Folded short azapeptide for conformation switching based fluorescent sensing

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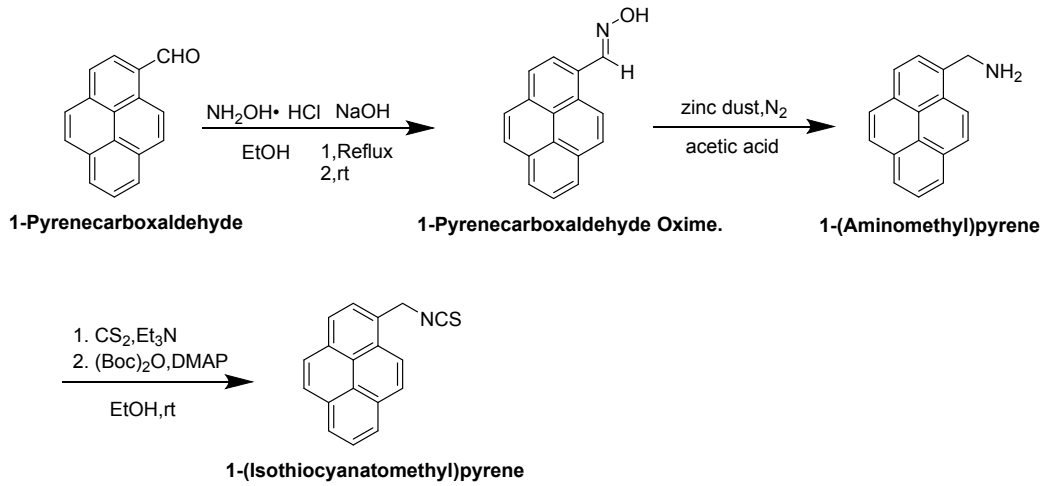
1. Syntheses and characterization



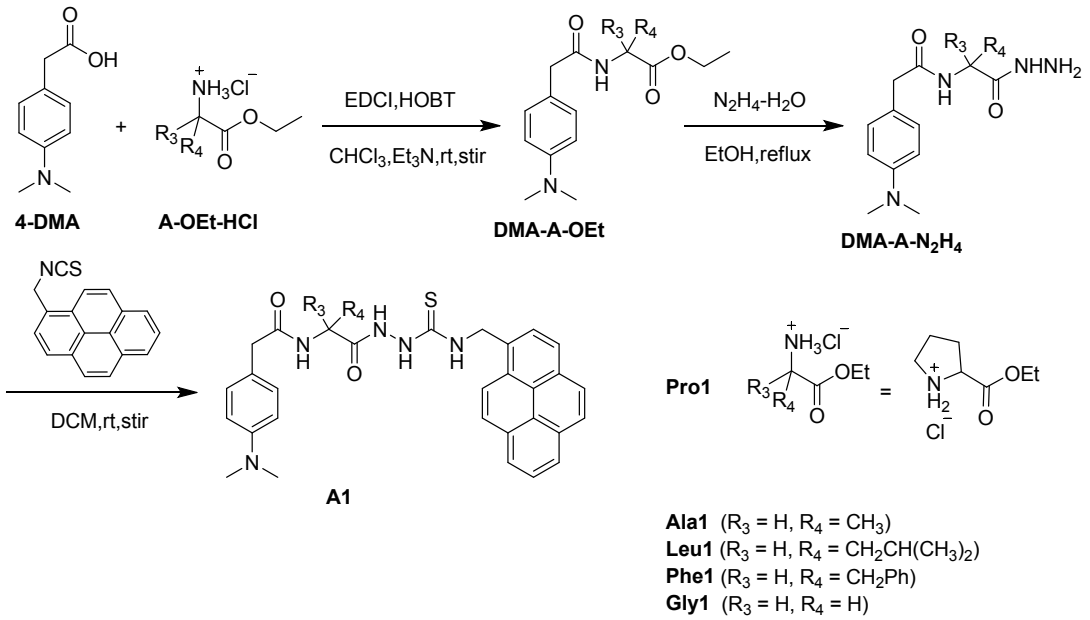
Scheme S1. General procedures for the syntheses of **1-(Isothiocyanatomethyl)pyrene**

1-Pyrenecarboxaldehyde Oxime: To a solution of **1-pyrenecarboxaldehyde** (1.0 g, 4.4 mmol) and hydroxylamine hydrochloride (0.48 g, 6.8 mmol) in ethanol (20 mL) and water (4 mL) was added NaOH (0.86 g). The mixture was heated at reflux for 5 min followed by stirring at room temperature for 2 h and poured into dilute HCl solution. The precipitate was collected by filtration and crystallized from toluene to yield the **1-Pyrenecarboxaldehyde Oxime** 0.59 g, 54%.

1-(Aminomethyl)pyrene: A solution of the **1-Pyrenecarboxaldehyde Oxime** (0.59 g, 2.4 mmol) in acetic acid (20 mL) was stirred with zinc dust(0.2 g, 3 mmol) at room temperature for 15 hours under N_2 atmosphere. The mixture was filtered and the filtrate was neutralized with NaOH. The resulting precipitate was dissolved in AcOEt and solvent was evaporated in vacuo. The residue was crystallized from toluene to give the **1-(Aminomethyl)pyrene** as a white solid 0.26 g, 46%.

1-(Isothiocyanatomethyl)pyrene: CS_2 (300 μL, 5 mmol) was added to a mixture of **1-(aminomethyl)pyrene** (0.1 g, 0.42 mmol) and triethylamine (1 mL) in EtOH (10 mL). The solution was cooled to 0 °C, to which $(Boc)_2O$ (99% eq.) and DMAP (catal.) in EtOH (10 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature, stirred for 5 hours and evaporated. CH_2Cl_2 (100 mL) and water (100 mL) were added to the resulting residue and the aqueous layer was separated and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic fractions were dried over

sodium sulfate and evaporated. The residue was purified by silica gel column chromatography to give a yellow powder **1-(Isothiocyanatomethyl)pyrene** 0.07 g, 62%.



Scheme S2. General procedures for the syntheses of **A1**

DMA-A-OEt: To a chilled solution of **4-DMA** (0.18 g, 1 mmol) and Et_3N (0.5 mL, 3.6 mmol) in $CHCl_3$ (10 mL) was added EDCI (0.29 g, 1.5 mmol) and HOBT (0.20 g, 1.5 mmol) at 0℃. After 30 min, a solution of (L or D) **A-OEt-HCl** (1.5 mmol) and Et_3N (0.5 mL, 3.6 mmol) in $CHCl_3$ (10 mL) was added. The mixture was left to stand at room temperature for 4 hours, evaporated in vacuo, and the solid residue was dissolved in AcOEt. The solution was washed successively with 1% $NH_3 \cdot H_2O$, saturated NH_4Cl and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, and purified by flash chromatography on silica gel using DCM:MeOH =50:1 as a mobile phase, affording a white powder.
(**DMA-Phe-OEt**: 0.28 g, 79%; **DMA-Pro-OEt**: 0.24 g, 79%; **DMA-Ala-OEt**: 0.21 g, 76%; **DMA-Leu-OEt**: 0.26 g, 81%; **DMA-Gly-OEt**: 0.22 g, 83%)

DMA-A-N_2H_4: Excess aqueous hydrazine (80%) was added to **DMA-A-OEt** in ethanol (15 mL) and refluxed overnight, evaporated in vacuo. The viscous liquid was dissolved in ethyl acetate. The solution was added with petroleum ether, and viberated in ultrasonic instrument. Then filter the white solid, which was washed by water to obtain white solid. (**DMA-Phe-N_2H_4**: 0.24 g, 92%; **DMA-Pro-N_2H_4**: 0.20 g, 87%;

DMA-Ala-N_2H_4: 0.16 g, 80%; **DMA-Leu-N_2H_4**: 0.19 g, 77%; **DMA-Gly-N_2H_4**: 0.15 g, 72%)

A1: To a solution of **DMA-A-N_2H_4** (0.3 mmol) in CH_2Cl_2 (10 mL), CH_2Cl_2 (10 mL) with 0.1 g 1-(isothiocyanatomethyl)pyrene (0.36 mmol) was added. The mixture was stirred at room temperature overnight and filtered the solid, which after washing by CH_2Cl_2 gave **A1** as white solid. (**Phe1**: 0.23 g, 54%; **Pro1**: 0.1 g, 59%; **Ala1**: 0.09 g, 56%; **Leu1**: 0.1 g, 57%; **Gly1**: 0.08 g, 51%)

L-**Phe1:**

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 10.26 (s, 1H), 9.6 (s, 1H), 8.43 (d, J = 9.2 Hz, 1H), 8.30 (t, J = 9.2 Hz, 3H), 8.23 (dd, J = 8.6, 4.1 Hz, 2H), 8.16 (d, J = 1.4 Hz, 2H), 8.08 (t, J = 7.6 Hz, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.09 – 7.28 (m, 5H), 6.77 (d, J = 8.0 Hz, 2H), 6.39 (d, J = 8.0 Hz, 2H), 5.49 (dd, J = 15.6, 5.9 Hz, 1H), 5.29 (dd, J = 15.6, 5.9 Hz, 1H), 4.35 (s, 1H), 3.20 (d, J = 14.4 Hz, 1H), 3.09 (d, J = 14.4 Hz, 1H), 3.20 (d, J = 10.0 Hz, 1H), 2.84 (dd, J = 14.2, 9.1 Hz, 1H), 2.70 (s, 6H).

^{13}C NMR (101 MHz, CD_3CN): δ (ppm) 183.02, 173.00, 171.81, 149.78, 137.68, 132.81, 131.69, 131.20, 130.95, 130.12, 129.80, 128.81, 128.02, 127.52, 127.06, 126.79, 125.68, 125.20, 123.78, 113.05, 54.68, 46.17, 41.37, 36.99.

HRMS (ESI): calcd for $[C_{37}H_{36}N_5O_2S]^+$: 614.2590, found: 614.2588.

D-**Phe1:**

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 10.26 (s, 1H), 9.6 (s, 1H), 8.43 (d, J = 9.2 Hz, 1H), 8.30 (t, J = 9.2 Hz, 3H), 8.23 (dd, J = 8.6, 4.1 Hz, 2H), 8.16 (d, J = 1.4 Hz, 2H), 8.08 (t, J = 7.6 Hz, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.09 – 7.28 (m, 5H), 6.77 (d, J = 8.0 Hz, 2H), 6.39 (d, J = 8.0 Hz, 2H), 5.49 (dd, J = 15.6, 5.9 Hz, 1H), 5.29 (dd, J = 15.6, 5.9 Hz, 1H), 4.35 (s, 1H), 3.20 (d, J = 14.4 Hz, 1H), 3.09 (d, J = 14.4 Hz, 1H), 3.20 (d, J = 10.0 Hz, 1H), 2.84 (dd, J = 14.2, 9.1 Hz, 1H), 2.70 (s, 6H).

^{13}C NMR (101 MHz, CD_3CN): δ (ppm) 183.13, 173.11, 171.84, 149.97, 137.69, 132.81, 131.73, 131.23, 130.99, 130.13, 129.83, 128.86, 128.10, 127.98, 127.55, 127.10,126.90,126.72, 125.73, 125.67,125.23, 124.90, 124.87,123.80, 54.75, 46.23, 41.42, 40.44, 37.01.

HRMS (ESI): calcd for $[C_{37}H_{36}N_5O_2S]^+$: 614.2590, found: 614.2593.

L-**Pro1:**

^{1}H NMR (650 MHz, acetonitrile-d_3-DMSO-d_6 (8/2, v/v)): δ (ppm) 10.13 (s, 1H), 9.22 (s, 1H), 8.54 (s, 1H), 8.45 (d, J = 9.2 Hz, 1H), 8.26 (t, J = 7.2 Hz, 2H), 8.18 (t, J = 8.2 Hz, 2H), 8.11 (s, 2H), 8.06 (t, J = 7.6 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 6.65 (d, J = 7.8 Hz, 2H), 6.21 (d, J = 7.8 Hz, 2H), 5.50 (dd, J = 15.4, 6.1 Hz, 1H), 5.31 (dd, J = 15.4, 5.5 Hz, 1H), 4.10 (t, J = 6.3 Hz, 1H), 3.60 – 3.50 (m, 2H), 3.33 (q, J = 15.5 Hz, 2H), 2.57 (s, 6H), 2.15 – 2.09 (m, 1H), 2.04 (m, 1H), 1.91 – 1.83 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 182.40, 171.97, 171.36, 149.31, 132.87, 131.28, 130.81, 130.31, 130.02, 128.19, 127.89, 127.27, 126.61, 126.30, 125.59, 125.51, 125.08, 124.44, 124.38, 123.67, 122.46, 112.46, 59.31, 47.80, 45.65, 29.30, 25.31.

HRMS (ESI): calcd for $[C_{33}H_{33}N_5O_2S]^+$: 564.2433, found: 564.2433.

L-**Ala1:**

^{1}H NMR (650 MHz, acetonitrile-d_3-DMSO-d_6 (8/2, v/v)): δ (ppm) 9.89 (s, 1H), 9.07 (s, 1H), 8.44 (d, J = 9.2 Hz, 1H), 8.32 (t, J = 5.2 Hz, 1H), 8.27 (t, J = 7.2 Hz, 2H), 8.20 (d, J = 9.5 Hz, 2H), 8.13 (s, 2H), 8.07 (t, J = 7.6 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.91 (s, 1H), 6.78 (d, J = 7.2 Hz, 2H), 6.39 (d, J = 7.1 Hz, 2H), 5.53 (dd, J = 15.2, 6.0 Hz, 1H), 5.29 (dd, J = 15.2, 5.2 Hz, 1H), 3.99 (s, 1H), 3.18 (d, J = 14.5 Hz, 1H), 3.07 (d, J = 14.3 Hz, 1H), 2.71 (s, 6H), 1.25 (d, J = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 182.43, 172.64, 172.46, 149.49, 132.95, 131.30, 130.81, 130.37, 129.90, 128.27, 127.91, 127.33, 126.65, 126.44, 125.63, 125.55, 125.07, 124.45, 124.41, 123.67, 112.65, 48.54, 45.66, 40.97, 17.35.

HRMS (ESI): calcd for $[C_{31}H_{32}N_5O_2S]^+$: 538.2277, found: 538.2279.

