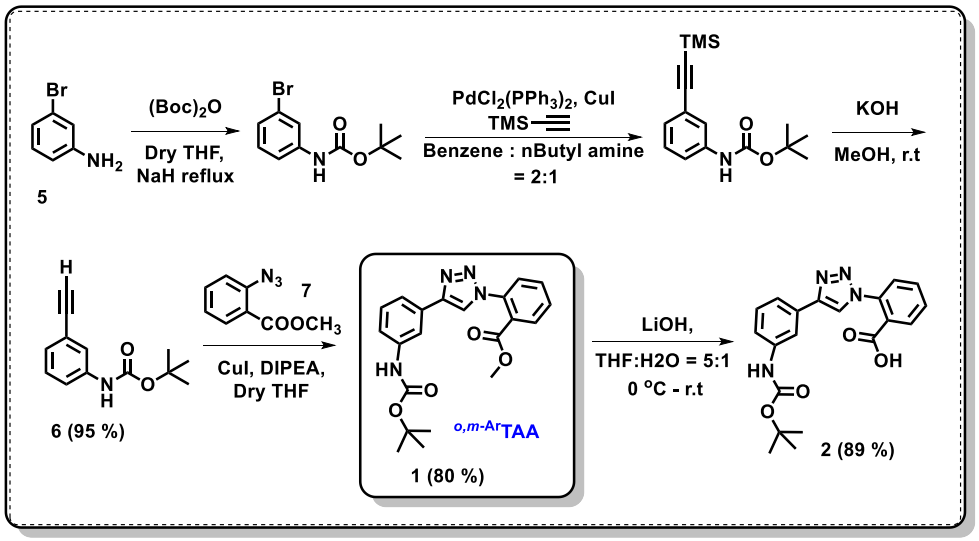
^{1}H NMR spectra were recorded either at 400 MHz or at 600 MHz and ^{13}C NMR spectra were recorded either at 100 MHz or at 150 MHz (mentioned accordingly). Coupling constants (*J* value) were reported in hertz (Hz). The chemical shifts were shown in ppm downfield form tetramethylsilane, using residual 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 analyzed 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 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 parameters are given below.

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.

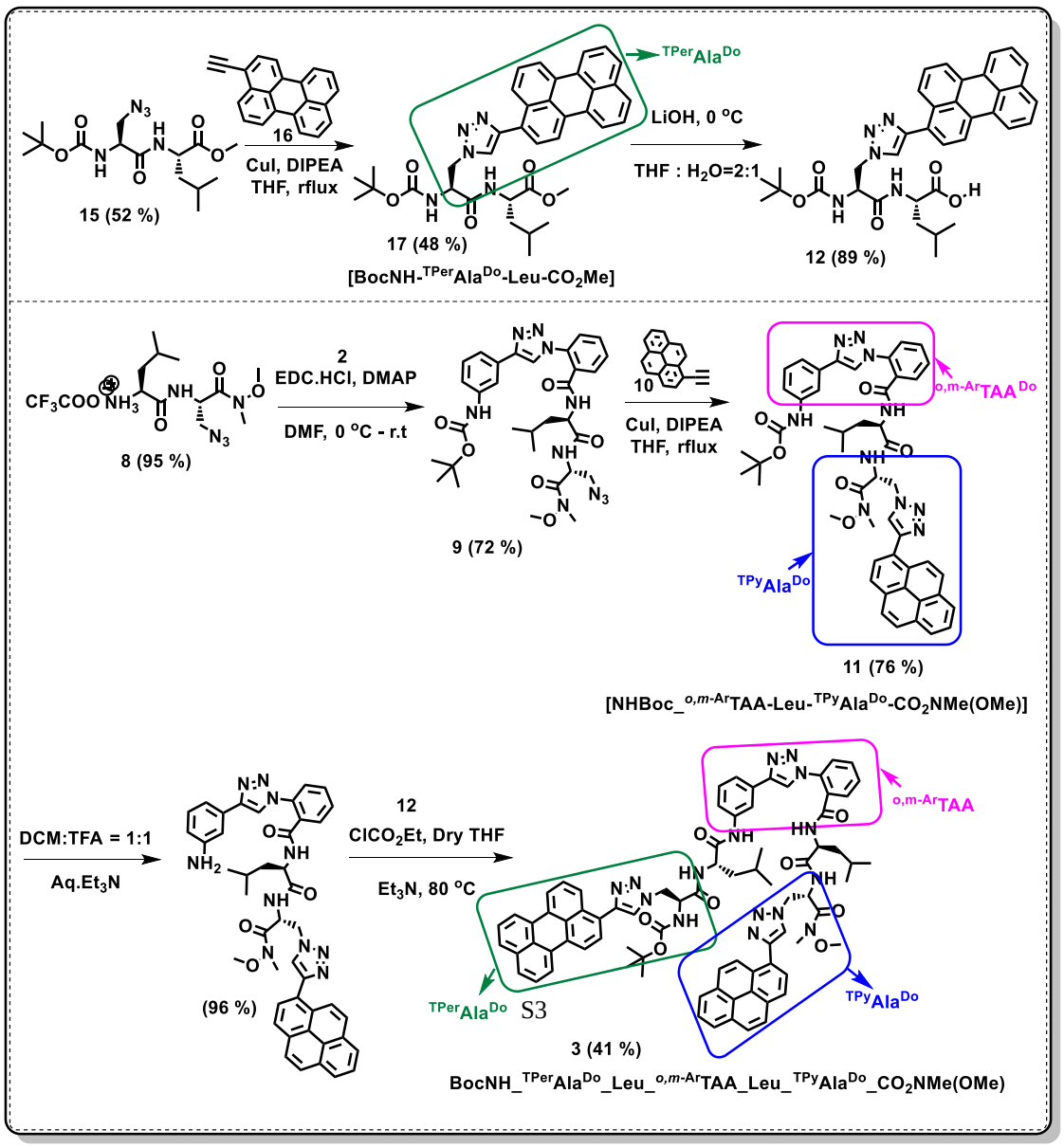
ROESY: Free induction decay (FID) with NS = 16 and DS =16, relaxation delay (D1) 2s, mixing time (P15) 0.02s, acquisition time (AQ) 0.085s, spectral width (SWH) 12019 Hz.

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.

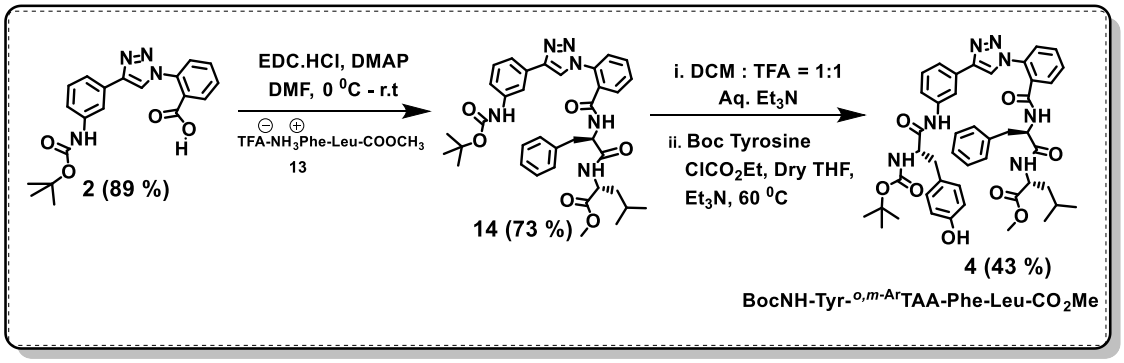
1.2. Synthesis of *ortho,meta*-Triazolo Aromatic Amino Acids Scaffold ($^{o,m\text{-Ar}}$TAA) and Corresponding Peptides-The Synthetic Schemes



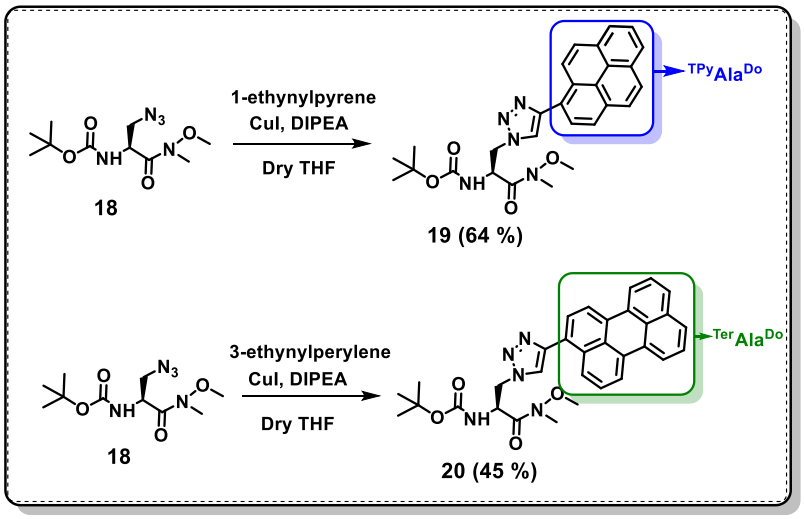
Scheme S1. Synthesis of *o*, *m*-aromatic triazolyl amino acid scaffold **1** ($^{o,m\text{-Ar}}$**TAA**).



Scheme S2. Synthetic scheme of aromatic triazolyl amino acid scaffold based unnatural **pentapeptide 3** [**BocNH-^{TPer}Ala^{Do}–Leu–$^{o,m\text{-}Ar}$TAA–Leu–^{TPy}Ala^{Do}-CONMe(OMe)].**



Scheme S3. Synthetic scheme of *ortho,meta*-triazolyl aromatic amino acid scaffold based Leu-enkephalin analogue natural **Tetrapeptide 4** [**BocNH-Tyr–$^{o,m\text{-}Ar}$TAA–Phe–Leu-COOMe**].



Scheme S4. Synthetic scheme for monomeric amino acids ^{TPy}**Ala**^{Do} (**19**) and ^{TPer}**Ala**^{Do} (**20**) in *N*, *C*-protected form.

1.3. Synthetic Procedures and Characterisation

***General procedure for the peptide coupling*:** To a solution *N*-protected amino acids/peptides 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 ^{o}C. Then the amine salt of wienreb amide or methyl ester protected corresponding amino acids or dipeptides (1.0 equiv.) were added followed by diisopropylethylamine (DIPEA) (2.4 equiv.). The reaction mixture was stirred for another 18-20 h at 0 ^{o}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.

***General procedure of [3+2] cyclo-addition reaction*:** The azido derivative of compounds were taken in dry THF and degassed for 5 min with nitrogen gas. After adding alkyne (1.1 equiv.) degassing were continued for the next 5 min. Then, 1 mol% powderd CuI was added. Then 1.2 equiv. DIPEA was added and reaction mixture was degassed and allowed to precede for 12 h about 65 to 70 ^{o}C. After total consumption of the starting azide (Monitored by TLC), the reaction mixture was evaporated completely and work up was done by EtOAc and NH_4Cl solution. The organic layer was washed with brine and dried over Na_2SO_4. The targeted trizolyl compounds were separated by column chromatography and characterized.

***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 ^{o}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_2SO_4. The hydrolyzed compound was isolated by column chromatography (Si-gel, $CHCl_3$: MeOH = 10:1). Yield was 90-96%.

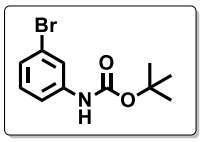
General procedure for the deprotection of the BocNH-group: The respective BocNH- protected amino acids and peptides was dissolved in CH_2Cl_2 and cooled to 0 ^{o}C. TFA (equal amount as the solvent) was added and the solution was allowed to warm to room temperature. The stirring was continued at room temperature until the starting material was consumed (monitored by TLC). The reaction mixture was evaporated in vacuum. 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 ^{o}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 vacuum to yield the crude product in quantitative yield to use for next step.

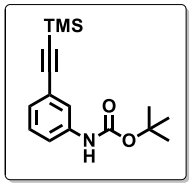
Synthesis of tert-butyl (3-ethynylphenyl)carbamate (6):

Synthesis of tert-butyl (3-bromophenyl)carbamate: In a dry THF, NaH (1.1 eqv.) (washed by hexane) and 3-bromo aniline **5** (2000 mg, 5.8 mmol) were taken. The reaction mixture was heated to reflux for one hour then cooled to room temperature.



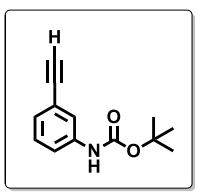
Boc anhydride (2.42 ml, 6.4 mmol) was added and reaction mixture was stirred for 30 minute. A second portion of sodium hydride (same eqv. amount again) was added to the reaction mixture and refluxed overnight. The reaction mixture was cooled to room temperature, carefully quenched by water. The reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4. The Pure product was isolated by column chromatography (Si-gel, PE : EtOAc = 10:1). (1300 mg, 4.8 mmol, Yield 82 %).

Synthesis of tert-butyl (3-((trimethylsilyl)ethynyl)phenyl)carbamate: In a dry R.B, compound tert-butyl (3-bromophenyl)carbamate (1300 mg, 4.8 mmol) was taken with dry solvent benzene : n-butyl amine = 2:1 then catalyst $PdCl_2(PPh_3)_2$ (168 mg, 0.24 mmol) was added and degassed by N_2 gas. After fifteen minute of stirring the



reaction mixture, TMS acetylene (1.02 ml, 7.2 mmol) and CuI (46 mg, 0.24 mmol) was added and heated to 80 ^oC temperature for 12 hour. Then solvent was dried by rotary evaporator, and it was partitioned between EtOAc and aqueous NH_4Cl solution (20 ml each). The organic layer was washed with brine solution. Pure product (1124 mg, 3.9 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 10:1). Yield 81 %. ^1H 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.

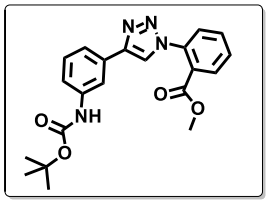
Synthesis of tert-butyl (3-ethynylphenyl)carbamate (6): Tert-butyl (3-((trimethylsilyl)ethynyl)phenyl)carbamate, (500 mg, 1.73 mmol) was treated with



methanol and KOH (145 mg, 2.6 mmol) at room temperature and stirrer for 1 hour. Then solvent was dried by rotary evaporator, work up was done with EtOAc and water. Pure product (360 mg, 1.64 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 10:1). Yield 95 %. ^1H 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.

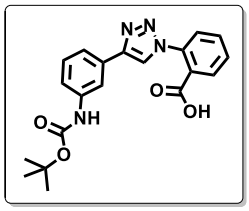
Synthesis of $^{o,m\text{-}Ar}$TAA (1): Tert-butyl (3-ethynylphenyl) carbamate **6**, (360 mg, 1.64 mmol) was taken in dry THF and degassed for 5 min with nitrogen gas. Methyl 2-azidobenzoate **7**, 320 mg (1.8 mmol), 1 mol % CuI, and 1.2 eqv. DIPEA was added

and reaction mixture was refluxed for 12 hour. After completion of reaction (monitored by TLC) the reaction mixture was evaporated completely and the work up



was done by EtOAc and NH_4Cl solution. The organic layer was washed with brine, dried over Na_2SO_4 and concentrates under high vacuum. Pure product **1** (520 mg, 1.3 mmol) was isolated by column chromatography (Si-gel, PE : EtOAc = 2:1). Yield 80 %. IR (KBr) 3346, 2978, 1726, 1598, 1529, 1437, 1299, 1159, 1051, 761 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz) δ 1.52 (9H, s). 3.70 (3H, s), 6.66 (1H, s), 7.36 (2H, d, J = 4.4 Hz), 7.51 (1H, d, J = 8.2 Hz), 7.60 (2H, t, J = 7.5 Hz), 7.68 (1H, t, J = 7.5 Hz), 7.95 (1H, s), 8.01 (1H, d, J = 7.7 Hz), 8.08 (1H, s); ^{13}C NMR ($CDCl_3$,150 MHz) δ 15.5, 28.5, 52.8, 80.9, 115.9, 118.5, 120.7, 121.8, 126.8, 127.8, 129.8, 130.1, 131.3, 131.5, 132.9, 136.3, 139.2, 147.6, 152.9, 165.9; HRMS calcd for $C_{21}H_{23}N_4O_4$ ($[M + H]^+$) 395.1712, found 395.1737.

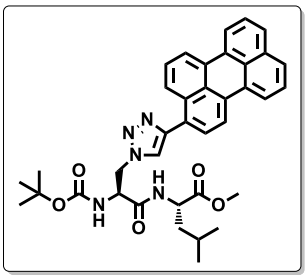
Synthesis of BocNH-$^{o,m\text{-}Ar}$TAA-COOCH$_3$ (2): Using the general procedure of methyl ester hydrolysis, starting from 600 mg (1.52 mmol) of **1**, 515 mg (1.36 mmol) of the title compound **2** was isolated as a white solid material in pure form by column chromatography ($CHCl_3$: MeOH = 50 : 1). Yield 89 %. IR (KBr) 3307, 2979, 1717, 1603, 1239, 1159, 1055, 761; ^1H NMR (CD_3OD, 600 MHz) δ 1.50 (9H, s),



7.30 – 7.25 (2H, m), 7.42 – 7.36 (1H, bs), 7.48 (1H, d, J = 7.5 Hz), 7.52 (1H, d, J = 7.6 Hz), 7.58 (1H, t, J = 7.6 Hz), 7.67 (1H, t, J = 7.7 Hz), 8.06 (1H, s), 8.07 (1H, s); ^{13}C NMR (CD_3OD, 150 MHz) δ 28.5, 39.7, 81.2, 115.6, 116.2, 118.5, 118.6, 120.8, 121.3, 122.6, 125.8, 127.1, 128.3, 129.7, 129.9, 130.2, 130.6, 130.9, 131.9, 132.1, 133.1, 134.9, 136.5, 139.1, 140.9, 147.4, 167.9, 173.3, 176.4.

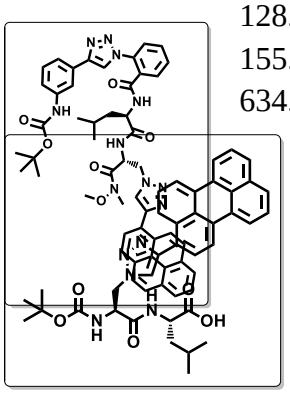
Synthesis of di, tri, tetrapeptides and triazolyl donor/acceptor unnatural pentapeptide (3): The targeted peptides were synthesized following the general procedure of peptide coupling protocol as was earlier discussed. The pure product was isolated by column chromatography.

Synthesis of BocNH-^{TPer}Ala^{Do}-Leu-COOCH$_3$ (17): Using general procedure of [3+2] cyclo-addition reaction, starting from 200 mg (0.52 mmol) of azide derivative of dipeptide **15** (Synthetic procedure described in our previous journal: Bag, S. S.; Yashmeen, A. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 5387.) and 157 mg (0.57 mmol) of 1-ethynyl Perylene **16**, (160 mg, 0.252 mmol) of the title compound **17** was isolated



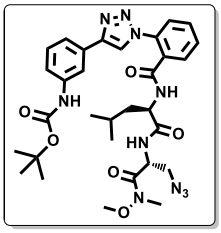
as a dark brown solid material (Si-gel, PE : EtOAc = 1:1). Yield 48 %; IR (KBr) 3298, 2957, 1477, 1721, 1661, 1520, 1249, 1163, 810, 765 cm^{-1}. ^1H NMR ($CDCl_3$; 600 MHz); δ 0.76 (6H, s). 1.41 (2H, s), 1.48 (9H, s), 1.60 – 1.52 (1H, m), 3.70 (3H, s), 4.56 (1H, d, J = 6.9 Hz), 4.82 (2H, d, J = 14.6 Hz), 5.03 (1H, d, J = 11.7 Hz), 5.96 (1H, d, J = 6.3 Hz), 7.07 (1H, s), 7.47 (3H, dd, J = 15.7, 7.7 Hz), 7.66 (3H,

d, *J* = 7.1 Hz), 7.97 (1H, s), 8.17 (4H, dd, *J* = 18.2, 7.2 Hz), 8.24 (1H, d, *J* = 8.6 Hz); ^{13}C NMR ($CDCl_3$; 150 MHz); *δ* 21.8, 22.9, 24.8, 28.5, 29.9, 41.3, 51.2, 52.6, 54.6, 81.3, 117.3, 119.9, 120.7, 122.9, 125.4, 126.7, 126.8, 127.3, 128.0, 128.0, 128.2, 128.6, 129.3, 131.0, 131.4, 131.6, 131.9, 132.4, 134.7, 147.0, 155.6, 169.1, 172.9. HRMS calcd. for $C_{37}H_{40}N_5O_5$ ([M + H]$^+$) 634.3024, found 634.3020.



Synthesis of (BocNH-^{TPer}Ala^{Do}-Leu-COOH (12): Using the general procedure of ester hydrolysis, starting from 160 mg (0.252 mmol) of **17**, 140 mg (0.224 mmol) of the title compound **12** was isolated as a dark brown solid material. Yield 89%. This compound is used for the next step without further characterisation.

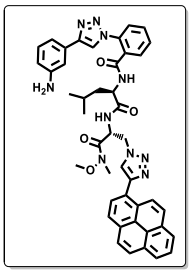
Synthesis of BocNH-$^{o,m\text{-}Ar}$TAA-Leu-Ser(N$_3$)-CONMe(OMe) (9): $^{o,m\text{-}Ar}$**TAA** scaffold **2** (116 mg, 0.266 mmol) was taken in dry DMF, then EDC.HCl (77 mg, 0.4 mmol), followed by DMAP (81 mg, 0.665 mmol) were added at 0 ^{o}C. Then Boc deprotected Leucine-serine dipeptide **8** (Synthetic procedure described in our previous journal: Bag, S. S.; Yashmeen, A. *Bioorg. Med. Chem. Lett*. **2017**, *27*, 5387) (100 mg, 0.266 mmol), was added and the reaction mixture and it was stirred for 1h at 0 ^{o}C, after that ice bath is removed and the reaction mixture was allowed to stir at room temperature for 18h. After completion of the reaction (monitored by TLC), the solvent was dried. Work up was done with EtOAc and $NaHCO_3$ solution. Combined organic layer was washed with brine solution and concentrated under reduced pressure. The product tripeptide azide **9** (124 mg, 0.19 mmol) was isolated in pure form by column chromatography (Si-gel, PE : EtOAc = 1:1) as colorless gummy material. Yield 72 %; IR (KBr) 3450, 2958, 2928, 2102, 1653, 1509, 1390, 1167, 1049, 848 cm^{-1}. ^{1}H NMR ($CDCl_3$; 600 MHz); *δ* 0.75 (6H, t, *J* = 6.8 Hz), 1.50 (9H, m), 1.41 (2H, m), 1.51 (1H, s), 3.19 (3H, s), 3.49 (2H, d, *J* = 5.0 Hz), 3.70 (3H, s), 4.51 (1H, dd, *J* = 14.2, 8.3 Hz), 4.99 (1H, bs), 6.68 (1H, d, *J* = 7.9 Hz), 6.98 (1H, bs), 7.20 (1H, d, *J* = 7.9 Hz), 7.32 (1H, t, *J* = 7.9 Hz), 7.59 – 7.45 (5H, m), 7.68 (1H, d, *J* = 7.2 Hz), 7.84 (1H, s), 8.17 (1H, s); ^{13}C NMR ($CDCl_3$; 150 MHz); *δ* 15.4, 21.8, 23.0, 24.8, 28.5, 32.5, 41.1, 49.7, 51.8, 52.7, 61.9, 66.0, 80.8, 116.0, 119.1, 120.6, 122.3, 126.3, 129.4, 129.7, 130.2, 130.8, 131.5, 131.6, 132.3, 134.2, 138.9, 148.0, 152.8, 166.7, 169.0, 171.6. HRMS calcd. for $C_{31}H_{41}N_{10}O_6$ ([M + H]$^+$) 649.3205, found 649.3211.



