Supporting information for

Discrimination of Redox-Responsible Biomolecules by a Single Molecular Sensor

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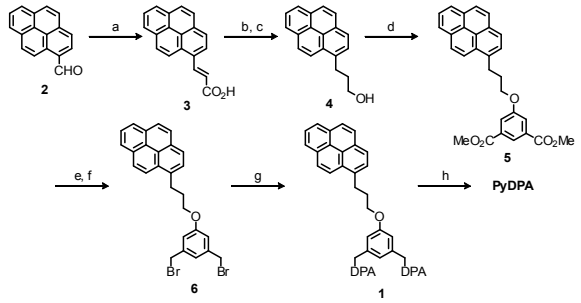
General information

All the chemicals were purchased from either Sigma-Aldrich or TCI and were used as received except anhydrous solvents. Thin layer chromatography was performed over Merck silica gel 60 F254 on aluminum foil. Merck silica gel 60 was used for stationary phase in chromatographic separation or silica pad filtration. Celite® 545 was used for celite filtration. All the ^{1}H and ^{13}C NMR spectra were obtained from Bruker DRX 300 NMR spectrometer. Fluorescence emission spectra were obtained on a JASCO FP-6500 spectrometer and the slit width was 3 or 5nm for excitation and 5nm for emission. HRMS were taken by Agilent 6890 Series, with FAB-positive mode. The **PyDPA** solution for all the photophysical experiment were prepared from 20mM stock solution in DMSO, diluted with 1mM HEPES buffer (pH 7.40). Obtained data were subjected to graph with Origin 8 program and further calculation studies were performed with Sigmaplot 8.0 program.

Synthesis of compounds

Tetrahydrofuran was distilled over sodium/benzophenone ketyl. Dichloromethane was purified by distillation over calcium hydride. Trace of water in pyridine and acetonitrile were removed by 4 Å molecular sieves prior to use. For NMR spectra analysis, samples were prepared by dissolving in DMSO-d_6, $CDCl_3$ or acetone-d_6, and multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

Scheme S1. Synthetic route to **PyDPA**



a) malonic acid, cat. piperidine, Pyridine, reflux; b) Pd/C, H_2(1atm), THF/*i*PrOH; c) $LiAlH_4$, THF; d) dimethy-5-hydroxyisophthalate, DIAD, PPh_3, THF, -78^{o}C then reflux; e) $LiAlH_4$, THF; f) PBr_3, DCM; g) di(2-picolyl)amine, K_2CO_3, KI, Acetonitrile, reflux; h) $Zn(NO_3)_2 \cdot 6H_2O$, Acetonitrile. THF = tetrahydrofuran, *i*PrOH = 2-propanol, DIAD = diisopropylazodicarboxylate, DCM = dichloromethane.

Synthesis of **3**[1] : 3-(pyren-1-yl)acrylic acid

To a stirred solid mixture of 1-pyrenecarboxaldehyde (1100 mg, 4.78 mmol) and malonic acid (920 mg, 8.8 mmol), pyridine (8mL) and catalytic amount of piperidine (8 drops) were successively added, and then refluxed for 3 hours. The mixture was poured into 3 M HCl solution cooled to 0 ^{o}C. The resulting solid was filtered, washed with cold water and recrystallized from $CHCl_3$-MeOH to give 1.1g (84%) of pure yellow solid product of **3**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.79 (1H, d, *J*=15.8Hz, ArCH=C*H*CO_2H), 8.08 (1H, t, *J* = 7.6 Hz), 8.16-8.34 (6H, m, Ar*H*), 8.46 (1H, d, *J*=4.4Hz, Ar*H*), 8.49 (1H, *J*=5.7Hz, Ar*H*), 8.66 (1H, d, *J*=15.7Hz, ArC*H*=CHCO$_2$H), 12.6 (1H, br, -CO$_2$*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ 122.050, 122.649, 124.193, 124.433, 124.978, 125.696, 126.237, 126.502, 126.986, 127.735, 128.266, 128.846, 129.050, 129.313, 130.588, 131.252, 132.493, 140.335, 168.095. HRMS (FAB$^+$) [M = $C_{19}H_{12}O_2$]$^+$, calculated 272.0837, found 272.0837.