L-**Leu1:**

^{1}H NMR (650 MHz, acetonitrile-d_3-DMSO-d_6 (8/2, v/v)): δ (ppm) 9.98 (s, 1H), 9.07 (s, 1H), 8.43 (d, J = 9.2 Hz, 1H), 8.27 (t, J = 7.4 Hz, 3H), 8.20 (dd, J = 8.5, 3.4 Hz, 2H), 8.12 (s, 2H), 8.07 (t, J = 7.6 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.83 (s, 1H), 6.77 (d, J = 7.1 Hz, 2H), 6.39 (d, J = 7.7 Hz, 2H), 5.55 (dd, J = 15.2, 6.1 Hz, 1H), 5.23 (dd, J = 15.0, 5.0 Hz, 1H), 3.97 (s, 1H), 3.17 (d, J = 14.5 Hz, 1H), 3.04 (d, J = 14.2 Hz, 1H), 2.71 (s, 6H), 1.59 (tt, J = 13.2, 6.6 Hz, 1H), 1.49 (t, J = 7.3 Hz, 2H), 0.87 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 182.44, 173.42, 172.96, 149.36, 148.25, 138.98, 132.96, 131.30, 130.80, 130.34, 129.89, 128.25, 128.23, 127.92, 127.82, 127.28,

126.60, 126.44, 125.59, 125.51, 125.06, 124.46, 124.38, 123.69, 123.40, 112.46, 99.99, 55.60, 45.44, 41.21, 25.47.

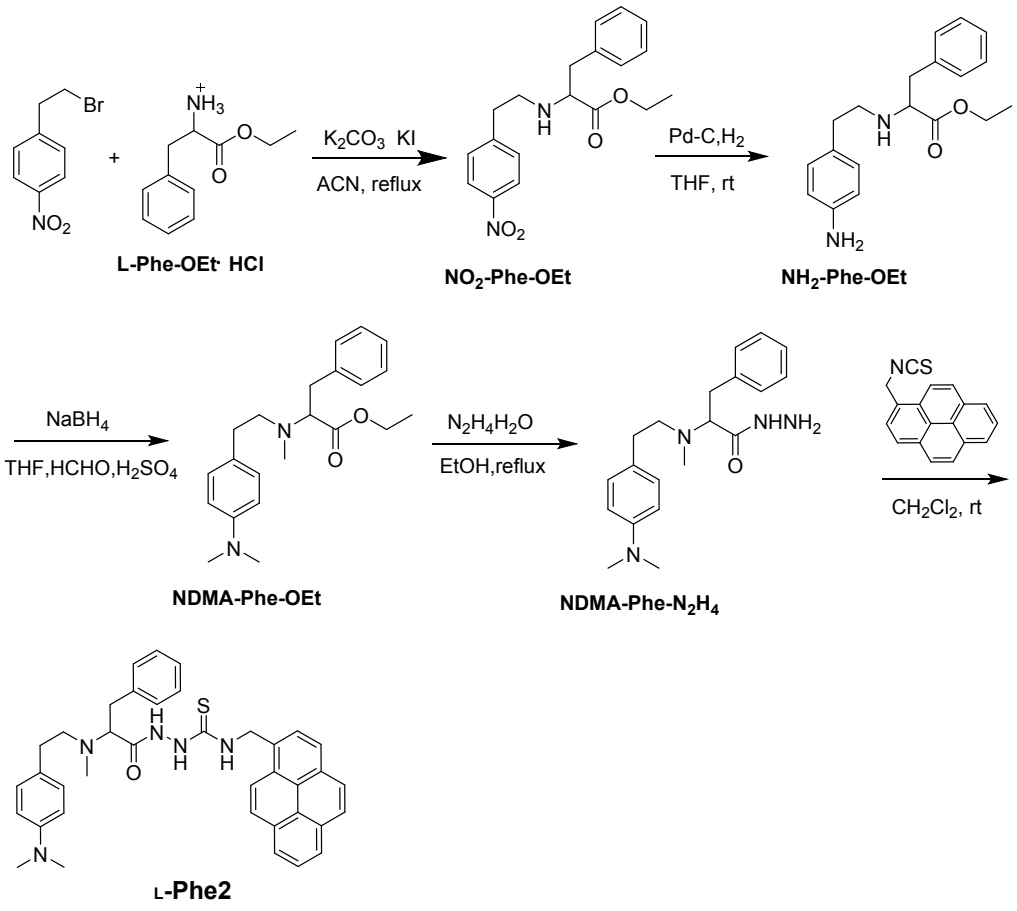
HRMS (ESI):calcd for $[C_{34}H_{38}N_5O_2S]^+$: 580.2746, found:580.2749.

L-Gly1:

^{1}H NMR (650 MHz, acetonitrile-d_3-DMSO-d_6 (8/2, v/v)): δ (ppm) 9.81 (s, 1H), 9.10 (s, 1H), 8.45 (d, J = 9.2 Hz, 1H), 8.28 (t, J = 7.2 Hz, 3H), 8.22 (d, J = 8.8 Hz, 2H), 8.13 (s, 2H), 8.07 (t, J = 7.6 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.79 (s, 1H), 6.91 (d, J = 7.3 Hz, 2H), 6.50 (d, J = 7.6 Hz, 2H), 5.47 (d, J = 5.5 Hz, 2H), 3.68 (d, J = 4.5 Hz, 2H), 3.24 (s, 2H), 2.77 (s, 6H).

^{13}C NMR (214 MHz, DMSO-d_6) δ 172.29, 171.56, 169.37, 149.62, 133.08, 131.29, 130.79, 130.31, 130.01, 128.12, 127.91, 127.90, 127.31, 126.66, 126.03, 125.64, 125.56, 125.05, 124.42, 124.37, 123.91, 123.56, 112.91, 112.79, 45.60, 41.78, 41.40, 40.69, 40.45.

HRMS (ESI): calcd for $[C_{30}H_{30}N_5O_2S]^+$: 524.2120, found: 524.2122.



Scheme S3. General procedures for the syntheses of **L-Phe2**

NO_2-Phe-OEt: To a solution of L-**Phe-OEt·HCl** (1.15 g, 5 mmol) and K_2CO_3 (1.38 g, 10 mmol) in ACN (10 mL) was added 4-Nitrophenethyl bromide (0.9 g, 4 mmol) in ACN (10 ml) with KI (0.08 g, 0.5 mmol). The reaction was refluxed for 2 days, then evaporated in vacuo. The solid was dissolved in ethyl acetate, and washed by water, dried over anhydrous Na_2SO_4 then purified by chromatography on a silica gel column. Elution with PE/EA from 20:1 to 10:1 furnished pure **NO_2-Phe-OEt** as white solid 0.56 g, 41%.

NH_2-Phe-OEt: NO_2-Phe-OEt (0.56 g, 1.6 mmol) and 10% Pd/C (0.05 g) were dissolved/suspended in 20 ml of THF. The reaction was stirred under hydrogen atmosphere at room temperature. After 10h of stirring, the solution was filtered to remove Pd/C, and the filtrate was evaporated under the reduced pressure to give the yellow crude product without further purification, 0.53 g, 96%.

NDMA-Phe-OEt: A solution of **NH_2-Phe-OEt** (0.53 g, 1.7 mmol) in 15 ml THF and 0.4 g sodium borohydride was added dropwise over a period of 10 min to a stirred mixture of 1.6 ml 37% aqueous formaldehyde and 1.5 ml 20% H_2SO_4 in 10ml THF. The reaction was left to stand at room temperature for 1 h, evaporated in vacuo, and the oily residue was dissolved in water. The mixture was made basic by dropwise addition of aqueous solution of ammonia. **NDMA-Phe-OEt** was extract by CH_2Cl_2 and purified by chromatography on silica gel (PE/EA=10:1), gave **NDMA-Phe-OEt** as oily solid, 0.25 g, 46%.

NDMA-Phe-N_2H_4: Excess aqueous hydrazine (80%) was added to **NDMA-Phe-OEt** in ethanol (10 mL) and refluxed overnight, evaporated in vacuo. The oily solid was dissolved in ethyl acetate. The solution was washed by water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, to obtained 0.23 g white solid, 96%.

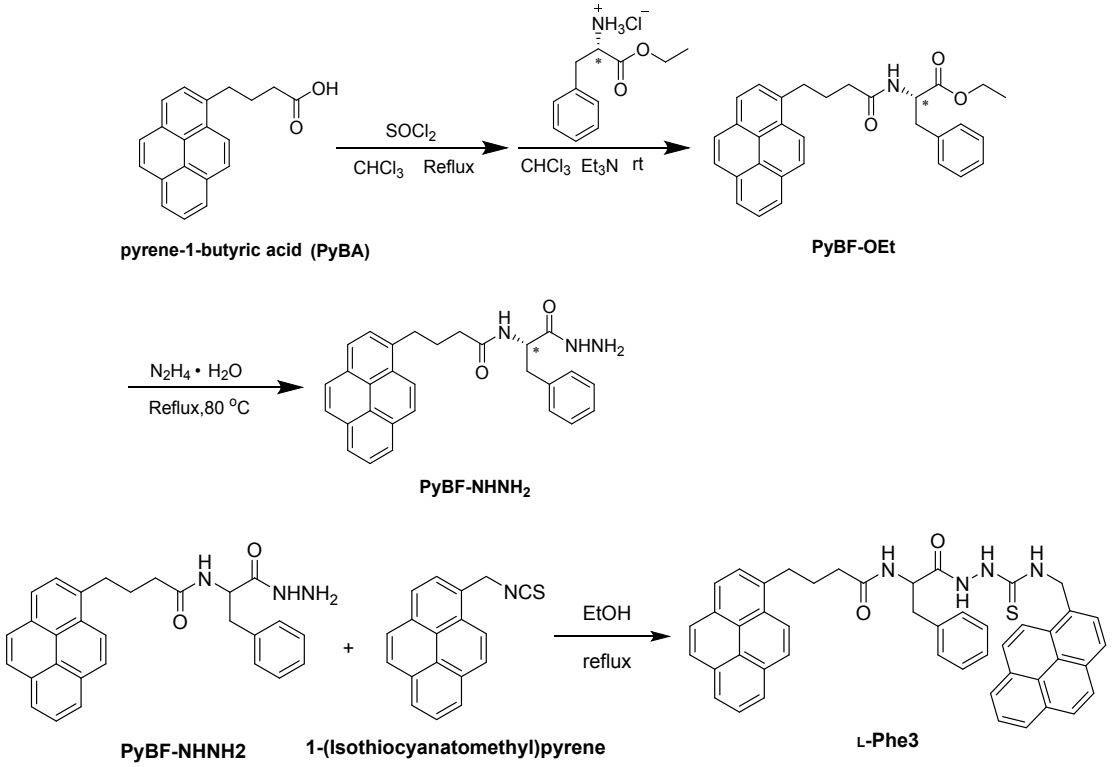
L-**Phe2:** To a solution of **NDMA-Phe-N_2H_4** (0.23 g, 0.68 mmol) in CH_2Cl_2 (10 mL), 0.19 g **1-(isothiocyanatomethyl)pyrene** (0.62 mmol) in CH_2Cl_2 (10 ml) was added. The mixture was stirred at room temperature overnight and filtered the cotton-like solid, which after washing by CH_2Cl_2 gave L-**Phe2** as yellow solid 0.23 g, 60%.

L-**Phe2:**

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.17 – 8.25 (m, 3H), 8.13 (t, J = 7.43 Hz, 2H), 8.01 – 8.10 (m, 3H), 7.96 (d, J = 7.8 Hz, 1H), 7.03 – 7.12 (m, 3H), 7.01 (d, J = 7.8 Hz, 2H), 6.73 (d, J = 8.4 Hz, 2H), 6.52 (d, J = 8.4 Hz, 2H), 6.31 (s, 1H), 5.42 (ddd, J = 20, 14.4, 4.8 Hz, 2H), 3.26 (t, J = 6.51 Hz, 1H), 2.97 – 3.06 (m, 1H), 2.88 (s, 6H), 2.59 – 2.66 (m, 1H), 2.31 – 2.47 (m, 4H), 2.06 (s, 3H).

^{13}C NMR (101 MHz, CD_3CN): δ (ppm) 181.65, 149.41,139.11, 131.28, 131.23, 130.72, 130.21, 129.15, 129.06, 128.97, 128.31, 127.58, 127.35, 127.16, 126.15, 126.10, 125.42, 124.93, 124.78, 124.63, 122.93, 112.82, 56.34, 47.25, 40.78, 37.87, 32.77, 31.05.

HRMS (ESI): calcd for $[C_{38}H_{40}N_5OS]^+$: 614.2954, found: 614.2962.



Scheme S4. General procedures for the syntheses of **L-Phe3**

PyBF-OEt: To a solution of **pyrene-1-butyric acid** (1.45 g, 5 mmol) in $CHCl_3$ (10 ml), $SOCl_2$ was added dropwise (1.19 g, 10 mmol) at 0^0C. Solvent was evaporated in vacuo after refluxing for 3 hours. Dissolve the pyrene-1-butyryl chloride with 10 ml fresh $CHCl_3$ and Et_3N (2 mL, 15 mmol), a solution of **L- and D- Phe-OEt·HCl** (1.15 g, 5 mmol) and Et_3N (2 mL, 15 mmol) in $CHCl_3$ (20 mL) was added. The mixture was left to stand at room temperature for 4 hours, evaporated in vacuo, and the solid

residue was dissolved in AcOEt. The solution was washed successively with 1% $NH_3 \cdot H_2O$, saturated NH_4Cl and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, to obtain 1.8 g white solid product 78.0%.

PyBF-NHNH$_2$: Excess aqueous hydrazine (80%) was added to **PyBF-OEt** in ethanol (15 mL) and refluxed for 24 hours, evaporated in vacuo. The viscous liquid was dissolved in water. The solution was extracted by CH_2Cl_2, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, to obtain 1.45 g white solid, 83%.

L-**Phe3:** To a solution of **PyBF-NHNH$_2$** (0.14 g, 0.31 mmol) in refluxed ethanol (20 mL), **1-(isothiocyanatomethyl)pyrene** (0.07 g, 0.26 mmol) in ethanol (10 mL) was added and cotton-shaped precipitates emerged after refluxing overnight. Filtering and washing by ethanol affords 0.06 g solid in 35% yield.