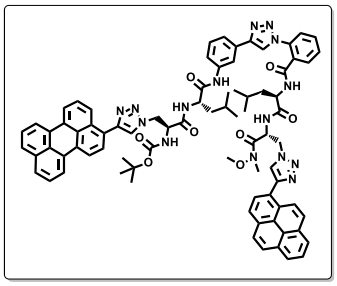
Synthesis of BocNH-$^{o,m\text{-}Ar}$TAA-Leu-^{TPy}Ala^{Do}-CONMe(OMe) (11): Using the general procedure of [3+2] cyclo-addition reaction, starting from (120 mg, 0.185 mmol) of azide derivative of tripeptide **9** and 44 mg (0.2 mmol) of 1-ethynyl pyrene **10**, (122 mg, 0.14 mmol) of the title compound **11** was isolated as a light brown solid material (Si-gel, PE : EtOAc = 1:2). Yield 76 %; IR (KBr) 3296, 2958, 1725, 1653, 1531, 1437, 1158, 849, 762 cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz) *δ* 0.77 (6H, dd, *J* =

10.1, 6.6 Hz), 1.47 (9H, s), 1.54 (3H, m), 3.77 (3H, s), 3.25 (3H, s), 4.49 (1H, d, *J* = 6.0 Hz), 4.83 (2H, d, *J* = 14.9 Hz), 5.39 (1H, bs), 6.83 (1H, s), 6.96 (1H, s), 7.09 (1H, s), 7.14 (1H, s), 7.16 (1H, s), 7.23 – 7.18 (1H, m), 7.40 – 7.32 (2H, m), 7.43 (1H, d, *J* = 5.2 Hz), 7.52 (1H, s), 7.70 (1H, s), 7.91 (1H, s), 7.99 – 7.92 (3H, m), 8.02 (2H, t, *J* = 8.2 Hz), 8.09 (2H, d, *J* = 7.0 Hz), 8.14 (1H, d, *J* = 7.1 Hz), 8.20 (1H, s), 8.60 (1H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 15.4, 21.8, 23.0, 249, 28.5, 32.8, 40.6, 50.5, 50.7, 52.8, 62.1, 66.0, 77.0, 77.2, 77.4, 80.5, 115.9, 118.5, 120.2, 121.8, 124.8, 124.9, 125.0, 125.2, 125.2, 125.3, 126.1, 127.3, 127.5, 127.8, 128.1, 128.5, 128.9, 129.6, 129.6, 130.6, 131.0, 131.1, 131.2, 131.5, 131.6, 134.1, 139.3, 147.3, 147.8, 152.9, 157.2, 167.0, 168.4, 172.0; HRMS calcd for $C_{49}H_{51}N_{10}O_6$ ($[M + H]^+$) 875.3988, found 875.3990.

Synthesis of NH_2-$^{o,m\text{-}Ar}$TAA-Leu-^{TPy}Ala^{Do}-CONMe(OMe): Using the general procedure of BocNH-deprotection, we have deprotected compound **11**. Then the reaction mixture was concentrates under vacuum, diluted with EtOAc and neutralized with Et_3N. The organic layer was then partitioned by EtOAc and water. Combined organic layer was washed with brine solution and dried over Na_2SO_4. Mixture concentrates under high vacuum. The product was obtained in quantitative yield and was used without further purification and characterization.



Synthesis of BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA-Leu-^{TPy}Ala^{Do}-CONMe(OMe) (3): The acid compound **12,** 120 mg (0.2 mmol) was taken in a dry 50 ml RB. Then dry THF was added to the RB under nitrogen atmosphere maintaining the solution to stir at 0 ^{o}C temperature. Then ethyl chloroformate (0.028 ml, 0.3 mmol) and tri-ethylamine (0.04 ml, 0.3 mmol) were added maintaining 0 ^{o}C. Then free amine of tripeptide, 150 mg (0.2 mmol) was added to the reaction mixture and allowed to stirrer about half an hour. Then the ice bath was removed and the reaction mixture was refluxed about 6 hours at 80 ^{o}C. Completion of reaction was monitored by TLC. Pure product **3**, 113 mg (0.12 mmol) was isolated as a dark brown solid material (Si-gel, PE : EtOAc = 1:3). Yield 41 %. IR (KBr) 3294, 2923, 1722, 1655, 1532, 1223, 1159, 1050, 849, 763 cm^{-1}. ^{1}H NMR (d_6-DMSO; 600 MHz) δ 0.65 (6H, s), 0.75 – 0.90 (6H, m), 1.21 (9H, s), 1.28 (4H, d, *J* = 18.2 Hz,), 1.42 (2H, s), 3.15 (3H, s), 3.76 (3H, s), 4.34 (1H, s), 4.72 (3H, s), 4.79 (1H, s), 5.33 (1H, s), 7.09 (2H, d, *J* = 32.3 Hz), 7.37 – 7.11 (3H, m), 7.41 (1H, s), 7.60 (7H, dd, *J* = 73.1, 43.1 Hz), 7.78 (1H, s), 8.08 (2H, s), 8.14 (5H, d, *J* = 34.0 Hz), 8.27 (6H, dd, *J* = 23.1, 16.5 Hz), 8.37 (2H, s), 8.48 (1H, s), 8.72 – 8.51 (4H, m), 8.78 (2H, s), 9.70 (1H, s), 10.29 (1H, s); ^{13}C NMR (d_6-DMSO; 150 MHz) δ 14.0, 14.6, 21.2, 22.1, 23.0, 24.0, 28.1, 29.1, 31.4, 32.1, 37.6, 49.7, 51.9, 59.2, 61.6, 78.6, 119.2, 121.1, 122.6, 123.9, 124.3, 125.1, 125.5, 126.5, 127.2, 127.4, 127.7, 127.9, 127.9, 128.3, 129.1, 129.3, 130.4, 130.6, 130.9, 132.4, 134.0, 134.3, 136.4, 138.2,



139.8, 146.0, 146.4, 153.5, 154.2, 155.1, 159.6, 162.2, 165.6, 166.3, 168.5, 168.8, 172.3, 173.5; HRMS calcd for $C_{80}H_{78}N_{15}O_8$ ($[M + H]^+$) 1376.6152, found 1376.6139.

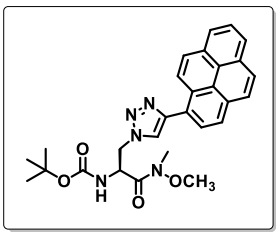
Synthesis of BocNH-$^{o,m\text{-}Ar}$TAA-Phe-Leu-COOCH$_3$ (14): A solution of N-protected aromatic triazolyl amino acid **$^{o,m\text{-}Ar}$TAA 2,** (116 mg, 0.266 mmol) in dry DMF**,** was taken in a dry R.B. Under ice cold condition (EDC.HCl) (76 mg, 0.4 mmol), DMAP (81 mg, 0.66 mmol) were added and stirred for 5 minutes then the amine salt of methyl ester protected phenyl alanine leucine dipeptide was added and stirring was continued for another 30 minutes maintaining 0 ^oC. Then the reaction mixture was bringing to room temperature and stirred for another 18 h. After completion of the reaction, the solvent was dried. Work up was done with EtOAc and water. Combined organic layer was washed with brine solution and purified by column chromatography (Si-gel, PE: EtOAc = 1:1). Pure product **14** (125 mg, 0.19 mmol) was isolated as solid compound. Yield 73 %; IR (KBr) 3302, 2957, 1730, 1652, 1531, 1236, 1158, 1051, 761 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz) δ 0.75 (6H, t, J = 6.8 Hz), 1.44 (2H, d, J = 9.2 Hz), 1.51 (10H, s), 3.49 (2H, d, J = 5.0 Hz), 3.70 (3H, s), 4.51 (1H, dd, J = 14.2, 8.3 Hz), 4.99 (1H, bs), 6.68 (1H, d, J = 7.9 Hz), 6.98 (1H, s), 7.20 (1H, d, J = 7.9 Hz), 7.32 (1H, t, J = 7.9 Hz), 7.58 – 7.44 (5H, m), 7.68 (1H, d, J = 7.2 Hz), 7.84 (1H, s), 8.17 (1H, s); ^{13}C NMR (d_6-DMSO; 150 MHz); δ 21.9. 22.8, 24.9, 28.5, 29.9, 37.8, 40.9, 51.4, 52.4, 54.9, 80.7, 116.0, 118.6, 120.6, 121.8, 125.7, 127.2, 128.8, 128.9, 129.5, 129.7, 129.9, 130.9, 131.5, 131.9, 134.5, 136.5, 139.3, 147.9, 153.1, 166.7, 170.5, 173.0; HRMS calcd for $C_{36}H_{43}N_6O_6$ ($[M + H]^+$) 655.3239, found 655.3241.

Synthesis of NH$_2$-$^{o,m\text{-}Ar}$TAA-Phe-Leu-COOCH$_3$: Using the general procedure of BocNH-deprotection followed by washing with triethyl amine, we are getting the free amine derivative of compound **14** (100 mg, 0.18 mmol). The product was obtained in quantitative yield and was used without further purification and characterization.

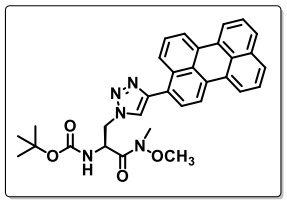
Synthesis of BocNH-Tyr-$^{o,m\text{-}Ar}$TAA-Phe-Leu-COOCH$_3$ (4): In a solution of *N*-Boc tyrosine (60.93 mg, 0.213 mmol) in dry THF at 0 ^0C temperature, ethyl chloroformate (24µl , 0.256 mmol) and triethyl amine (0.035 ml, 0.256 mmol) were added. After five minutes free amine and *C*-terminal protected tripeptide (100 mg, 0.19 mmol) was added and stirred at half an hour at cold condition. Then reaction mixture was refluxed about 6 hours. Completion of the reaction (monitored by TLC), the solvent was dried by rotary evaporator, Work up was done with EtOAc and water. Combined organic layer was washed with brine solution and concentrates under high vacuum. Pure product **4** (70 mg, 0.08 mmol) was isolated by column chromatography (Si-gel, PE : EtOAc = 1:1) as white solid. Yield 43 %; IR (KBr) 3320, 2958, 1743,

1658, 1618, 1537, 1516, 1368, 1254, 1164, 1030, 790 cm^{-1}. ^1H NMR (d_6-DMSO; 600 MHz) δ 0.70 (3H, d, J = 4.2 Hz). 0.81 (3H, d, J = 4.3 Hz), 1.32 (9H, s), 1.48 (1H, bs), 1.58 (2H, d, J = 8.2 Hz), 2.78 – 2.68 (1H, m), 2.86 (2H, dd, J = 29.4, 16.4 Hz), 3.13 (2H, dd, J = 22.0, 9.1 Hz), 3.62 (3H, s), 4.28 (2H, s), 4.58 (1H, s), 6.64 (1H, d, J = 7.4 Hz), 7.01 (1H, d, J = 7.8 Hz), 7.10 (1H, d, J = 7.5 Hz), 7.17 – 7.12 (1H, m), 7.22 – 7.18 (1H, m), 7.30 (3H, d, J = 7.9 Hz), 7.34 (2H, d, J = 6.7 Hz), 7.39 (1H, t, J = 7.4 Hz), 7.60 – 7.54 (2H, m), 7.63 (1H, d, J = 7.7 Hz), 7.67 (1H, t, J = 7.6 Hz), 7.74 (1H, d, J = 7.7 Hz), 8.22 (1H, s), 8.40 (1H, d, J = 6.3 Hz), 8.67 (1H, s), 9.00 (1H, d, J = 7.9 Hz), 9.16 (1H, s), 10.11 (1H, s); ^{13}C NMR (d_6-DMSO; $CDCl_3$; 150 MHz), δ 21.9, 23.2, 24.8, 28.8, 37.4, 39.7, 39.9, 40.0, 40.2, 40.3, 40.4, 40.6, 51.4, 52.5, 54.8, 57.6, 78.7, 115.5, 116.5, 119.6, 121.3, 122.9, 125.8, 125.9, 127.0, 128.6, 128.8, 128.9, 129.3, 129.6, 129.7, 129.9, 130.8, 131.4, 131.6, 131.9, 134.6, 138.6, 140.2, 147.1, 156.1, 156.4, 166.8, 171.8, 172.1, 173.5; HRMS calcd for $C_{45}H_{52}N_7O_8$ ($[M + H]^+$) 818.3872, found 818.3876.

***Synthesis of $^{TPy}Ala^{Do}$ (19)*:** Using general procedure of [3+2] cyclo-addition reaction 48 mg (0.18 mmol) of chiral serine azide **18** and 44 mg (0.19 mmol) of 1-ethynyl pyrene reacted each other gives 68 mg (0.137 mmol) of the title compound **19** was isolated as a light brown solid. Yield 72.4%; IR (KBr) 3421, 2976, 2930, 2103, 1712, 1663, 1164, 849, 757 cm^{-1}. ^1H NMR ($CDCl_3$; 400 MHz) δ 1.39 (9H, s), 3.24 (3H, s), 3.77 (3H, s), 4.84-4.85 (2H, d, J = 4.8 Hz), 5.18-5.20 (1H, m), 5.73-5.75 (1H, d, J = 7.6 Hz), 7.95-8.00 (2H, m), 8.03-8.06 (2H, m), 8.07 (1H, s), 8.12-8.12 (3H, m), 8.61-8.63 (1H, d, J = 9.2 Hz), ^{13}C NMR ($CDCl_3$; 100 MHz) δ 22.9, 28.5, 29.9, 32.6, 51.4, 62.0, 77.1, 77.4, 77.7, 124.3, 124.8, 125.0, 125.2, 125.3, 125.5, 126.2, 127.4, 127.5, 127.9, 128.2, 128.7, 131.0, 131.4, 131.5, 147.5, 155.4, 169.2. HRMS calcd. for $C_{28}H_{30}N_5O_4$ ($[M + H]^+$) 500.2376, found 500.2323.



Synthesis of $^{TPer}Ala^{Do}$ (20): Using general procedure of [3+2] cyclo-addition reaction, 0.060 g (0.219 mmol) of azide derivative of serine **18** and 0.072 g (0.263 mmol) of 1-ethynyl perylene was reacted. After completion of reaction (monitored by TLC) 0.058 g (0.105 mmol) of the title compound **20** was isolated in pure form as a brown solid (Si-gel, PE : EtOAc = 1:1). Yield 79%. IR (KBr) 3296, 2925, 2103, 1718, 1662, 1498, 1367, 1164, 1023, 809, 765 cm^{-1}. ^1H NMR ($CDCl_3$; 400 MHz) δ 1.40 (9H, s); 3.25 (3H, s); 3.78 (3H, s); 4.86–4.77 (2H, m); 5.14 (1H, bs); 5.62 (1H, d, J = 5.6 Hz); 7.43 (2H, d, J = 7.6 Hz); 7.51–7.47 (1H, m); 7.65 (2H, m); 7.82 (1H, s); 7.88 (1H, d, J = 8 Hz); 8.01 (1H, d, J = 8.4 Hz); 8.17–8.05 (4H, m); ^{13}C NMR ($CDCl_3$; 100 MHz); δ 28.4, 32.7, 51.4, 51.5, 62.1, 80.7, 120.1, 120.6, 120.7, 125.6, 126.7, 126.8, 127.2, 128.0, 128.1, 128.2, 128.7, 129.3, 131.1, 131.4, 131.6, 131.8, 134.8, 147.0, 155.3, 169.1; HRMS calcd for $C_{32}H_{32}N_5O_4$ ($[M+H]^+$) 550.2449, found 550.2450.



2. Conformational Study of Pentapeptide 3 and Tetrapeptide 4 using CD, IR, NMR Spectroscopic Techniques.

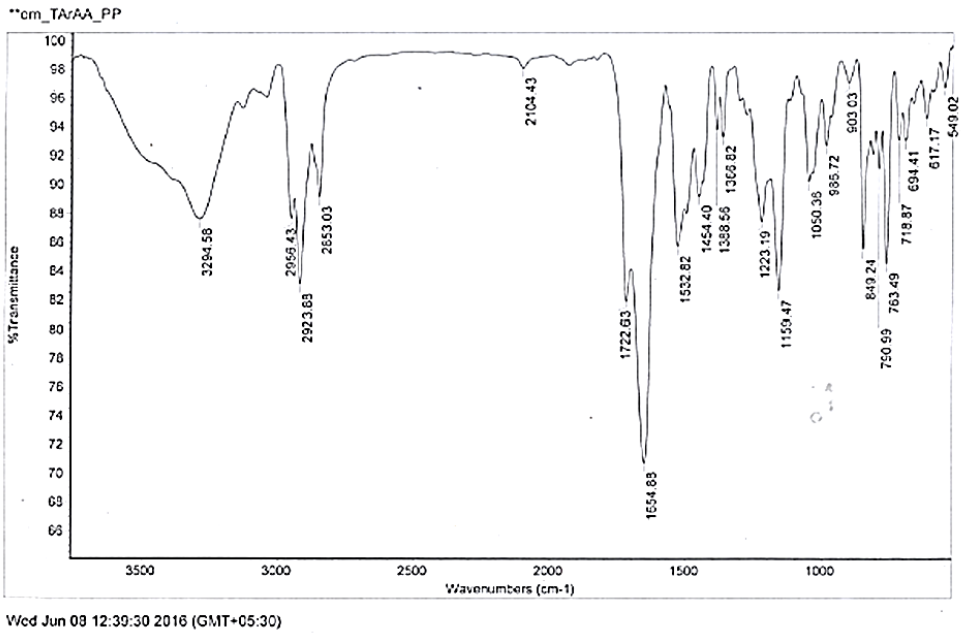
2.1. ***Study of Circular Dichroism Spectroscopy:*** CD spectra were recorded using a CD spectropolarimeter with a cell path length of 1 mm at in different solvent at room temperature. All the samples were with 50 μM concentration and prepared in spectroscopic grade solvents.

2.2. Infrared Spectroscopic study of BocNH-^{TPer}Ala^{Do}-Leu-$^{o.m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe) [Pentapeptide 3] and BocNH-Tyr-$^{o.m\text{-}Ar}$TAA^{Do}-Phe-Leu-COOMe [Tetrapeptide 4]

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

Table S1. Summary table of IR spectra

Peptides	Free N-H (cm^{-1})	H bonded N-H (cm^{-1})
Pentapeptide 3	3463	3294
Tetrapeptide 4	3480	3320



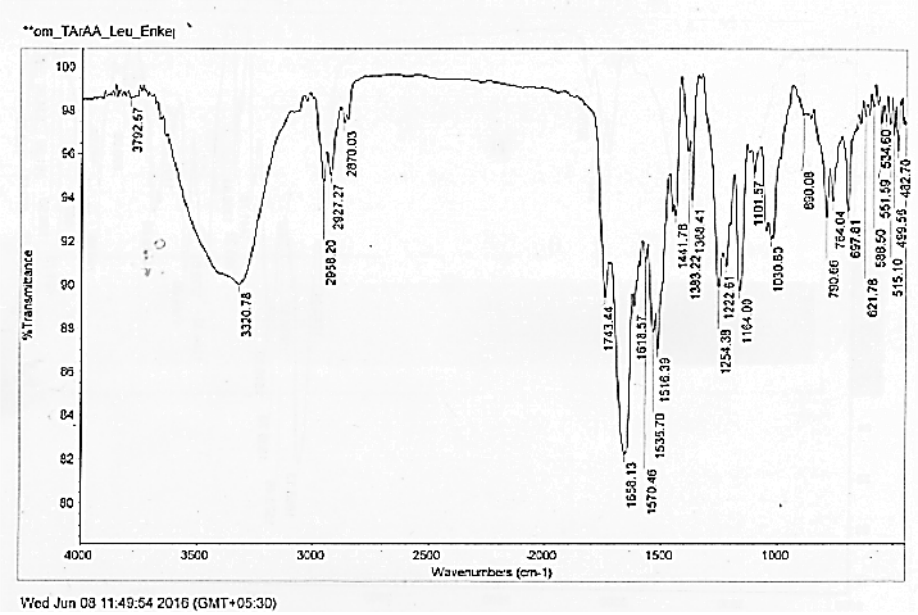


Figure S1. IR spectra of **BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe) Pentapeptide 3 (top) and BocNH_Tyr_$^{o,m\text{-}Ar}$TAA$_{Do}$_Phe_Leu_COOMe Tetrapeptide 4 (bottom)**

2.3. Variable Temperature ^{1}H NMR Data of Pentapep. 3 and Tetrapep. 4.

In our synthesized peptides the presence of intramolecular H-bonds was assessed by determining the variation of chemical shifts of the various NHs with temperature, in d_6-DMSO in which all NHs exhibited different chemical shifts. The Triazole C-H of N-terminal amino acid **pentapeptide 3 [BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe)]** also exhibited temperature effect. All the amide NH's, and triazole C-H exhibited ($\Delta\delta/\Delta$T) values that are moderate to close to Kessler limit of -3 to -6 ppb/K indicating presence of strong to moderate intramolecular H-bonding and supported the predominant sheet like structure of the peptides.

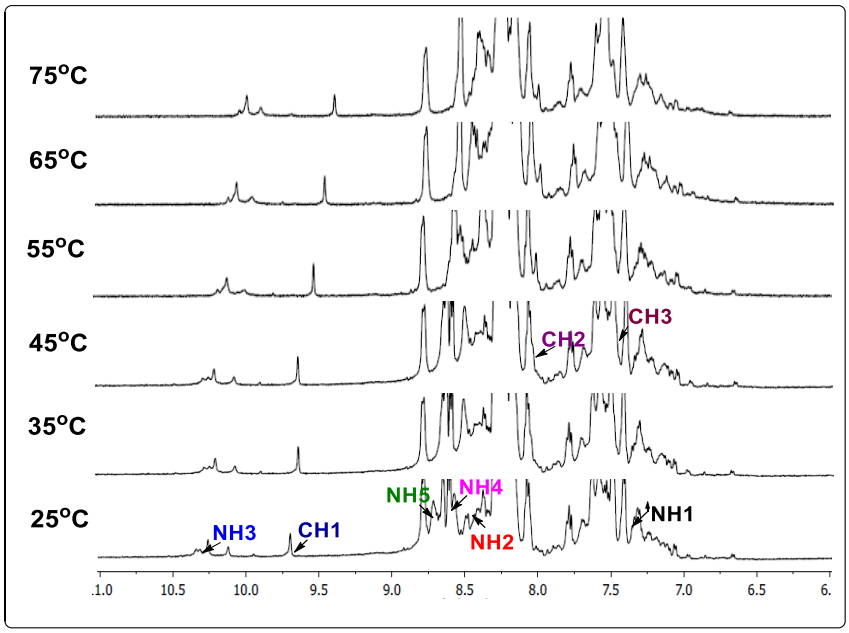


Figure S2. Variable temperature ^{1}H NMR spectra of **peptide 3** in d_6-DMSO showing temperature dependence of amide-NH/triazole-CH chemical shift.

Table S2. Values of temperature coefficients of chemical shifts of amide NHs or triazole-CH in **Pentapep. 3 [BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe)].**

Kessler limit ($\Delta\delta/\Delta T$) ppb/k	
N_terminal_Ser_NH1	- 5.00 ppb/k
N_terminal_Leu_NH2	- 5.43 ppb/k
$^{o,m\text{-}Ar}$TAA_NH3	- 4.63 ppb/k
C_ terminal _Leu_NH4	- 5.26 ppb/k
C_ terminal _Ser_NH5	- 6.31 ppb/k
$^{o,m\text{-}Ar}$TAA_CH1	- 6.00 ppb/k
N_terminal_^{Per}Tz_CH1	- 1.00 ppb/k
C_ terminal_^{Py}Tz_CH3	- 1.07 ppb/k

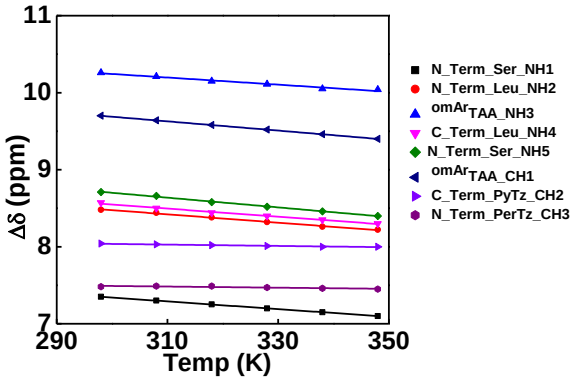


Figure S3. Temperature dependence of amide-NH/triazole-CH chemical shift of **pentapeptide 3 [BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe)]**

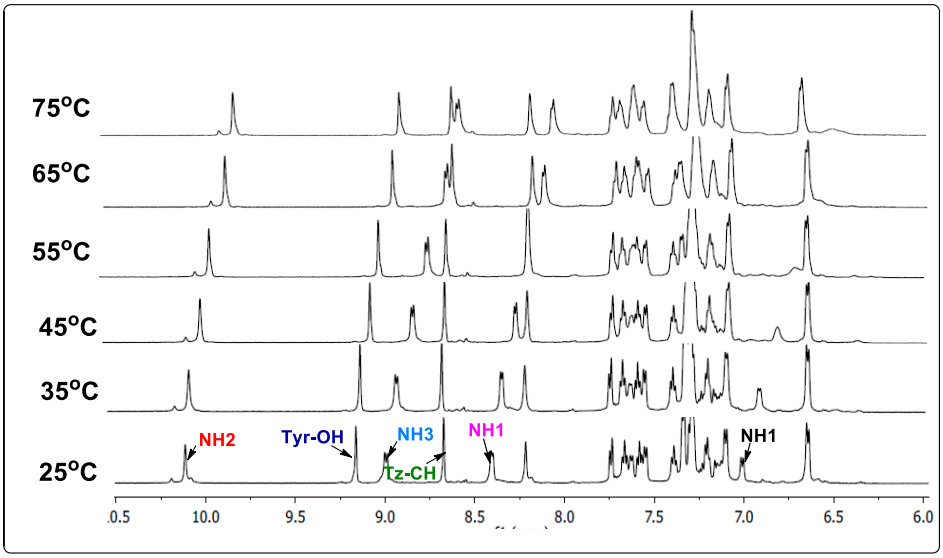


Figure S4. Variable temperature ^{1}H NMR spectra of **Tetrapeptide 4** in d_6-DMSO showing temperature dependence of amide-NH/triazole-CH chemical shift.