Synthesis of **4** : 3-(pyren-3-yl)propan-1-ol

To a stirred solution of **3** (1.0 g, 4.04 mmol) in tetrahydrofuran:2-propanol mixture (40 mL:20 mL) was added a suspension of 10 wt% Pd/C (20 mg in 10 mL 2-propanol). Several balloons of molecular hydrogen were adapted for few hours, until the characteristic ^{1}H NMR signal at δ 6.8 (olefinic proton) were completely disappeared. Then the solution was filtered through celite pad, volatiles were evaporated under reduced pressure, then trace of solvents was azeotropically removed with hexanes. The resulting material dissolved in 40 mL of anhydrous tetrahydrofuran was added to the pre-chilled suspension of $LiAlH_4$ (600 mg, 15.8 mmol, 3.6 equiv.) in anhydrous tetrahydrofuran. The mixture was stirred overnight at room temperature. Excessive hydride was destroyed by successive addition of 1 mL water, 2 mL 15% NaOH in water, and 3 mL water. The mixture was filtered through celite pad and concentrated under reduced pressure to give 880 mg (84% over two steps) of semi-solid product, **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (2H, m, *J*=7.4Hz), 2.97 (1H, br s, CH_2O*H*), 3.38 (2H, t, *J*=8.0Hz), 3.76 (2H, t, *J*=6.4Hz), 7.80 (1H, d, *J*=7.8Hz), 7.9-8.08 (5H, m, Ar*H*), 8.16 (2H, t, *J*=7.9Hz), 8.23 (1H, d, *J*=9.2Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.655, 34.511, 62.293, 123.357, 124.801, 124.897, 124.941, 125.059, 125.120, 125.871, 126.669, 127.194, 127.315, 127.574, 128.692, 129.884, 130.962, 131.489, 136.227. HRMS (FAB$^+$) [M = $C_{19}H_{16}O$]$^+$, calculated 260.1201, found 260.1201.

Synthesis of **5** : dimethyl 5-(3-(pyren-1-yl)propoxy)isophthalate

To a pre-chilled, stirred solution of **4** (781 mg, 3.0 mmol), dimethyl-5-hydroxyisophthalate (945 mg, 4.5 mmol, 1.5 equiv.), and triphenylphosphine (944 mg, 3.6 mmol, 1.2 equiv.) in anhydrous tetrahydrofuran (50 mL) was added diisopropylazodicarboxylate (0.79 mL, 3.9 mmol, 1.3 equiv.) dropwise during 5 minutes at -78 ^{o}C. After finishing the addition, the mixture was refluxed for three hours. Water (1 mL) was then added,

[1] S. Kumar; *et. al.*, *J. Org. Chem.*, **1989**, *54*, 5272

volatiles were removed and 10 mL of ethyl ether-ethyl acetate mixture was added, and stood at 0 ^{o}C overnight. Precipitates were collected to give 832 mg (61%) of pure **5**. Resulting residue was chromatographed on silica gel by 20% ethyl acetate in hexanes to give more products (230 mg, 17%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (2H, m, *J*=6.2Hz), 3.59 (2H, t, *J*=7.7Hz), 3.94 (6H, s), 4.14 (2H, t, *J*=5.9Hz), 7.79 (2H, d, *J*=1.4Hz), 7.88 (1H, d, *J*=7.8Hz), 8.0-8.05 (3H, m, Ar*H*), 8.10 (2H, dd, J=6.4Hz, 9.3Hz), 8.17 (2H, d, *J*=7.7Hz), 8.30 (1H, s), 8.31 (1H, d, *J*=7.6Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.651, 31.012, 52.403, 67.558, 119.878, 122.981, 123.189, 124.810, 124.876, 124.959, 124.984, 125.123, 125.861, 126.750, 127.377, 127.454, 128.768, 130.013, 130.888, 131.424, 131.777, 135.446, 159.111, 166.180. HRMS (FAB$^+$) [M = $C_{29}H_{24}O_5$]$^+$, calculated 452.1624 , found 452.1624.