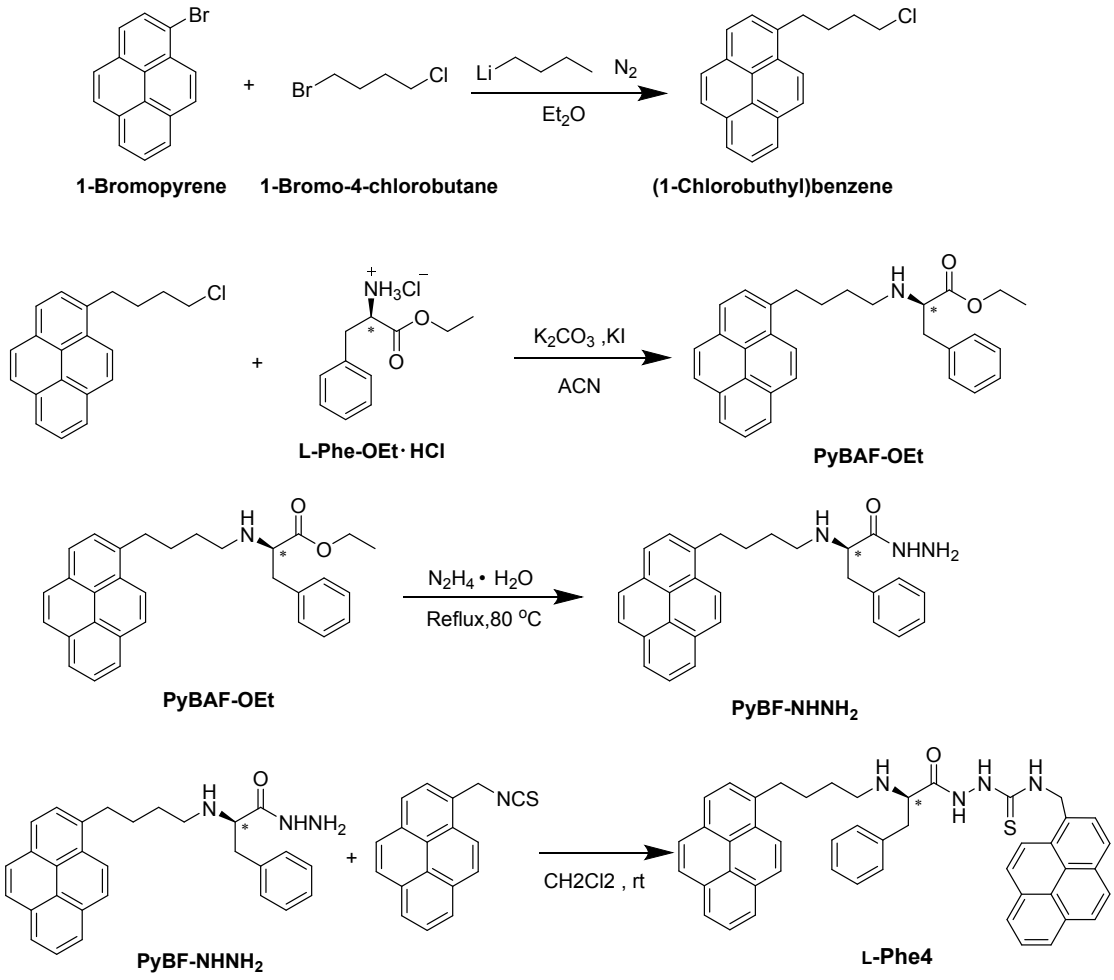
L-**Phe3:**

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 10.27 (s, 1H), 9.61 (s, 1H), 8.38 (d, J = 9.3 Hz, 1H), 8.31 (dd, J = 9.9, 5.6 Hz, 2H), 8.25 (dd, J = 15.7, 7.7 Hz, 3H), 8.18 – 7.95 (m, 13H), 7.61 (s, 1H), 7.29 – 7.18 (m, 4H), 7.13 (t, J = 6.9 Hz, 1H), 5.54 – 5.38 (m, 2H), 4.40 (s, 1H), 3.05 (dd, J = 10.1, 4.3 Hz, 1H), 3.01 – 2.93 (m, 2H), 2.85 (dd, J = 13.9, 9.2 Hz, 1H), 2.05 (d, J = 7.3 Hz, 2H), 1.65 (s, 2H).

^{13}C NMR (101 MHz, CD_3CN): δ (ppm) 182.15, 177.96, 173.49, 171.10, 136.96, 135.99, 131.77, 130.74, 130.67, 130.24, 130.14, 129.91, 129.04, 128.83, 127.89, 127.82, 127.76, 127.02, 126.97, 126.91, 126.87, 126.60, 126.49, 126.13, 126.01, 125.70, 125.66, 125.59, 124.73, 124.66, 124.39, 124.29, 124.25, 124.17, 124.01, 123.98, 123.83, 123.81, 123.00, 122.63, 53.87, 45.27, 35.96, 34.07, 31.61, 26.48.

HRMS (ESI): calcd for $[C_{47}H_{38}N_4O_2S]^-$: 721.2643, found: 721.2632.

Scheme S5. General procedures for the syntheses of L-**Phe4**



(1-Chlorobuthyl)pyrene: To a stirred solution of **1-bromopyrene** (1.48 g, 5 mmol) in anhydrous diethyl ether (10 mL) under an Ar atmosphere, *n*-butyllithium(1.5 eq., 2.5 M in *n*-hexane) was slowly added at 0°C followed by the addition of **1-bromo-4-chlorobutane** (4.0 eq.), which was then refluxed for 2 hours. The reaction mixture was cooled to room temperature, partitioned between diethyl ether (40 mL) and water (30 mL). The aqueous phase was extracted with diethyl ether, the combined organic phase was dried over $MgSO_4$, evaporated in vacuo and purified by flash chromatography on silica gel using *n*-hexane as a mobile phase, affording a yellow powder 0.88 g, 60%.

PyBAF-OEt: To a solution of **L-Phe-OEt·HCl** (0.7 g, 3 mmol) in ACN (15 mL), 0.8 g K_2CO_3 and KI (catal.) was added and stirred for 15 minutes, to which **(1-chlorobuthyl)pyrene** (0.8 g, 2.7 mmol) in ACN (50 mL) was added and refluxed overnight. The solid residue obtained after solvent evaporation was dissolved in water and extracted by CH_2Cl_2. Thus obtained residue was purified by flash chromatography (SiO_2; CH_2Cl_2) to give viscous liquid 0.73 g, 59%.

PyBAF-NHNH$_2$: Excess aqueous hydrazine (80%) was added to **PyBAF-OEt** in ethanol (20 mL) and then refluxed for 24 hours, evaporated in vacuo, and the viscous liquid was dissolved in water. The solution was extracted by CH_2Cl_2, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, to give a viscous liquid 0.42 g, 60%.

L-Phe4: To a solution of **PyBAF-NHNH$_2$** (0.16 g, 0.36 mmol) in CH_2Cl_2 (10 mL), CH_2Cl_2 (10 mL) with 0.04 g **1-(isothiocyanatomethyl)pyrene** (0.15 mmol) was added. The mixture was stirred at room temperature overnight and filtered the cotton-like solid, which after washing by CH_2Cl_2 gave **L-Phe4** as yellow solid 0.03 g, 28%.

L-Phe4:

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 9.57 (s, 1H), 8.39 (d, J = 9.2 Hz, 1H), 8.32 – 8.17 (m, 7H), 8.17 – 8.01 (m, 8H),7.97 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.03 – 7.26(m, 5H), 5.44 (ddd, J = 21.0, 16.1, 5.7, Hz, 2H), 3.17 (s, 2H), 2.91 (dd, J = 13.3, 6.0 Hz, 1H), 2.72 (dd, J = 13.3, 6.0 Hz, 1H), 2.37 (s, 1H), 1.61 (s, 2H), 1.39 (s, 2H).

^{13}C NMR (101 MHz, CD_3CN): δ (ppm) 182.55, 137.90, 136.89, 131.52, 131.00, 130.85, 130.51, 130.34, 130.33, 129.23, 128.87, 128.05, 128.02, 127.87, 127.41, 127.14, 127.06, 127.00, 126.78, 126.71,126.09, 125.97, 125.93, 125.89, 125.70, 124.95, 124.87, 124.50, 124.36, 124.30, 124.26, 124.12, 123.98, 123.25, 122.65, 62.09, 47.13, 45.52, 38.34, 32.21, 29.17, 28.69.

HRMS (ESI): calcd for $[C_{47}H_{40}N_4OS]^+$: 709.3001, found: 709.3006.

2. Evidence of β-turn: DFT calculations and NMR temperature coefficients

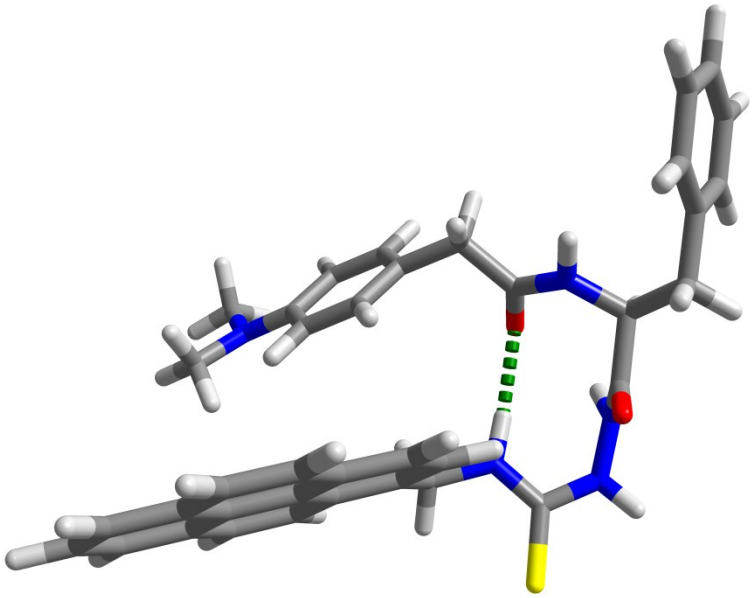


Fig. S1 DFT-optimized structure of L-**Phe1** at B3LYP/6-31G* level. Green dashed line highlights the intramolecular hydrogen bonding that is indicative of the β-turn structure.

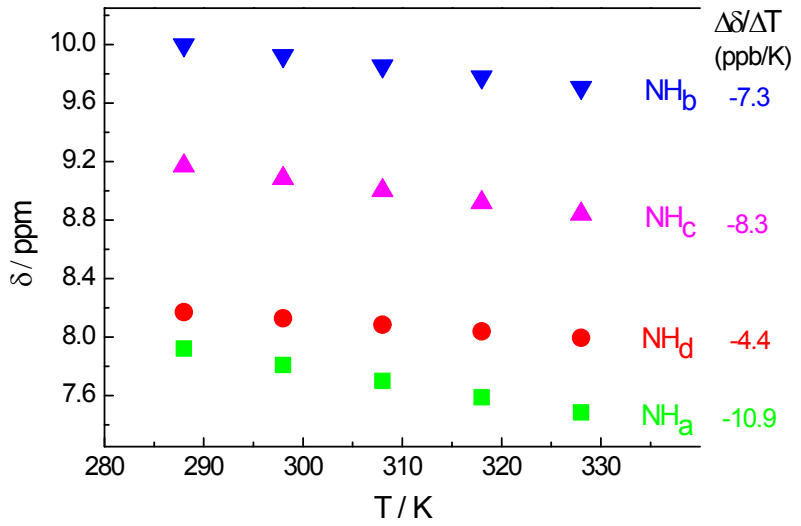


Fig. S2 Temperature dependence of the -NH proton resonances of L-**Phe1** in CD_3CN/DMSO-d_6 (v/v = 8/2) and the values of the fitted temperature coefficients. [L-**Phe1**] = 4 mM.

3. Spectral data of D-Phe1

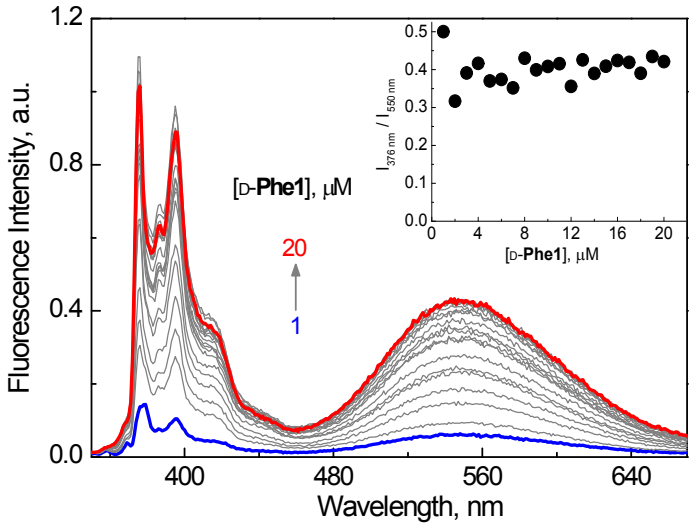


Fig. S3 Fluorescence spectra of **D-Phe1** of varying concentration in acetonitrile. Inset shows the ratio of the excimer to monomer intensity as a function of concentration of **D-Phe1**. λ_{ex} = 340 nm.

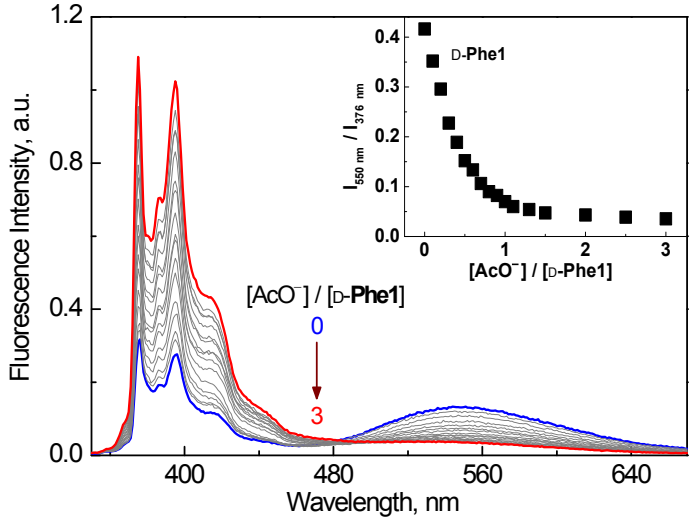


Fig. S4 Fluorescence spectra of **D-Phe1** in acetonitrile in the presence of acetate anion of 0 to 30 μM. Inset shows the ratio of the excimer (550 nm) to monomer (376 nm) intensity of **D-Phe1** versus concentration of acetate. [**D-Phe1**] = 10 μM. λ_{ex} = 340 nm.