Table S3. Values of temperature coefficients of chemical shifts of amide NHs or triazole-CH in **Tetrapeptide 4 [BocNH-Tyr-$^{o,m\text{-Ar}}$TAA^{Do}-Phe-Leu-COOMe)]**

Kessler limit ($\Delta\delta/\Delta$T) ppb/k	
N_terminal_Tyr_NH1	- 12.5 ppb/k
$^{o,m\text{-Ar}}$TAA_NH2	- 6.49 ppb/k
C_terminal_Phe _NH3	-9.0 ppb/k
C_ terminal _ Leu_NH4	-7.54 ppb/k
^{Ar}TAA_triazole_CH1	-1.63 ppb/k
Tyr_OH	-5.63 ppb/k

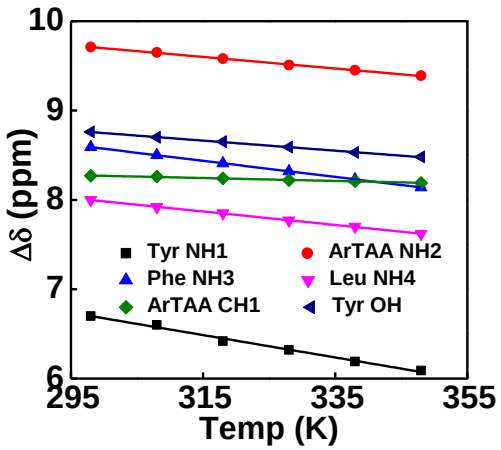


Figure S5. Temperature dependence of amide-NH/triazole-CH chemical shift of $^{o,m\text{-Ar}}$TAA leucine Enkephaline (**Tetrapep. 4**)

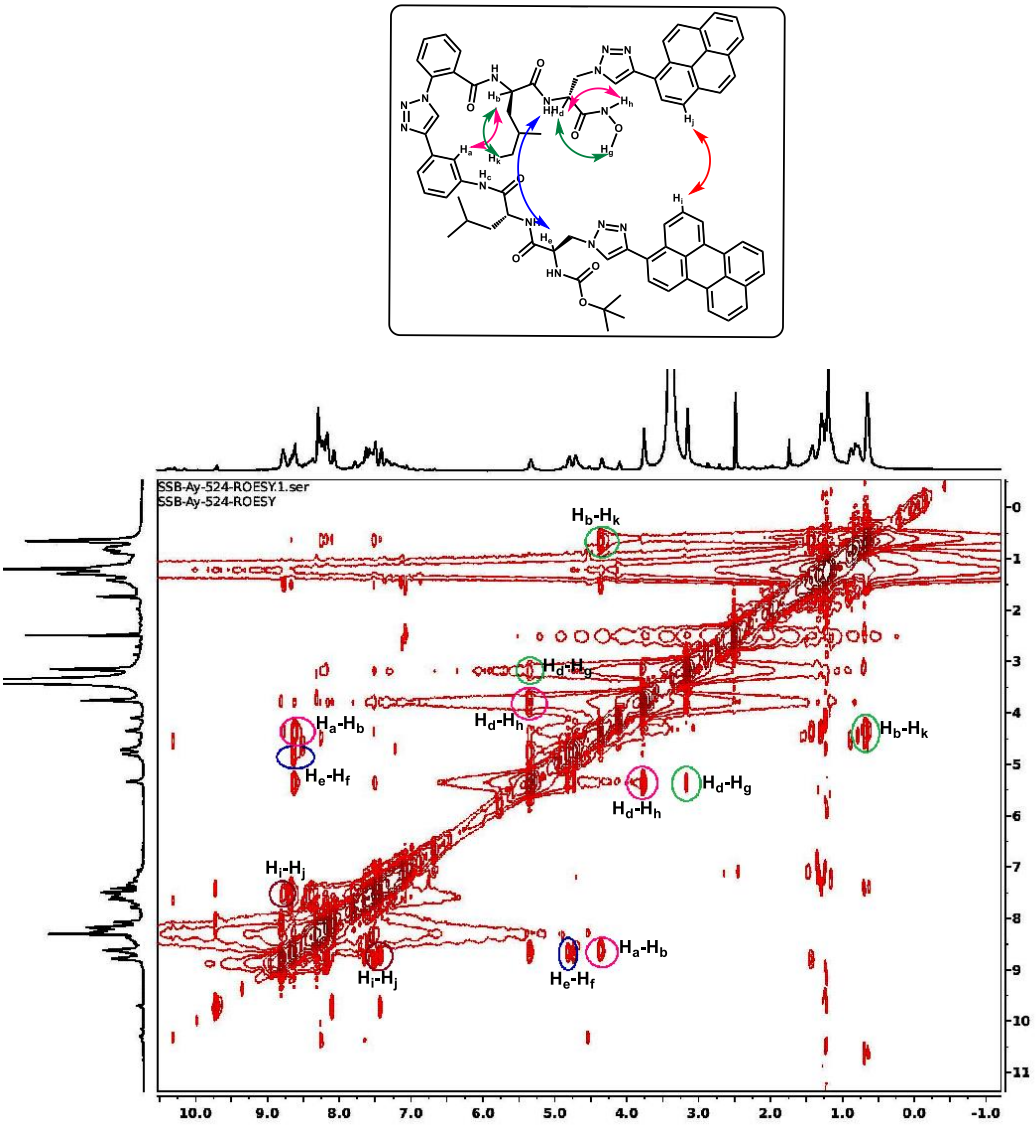


Figure S6. NOSEY Spectra of synthesized **pentapeptide 3 [BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA^{Do}-Leu-^{TPy}Ala^{Do}-CONMe(OMe)].**

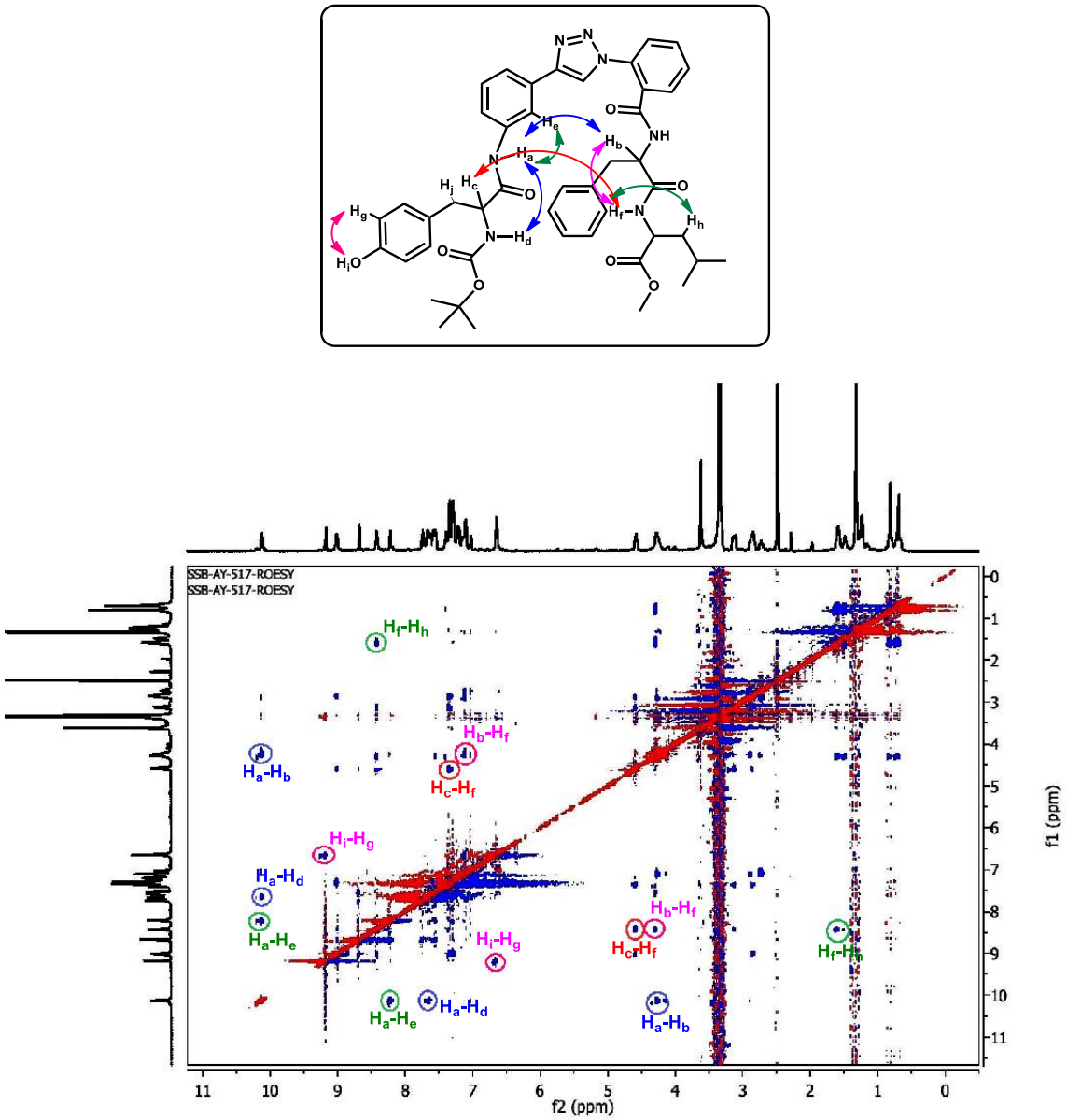


Figure S7. NOESY Spectra of synthesized $^{o,m\text{-Ar}}$**TAA Leu-Enkephaline**

2.4. Macro Model Study and Molecular Dynamics Simulation for Peptide 3 and 4.

Optimization of the Peptides and Conformational Search of Optimized Structures: We have carried out the molecular modeling study of peptide 3 and 4 using Schrodinger MacroModel (Maestro vs. 9.1) software with OPLS 2005 force field in water. The Polak-Ribiere first derivative method was used for the conjugate gradient minimization scheme [PRCG (Polak-Ribiere Conjugate Gradient)] to minimize the peptides.

Using OPLS 2005 force field at constant dielectric in water the conformational search was carried out with "large scale low-frequency-mode conformational search" (Mixed torsional/Large scale low-mode sampling = MCMM/LMCS) method.

Out of 50 minimized and well converged conformers, one conformer appeared 4 times (for **Pentapeptide 3**) and 7 times (**Tetrapeptide 4**) which remained within 1.00 k.cal/mole (4.18 kJ/mole) global minimum with a convergence threshold of 0.04 to 0.049 RMSD (threshold cutoff = 0.05).

Molecular Dynamics Simulation of Optimized Structure of the Peptides: Next MD simulations for the peptides were carried out using an OPLS 2005 force field. The starting structures for the duplexes were the global minimum conformers. The minimization method was chosen for minimizing the generated structures (with maximum iteration of 1000) with gradiant convergence threshold of 0.05.

The triazolyl pyrene and perylene moiety in **Pentapeptide 3**, were chosen as freely moving moieties during the simulation.

The peptide backbone containing orthometa triazolo aromatic amino acid scaffold (**$^{o,m\text{-Ar}}$TAA**) supports the β-sheet conformation. The observed H-bonding possibility revealed from the Modeling study was also supported from the VT-NMR study in all cases. The close proximity of triazolyl pyrene and perylene units was revealed from NMR studies was also supported by the MD simulation.

3. Study of Photophysical Properties

UV-visible: UV –visible spectra of the synthesized compounds with concentration 10 µm, were measured in different solvents using a UV-Visible spectrophotometer with a cell of 1 cm path length at 25 ^{o}C. Experiments were carried out with freshly prepared solutions made with spectroscopic grade solvents. The measurements were carried out in absorbance mode. The absorbance values of the sample solutions were measured in the wavelength regime of 200–550 nm.

Steady State Fluorescence: All the sample solutions with 10 µm concentration, was prepared freshly with spectroscopic grade solvents and were used for measuring *steady state fluorescence*. Fluorescence emission and excitation spectra were obtained using a fluorescence spectrophotometer at 25 °C using 1 cm path length cell. The excitation wavelengths for the monomers were set at λ^{abs}_{max}, emission spectra were recorded in the wavelength regime of 300–700 nm with an integration time of 0.2 sec. All the sample solutions were prepared freshly just before doing the experiment. Fluorescence emission spectrum was recorded exciting at their absorption maxima. Steady-state fluorescence emission spectra were recorded at room temperature as an average of 10 scans using an excitation slit of 5.0 nm, emission slit 5.0 nm, and scan speed of 120 nm/min.The fluorescence quantum yields (Φ_f) were determined using quinine sulphate as a reference with the known Φ_f $(0.54)^3$ in 0.1 molar solution in sulphuric acid. The following equation was used to calculate the quantum yield,

$$\Phi_S = \Phi_R \frac{Fl_S^{Area}}{Fl_R^{Area}} \frac{Abs_R}{Abs_S} \frac{n_S^2}{n_R^2}$$

where, Φ_R is the quantum yield of standard reference, Fl_S^{Area} (sample) and Fl_R^{Area} (reference) are the integrated emission peak areas, Abs_S (sample) and Abs_R (reference) are the absorbances at the excitation wavelength, and n_S (sample) and n_R (reference) are the refractive indices of the solutions.

Time resolved fluorescence: A time resolved fluorescence spectrophotometer (*Eddinburg Instruments FSP920*) was utilized to perform fluorescence lifetime experiments. Working condition was maintained at 25^{o}C and results were recorded at an excitation wavelength of 290 nm LED and 375 nm laser using a cuvette having path length 1 cm. To analyze the lifetime data time correlated single photon counting (TCSPC) method was used and analysis was done using a software package with range 205 – 4000 channels.

All the experiments were done four times. The experimental errors were found within 1-2 nm. The experimental standard errors were calculated based the following equations for four consecutive run for the same experiment at same condition.

$$SD = \sqrt{\sum_{i=1}^{n} \frac{1}{n-1}(x - \overline{x})^2}$$

……Equation 1

where SD is standard deviation, *x* is individual data points, $\overline{x}$ is the mean value of the experiments and n is the total number of observations.

The standard error (*SE*) was measured by sample standard deviation obtained divided by the square root of number of observations

$$SE = \frac{SD}{\sqrt{n}}$$

……..……....Equation 2

The experimental errors in wavelength for both the UV-Visible and fluorescence measurement were found to be in the range of 1-2 nm. Error in Quantum yield calculation lies in the range of 8-10%. The experimental error for lifetime measurement lies between $\pm$ 0.4 ns range.

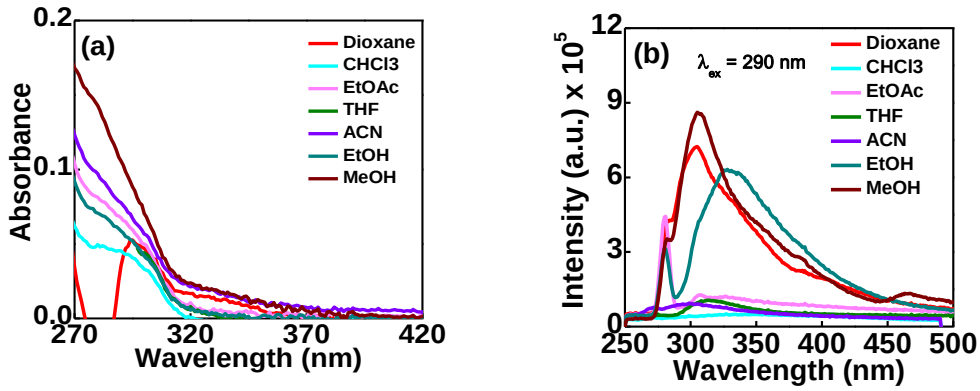


Figure S8. (a) UV-Visible, (b) fluorescence emission spectra of scaffold $^{o,m\text{-Ar}}$**TAA (1),** in different solvents [10 µM, r.t.; $\lambda_{ex} = \lambda_{max} \approx 290$ nm in each solvent].

Table S4. Summary table of photophysical properties of the $^{o,m\text{-Ar}}$**TAA, 1.**

Entry	**Solvents**	**UV-Vis & Fluorescence**		
		λ_{max}^{abs} **(nm)**	λ_{max}^{fl} **(nm)**	Φ_f
BocNH_$^{o,m\text{-Ar}}$TAA _COOMe (1)	Dioxane	294	304	0.23
	$CHCl_3$	290	326	0.05
	EtOAc	290	280, 308, 327	0.09
	THF	290	312	0.07
	ACN	290	272, 297	0.07
	EtOH	290	280, 330	0.23
	MeOH	290	282,306	0.25

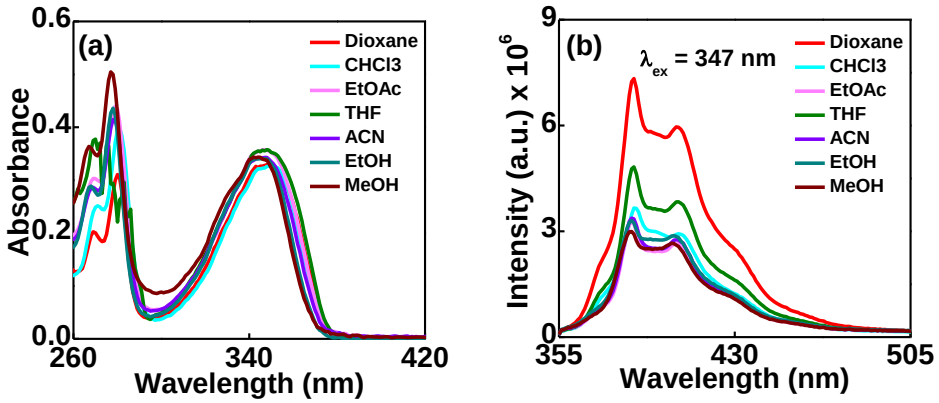


Figure S9. (a) UV-Visible, (b) fluorescence emission spectra of **UNNA 19** [(^{TPy}**Ala**^{Do})**;** Considered as the monomeric acceptor unit in FRET study] in different solvents [10 µM, r.t.; $\lambda_{ex} = \lambda_{max} \approx 347$ nm in each solvent].

Table S5. Summary table of photophysical properties of ^{TPy}**Ala**^{Do}**, 19.**

Entry	Solvents	UV-Vis & Fluorescence		
		λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
^{TPy}**Ala**^{Do} (**19**)	Dioxane	270, 280, 347	384, 405, 427	0.29
	$CHCl_3$	270, 281, 347	385, 405, 428	0.15
	EtOAc	269, 279, 347	384, 405, 427	0.14
	THF	270, 280, 347	384, 405, 427	0.19
	ACN	267, 278, 347	384, 405, 427	0.14
	EtOH	268, 278, 345	384, 405, 427	0.15
	MeOH	267, 277, 343	384, 405, 427	0.14

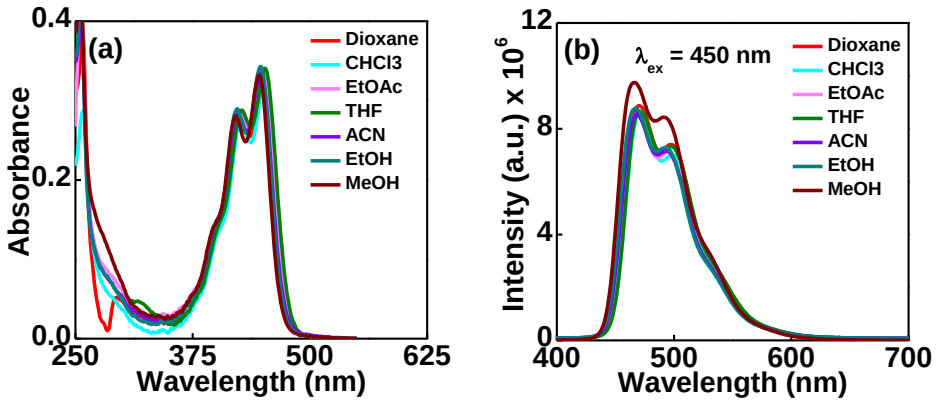


Figure S10. (a) UV-Visible, (b) fluorescence emission spectra of **UNNA 20** [(^{TPer}**Ala**^{Do})**,** Considered as the monomeric acceptor unit in FRET study] in different solvents [10 µM, r.t.; $\lambda_{ex} = \lambda_{max} \approx 450$ nm in each solvent].

Table S6. Summary table of photophysical properties of the **UNNA 20** (^{TPer}**Ala**^{Do})

Entry	Solvents	UV-Vis & Fluorescence		
		λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
(^{TPer}**Ala**^{Do}) (**20**)	Dioxane	397, 422, 450	470, 497	0.68
	$CHCl_3$	397, 422, 452	470, 497	0.65
	EtOAc	397, 422, 450	470, 497	0.65
	THF	397, 422, 450	470, 497	0.67
	ACN	397, 422, 450	470, 497	0.65
	EtOH	397, 422, 450	470, 497	0.67
	MeOH	397, 422, 448	466, 497	0.74

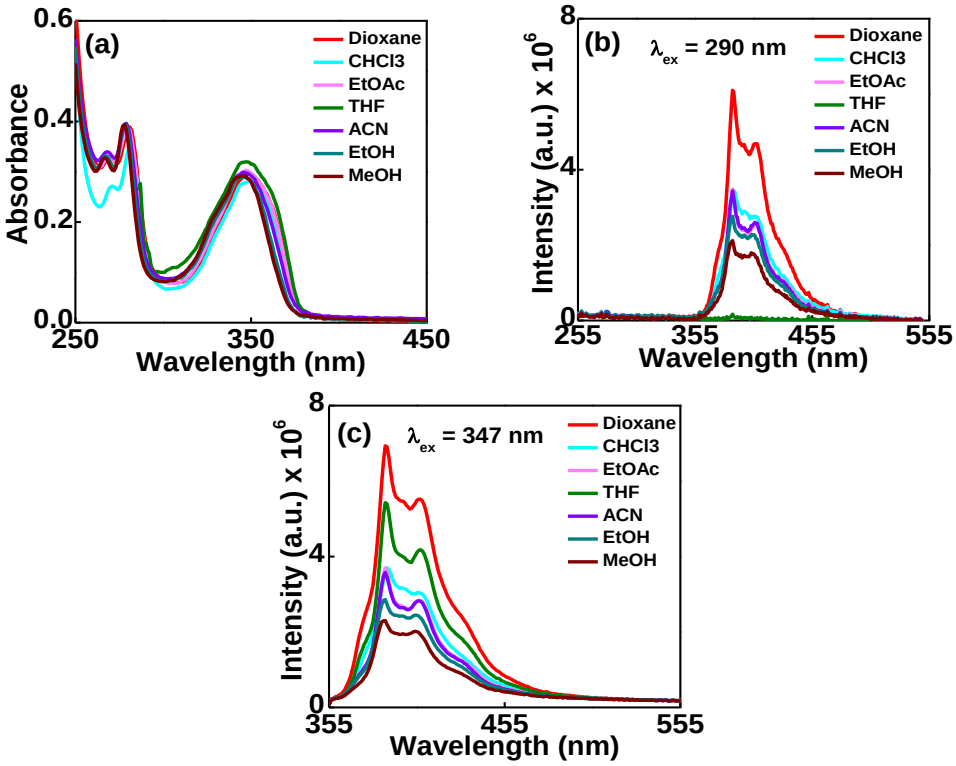


Figure S11. (a) UV-Visible, (b) fluorescence emission at $\lambda_{ex} \approx 290$ nm, fluorescence emission at $\lambda_{ex} \approx 347$ nm (c) of **Tripeptide 11** [**BocNH-**$^{o,m\text{-}Ar}$**TAA–Leu–**^{TPy}**Ala**^{Do}**-CONMe(OMe)**] in different solvents [10 µM, r.t.; $\lambda_{ex} = \lambda_{max} \approx 290$ and 347 nm in each solvent]

Table S7. Summary table of photophysical properties of **Tripeptide 11** [**BocNH–**$^{o,m\text{-}Ar}$**TAA–Leu–**^{TPy}**Ala**^{Do}**-CONMe(OMe)**]

Entry	**Solvents**	**UV-Vis & Fluorescence**		
		λ_{max}^{abs} **(nm)**	λ_{max}^{fl} **(nm)**	Φ_f
[**BocNH–**$^{o,m\text{-}Ar}$**TAA–Leu-**^{TPy}**Ala**^{Do}**–CONMe(OMe)**](**11**)	Dioxane	268, 278, 348	386, 407, 429	0.33
	$CHCl_3$	268, 279, 348	386, 407, 429	0.2
	EtOAc	268, 278, 347	386, 406, 429	0.18
	ACN	268, 278, 347	386, 406, 429	0.18
	EtOH	268, 278, 347	386, 407, 429	0.16
	MeOH	268, 278, 347	386, 406, 429	0.14

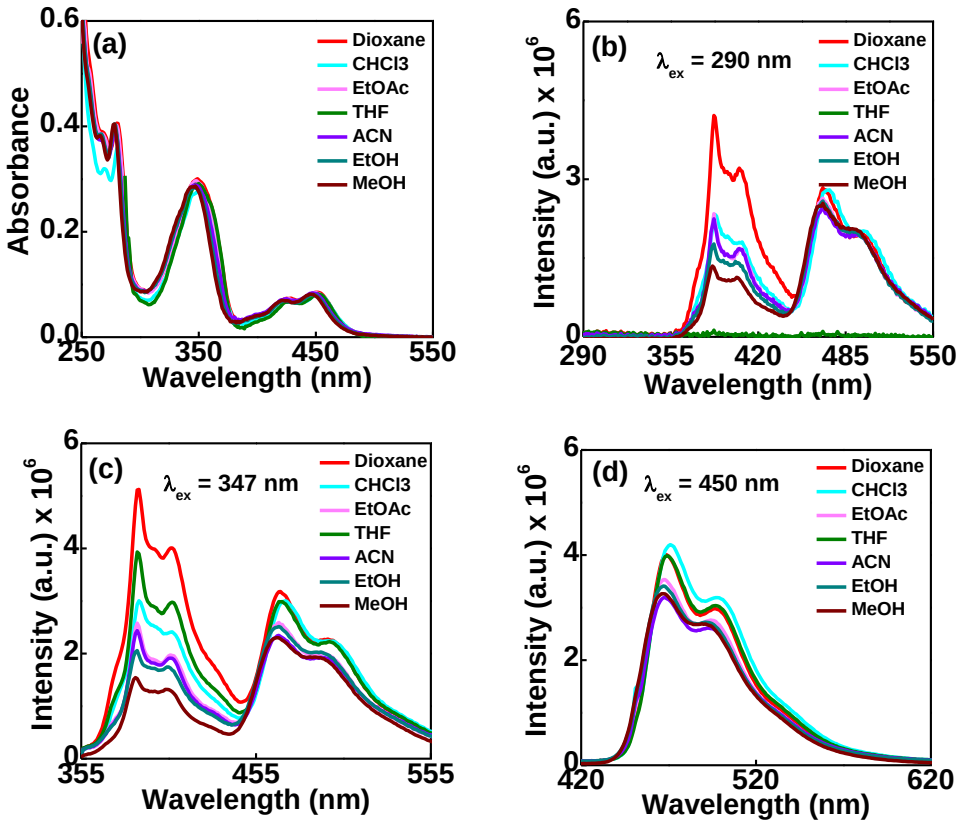


Figure S12. (a) UV-Visible, (b) fluorescence emission at $\lambda_{ex} \approx 290$ nm (c) fluorescence emission at $\lambda_{ex} \approx 347$ nm, (d) fluorescence emission at $\lambda_{ex} \approx 450$ nm of Fluorescent **Pentapeptide 3** [**BocNH-^{TPer}Ala^{Do}–Leu–$^{o,m\text{-}Ar}$TAA–Leu–^{TPy}Ala^{Do}-CONMe(OMe)**] in different solvents [10 μM, r.t.].