Synthesis of **6**[2] : 5-(3-(pyren-1-yl)propoxy)α,α'-dibromo-*m*-xylene

To a suspension of $LiAlH_4$ (258 mg, 6.8 mmol, 4 equiv.) in anhydrous tetrahydrofuran (50mL), a solution of **5** (770 mg, 1.7 mmol) in anhydrous tetrahydrofuran (5 mL) was added dropwise. The mixture was then stirred for 5 hours, until all the starting material was completely disappeared in TLC analysis. Then the mixture was quenched by successive addition of 0.5 mL water, 1 mL of 15% NaOH and 1.5 mL of water, and then filtered through celite pad. Volatiles were removed under reduced pressure, and dried under vacuum. The resulting residue was dissolved in 2:1 mixture of anhydrous tetrahydrofuran-dichloromethane (30 mL), and then 3.4 mL of 1 M phosphorous tribromide in dichloromethane was added under nitrogen atmosphere, at 7 ^{o}C. The mixture was further stirred for 3 hours at room temperature, quenched with 1 mL of MeOH, diluted with 50 mL dichloromethane, added half-saturated sodium bicarbonate and extracted twice with 50 mL dichloromethane each. Combined organic layer was dried over magnesium sulfate, evaporated under reduced pressure. The resulting residue was filtered through small quantity of silica gel pad to give 710 mg of **6** (80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (2H, m, *J*=7.0Hz), 3.56 (2H, t, *J*=7.7Hz), 4.05 (2H, t, *J*=6.0Hz), 4.43 (4H, s, ArCH_2Br), 6.89 (2H, s), 7.02 (1H, s), 7.89 (1H, d, *J*=7.8Hz), 8.02-8.05 (3H, m), 8.08 (2H, dd, *J*= 9.1Hz, 9.0Hz), 8.18 (2H, d, *J*=7.6Hz), 8.30 (1H, d, *J*=9.3Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.690, 31.064, 32.935, 67.160, 115.323, 121.837, 123.250, 124.832, 124.897, 124.974, 125.005, 125.134, 125.891, 126.765, 127.392, 127.467, 127.516, 127.784, 130.009, 130.917, 131.443, 135.599, 139.622, 159.444. HRMS (FAB$^+$) [M = $C_{27}H_{22}Br_2O$]$^+$, calculated 520.0037, found 520.0037.

Synthesis of **1**[2] : 5-(3-(pyren-1-yl)propoxy)α,α'-bis((di-2-picolyl)amino)*m*-xylene

6 (350 mg, 0.67 mmol, 1 equiv.), di-(2-picolyl) amine (0.26 mL, 1.47 mmol, 2.2 equiv.), potassium iodide (244 mg, 1.47 mmol, 2.2 equiv.), and potassium carbonate (276 mg, 3.0 equiv.) were dissolved in acetonitrile (15 mL). The resulting mixture was stirred at slightly raised temperature for 16 hours. Volatiles were

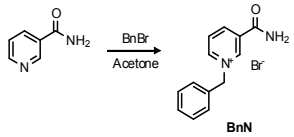
[2] Rhee, H.-W.; Lee, C.-R.; Cho, S.-H.; Song, M.-R.; Cashel, M.; Choy, H. E.; Seok, Y. J.; Hong, J.-I., *J. Am. Chem. Soc.* **2008,** *130* (3), 784-5.

removed under reduced pressure, diluted with ethyl ether, half-saturated sodium bicarbonate solution, and extracted twice to 50 mL of ethyl ether each. Combined organic layer was dried over magnesium sulfate, evaporated under reduced pressure, and then subjected to flash silica gel column chromatography. Gradient elution from chloroform to 10% methanol in chloroform gave 300 mg of **1** (43%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (2H, m, *J*=6.7Hz), 3.58 (2H, t, *J*=7.7Hz), 3.67 (4H, s), 3.83 (8H, s), 4.07 (2H, t, *J*=6.0Hz), 6.93(2H, s), 7.11(5H, m), 7.53(8H, m), 7.90(1H, d, *J*=7.8Hz), 7.96-8.18(7H, m), 8.33(1H, d, *J*=9.2Hz), 8.50(4H, d, *J*=4.7Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 29.718, 31.206, 58.607, 60.093, 66.834, 113.617, 121.517, 121.915, 122.696, 123.313, 124.771, 124.829, 124.918, 124.973, 125.101, 125.852, 126.711, 127.337, 127.415, 127.484, 127.792, 129.926, 130.867, 131.403, 135.814, 136.367, 140.667, 148.977, 159.214, 159.757. HRMS (FAB^+) $[M+H = C_{51}H_{47}N_6O]^+$, calculated 759.3811, found 759.3811.

Synthesis of **PyDPA** :

To an aliquot of **1** (3 micromoles) in acetonitrile (0.2 mL), zinc(II)nitrate hexahydrate (6.15 micromoles, 2.05 equiv.), in acetonitrile (0.1 mL) was added slowly, stirred for 1 hour, and then solvent was removed under reduced pressure. The resulting residue was dried under vacuum then used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36(2H, br), 3.63(2H, d, *J*=7.4Hz), 3.73(4H, d, *J*=16Hz), 3.87(4H, s), 4.20(2H, t, *J*=7Hz), 4.34(4H, d, *J*=16Hz), 6.93(1H, s), 7.06(2H, s), 7.48(4H, d, *J*=7.8Hz), 7.65(4H, t, *J*=6.5Hz), 8.03-8.5(13H, m), 8.68(4H, d, *J*=4.7Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.335, 31.162, 56.107, 57.411, 67.322, 118.190, 123.828, 123.892, 124.578, 124.720, 125.028, 125.331, 125.539, 126.695, 126.792, 127.116, 127.380, 127.769, 127.937, 128.110, 128.766, 129.903, 130.849, 131.359, 134.134, 136.472, 141.201, 148.374, 154.743, 159.334. HRMS (FAB^+) $[M\text{-}NO_3 = C_{51}H_{46}N_9O_{10}Zn_2]^+$, calculated 1072.1945, found 1072.1951.