4. ^{1}H NMR titrations

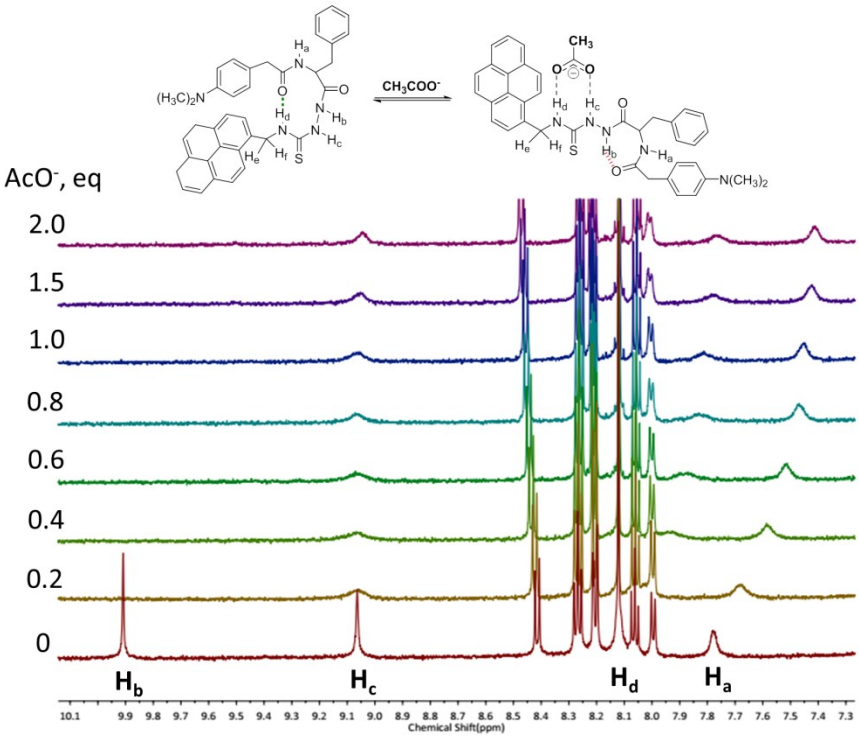


Fig. S5 ^{1}H NMR spectra of the portion of -NH protons in L-**Phe1** in CD_3CN/DMSO-d_6 (8:2, v/v) in the presence of acetate anion of increasing concentration from 0 to 2.0 equivalents. [L-**Phe1**] = 1 mM.

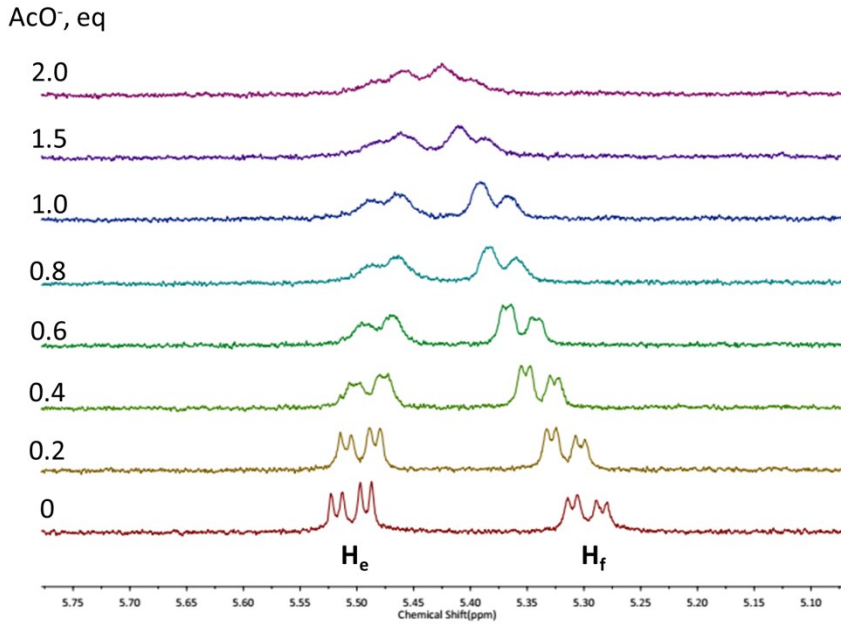


Fig. S6 ^{1}H NMR spectra of the portion of methylene -CH_eH_f in L-**Phe1** in CD_3CN/DMSO-d_6 (8:2, v/v) in the presence of acetate anion of increasing concentration from 0 to 2.0 equivalents. [L-**Phe1**] = 1 mM.

5. Fluorescence spectra of azapeptides able to form exciplex

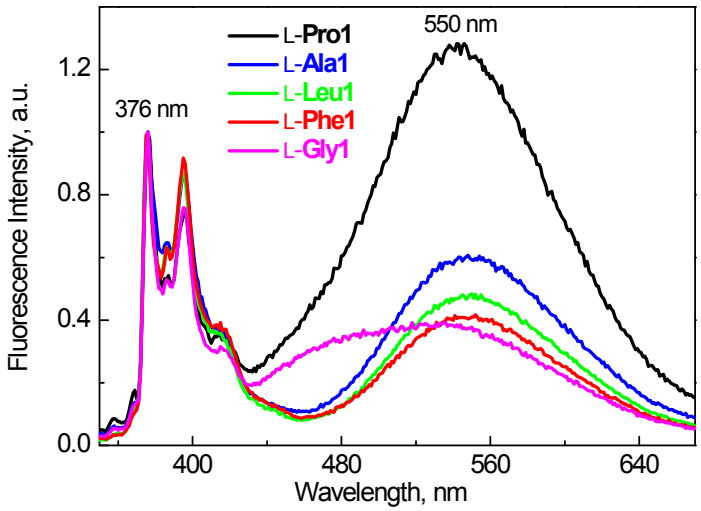


Fig. S7 Fluorescence spectra of L-**Pro1**, L-**Ala1**, L-**Leu1**, L-**Phe1** and L-**Gly1** in acetonitrile. [L-**Pro1**] = [L-**Ala1**] = [L-**Leu1**] = [L-**Phe1**] = [L-**Gly1**] =10 μM. λ_{ex} = 340 nm.

6. Crystallographic data

Table S1 Crystallographic data for **Pro1**

Compound reference	**Pro1**
Empirical formula	$C_{35}H_{36}N_6O_2S$
Formula weight	604.76
Crystal system	Monoclinic
a, Å	14.700(2)
b, Å	8.4865(12)
c, Å	26.024(4)
α, deg	90
β, deg	98.902(13)
γ, deg	90
V, Å^3	3207.3(8)
Temperature, K	119
Space group	$P2_1/n$
No. of formula units per unit cell, Z	4
Radiation type	CuKα
Absorption coefficient, mm^{-1}	1.220
Reflections collected / unique	18669 / 4909 [R(int) = 0.1085]
Final R indices[I>2sigma(I)]	R1 = 0.1428, wR2 = 0.3548
R indices (all data)	R1 = 0.1668, wR2 = 0.3757
Goodness of fit on F^2	1.046
CCDC number	1583614

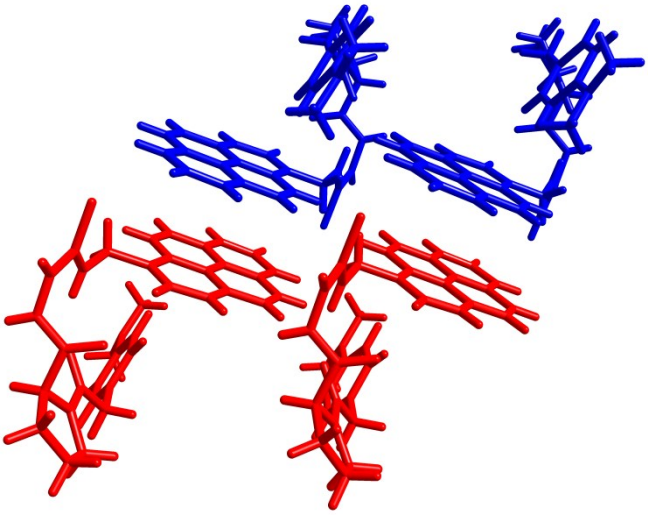


Fig. S8 X-ray crystal structures of **Pro1**. The crystals obtained from the racemic mixtures of L-**Pro1** and D-**Pro1**. One unit cell contains two L-**Pro1** molecules as depicted in blue and two D-**Pro1** molecules in red.

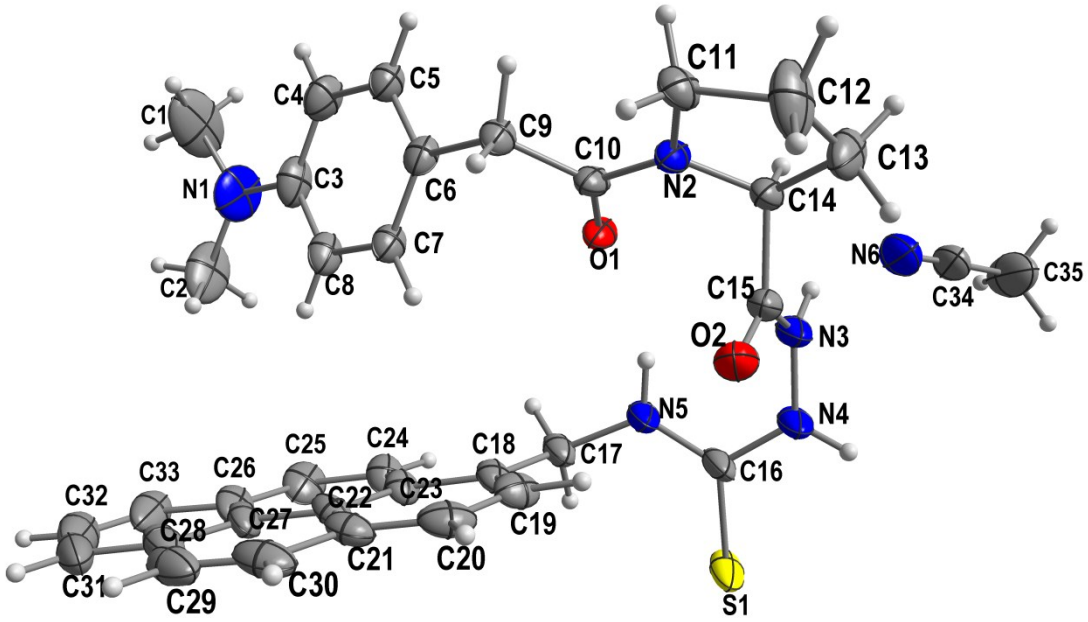


Fig. S9 A labelled ORTEP plot of **Pro1** with ellipsoids shown at the 20% probability level.

Table S2 Hydrogen bonding details for **Pro1**

D-H···A	d(D-H)	d(H···A)	d(D···A)	<DHA	symop for A
N3-H3···N6	0.880	2.123	2.901	147.15	[x+1, y+1, z]
N4-H4···S1	0.880	2.487	3.295	152.86	[-x+2, -y+1, -z+1]
N5-H5···O1	0.880	2.115	2.948	157.48	

7. Fluorescence spectral titrations by anions

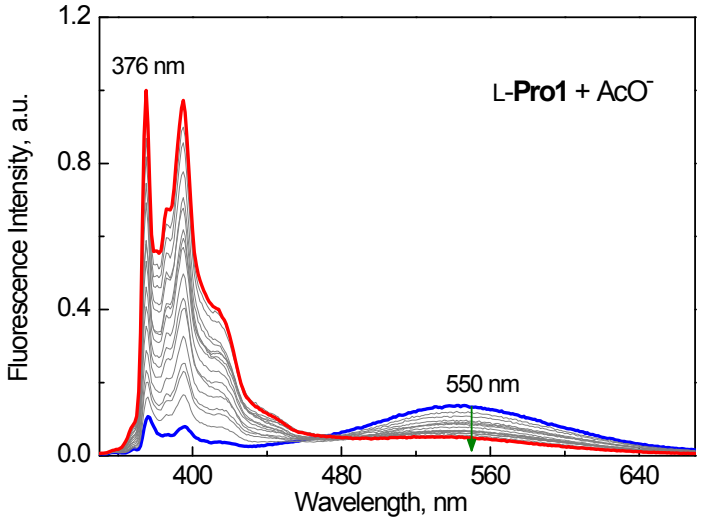


Fig. S10 Fluorescence spectra of L-**Pro1** in the presence of AcO^- in acetonitrile. [L-**Pro1**] = 10 μM. λ_{ex} = 340 nm.

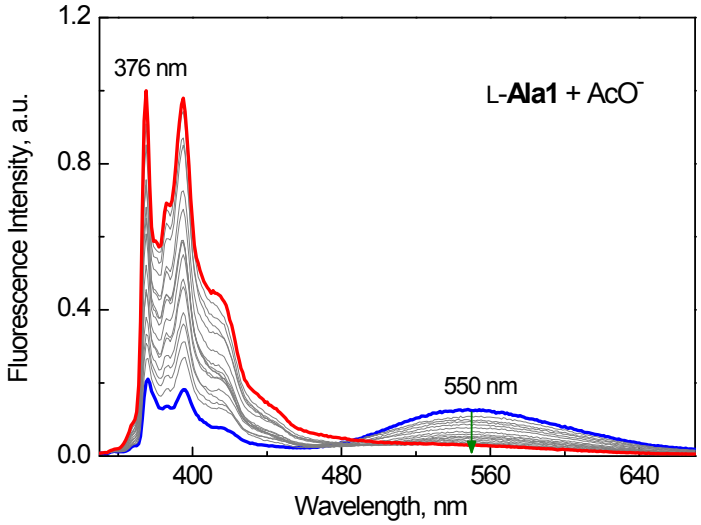


Fig. S11 Fluorescence spectra of L-**Ala1** in the presence of AcO^- in acetonitrile. [L-**Ala1**] = 10 μM. λ_{ex} = 340 nm.

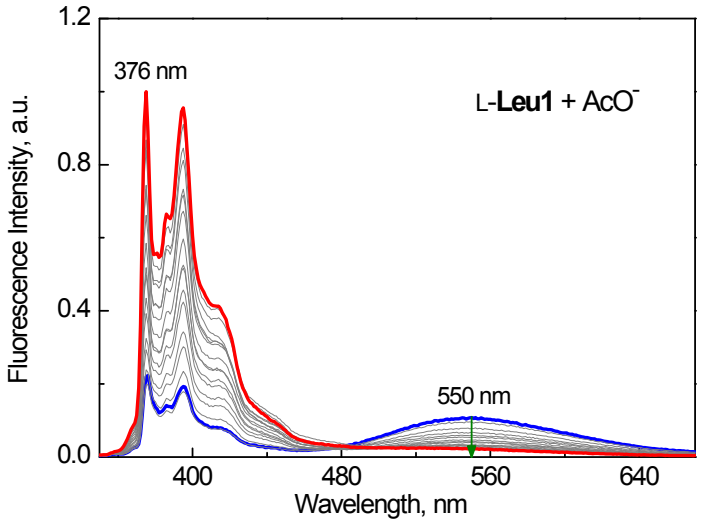


Fig. S12 Fluorescence spectra of L-**Leu1** in the presence of AcO^- in acetonitrile. [L-**Leu1**] = 10 μM. λ_{ex} = 340 nm.