Table S8. Summary table of photophysical properties of **Pentapeptide 3** [**BocNH-^{TPer}Ala^{Do}–Leu–$^{o,m\text{-}Ar}$TAA–Leu–^{TPy}Ala^{Do}-CONMe(OMe)**].

Entry	Solvents	UV-Vis & Fluorescence		
		λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
[**BocNH-^{TPer}Ala^{Do}–Leu–$^{o,m\text{-}Ar}$TAA–Leu–^{TPy}Ala^{Do}-CONMe(OMe)**] (**3**)	Dioxane	267, 279, 349, 425, 447	387, 407, 467, 497	0.51
	$CHCl_3$	267, 279, 350, 426, 452	388, 407, 468, 496	0.55
	EtOAc	267, 279, 347, 425, 448	386, 406, 468, 495	0.47
	THF	267, 279, 347, 426, 450	386, 406, 468, 495	0.52
	ACN	267, 279, 348, 426, 448	386, 406, 468, 495	0.45
	EtOH	267, 279, 346, 422, 447	386, 406, 468, 495	0.47
	MeOH	267, 279, 344, 422, 446	386, 406, 468, 495	0.43

4. Establishment of Relay FRET Process in Fluorescent Pentapeptide 3.

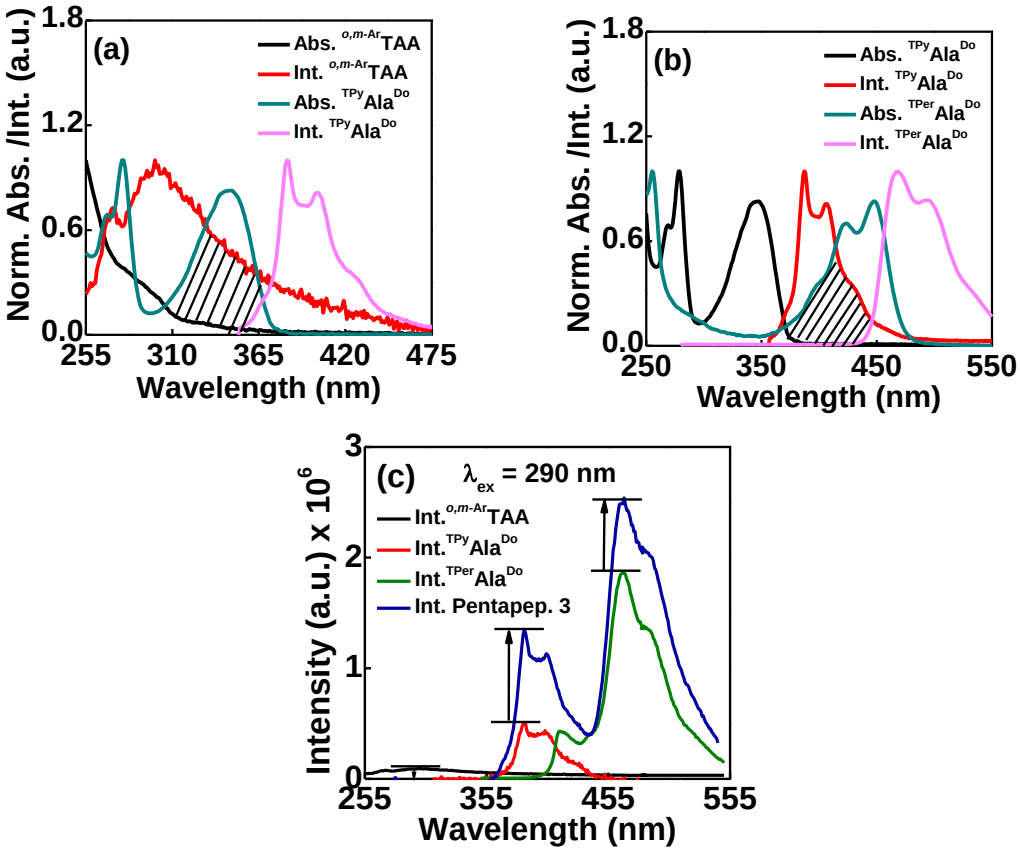


Figure S13. Overlap spectra of two monomers (a) $^{o,m\text{-Ar}}$**TAA,** ^{TPy}**Ala**^{Do} and (b) ^{TPy}**Ala**^{Do} , ^{TPer}**Ala**^{Do}. (c) Fluorescence emission spectra of donor $^{o,m\text{-Ar}}$**TAA** and acceptor ^{TPy}**Ala**^{Do}**,** ^{TPer}**Ala**^{Do} and the **Pentapeptide 3** which contain these three units (10 μM each, r.t.; λ_{ex} = 290 nm) in Acetonitrile solvent.

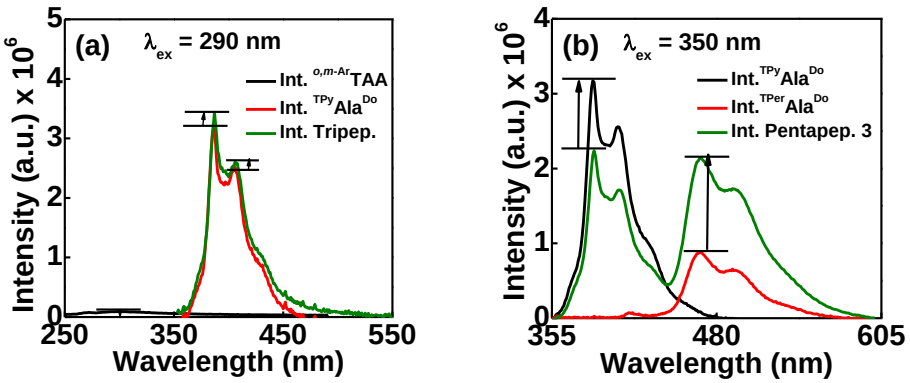


Figure S14. (a) Fluorescence emission spectra of individual donor $^{o,m\text{-Ar}}$**TAA** and acceptor amino acid ^{TPy}**Ala**^{Do} and the **Tripeptide 11,** $^{o,m\text{-Ar}}$**TAA_Leu_**^{TPy}**Ala**^{Do} which contain these two units in solvent Acetonitrile, **(b)** Fluorescence emission spectra of individual donor ^{TPy}**Ala**^{Do} and acceptor amino acid ^{TPer}**Ala**^{Do} and the **Pentapeptide** which contain these two units in solvent Acetonitrile. (10 μM each, r.t.; λ_{ex} = 300 nm).

4.1. Lifetime Traces and Tables

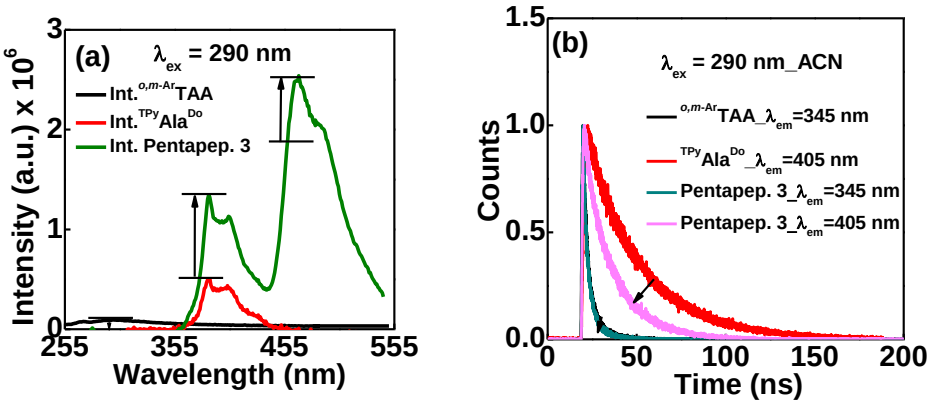


Figure S15. (a) Fluorescence emission spectra of donor $^{o,m\text{-Ar}}$**TAA** and acceptor ^{TPy}**Ala**^{Do} and the **Pentapeptide 3** which contain these two units (10 μM each, r.t.; λ_{ex} = 290 nm) in solvent Acetonitrile. (b) Time resolved fluorescence of donor and acceptor chromophore $^{o,m\text{-Ar}}$**TAA** and ^{TPy}**Ala**^{Do} and **Pentapeptide 3** using λ_{ex} = 290 nm λ_{em} = 405 nm in Acetonitrile solvent. Decrease in lifetime of $^{o,m\text{-Ar}}$**TAA** as well as increase in life time of ^{TPy}**Ala**^{Do} is an evidence of FRET from $^{o,m\text{-Ar}}$**TAA** to ^{TPy}**Ala**^{Do}.

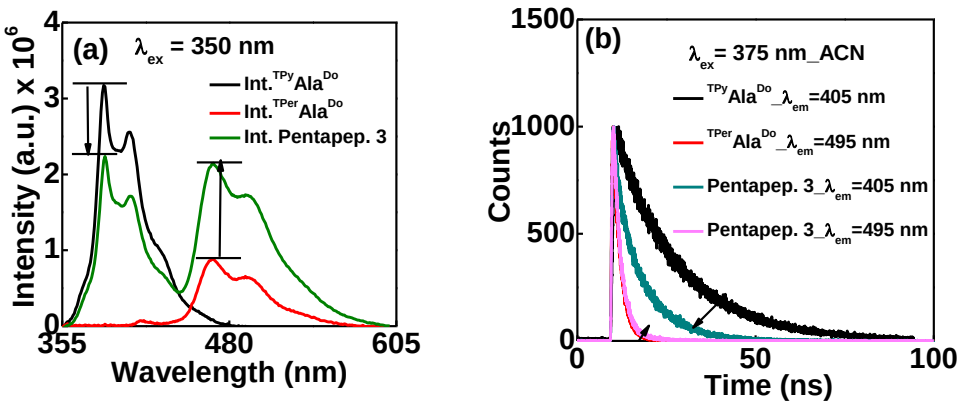


Figure S16. (a) Fluorescence emission spectra of donor ^{TPy}**Ala**^{Do} and acceptor ^{TPer}**Ala**^{Do} and the **Pentapeptide 3** which contain these two units (10 μM each, r.t.; λ_{ex} = 347 nm) in solvent Acetonitrile. (b) Time resolved fluorescence of donor and acceptor chromophore ^{TPy}**Ala**^{Do} and ^{TPer}**Ala**^{Do} and **Pentapeptide 3** using λ_{ex} = 375 nm λ_{em} = 405, 495 nm in Acetonitrile solvent. Decrease in lifetime of ^{TPy}**Ala**^{Do} as well as increase in life time of ^{TPer}**Ala**^{Do} is an evidence of FRET from ^{TPy}**Ala**^{Do} to ^{TPer}**Ala**^{Do}.

Table S9: Summary table of fluorescence lifetimes of ^{TPy}**Ala**^{Do}, ^{TPer}**Ala**^{Do}, $^{o,m\text{-}Ar}$**TAA and BocNH-** $^{o,m\text{-}Ar}$**TAA_Leu-**^{TPy}**Ala**^{Do}**-CONMe(OMe) 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
$^{o,m\text{-}Ar}$**TAA**	**Acetonitrile**	0.07	350	2.3 (59 %)	8.3 (41 %)	4.79	0.01	0.2	0.95
^{TPy}**Ala**^{Do}		0.14	405	15.2 (100 %)	---	15.2	0.011	0.055	1.05
^{TPer}**Ala**^{Do}		0.65	495	3.8 (100 %)	---	3.8	0.17	0.09	0.95
Tripep. 11		0.01	350	3.62 (100 %)	---	3.62	0.003	0.27	1.2
Tripep. 11		0.17	405	3.8 (3 %)	17.3 (97%)	16.98	0.01	0.049	1.03
PentaPep. 3		0.001	350	1.9 (58 %)	6.5 (42 %)	3.86	0.0002	0.26	0.94
PentaPep. 3		0.12	405	2.9 (6 %)	16.9 (94 %)	16.15	0.007	0.05	0.96
PentaPep. 3		0.33	495	3.67 (83 %)	9.5 (17 %)	4.66	0.07	0.14	0.94
For lifetimes of the fluorescent amino acids and peptides λ_{ex} = 290 nm; Concentration of each fluorescent amino acids and peptides = 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 S10: Summary table of fluorescence lifetimes of ^{TPy}**Ala**^{Do}, ^{TPer}**Ala**^{Do} **and BocNH-** $^{o,m\text{-}Ar}$**TAA_Leu-**^{TPy}**Ala**$_{Do}$**-CONMe(OMe) 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
^{TPy}**Ala**^{Do}	**Acetonitrile**	0.14	405	15.16 (100 %)	---	15.16	0.011	0.055	1.014
^{TPer}**Ala**^{Do}		0.65	495	3.8 (100 %)	---	3.8	0.17	0.09	1.04
Tripep. 11		0.17	405	4.7 (11 %)	17.1 (89 %)	15.78	0.011	0.052	1.01
PentaPep. 3		0.12	405	3.99 (13 %)	17.1 (87 %)	15.29	0.008	0.057	1.06
PentaPep. 3		0.33	495	3.54 (80 %)	9.38 (20 %)	4.71	0.07	0.14	0.98

For lifetimes of the fluorescent amino acids and peptides λ_{ex} = 375 nm; Concentration of each fluorescent amino acids and peptides = 10 μM; <τ>, 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 S11: Summary table of fluorescence lifetimes of **^{TPer}Ala^{Do}, Pentapep. 3 [BocNH-^{TPer}Ala^{Do}-Leu-$^{o,m\text{-}Ar}$TAA-Leu-^{TPy}Ala^{Do}-CONMe(OMe)] at λ_{ex} = 405 nm**

Entry	Solvents	Φ_f	λ [nm]	τ_1 [ns]	τ_2 [ns]	<τ> [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
^{TPer}Ala^{Do}	**Acetonitrile**	0.65	495	3.71 (100 %)	---	3.71	0.17	0.09	1.06
PentaPep. 3		0.33	495	2.85 (57 %)	5.2 (43 %)	3.85	0.086	0.17	1.05

For lifetimes of the fluorescent amino acids and peptides λ_{ex} = 405 nm; Concentration of each fluorescent amino acids and peptides = 10 μM; <τ>, 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 $k_{nr} = (1-\Phi_f)/<\tau>$.

4.2. Calculation of the Forster distance and FRET efficiency in Acetonitrile Solvent:

For the FRET the fluorescence resonance energy transfer (FRET) and the Förster distance 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 energy *v* transfer efficiency is 50%. The Förster distance R_0 (Å) was calculated by the following equation (2)

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

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)

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

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

Using the values of κ^2= 2/3, n = 1.34, Φ_D = 0.07, and the obtained overlap integral, J (λ) = 6.21 x 10^{16}, the R_0 and r values were calculated which were found to be R_0 = 65.5 Å and r = 46.1 Å. R_0 is the critical distance when the energy transfer efficiency is 89.6 % and r is the distance between the donor $^{o,m\text{-Ar}}$**TAA** and acceptor **TPy.**

Using the similar values of κ^2, n = 1.34 and Φ_D = 0.14, and the obtained overlap integral, J (λ) = 1.73 x 10^{17}, the R_0 and r values were calculated and found R_0 = 87.2 Å and r = 101.8 Å. R_0 is the critical distance when the energy transfer efficiency is 29.4 % and r is the distance between the donor **TPy** and acceptor **TPer**.

5. Studies on the Interaction of Pentapeptide 3 With BSA Protein

5.1. *General experimental (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. Milli-Q Water was taken for solution preparation. All solutions were prepared freshly before doing the experiments. The probe molecules (Pentapeptide **2** and **3**) was synthesized and purified according to the procedure described.

5.2. *Preparation of BSA Solution*

Phosphate buffer of pH 7.0 was used to prepare the solution of BSA (Merck). A 100 μM of stock BSA solution was prepared by dissolving 0.0198 gm of BSA in 3.00 mL phosphate buffer (5 mM) of pH 7.0. Because of the poor solubility of the fluorophores in buffer, 2-3% DMF is used to solubilize them. The presence of 2-3% DMF does not induce structural and spectral changes to the biomolecules. Each sample solution was mixed well before spectral measurements.

5.3. *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 (5 μM for peptide) was titrated with different concentrations of BSA (ranging from 0, 1.25, 2.5, 3.75, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 35, 40, 45 μM). The total volume of the final solution for each sample was 1 mL. The % of DMF content did not exceed 3%.

5.4. Photophysical Study: UV-Visible Study

Shimadzu UV- 2550 UV Visible spectrophotometer was used to carry out the UV–Visible absorbance measurements, using 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 7.0 containing solution at 298 K. 2-3 % DMF was used to solubilize the probe. Absorbance mode was used to take the measurements and the absorbance values of the sample solutions were measured in the wavelength regime of 200– 700 nm. Freshly prepared sample solutions was used to carry out all the experiments.

5.5. Photophysical Study: Fluorescence Study

Fluoromax 4 spectrophotometer was used to carry out all fluorescence and steady state anisotropy experiments, using with a cell of 1 cm path length at 298 K. All the fluorescence studies were carried out in 20 mM phosphate buffer of pH 7.0 solution at 298 K. 2-3 % DMSO was used to solubilize the probe. The excitation wavelength for probe (**Pentapep. 3**) was set at 280 nm, 350 nm and 340 nm. Emission spectra were measured in the wavelength regime of 290–650 nm. Fluoromax 4 spectrophotometer was used to measure Steady state anisotropy of the solutions. Life Space-II (Edinburgh Instruments) time resolved fluorescence spectrophotometer was used to measure Time resolved fluorescence anisotropy decay. The fluorescence quantum yields (Φ)

were determined using quinine sulphate and perylene as a reference with the known Φ (0.54) in 0.1 molar solution in sulphuric acid.

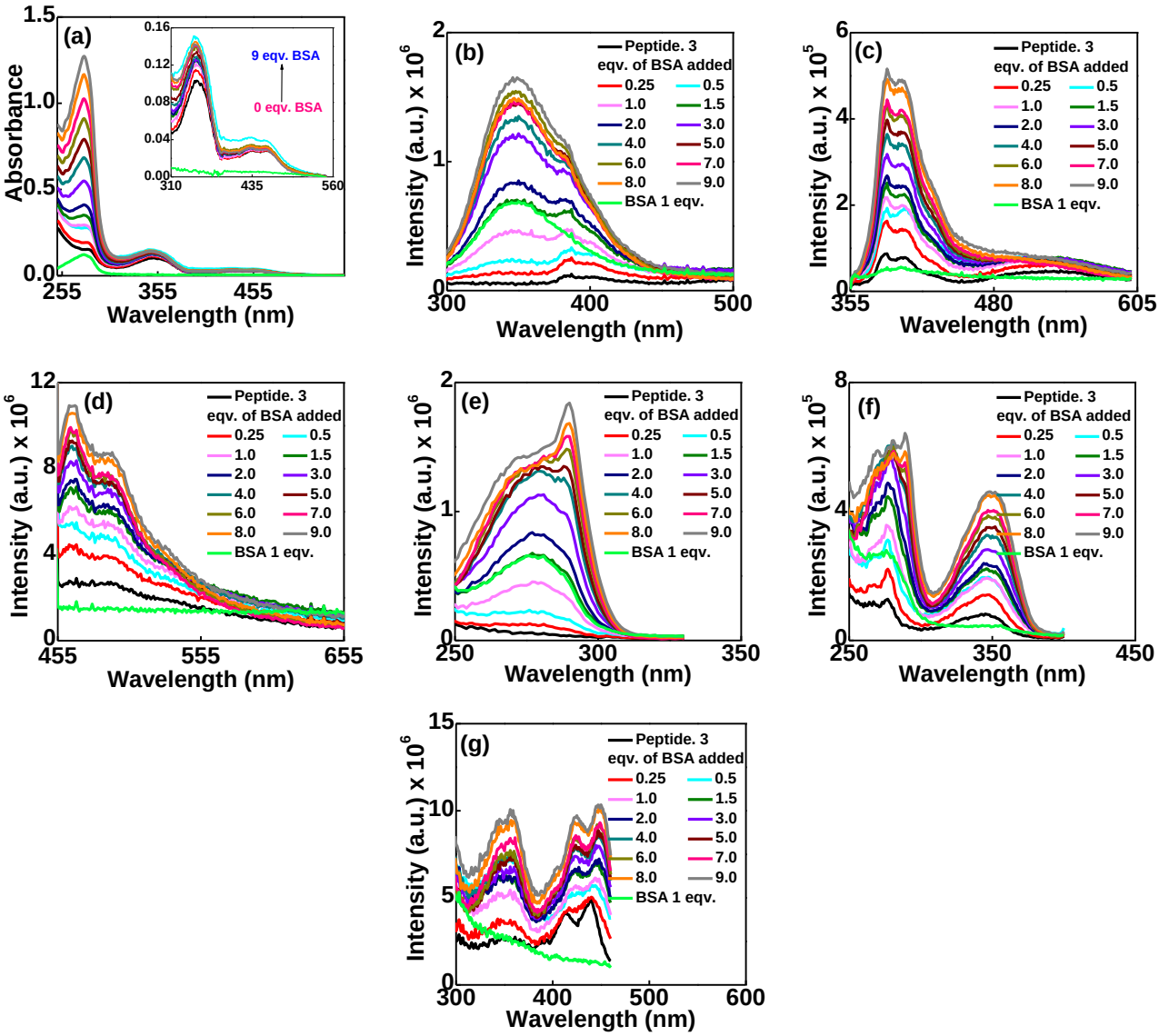


Figure S17. (a) UV-visible titration spectra and (b-d) emission titration spectra at λ_{ex} = 280, 350 and 450 nm respectively (e-g) excitation titration spectra at λ_{ex} =350, 405 and 495 nm, of **Pentapep. 3** in presence and absence of increasing concentration of BSA at 298K. [**Pentapep. 3**] = 5 μM and [BSA] = 0, 1.25, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45 μM.