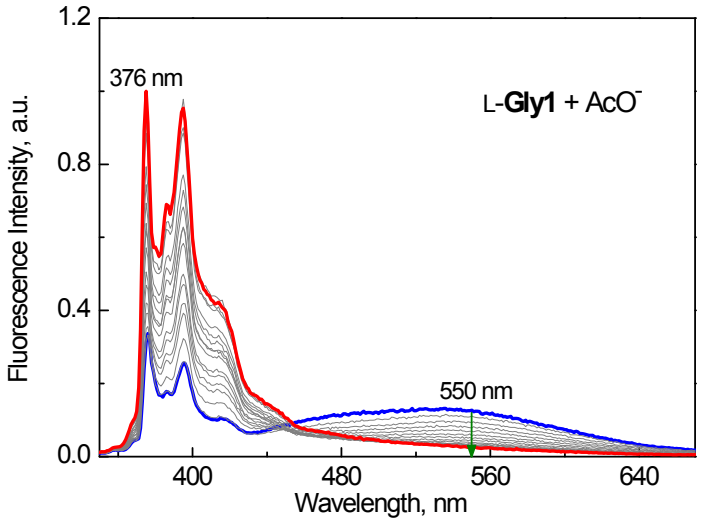


Fig. S13 Fluorescence spectra of L-**Gly1** in the presence of AcO^- in acetonitrile. [L-**Gly1**] = 10 μM. λ_{ex} = 340 nm.

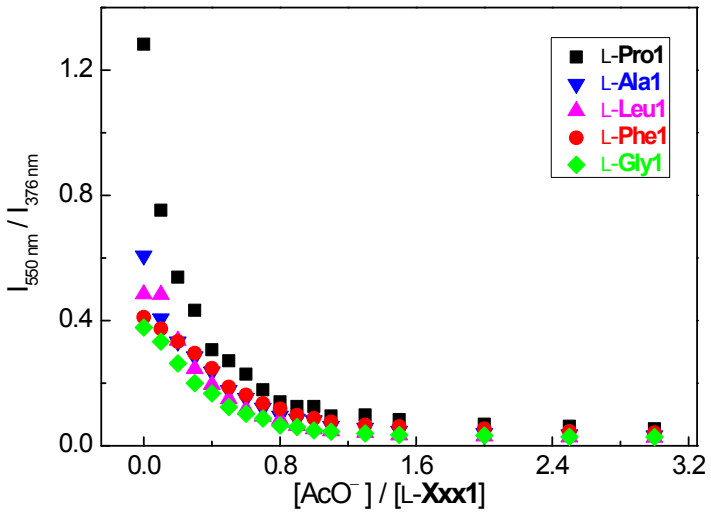


Fig. S14 Plots of $I_{550\ nm}/I_{376\ nm}$ of L-**Pro1**, L-**Ala1**, L-**Leu1**, L-**Phe1** and L-**Gly1** against the concentration of AcO^-. [L-**Pro1**] = [L-**Ala1**] = [L-**Leu1**] = [L-**Phe1**] = [L-**Gly1**] = 10 μM.

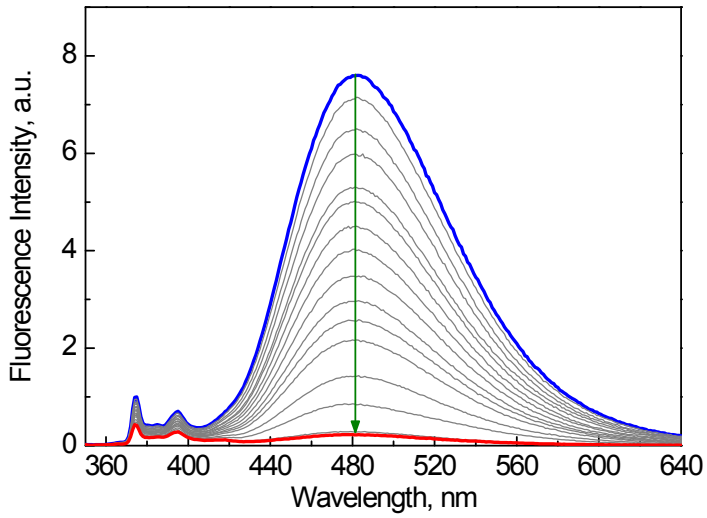


Fig. S15 Fluorescence spectra of L-**Phe3** in acetonitrile in the presence of F^-. [L-**Phe3**] = 10 μM. λ_{ex} = 340 nm.

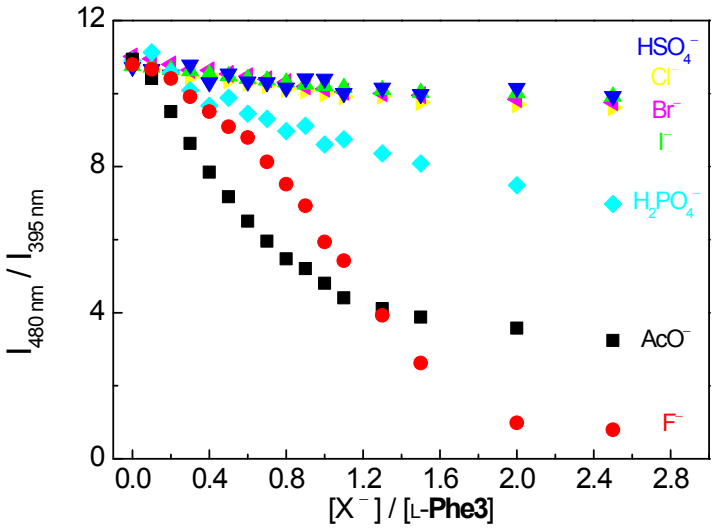


Fig. S16 Plots of $I_{480\ nm}/I_{395\ nm}$ of L-**Phe3** in acetonitrile against the concentration of anions. [L-**Phe3**] = 10 μM.

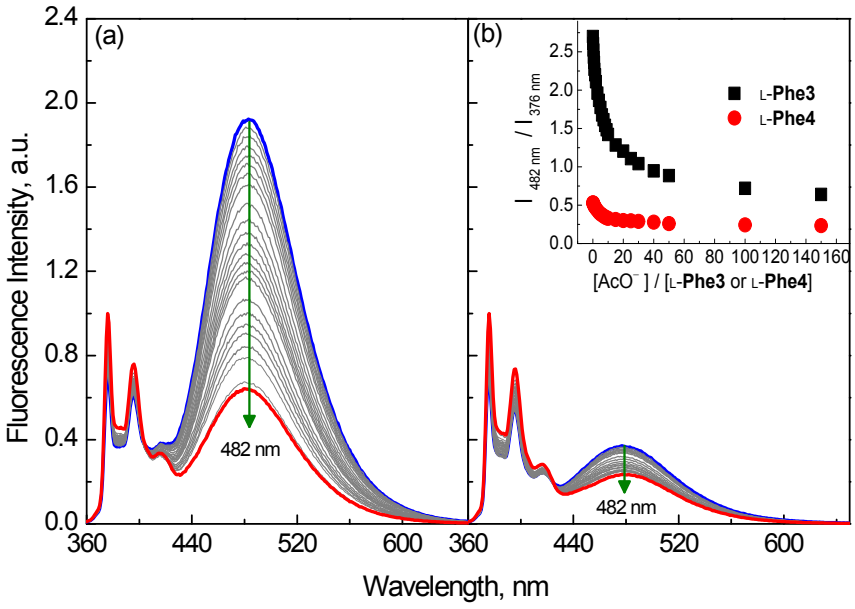
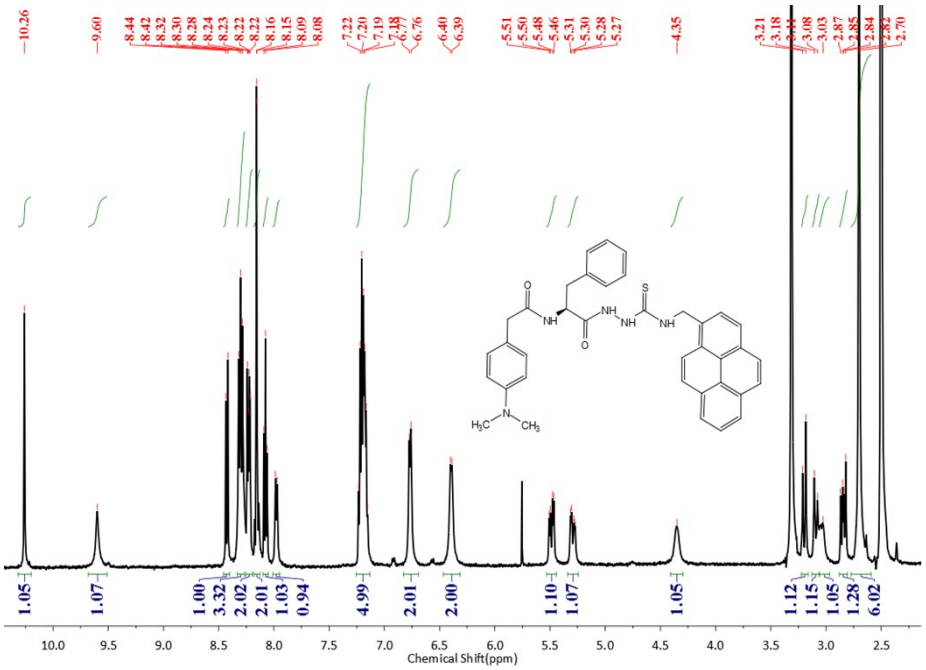
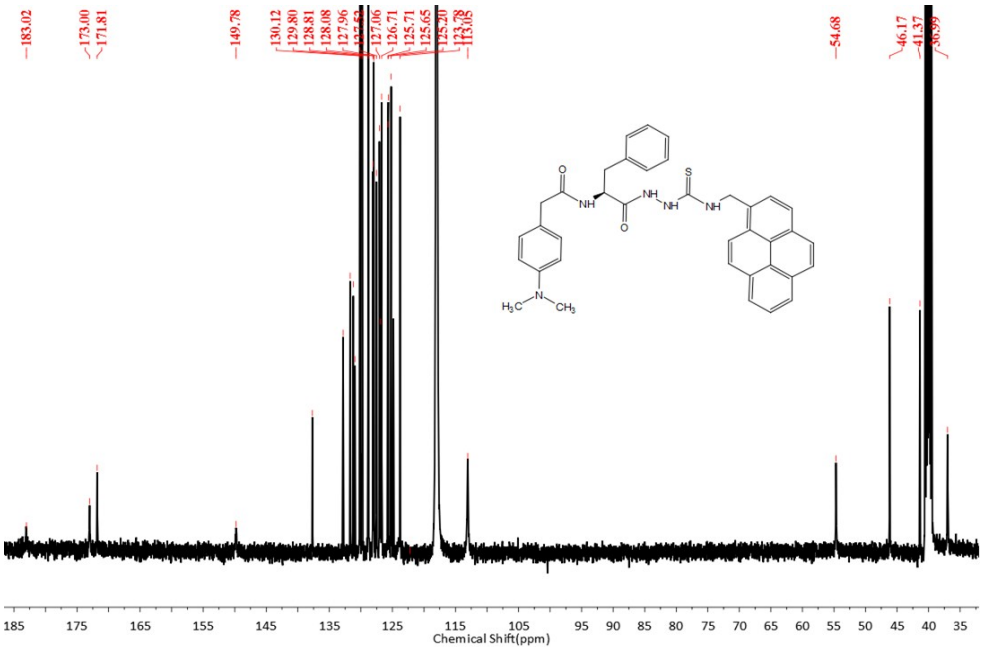


Fig. S17 Fluorescence spectra of L-**Phe3** (a) and L-**Phe4** (b) in 1 mM aqueous CTAB solutions in the presence of sodium acetate of 0 to 150 eq. Inset in (b) shows the ratio of the excimer (482 nm) to monomer (376 nm) intensity of L-**Phe3** and L-**Phe4** versus concentration of sodium acetate. λex = 340 nm, [L-**Phe3**] = [L-**Phe4**] = 10 μM.