Table S12: Summary table of photophysical properties of the **Pentapep. 3** in BSA in Na-phosphate buffer 20 mm (pH=7.0)

Entry	UV-Vis and Fluorescence		
	λ_{max}^{abs} (nm)	λ_{max}^{fl} (nm)	Φ_f
Pentapep. **3**	280, 250, 432, 455	350, 387, 405, 465, 492	0.017
0.25 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.027
0.50 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.037
1.00 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.035
1.50 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.039
2.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.041
3.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.046
4.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.049
5.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.052
6.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.054
7.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.055
8.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.061
9.0 eqv.BSA	280, 250, 432, 455	350, 387, 405, 465, 492	0.066
Only BSA	280	350	0.041

5.6. FRET study between Pentapep. 3 and BSA Protein

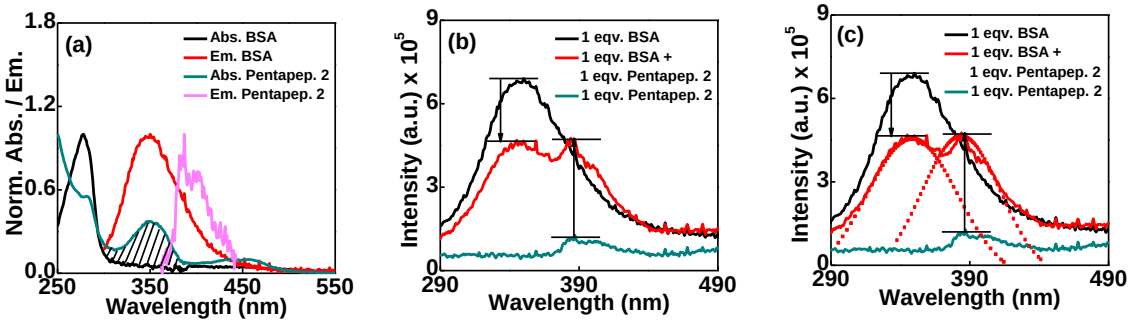


Figure S18. (a) Overlaping of emission spectra of BSA (act as a FRET donor) and the absorption spectra of **Pentapep. 3** (act as a FRET acceptor) (5 μM each, r.t.; λ_{ex} = 280 nm in Buffer). **(b)** Fluorescence spectra of individual donor (BSA) and acceptor **Pentapep. 3** and 1:1 mixture of both of them. The acceptor emission increased in presence of donor, whereas the donor emission decreases of the individual donor emission. This change in fluorescence intensity is visual evidence of FRET (5 μM each, r.t.; λ_{ex} = 280 nm in buffer).

5.7. Time Resolved Fluorescence Study

Table S13: Summary table of fluorescence lifetimes of the peptides **Pentapep. 3** with increasing BSA concentation **at λ_{ex} = 290 nm**

Entry	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	0.041	350	3.7 (14 %)	6.8 (87 %)	6.41	0.006	0.15	0.99
1:1 BSA: OmPP	0.018	350	3.6 (14 %)	6.8 (86 %)	6.35	0.003	0.155	0.95
Only OmPP	0.003	350	0.87 (27 %)	4.2 (73 %)	3.27	0.001	0.3	0.94
0.5 : 1 BSA: OmPP	0.013		3.4 (15 %)	6.8 (85 %)	6.25	0.002	0.16	0.94
1:1 BSA: OmPP	0.018		3.6 (14 %)	6.8 (86 %)	6.35	0.003	0.15	0.95
2:1 BSA: OmPP	0.029		4.2 (20 %)	6.9 (80 %)	6.39	0.005	0.15	0.98
Only OmPP	0.009	405	4.8 (29 %)	59.3 (71 %)	43.9	0.0002	0.022	1.04
1:0.5 BSA: OmPP	0.02		6.4 (61 %)	57.9 (39 %)	26.3	0.0007	0.037	1.03
1:1 BSA: OmPP	0.014		6.5 (73 %)	59.6 (27 %)	21.45	0.0006	0.046	1.06
1:2 BSA: OmPP	0.011		6.6 (77 %)	60.4 (23 %)	18.28	0.0006	0.054	1.04

Table S14: Summary table of fluorescence lifetimes of the **Pentapep. 3** with increasing BSA concentation at **λ_{ex} = 375 nm**

Entry	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 OmPP	0.009	405	3.78 (38 %)	38.12 (62 %)	25.31	0.0004	0.039	0.98
0.5:1 BSA: OmPP	0.02		4.37 (21 %)	39.03 (79 %)	31.62	0.0006	0.03	1.04
1:1 BSA: OmPP	0.014		5.15 (23 %)	39.8 (77 %)	31.8	0.0004	0.031	1.07
2:1 BSA: OmPP	0.011		5.185 (25 %)	40.02 (75 %)	31.44	0.0003	0.032	1.05
Only OmPP	0.005	495	0.64 (14 %)	4.83 (86 %)	4.23	0.001	0.23	0.92
0.5:1 BSA: OmPP	0.004		1.1 (25 %)	5.65 (75 %)	4.52	0.0009	0.22	0.96

1:1 BSA: OmPP	0.003		0.84 (40 %)	5.95 (60 %)	3.88	0.0008	0.26	0.98
2:1 BSA: OmPP	0.001		1.43 (40 %)	7.005 (60 %)	2.85	0.0004	0.35	1.05
For lifetimes of the fluorescent amino acids λ_{ex} = 375 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 S15: Summary table of fluorescence lifetimes of the **Pentapep. 3** with increasing BSA concentation at λ_{ex} **= 405 nm**

Entry	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 OmPP	0.005	495	5.18 (100 %)	---	5.18	0.001	0.19	0.95
0.5:1 BSA: OmPP	0.004		2.87 (18 %)	5.54 (82 %)	5.06	0.0008	0.2	0.97
2:1 BSA: OmPP	0.001		2.72 (18 %)	5.74 (82 %)	4.77	0.0002	0.21	0.98
For lifetimes of the fluorescent amino acids λ_{ex} = 375 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 $k_{nr} = (1- \Phi_f)/<\tau>$.								

5.8. Benesi-Hildebrand plot/Job's Plot and ITC experiment to evaluate binding therdynamics

The association constant (K) of the fluorophore with BSA was determind 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 **Pentapep. 3** in the absence of BSA, in the presence of an inetermediate and at infinite concentration of BSA respectively. From the slop of the $1/(I_{405} - I_0)$ and $1/(I_{495} - I_0)$ *vs.* $1/[BSA]$ plot of equation 1, binding constant K was determined.

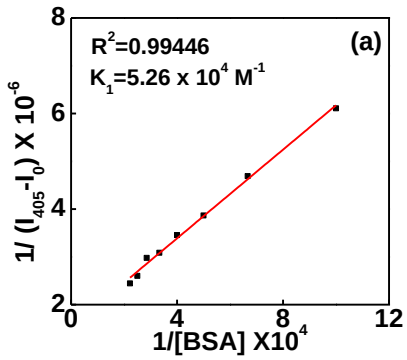


Figure S19: Benesi- Hildebrand plot of **Pentapep. 3** in presence of increasing BSA concentration. **[Pentapep. 3]** = 5 µM and [BSA] = 0, 1.25, 2.5, 5, 7.5, 10, 15, 20, 25, 30 µM [Na-phosphate buffer (pH=7.0)]

5.9. *Study of Circular Dichroism Spectroscopy:*

CD spectra were recorded using a CD spectropolarimeter with a cell path length of 10 nm at 25 ^{o}C. All the sample solutions were prepared in 20mm phosphate buffer solvent of pH=7.00. 3% DMSO is added in each sample to dissolve the peptide.

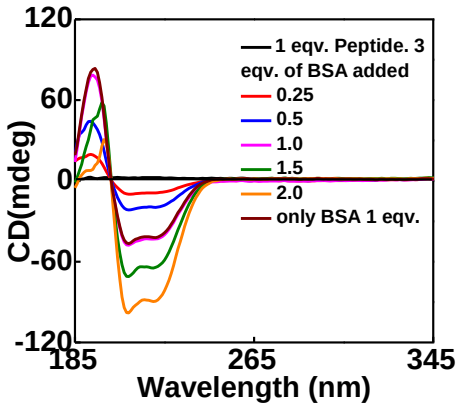


Figure S20. CD spectra of **Peptide 3** tritated with increasing concentration of BSA. [peptide] = 10 µM and [BSA] = 2.5, 5, 10, 15 and 20 µM.

5.10. Molecular Docking Calculation:

Docking calculations with BSA protein and pentapeptide **3** were carried out using Autodock4 (Bikadi, Hazai, 2009). Following is the BSA sequence which was used to generate the 3D model. >gi|3336842|emb|CAA76847.1| bovine serum albumin [Bos taurus]

MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQ
YLQQCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLR
ETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKF
WGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETM
REKVLTSSARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTD
LTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCI
AEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYA
VSVLLRLAKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQ
FEKLGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMP
CTEDYLSLILNRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPK
AFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKATEEQLKTVMENFVA
FVDKCCAADDKEACFAVEGPKLVVSTQTALA

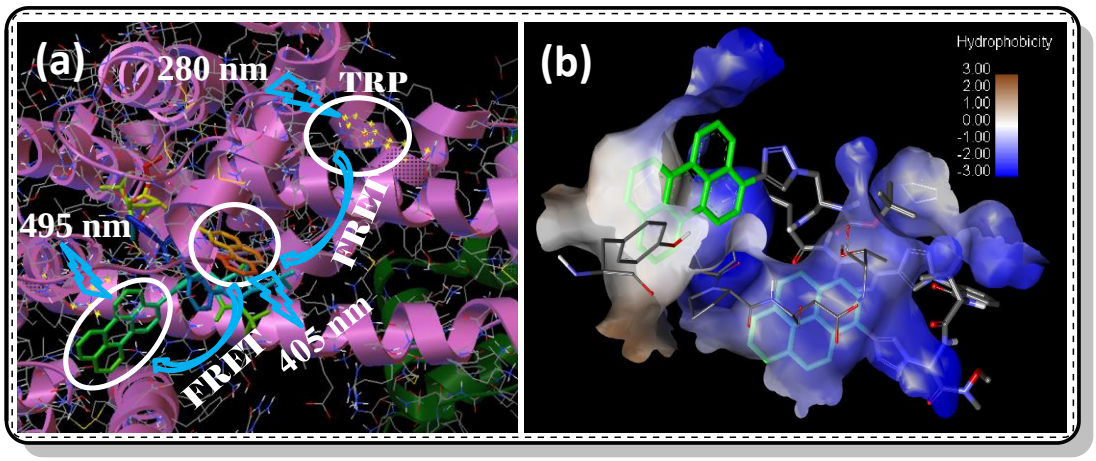


Figure S21. (a) Docking pose of **Pentapeptide 3** in presence of BSA. (b) **Pentapeptide 3** hydrophobic interaction with the hydrophobic pocket of BSA.

5.11. Steady State Anisotropy and Polarisation study

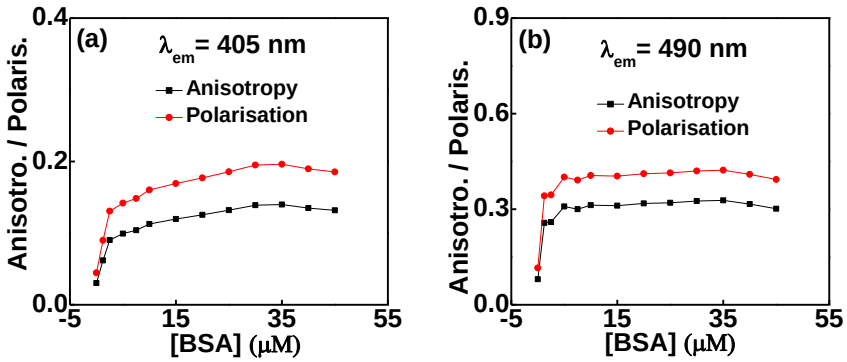


Figure S22: Steady-state Anisotropy and Polarisation plot (a) λ_{ex} = 350 nm, λ_{em} = 405 nm and (b) λ_{ex} = 450 nm, λ_{em} = 495 nm of **Pentapep. 3** in presence of increasing BSA concentration. **[Pentapep. 3]** = 5 μM and [BSA] = 0, 1.25, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45 μM.

5.12. Isothermal titration calorimetry measurement

Isothermal titration calorimetric measurement is a method to carry out to determine the characterization of noncovalent, equilibrium interactions involving small molecules or peptides with macromolecule (Protein) thermodynamically. Now we have carried out the ITC experiment of **Pentapeptide 3** with BSA in phosphate bufffer pH 7.00 at 298 K. 2 μm protien solution was taken in a sample cell and 30 μm **peptide 3** was used as titrant solution. In the spectra of ITC panel a each peak in binding isotherm shows a single injection of **peptide 3** into BSA solution and panel b shows the amount of heat liberated per injection as a function of molar ratio of peptide to BSA. Resulting data was fitted to a sigmoidal curve involving one binding site. Here panel a of **Figure S23** shows that BSA shows a negative deflection, which means that the binding is an exothermic process at 298 K. The negative value of enthalpy (ΔH = -5.063 x 10^7) indicates the binding is enthalpycally favoured but entropically opposed (ΔS = -1.7 x 10^5). The binding constant (K), binding stoichiomatry (N), enthalpy change (DH), entropy change (DS) and change in free energy were obtained from the fitted data.

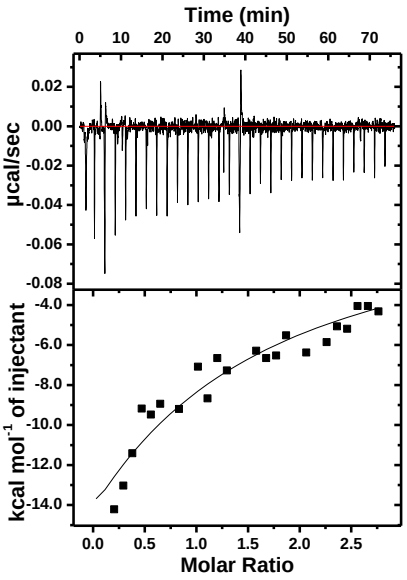


Figure S23: Plot of isothermal titration calorimetry (some bad data points are removed).

Table S16: Thermodynamical parameter of **BSA-peptide** system from ITC measurement

Model : One Sites binding				
K (M^{-1})	N	ΔH (cal/mol)	ΔS (cal/mol/deg)	ΔG_1 (Kcal)
1.15 x 10^5	0.0012	-5.063 x 10^7	-1.7 x 10^5	-3.00

6. ^{1}H and ^{13}C Spectra

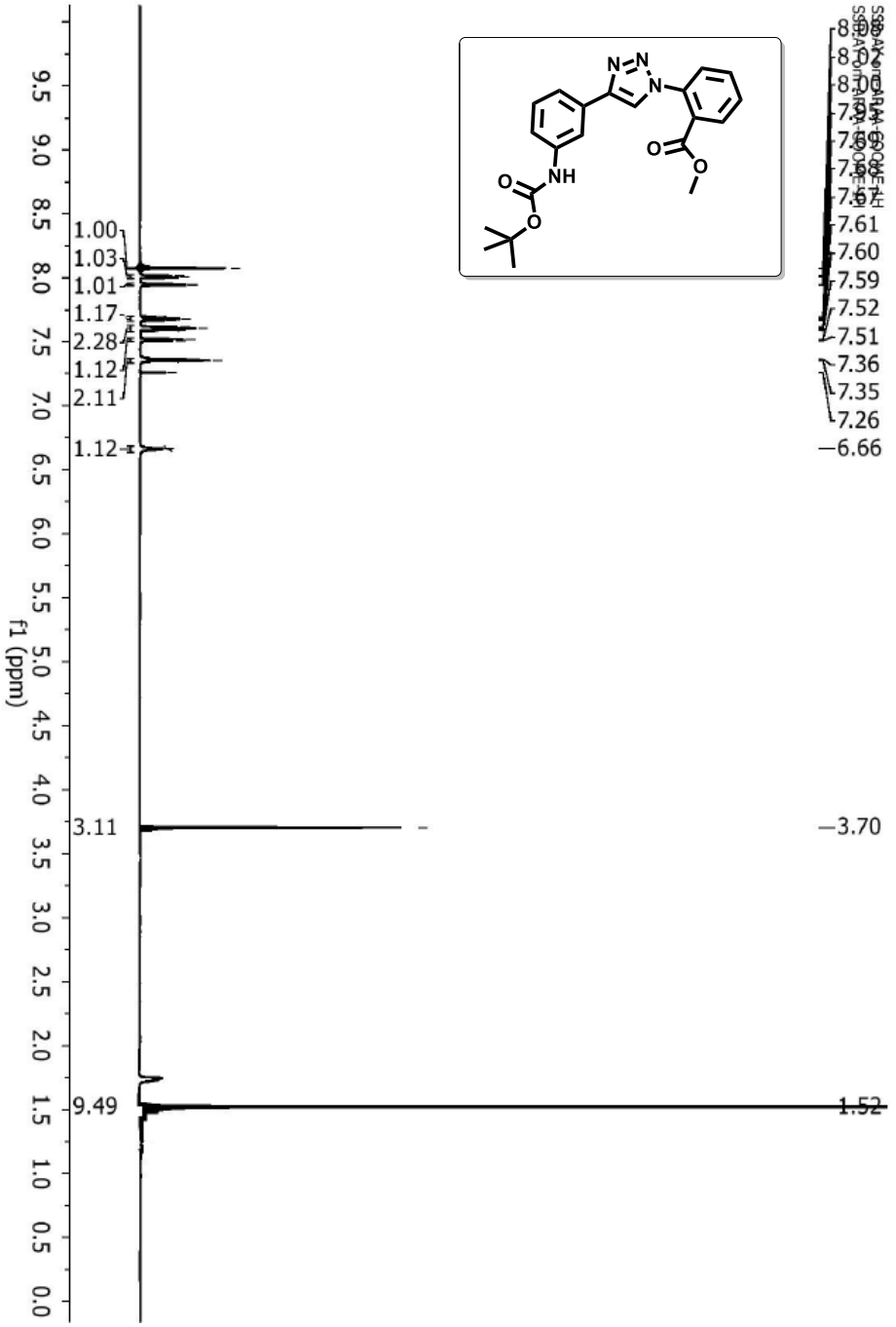


Figure S24: ^{1}H Spectra of synthesized compound **1.**

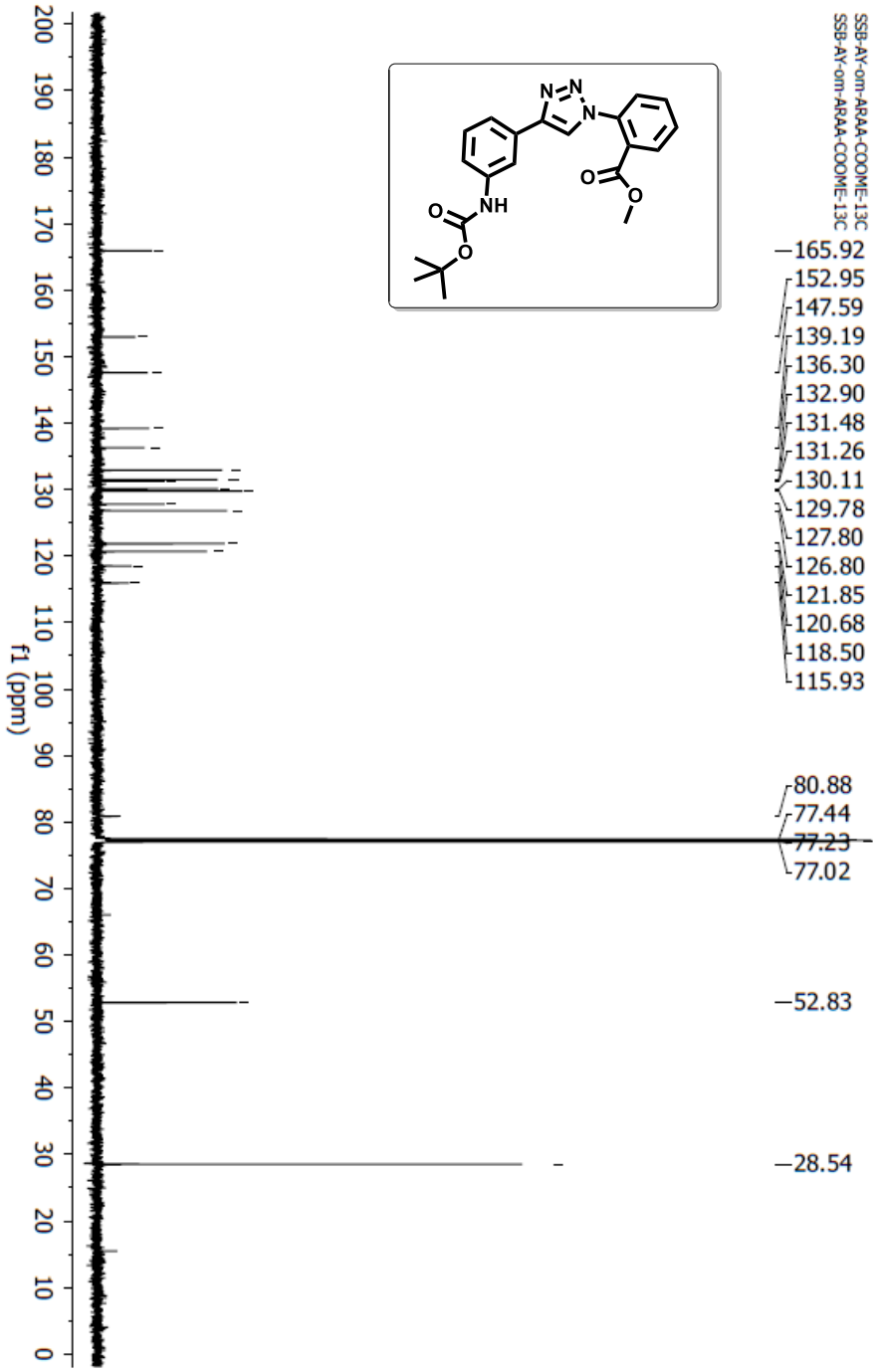


Figure S25: ^{13}C Spectra of synthesized compound **1.**

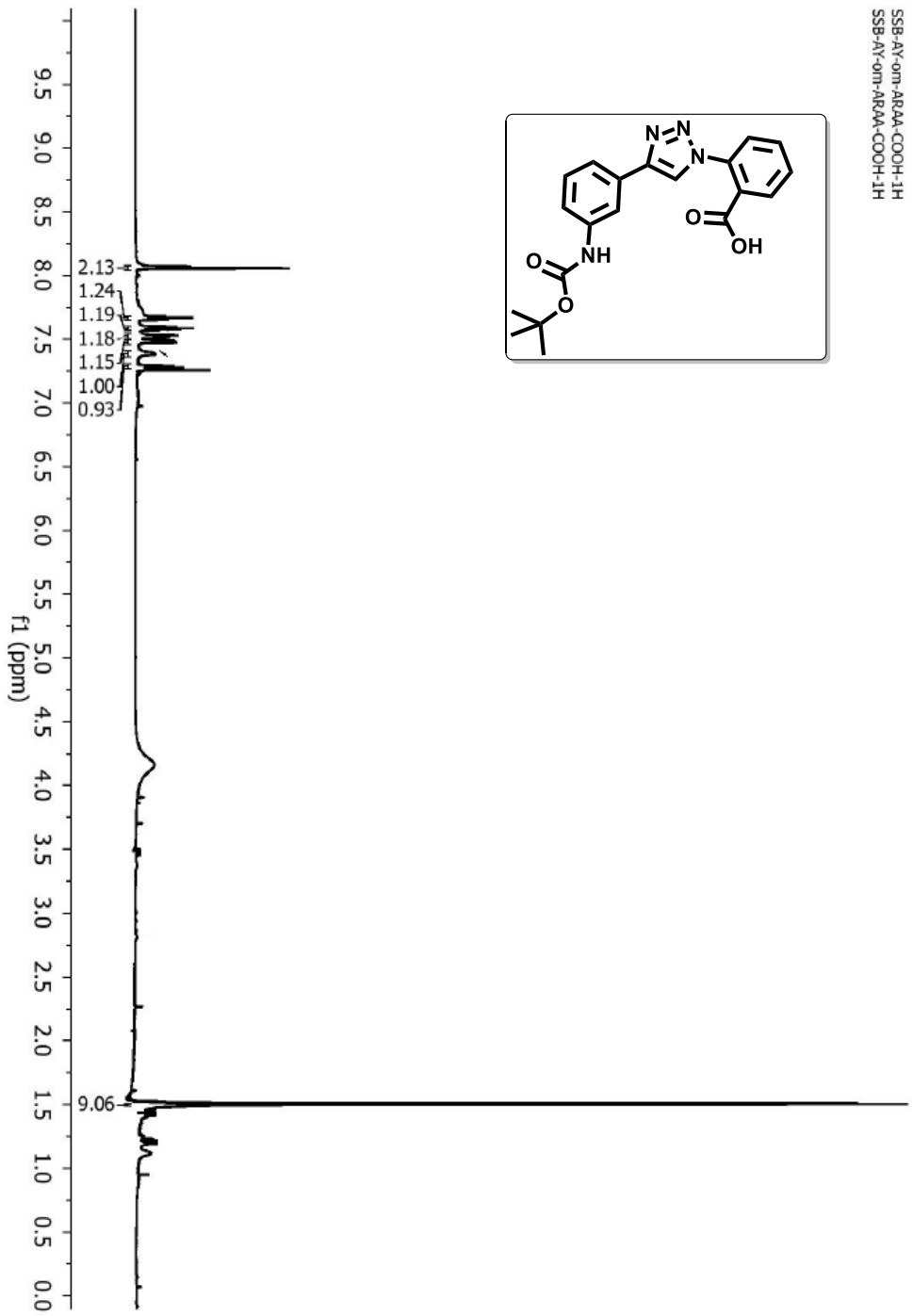


Figure S26: ^{1}H Spectra of synthesized compound **2.**

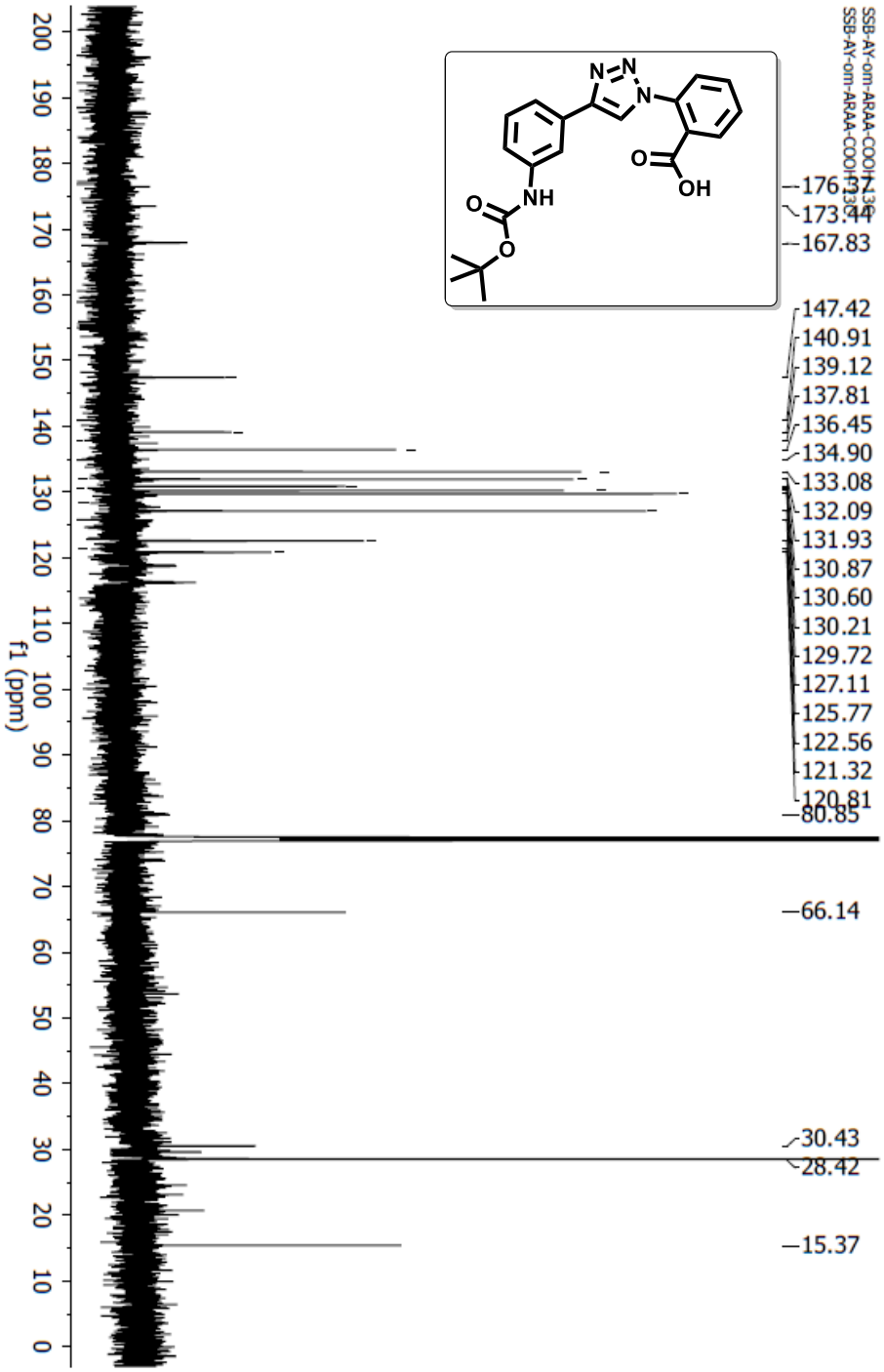


Figure S27: ^{13}C Spectra of synthesized compound **2.**

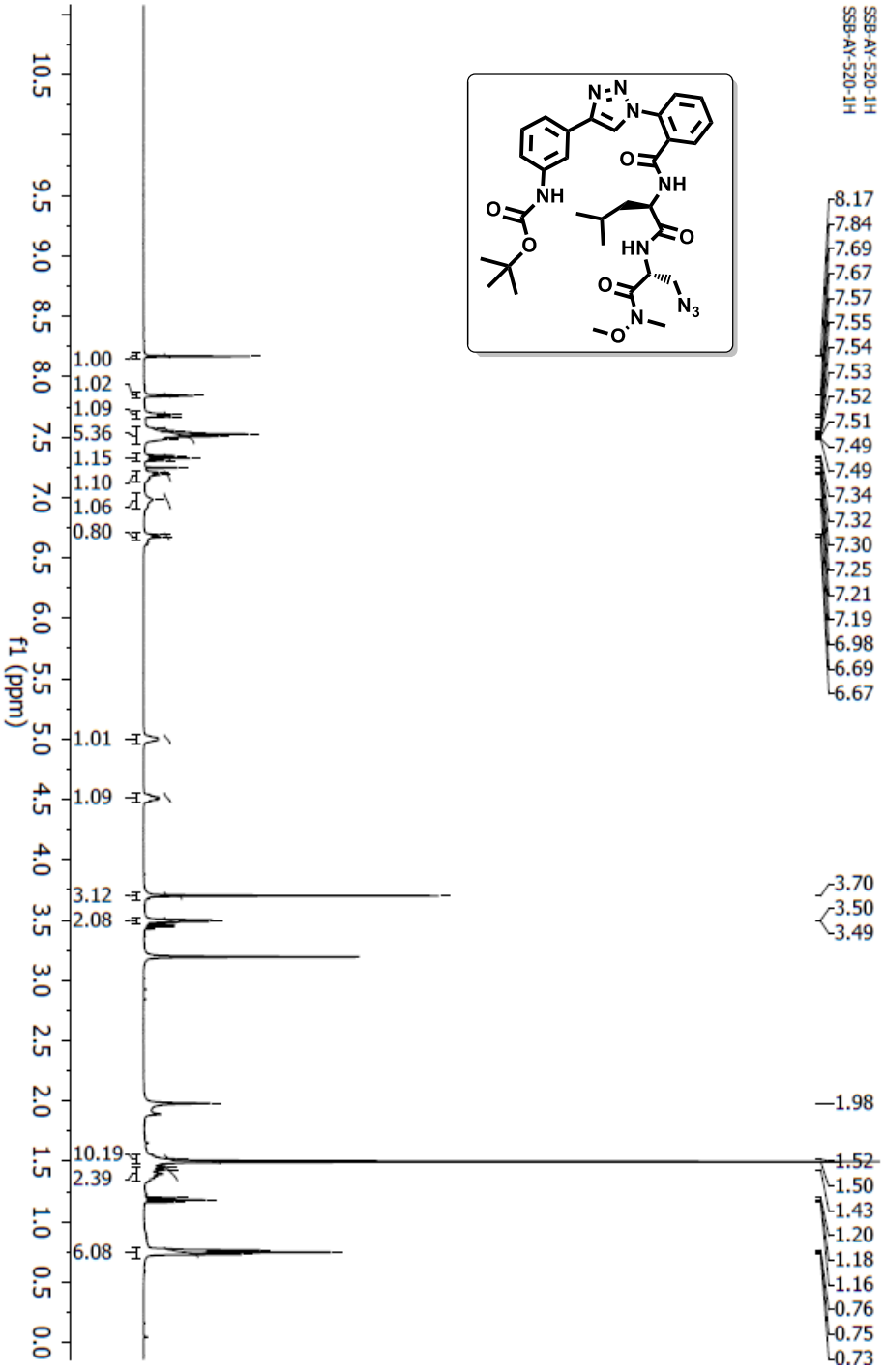


Figure S28. ^{1}H Spectra of synthesized compound **9.**

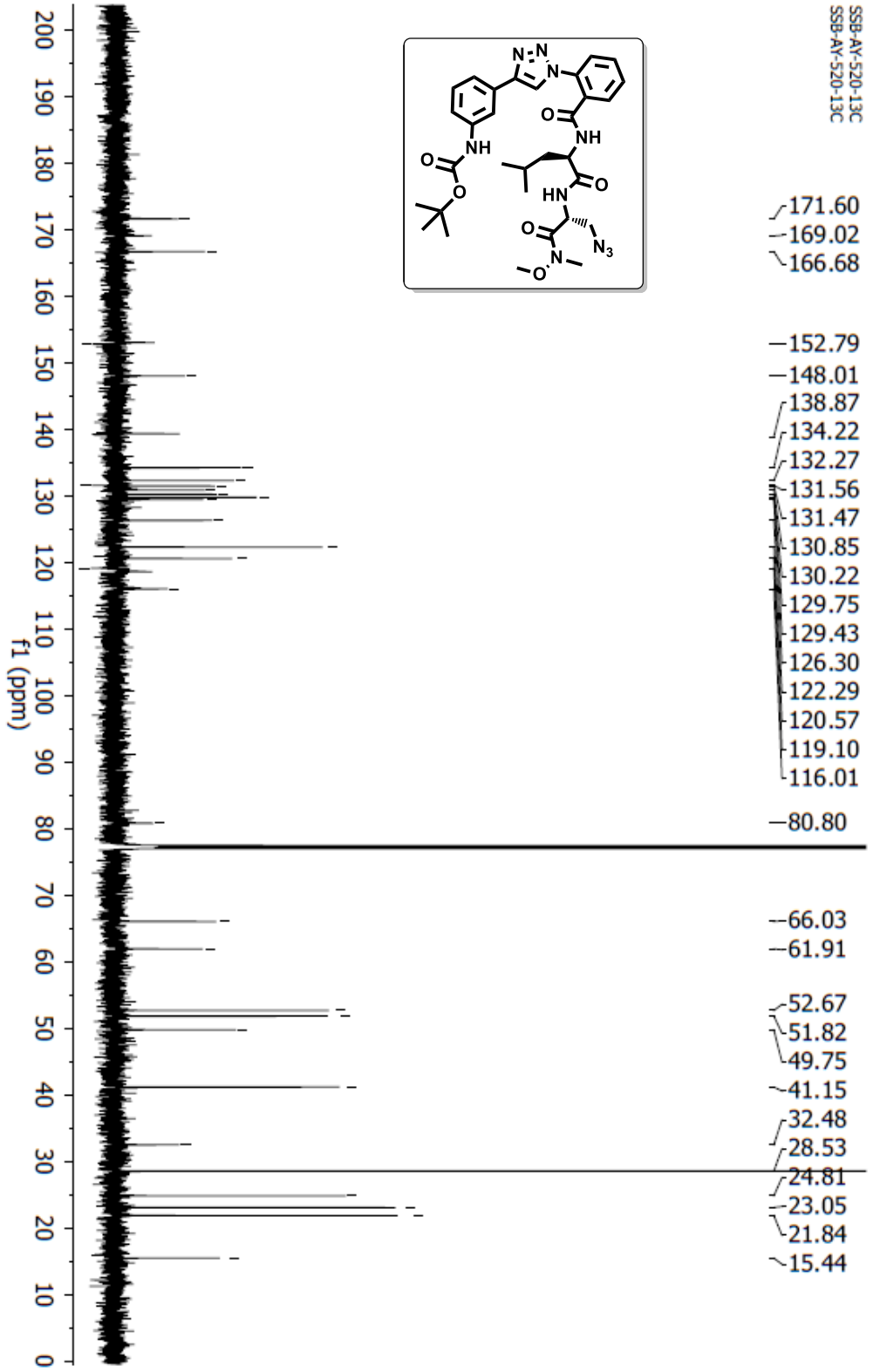


Figure S29. ^{13}C Spectra of synthesized compound **9.**

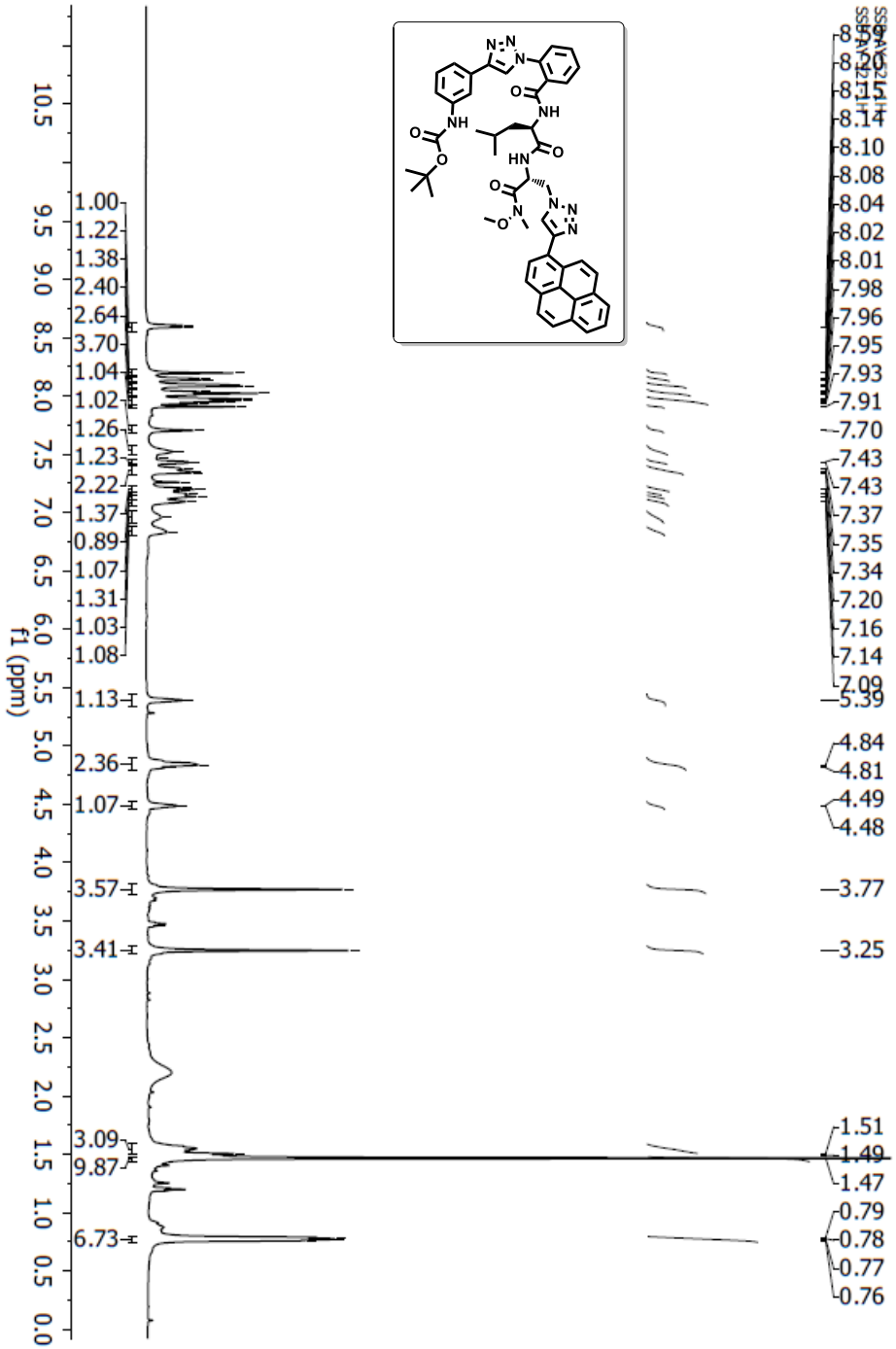


Figure S30. ^{1}H Spectra of synthesized compound **11.**