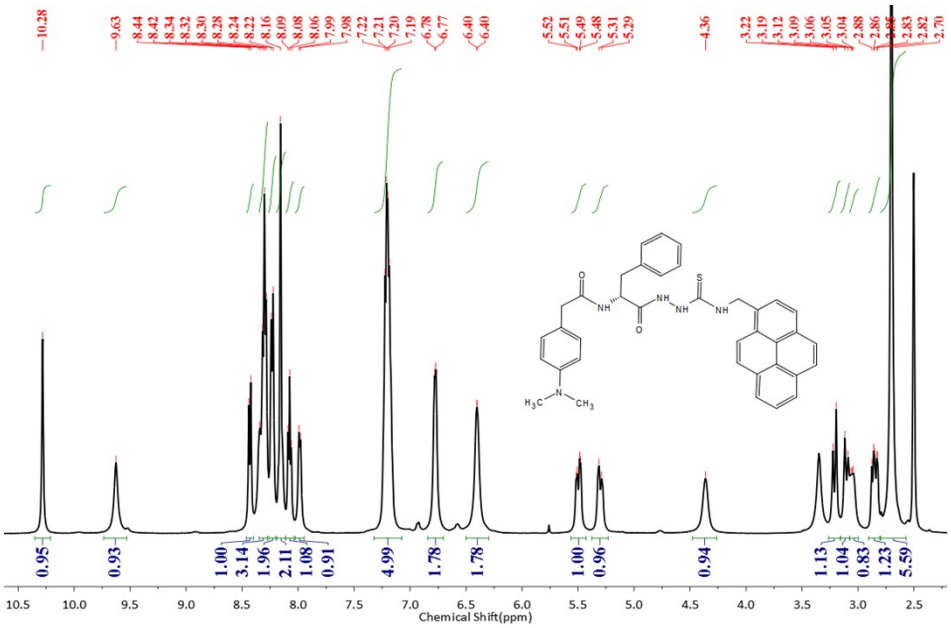
8. ^{1}H and ^{13}C NMR spectra

L-Phe1





D-**Phe1**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8.42
8.34
8.32
8.30
8.28
8.24
8.22
8.16
8.09
8.08
8.06
7.99
7.98
7.22
7.21
7.20
7.19
6.78
6.77
6.40
6.40
5.52
5.51
5.49
5.48
5.31
5.29
4.36
3.22
3.19
3.12
3.09
3.06
3.05
3.04
2.88
2.86
2.83
2.82
2.70
0.95
0.93
1.00
3.14
1.96
2.11
1.08
0.91
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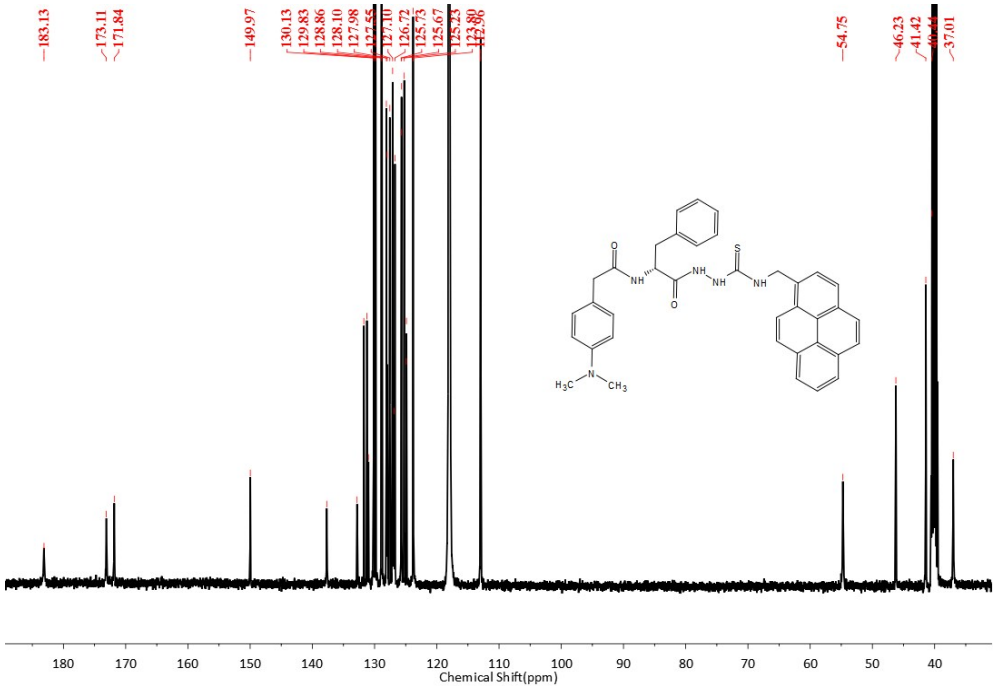
1.78
1.00
0.96
0.94
1.13
1.04
0.83
1.23
5.59
Chemical Shift(ppm)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171.84
149.97
130.13
129.83
128.86
128.10
127.98
127.55
127.10
126.72
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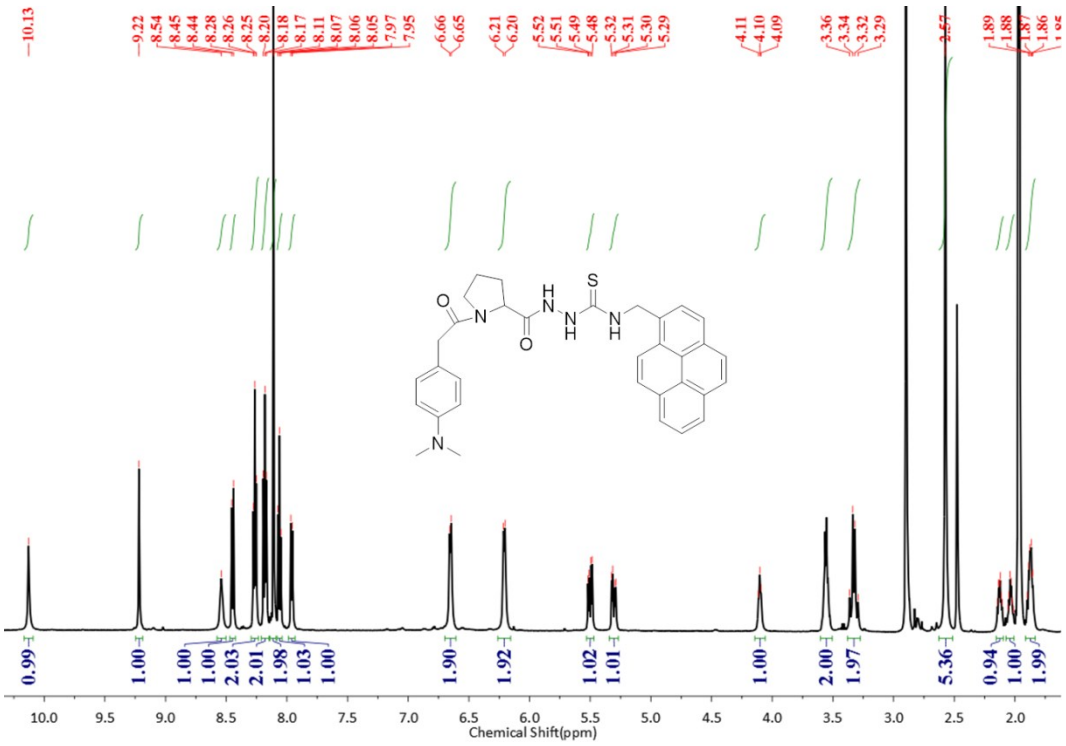
125.67
125.23
123.80
123.96
54.75
46.23
41.42
37.01
Chemical Shift(ppm)

L-**Pro1**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8.26
8.25
8.20
8.18
8.17
8.11
8.07
8.06
8.05
7.97
7.95
6.66
6.65
6.21
6.20
5.52
5.51
5.49
5.48
5.32
5.31
5.30
5.29
4.11
4.10
4.09
3.36
3.34
3.32
3.29
2.57
1.89
1.88
1.87
1.86
0.99
1.00
1.00
1.00
2.03
2.01
1.98
1.03
1.00
1.90
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1.01
1.00
2.00
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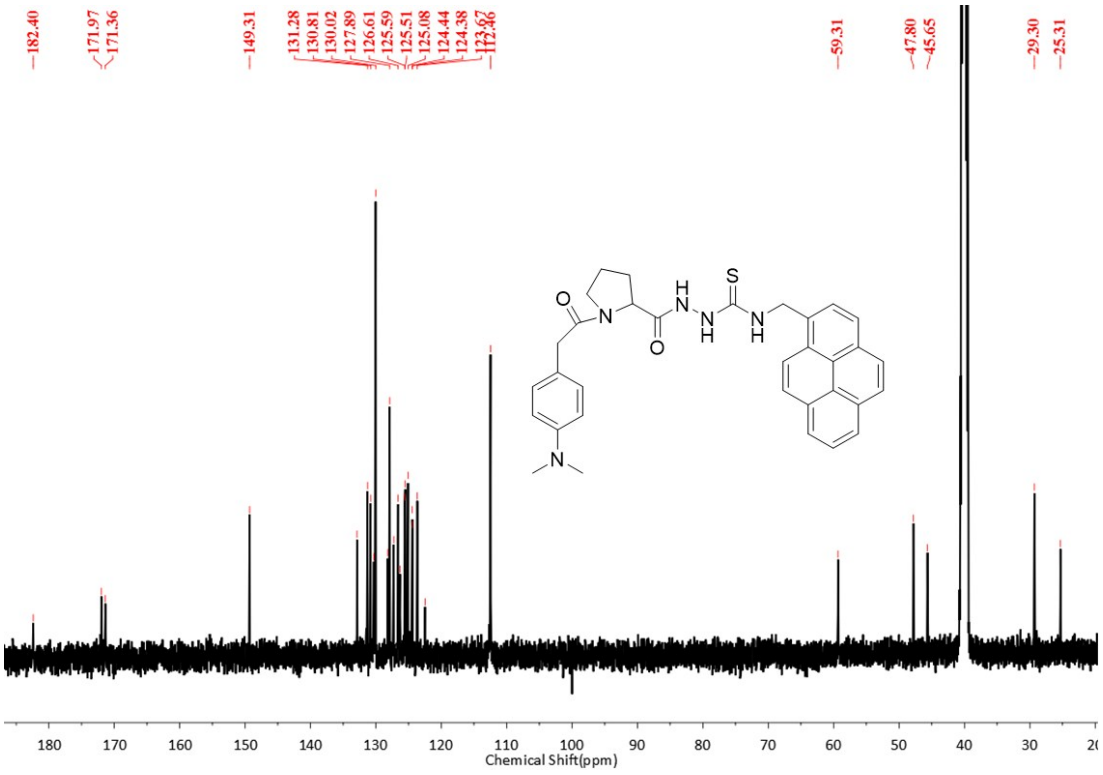
0.94
1.00
1.99
Chemical Shift(ppm)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171.97
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130.02
127.89
126.61
125.59
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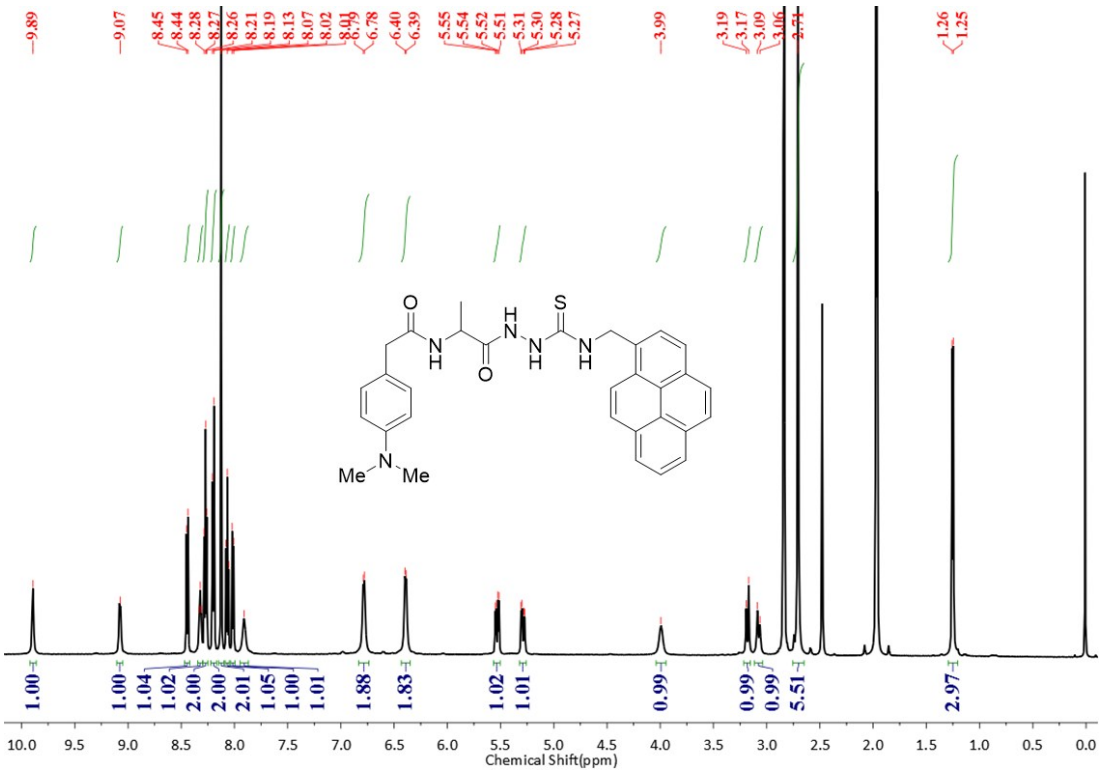
124.38
59.31
47.80
45.65
29.30
25.31
Chemical Shift(ppm)