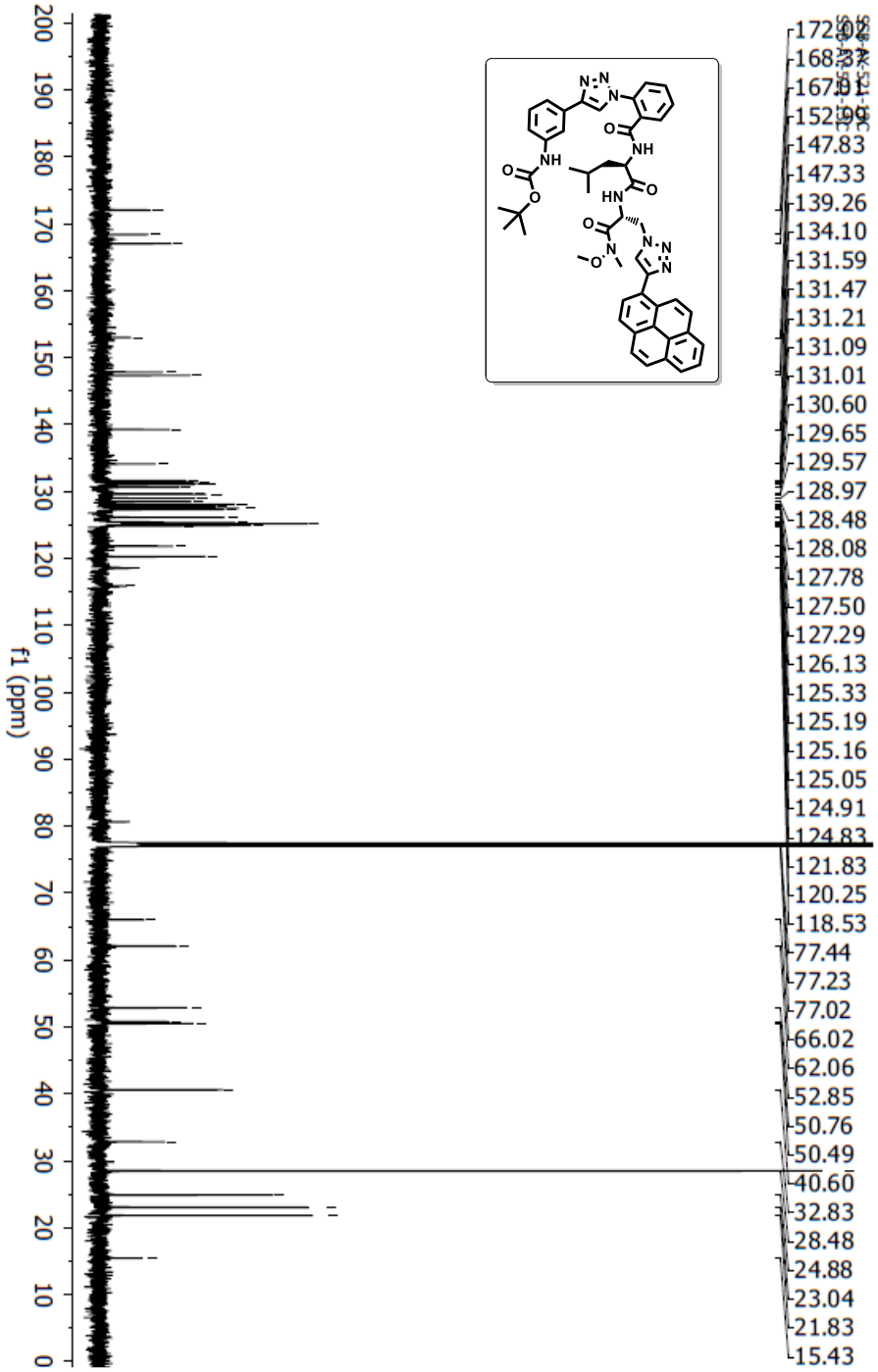


Figure S31. ^{13}C Spectra of synthesized compound **11.**

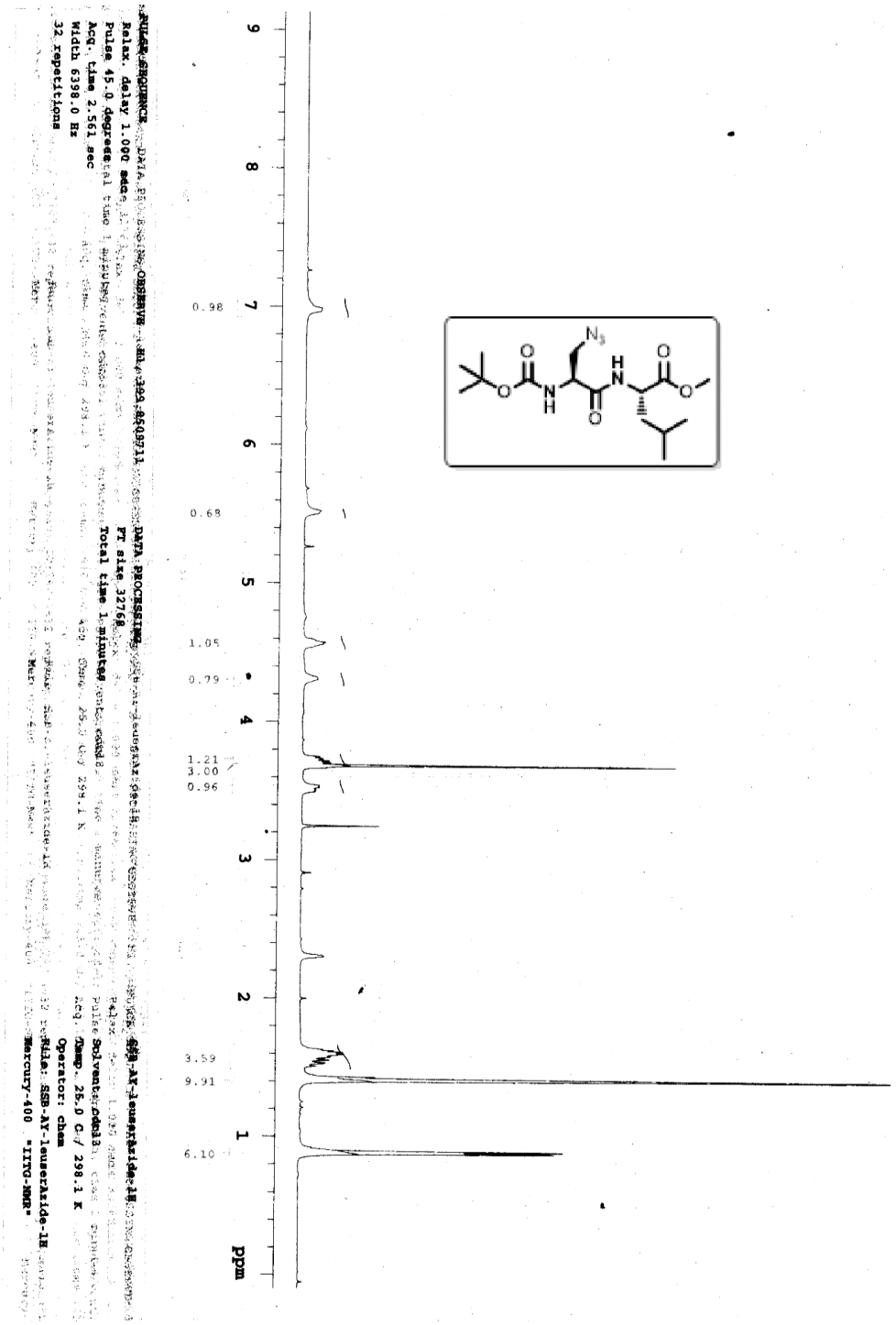


Figure S32. ^{1}H Spectra of synthesized compound **15.**

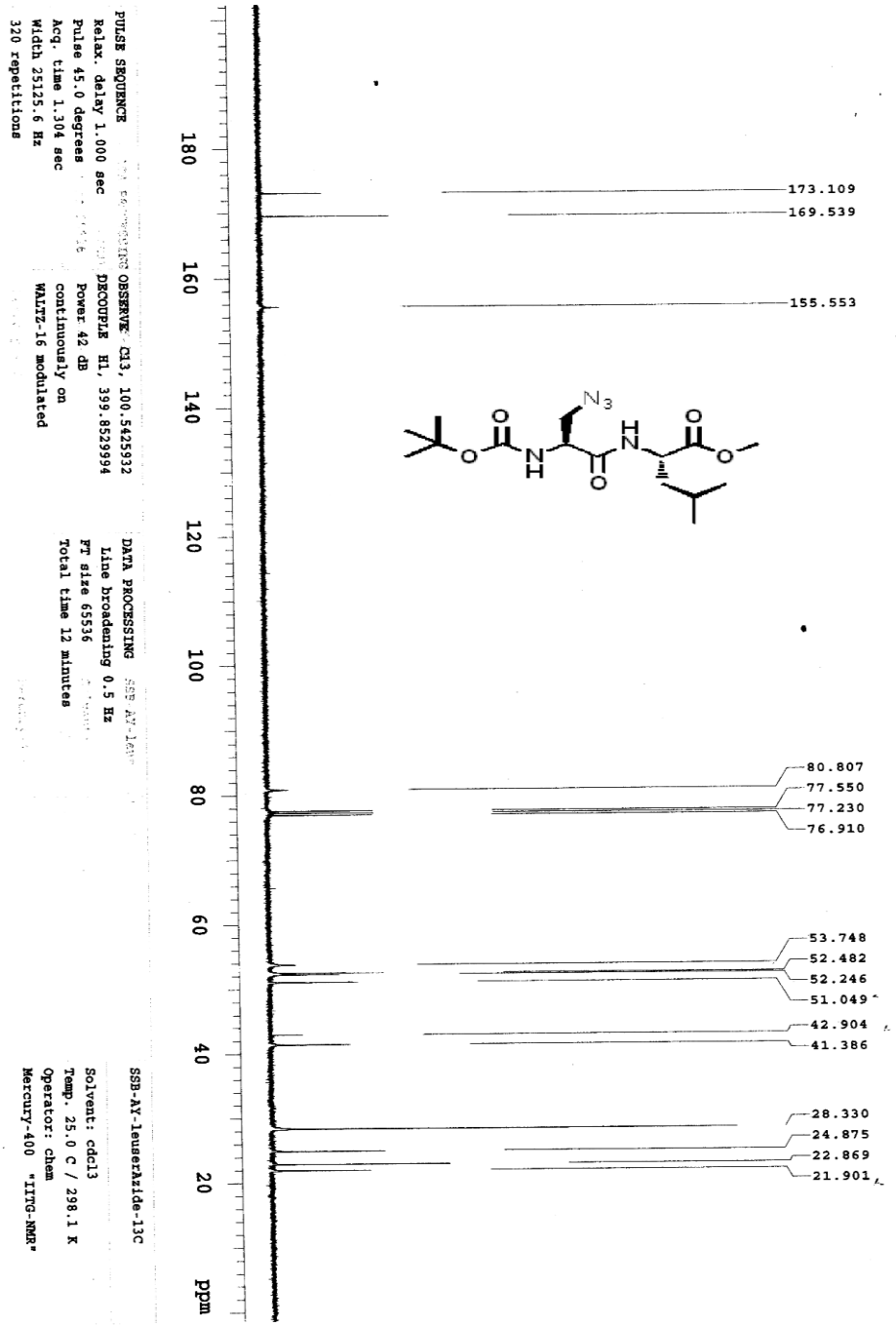


Figure S33. ^{13}C Spectra of synthesized compound **15.**

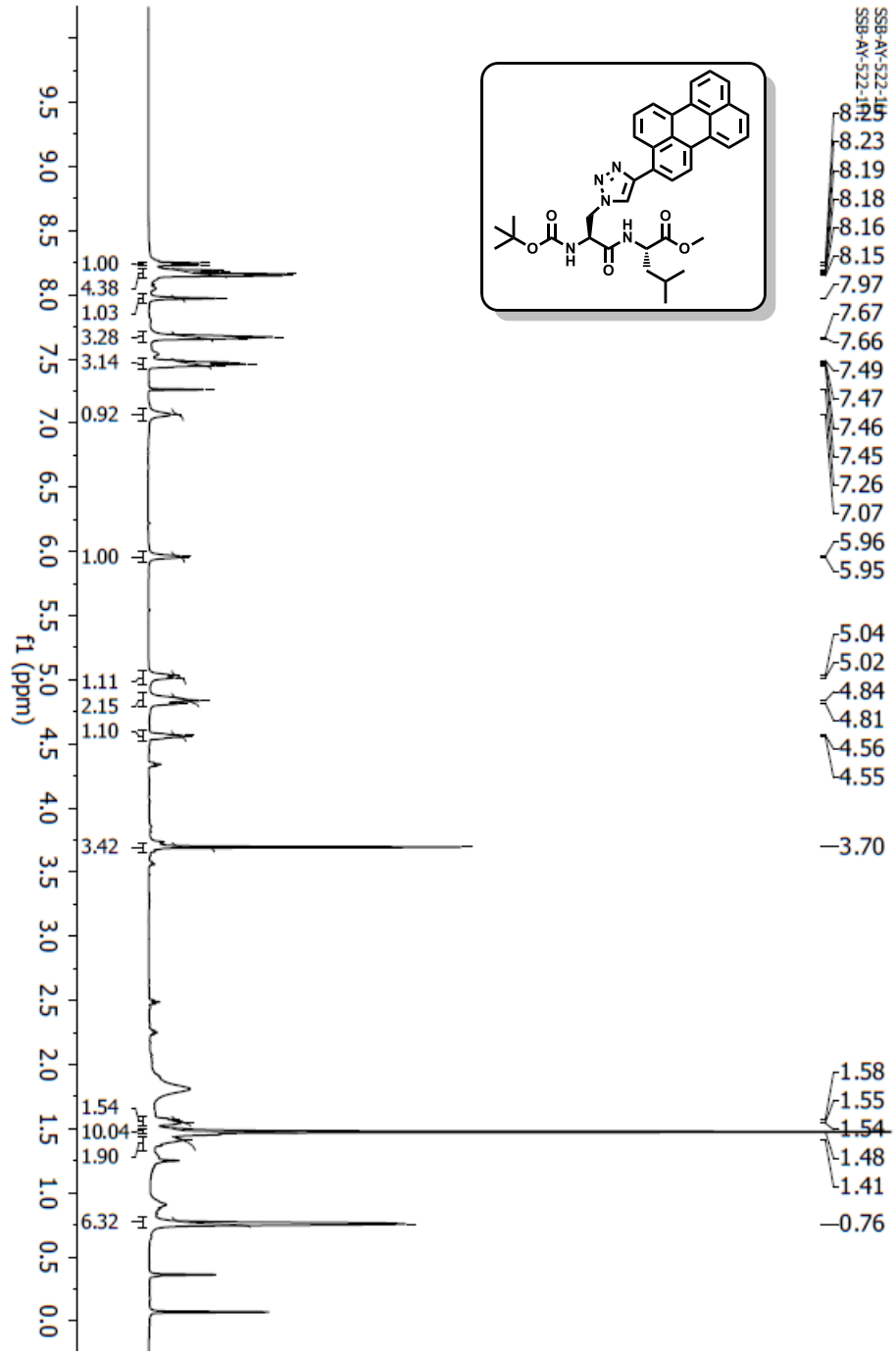


Figure S34. ^{1}H Spectra of synthesized compound **17.**

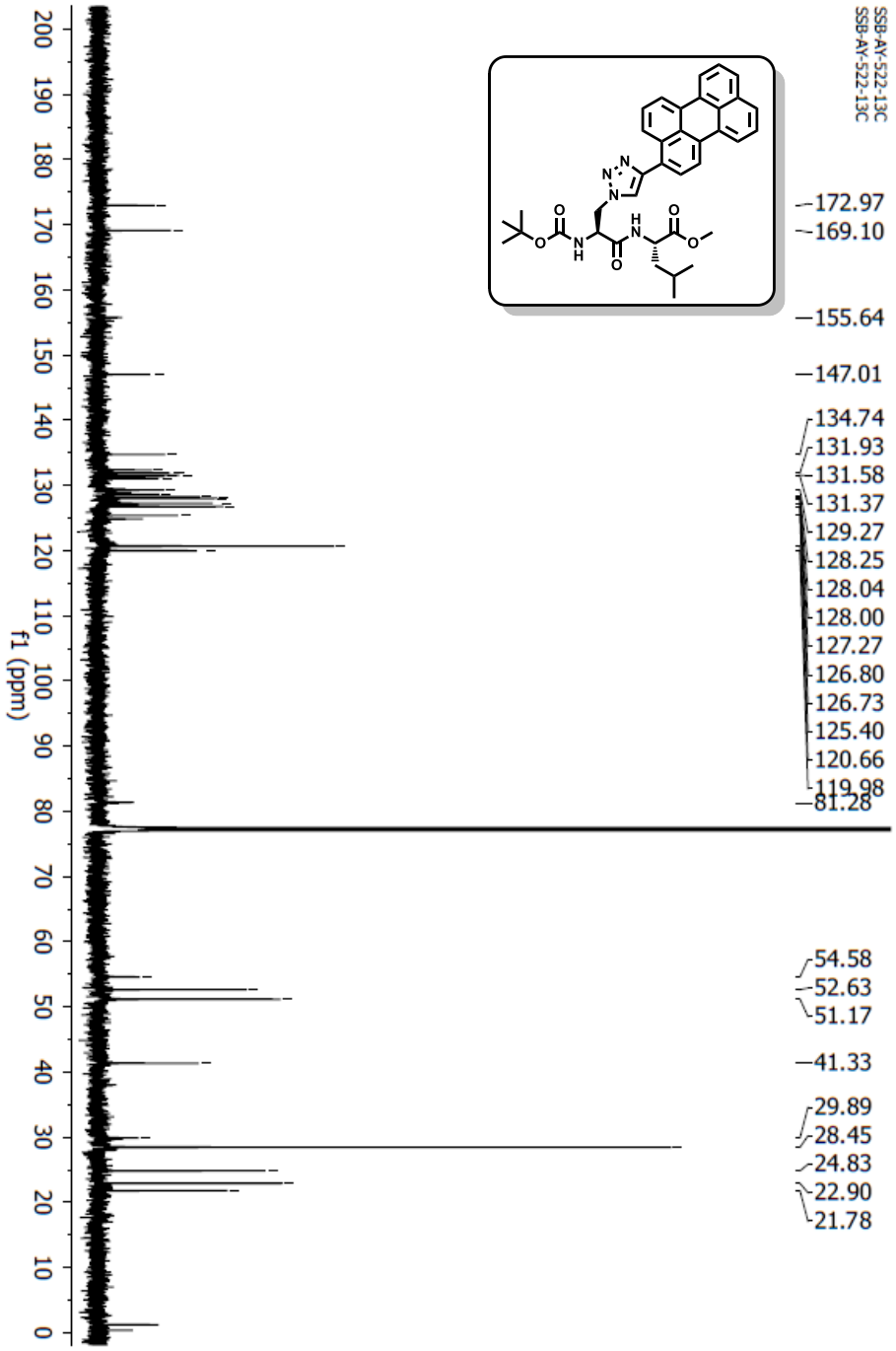


Figure S35. ^{13}C Spectra of synthesized compound **17.**

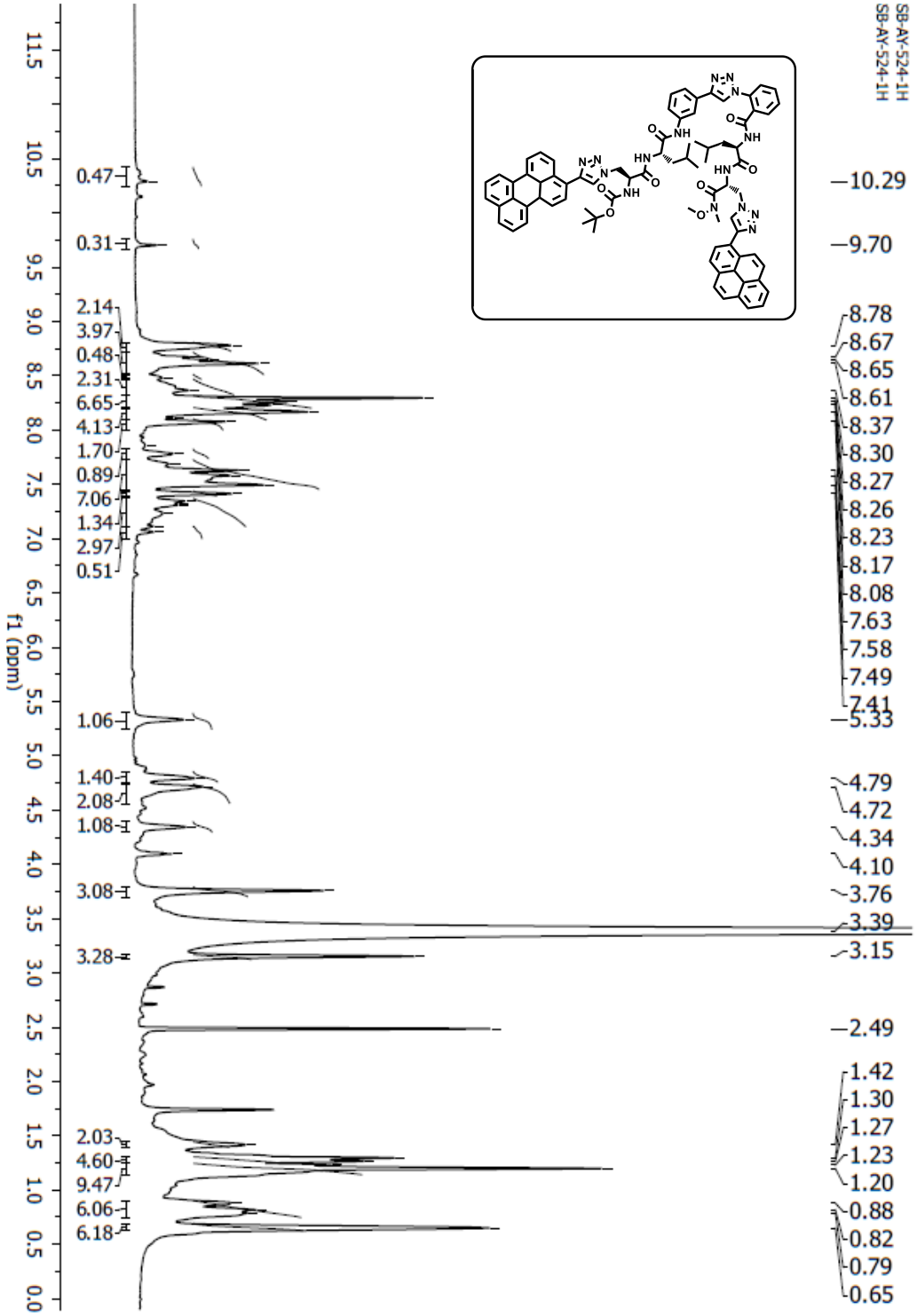


Figure S36. ^{1}H Spectra of synthesized compound **3.**

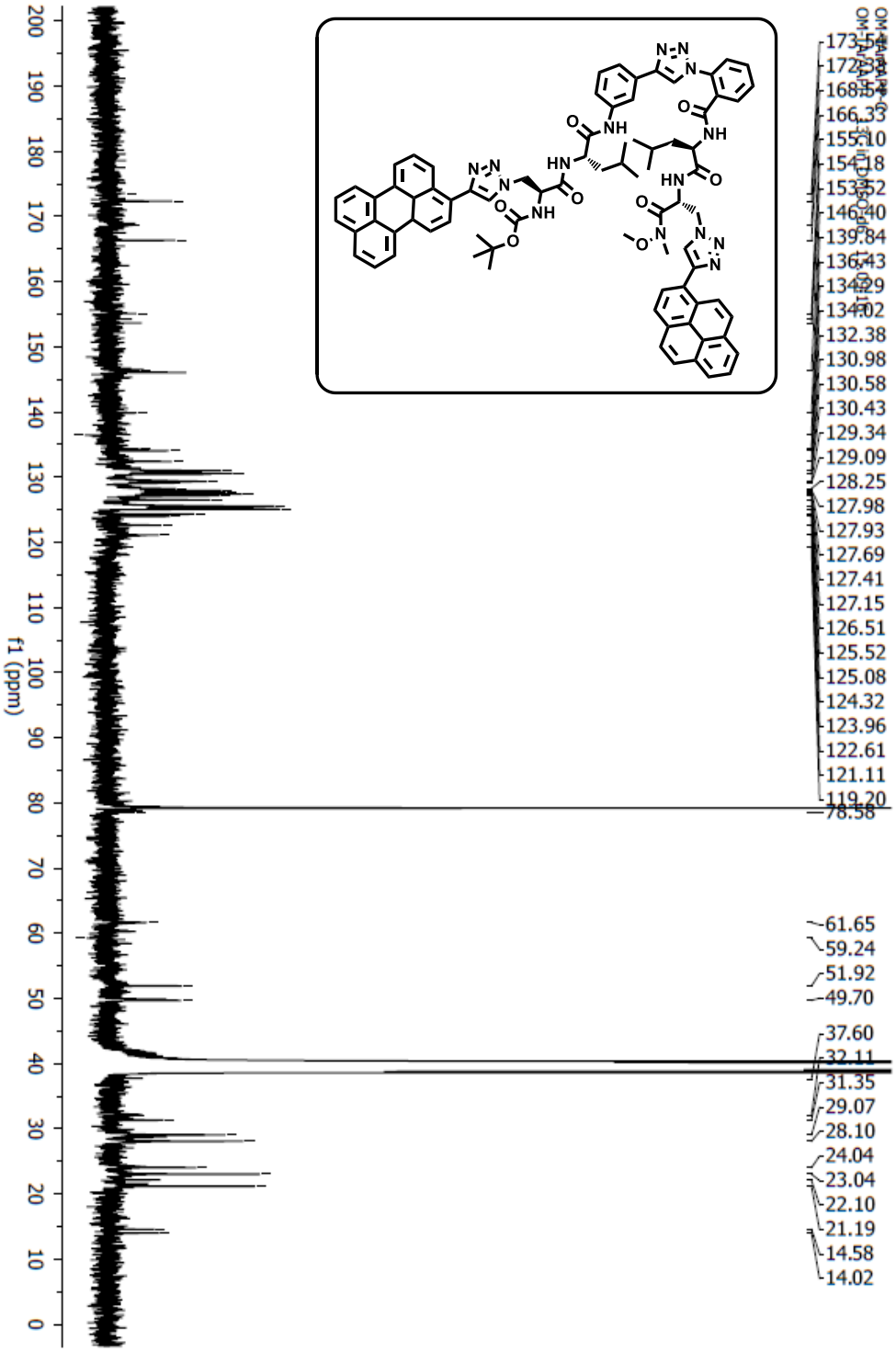


Figure S37. ^{13}C Spectra of synthesized compound **3.**

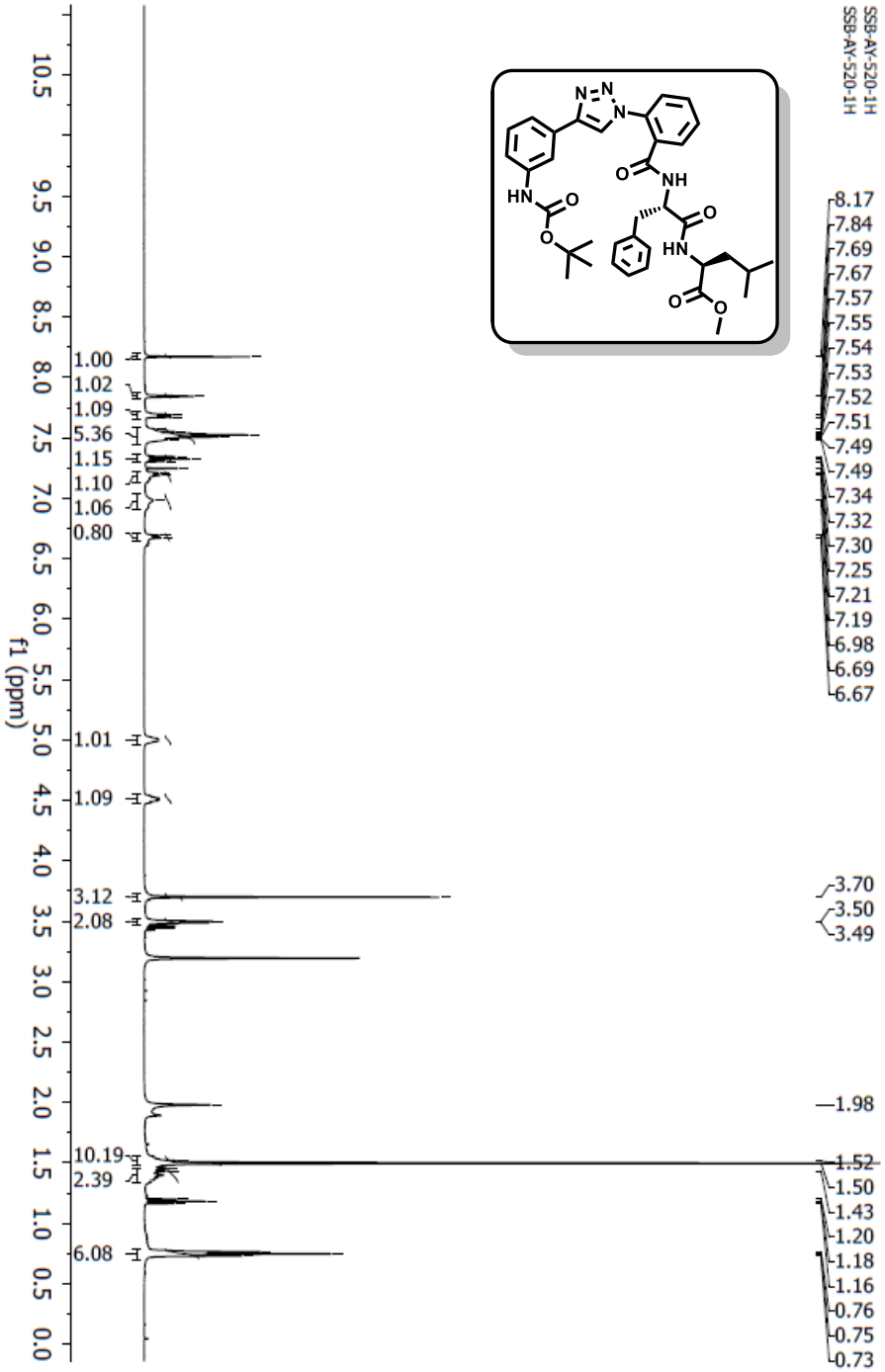