L-**Ala1**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8.21
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1.02
2.00
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2.01
1.05
1.00
1.01
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1.01
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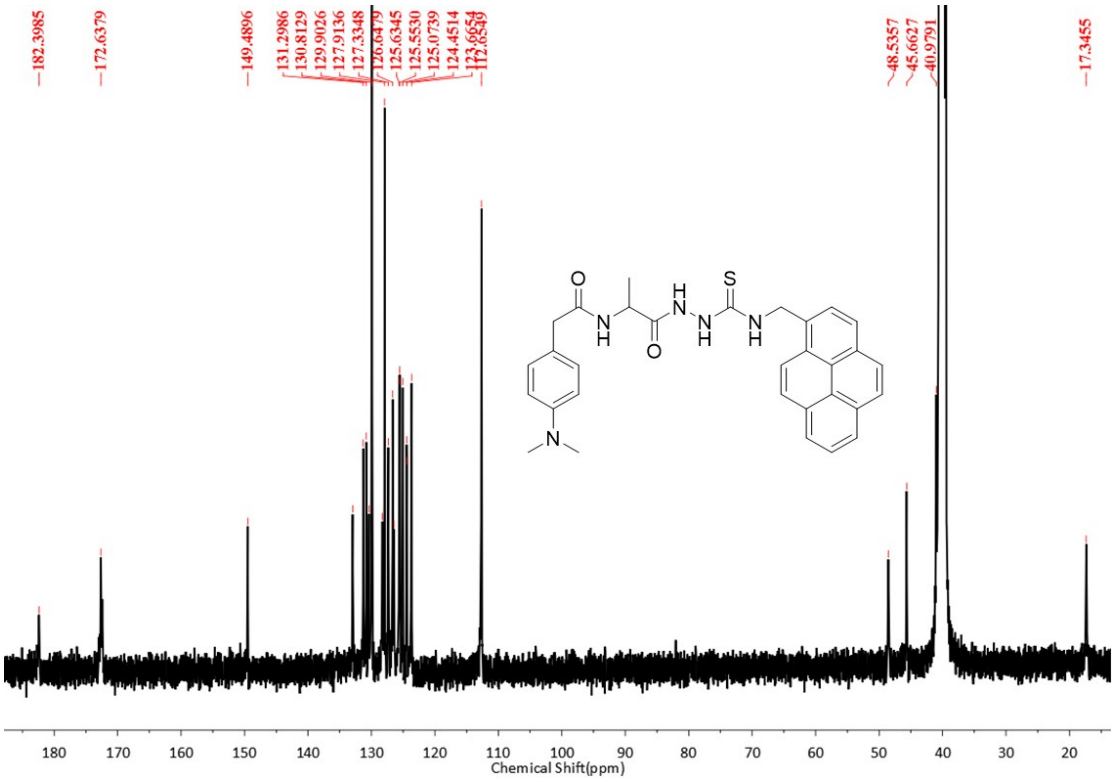
Me
N
Me
Chemical Shift(ppm)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125.0739
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48.5357
45.6627
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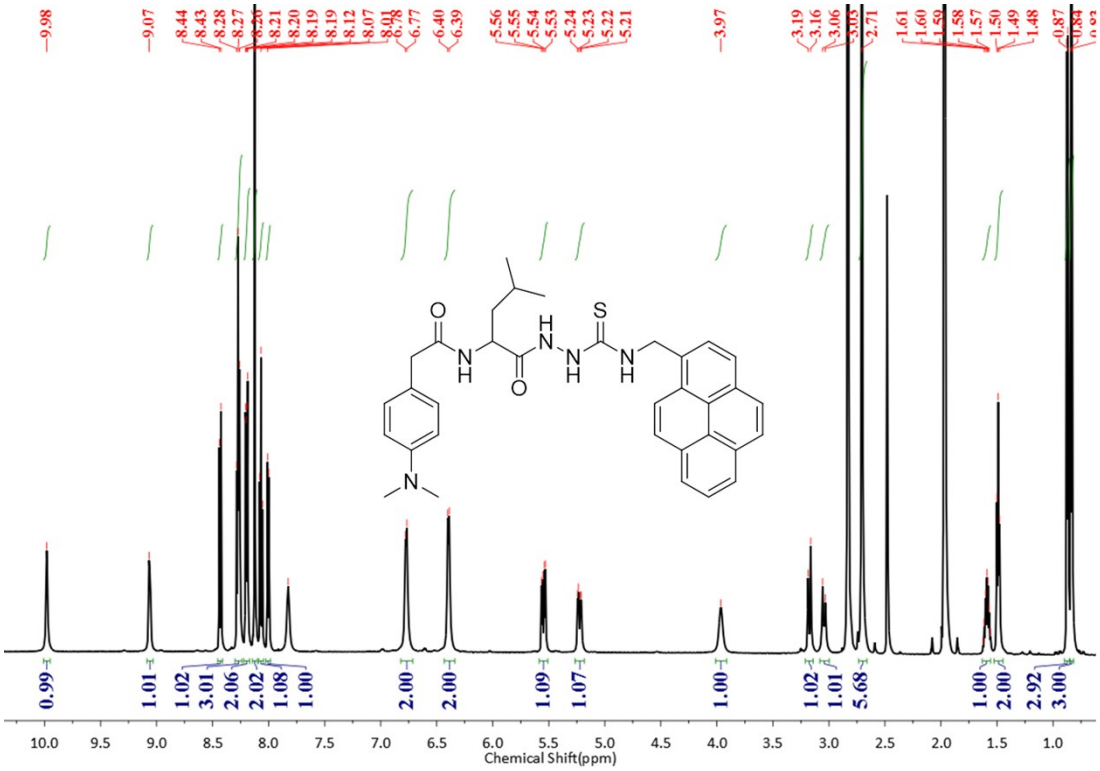
17.3455
N
Chemical Shift(ppm)

L-**Leu1**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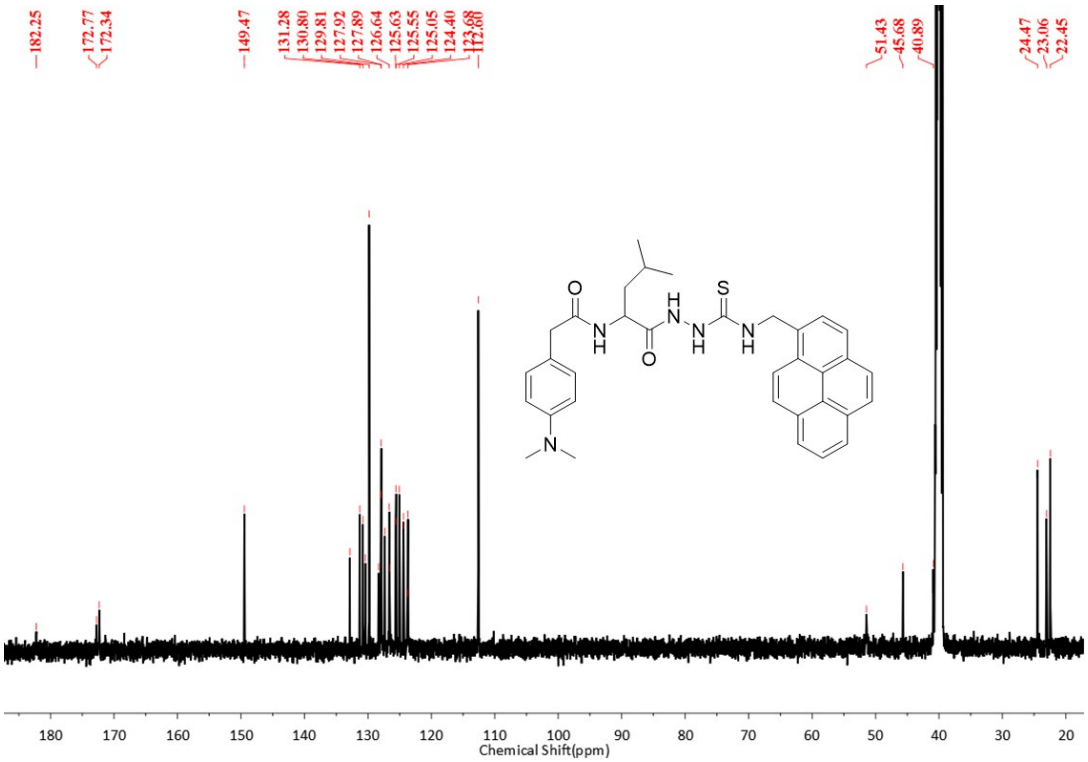
9.07
8.44
8.43
8.28
8.27
8.26
8.21
8.20
8.19
8.19
8.12
8.07
8.01
6.78
6.77
6.40
6.39
5.56
5.55
5.54
5.53
5.24
5.23
5.22
5.21
3.97
3.19
3.16
3.06
3.03
2.71
1.61
1.60
1.59
1.58
1.57
1.50
1.49
1.48
0.87
0.84
0.99
1.01
1.02
3.01
2.06
2.02
1.08
1.00
2.00
2.00
1.09
1.07
1.00
1.02
1.01
5.68
1.00
2.00
2.92
3.00
Chemical Shift(ppm)


182.25
172.77
172.34
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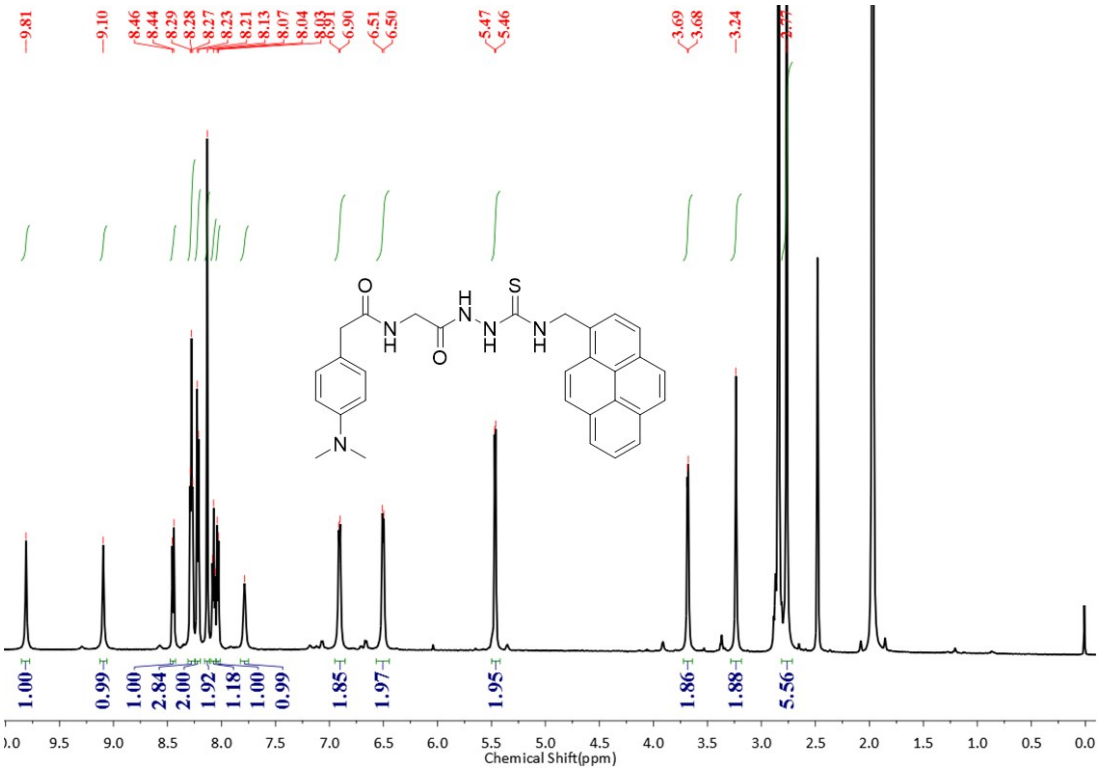
130.80
129.81
127.92
127.89
126.64
125.63
125.55
125.05
124.40
123.68
123.60
51.43
45.68
40.89
24.47
23.06
22.45
Chemical Shift(ppm)