Figure S38. ^{1}H Spectra of synthesized compound **14.**

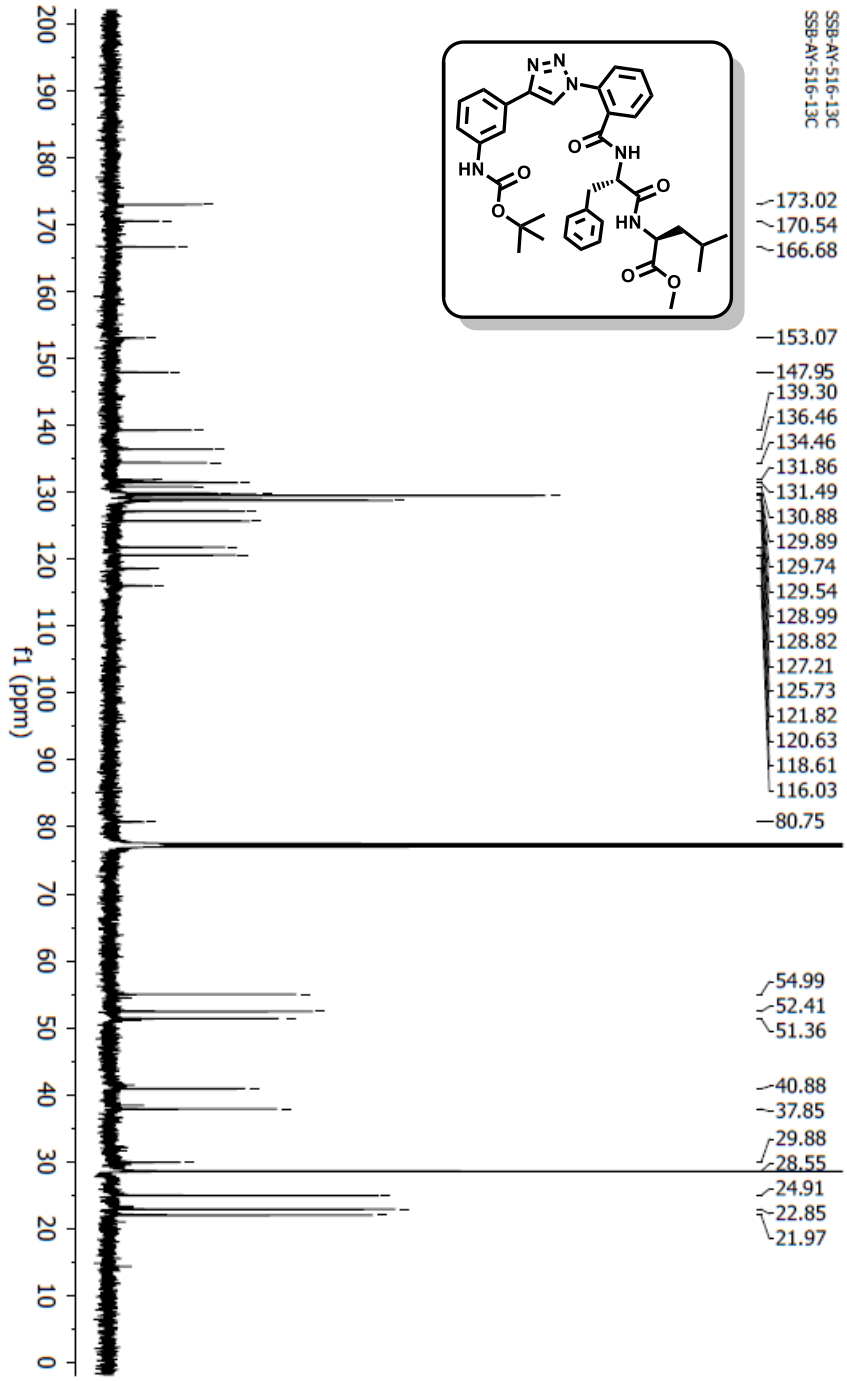


Figure S39. ^{13}C Spectra of synthesized compound **14.**

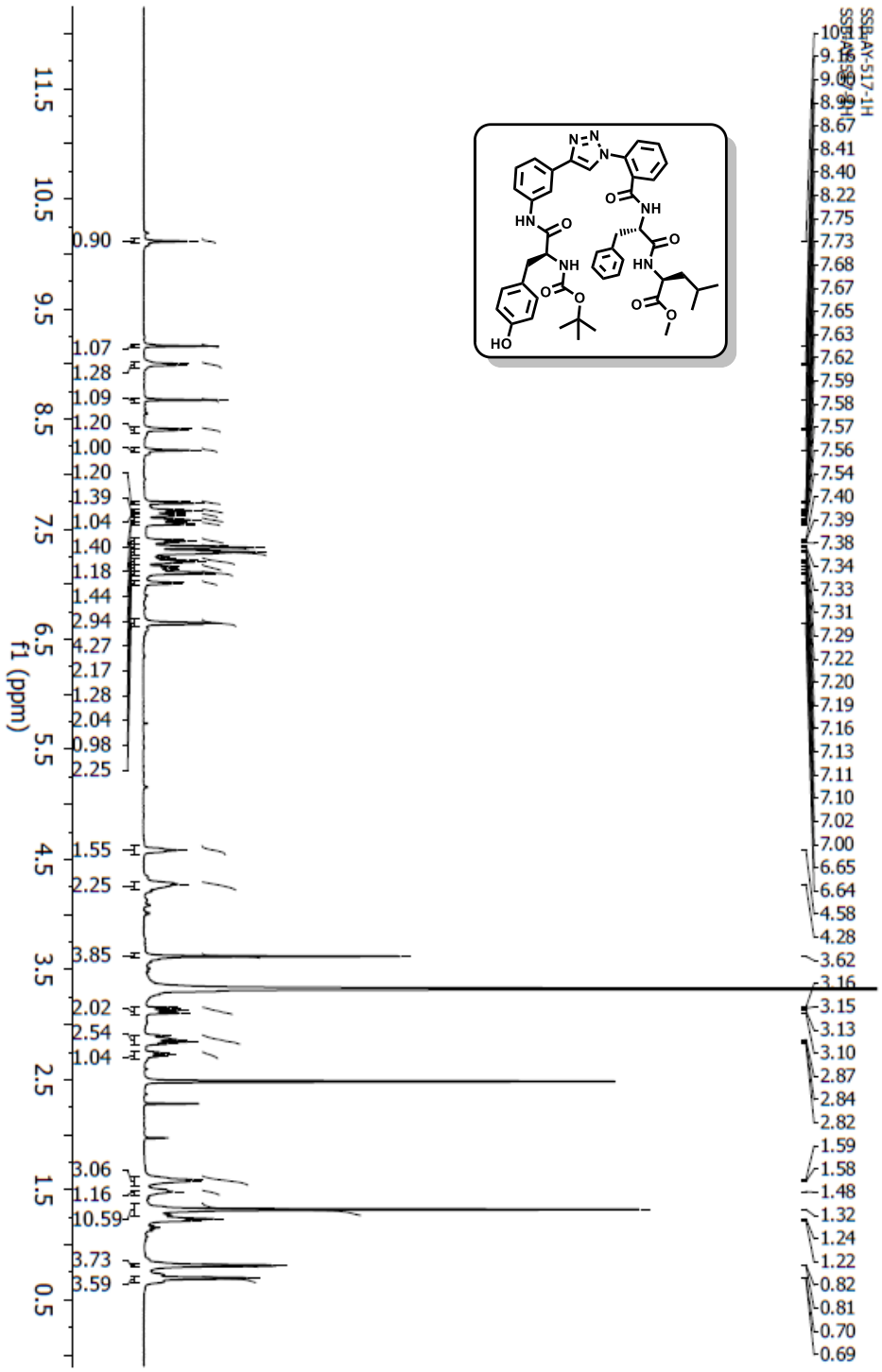


Figure S40. ^{1}H Spectra of synthesized compound **4.**

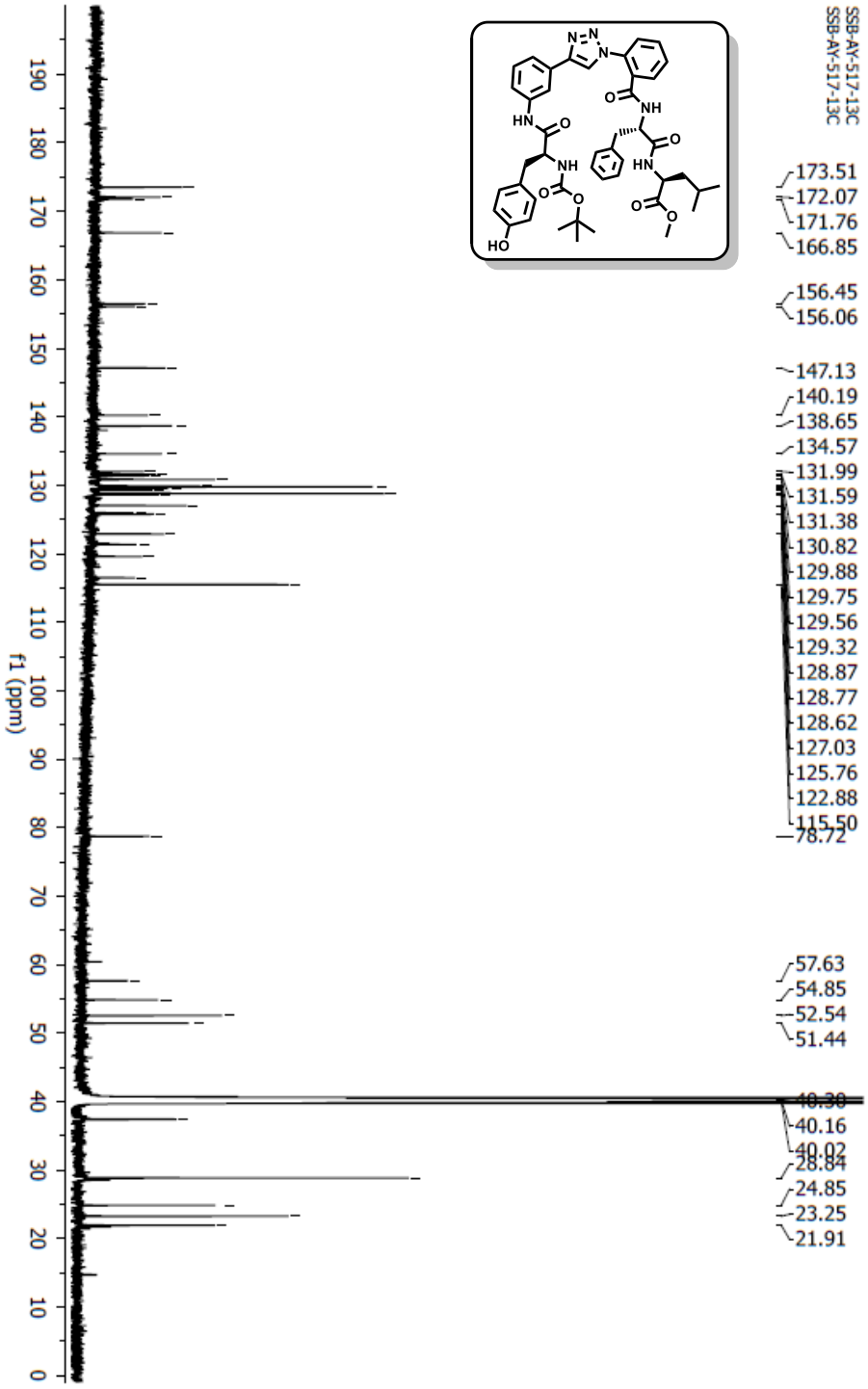


Figure S41. ^{13}C Spectra of synthesized compound **4.**

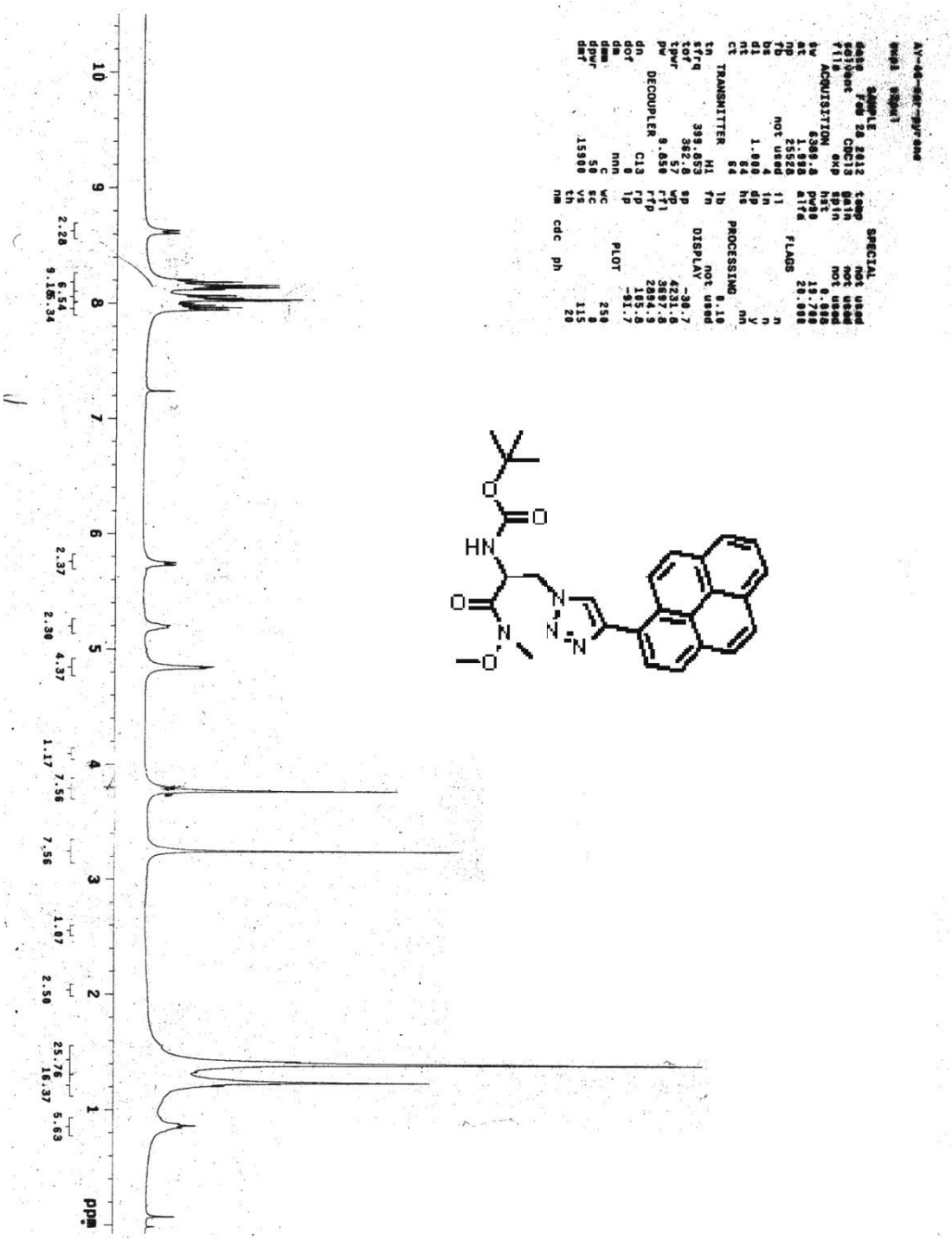


Figure S42. ^{1}H Spectra of synthesized compound **19.**

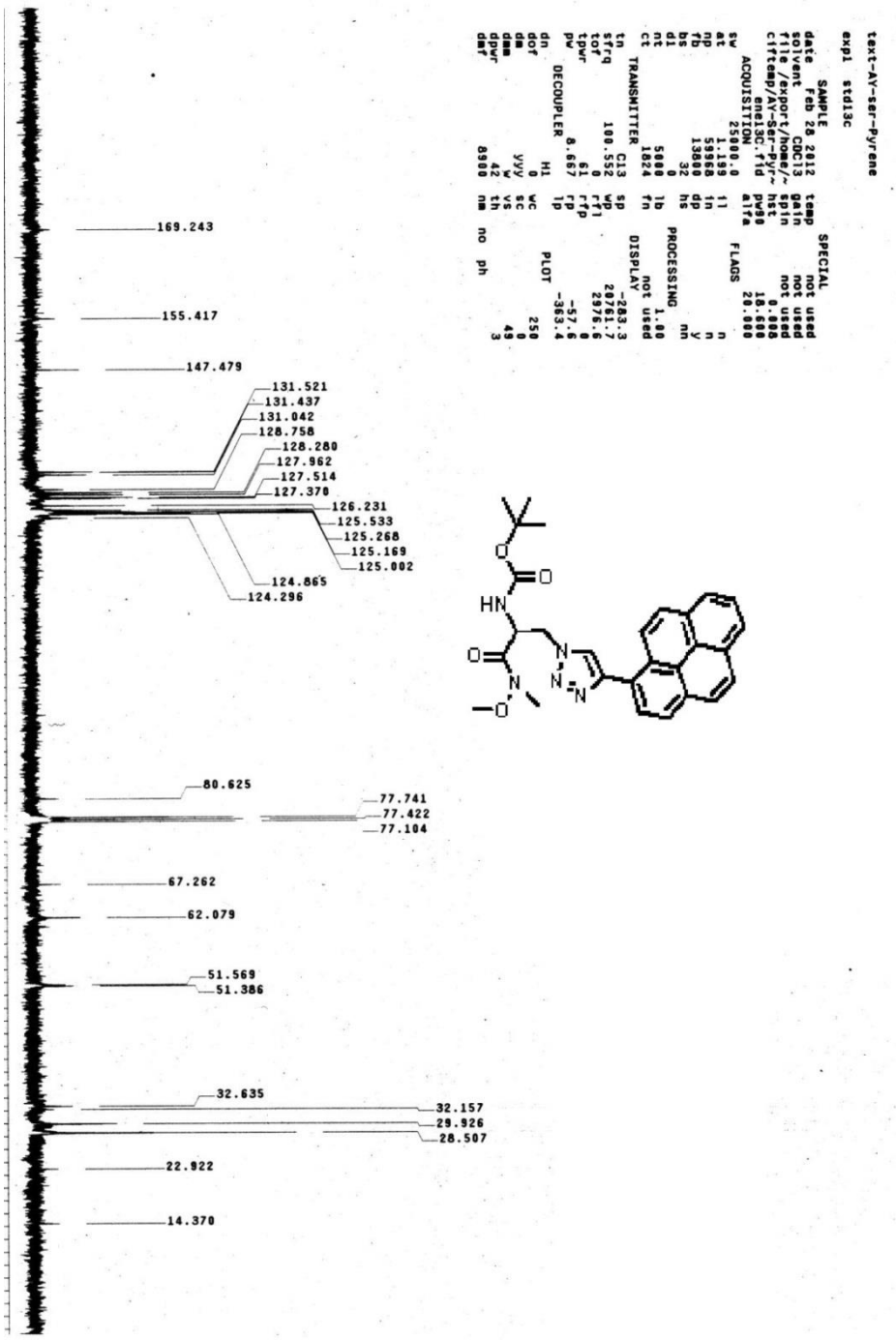


Figure S43. ^{13}C Spectra of synthesized compound **19.**

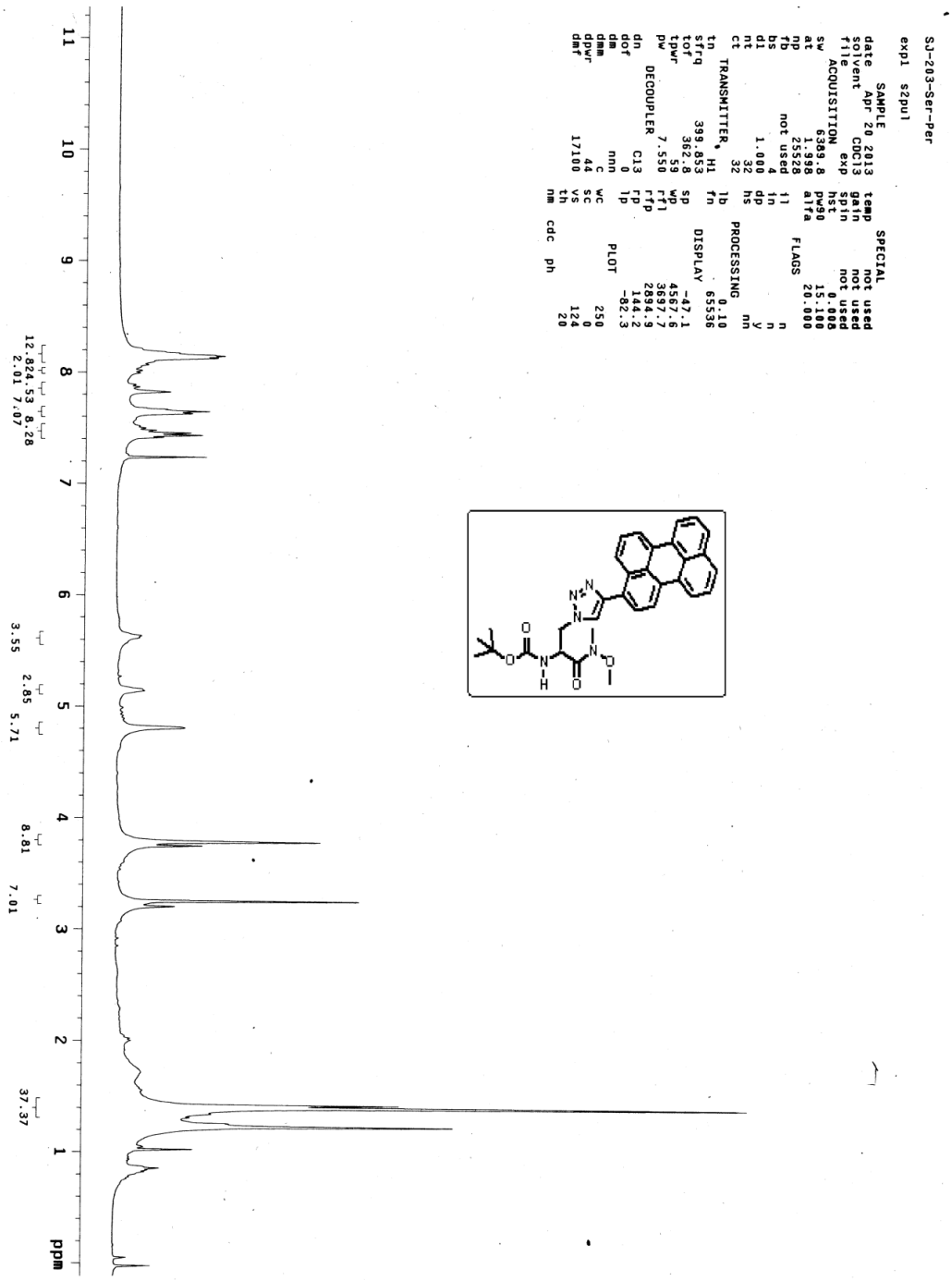


Figure S44. ^{1}H Spectra of synthesized compound **20.**

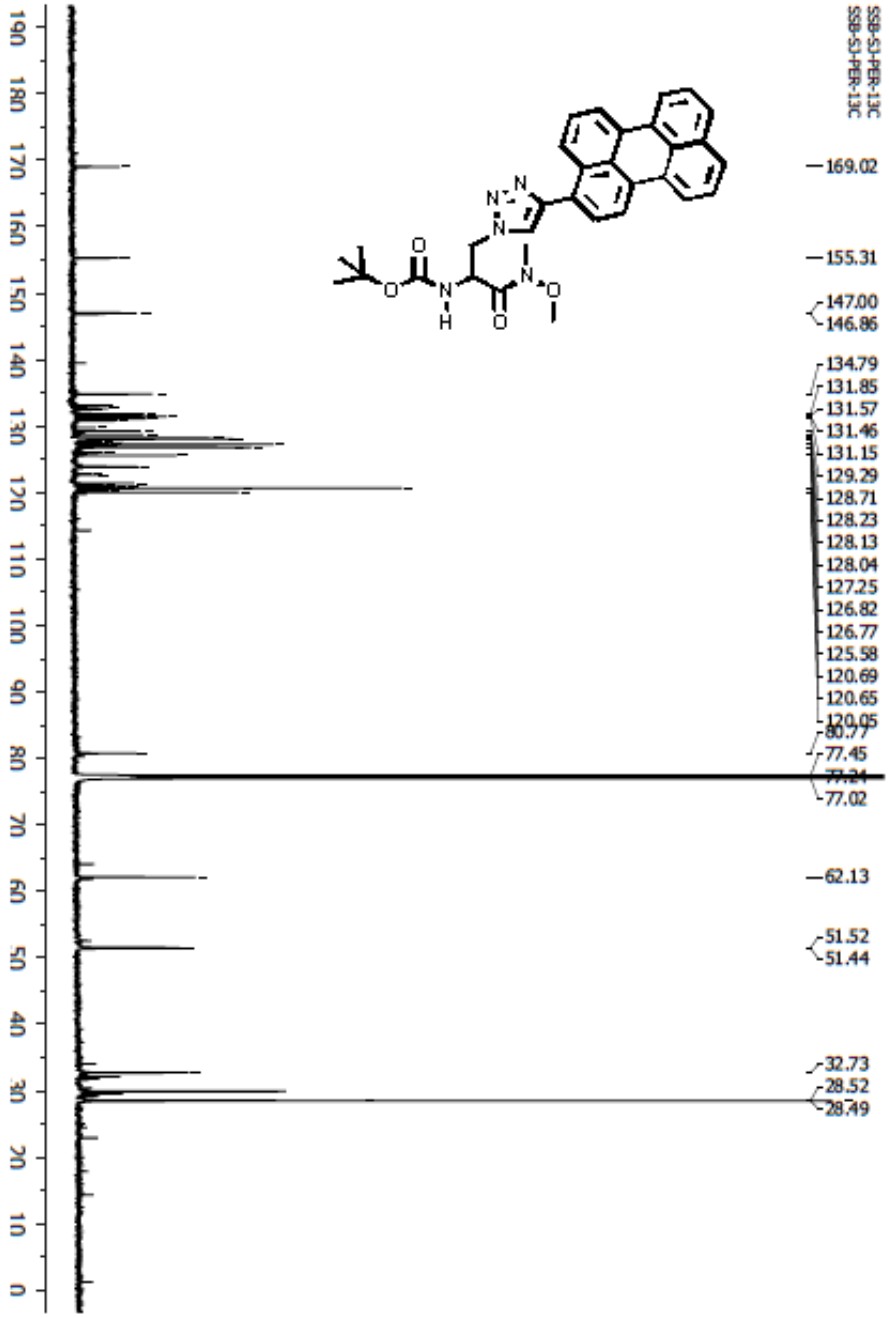


Figure S45. ^{13}C Spectra of synthesized compound **20.**