L-**Gly1**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8.23
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8.07
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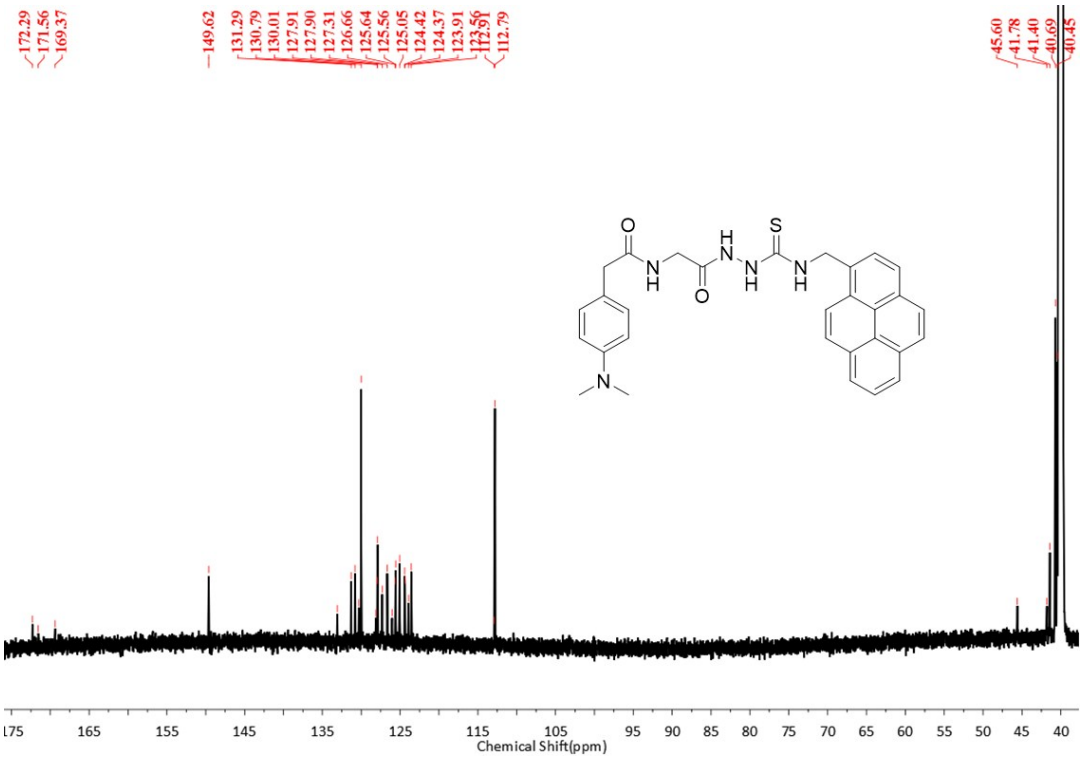
8.03
6.91
6.90
6.51
6.50
5.47
5.46
3.69
3.68
3.24
1.00
0.99
1.00
2.84
2.00
1.92
1.18
1.00
0.99
1.85
1.97
1.95
1.86
1.88
5.56
Chemical Shift(ppm)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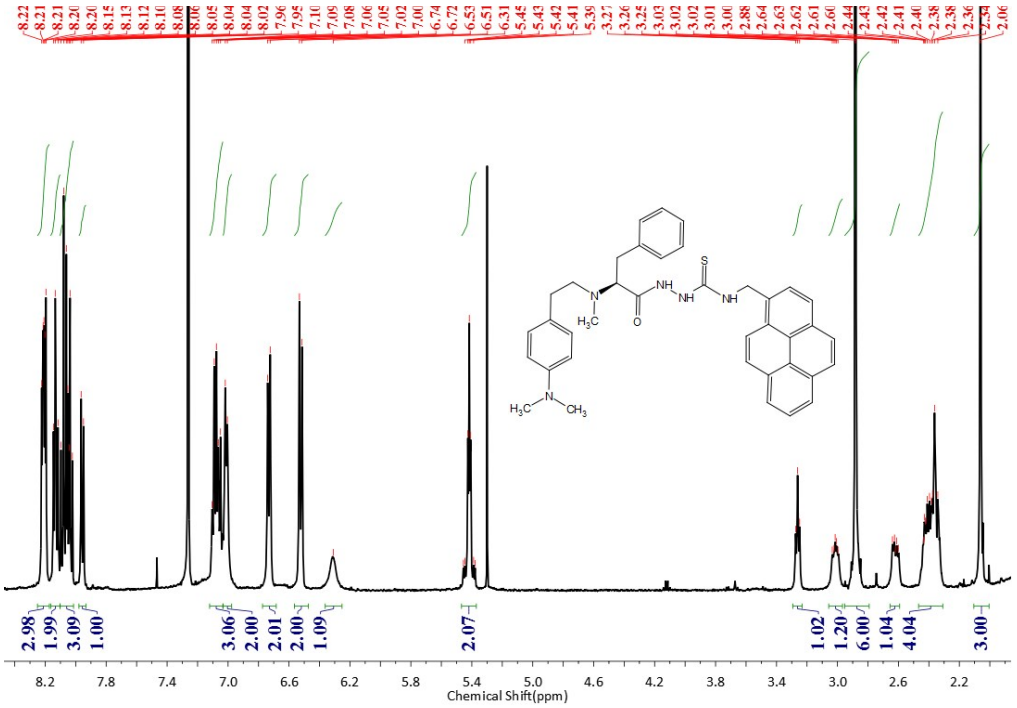
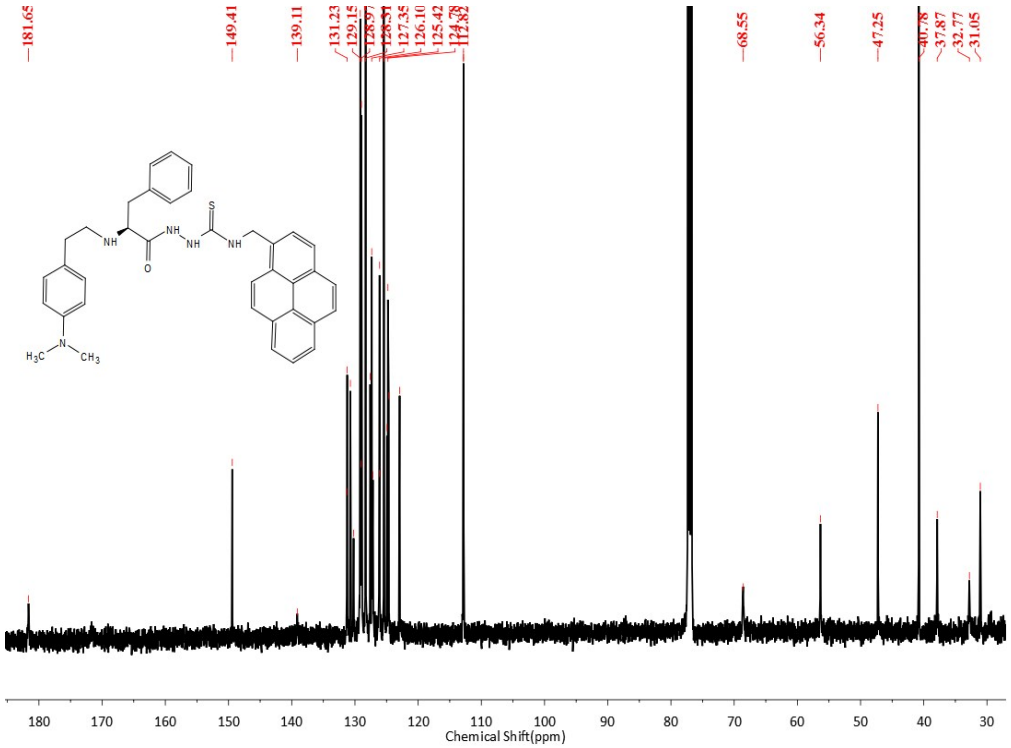
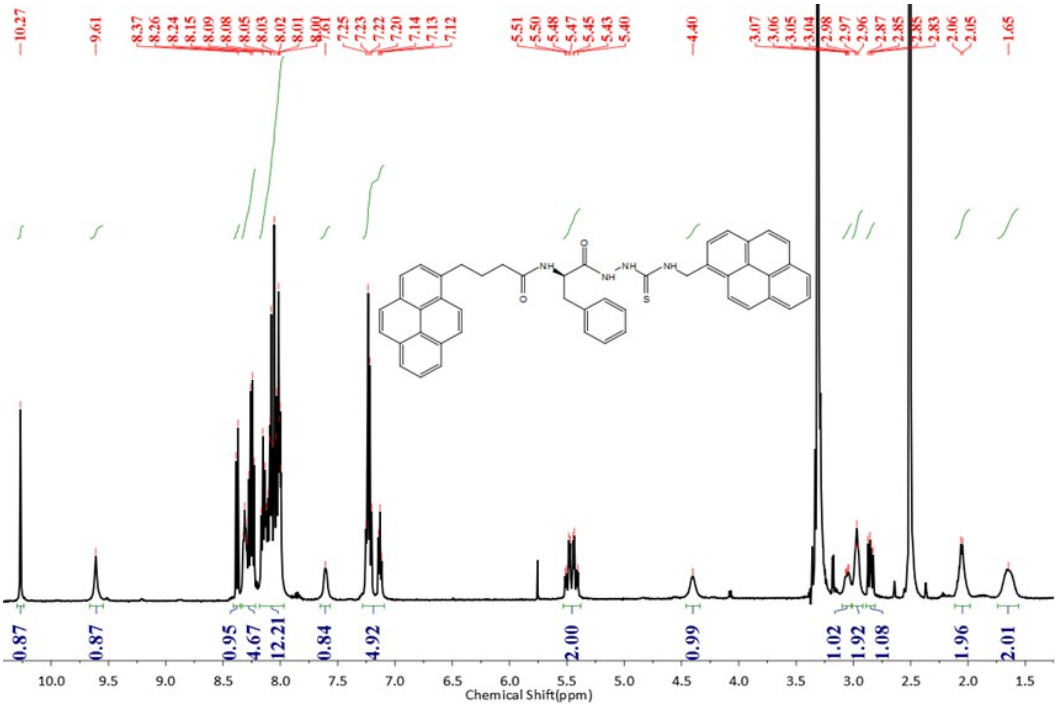
125.05
124.42
124.37
123.91
123.56
112.91
112.79
45.60
41.78
41.40
40.69
40.45
Chemical Shift(ppm)

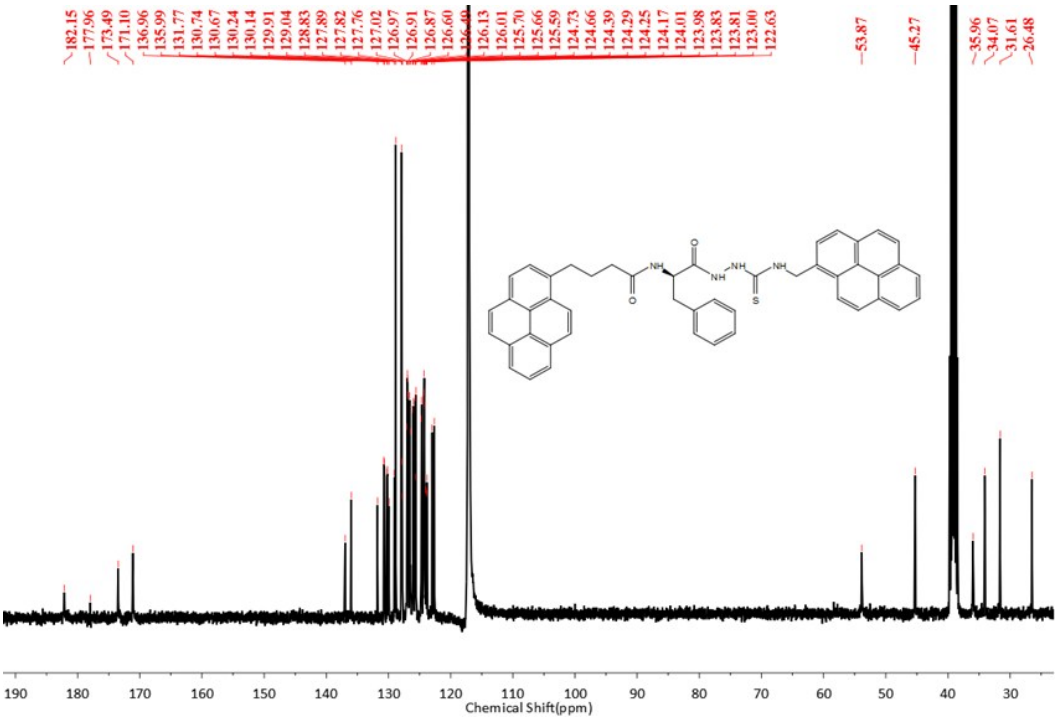
L-**Phe2**


2.98
1.99
3.09
1.00
3.06
2.00
2.01
2.00
1.09
2.07
1.02
1.20
6.00
1.04
4.04
3.00
Chemical Shift(ppm)

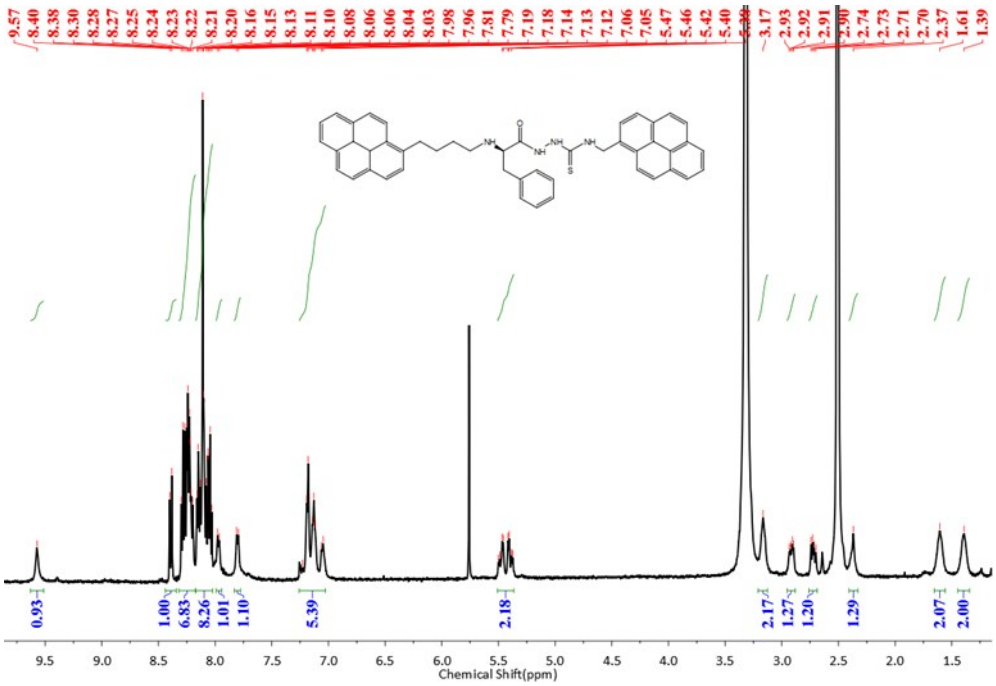

181.65
149.41
139.11
131.23
129.15
128.97
128.51
127.35
126.10
125.42
68.55
56.34
47.25
37.87
32.77
31.05
Chemical Shift(ppm)

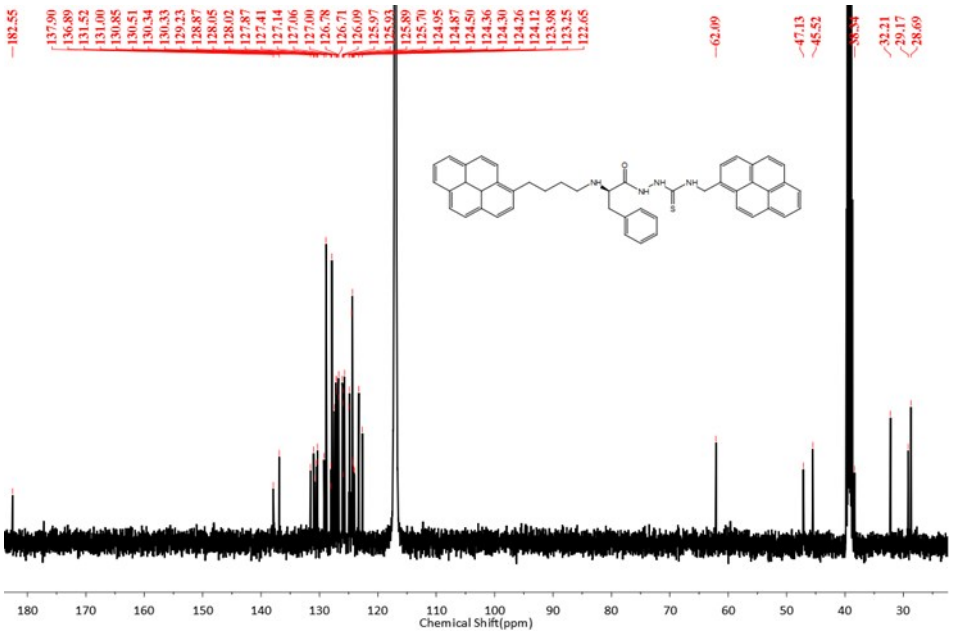
L-**Phe3**


10.27
9.61
8.37
8.26
8.24
8.15
8.09
8.08
8.05
8.03
8.02
8.01
8.00
7.81
7.25
7.23
7.22
7.20
7.14
7.13
7.12
5.51
5.50
5.48
5.47
5.45
5.43
5.40
4.40
3.07
3.06
3.05
3.04
2.98
2.97
2.96
2.87
2.85
2.83
2.06
2.05
1.65
0.87
0.87
0.95
4.67
12.21
0.84
4.92
2.00
0.99
1.02
1.92
1.08
1.96
2.01
Chemical Shift(ppm)


182.15
177.96
173.49
171.10
136.96
135.99
131.77
130.74
130.67
130.24
130.14
129.91
129.04
128.83
127.89
127.82
127.76
127.02
126.97
126.91
126.87
126.60
126.46
126.13
126.01
125.70
125.66
125.59
124.73
124.66
124.39
124.29
124.25
124.17
124.01
123.98
123.83
123.81
123.00
122.63
53.87
45.27
35.96
34.07
31.61
26.48
Chemical Shift(ppm)

L-**Phe4**


9.57
8.40
8.38
8.30
8.28
8.27
8.25
8.24
8.23
8.22
8.21
8.20
8.16
8.15
8.13
8.11
8.10
8.08
8.06
8.06
8.04
8.03
7.98
7.96
7.81
7.79
7.19
7.18
7.14
7.13
7.12
7.06
7.05
5.47
5.46
5.42
5.40
3.17
2.93
2.92
2.91
2.90
2.74
2.73
2.71
2.70
2.37
1.61
1.39
0.93
1.00
6.83
8.26
1.01
1.10
5.39
2.18
2.17
1.27
1.20
1.29
2.07
2.00
Chemical Shift(ppm)


182.55
137.90
136.89
131.52
131.00
130.85
130.51
130.34
130.33
129.23
128.87
128.05
128.02
127.87
127.41
127.14
127.06
127.00
126.78
126.71
126.09
125.97
125.93
125.89
125.70
124.95
124.87
124.50
124.36
124.30
124.26
124.12
123.98
123.25
122.65
62.09
47.13
45.52
32.21
29.17
28.69
Chemical Shift(ppm)