Scheme S2. Synthesis of model compound, **BnN**

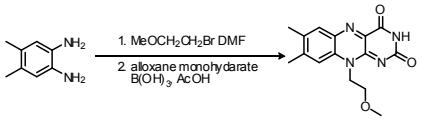


Synthesis of **BnN** :

To a stirred suspension of nicotinamide (610 mg, 5 mmol) in acetone (10 mL), benzyl bromide (0.77 mL, 6.5 mmol, 1.3 equiv.) were added, then gently heated for 2 days. Resulting suspension was filtered to give pure benzylated nicotinamide, **BnN** (1.2 g, 82%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.96 (2H, s, N^+CH_2Ph) 7.43-7.46 (3H, m, *H*s in Bn), 7.58-7.63 (2H, m, *H*s in Bn), 8.19 (1H, br s, N*H*), 8.27 (1H, dd, *J*=6.3Hz, 7.9Hz),

8.64 (1H, br s, N*H*), 8.98 (1H, dd, *J*=1.0Hz, 8.0Hz), 9.35 (1H, d, *J*= 5.0Hz), 9.71 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) 63.920, 128.747, 129.505, 129.678, 129.915, 134.441, 134.520, 144.310, 145.276, 146.837, 163.153. HRMS (FAB$^+$) [M-Br$^-$ = $C_{13}H_{13}N_2O]^+$, calculated 213.1022, found 213.1028.

Scheme S3. Synthesis of model compound, **MEI**



MEI

Synthesis of **MEI** :

To a stirred solution of 4,5-dimethylphenylene-1,2-diamine (1.02 g, 0.75 mmol) and potassium carbonate (1.0 g, 0.75 mmol) in DMF (20 mL), 2-bromoethyl methyl ether (0.6 mL, 0.68 mmol, 0.9 equiv.) were added at 60 ^{o}C. The mixture was stirred for 6 hours, then organic solvent were removed under vacuum. Resulting material was diluted with 150 mL of ethyl ether and washed with 3 portions of water. Ethereal layer were dried over magnesium sulfate, and ether were removed under reduced pressure to give dark brown liquid. Materials were redissolved by acetic acid (30 mL) then 3 g of boric acid and 2.0 g of alloxane monohydrate were added at room temperature. Resulting mixture was stirred overnight. Acetic acid was evaporated *in vacuo*, and resulting material were recrystallized from 25% MeOH in chloroform to give isoalloxazine derivative, **MEI** (0.75 g, 33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38(3H, s), 2.48(3H, s), 3.25(3H, s) 3.74(2H, t, *J*=5.7Hz), 4.77(2H, t, *J*=5.7Hz), 7.82(1H, s, Ar*H*), 7.84(1H, s, Ar*H*), 11.30(1H, br s, N*H*). ^{13}C NMR (75 MHz, DMSO-d_6) 19.211, 21.117, 44.338, 58.906, 68.662, 117.125, 131.216, 131.808, 134.077, 136.207, 137.503, 146.722, 150.653, 155.968, 160.315. HRMS (FAB$^+$) [M+H = $C_{15}H_{17}N_4O_3]^+$, calculated 301.1301, found 301.1301.

Additional spectral data and fitting results

1) Mathematical basis for fittings

A. 1:1 binding profile

$$\mathrm{H} + \mathrm{G} \rightleftharpoons \mathrm{HG} \text{ with } K_d = K_a^{-1} = \frac{[H] \cdot [G]}{[HG]} \tag{1}$$

$$H_0 = [H] + [HG], G_0 = [G] + [HG] \tag{2}$$

$$H_0 \cdot G_0 = [H] \cdot [HG] + [G] \cdot [HG] + [H] \cdot [G] + [HG]^2$$

$$[HG] = \frac{H_0 + G_0 + K_d - \sqrt{(H_0 + G_0 + K_d)^2 - 4 \cdot H_0 \cdot G_0}}{2} \tag{3}$$

$$F_{obs} = \alpha \cdot [H] + \beta[HG] = \alpha \cdot (H_0 - [HG]) + \beta \cdot [HG]$$

$$= \alpha \cdot H_0 + (\beta - \alpha) \cdot [HG] \tag{4}$$

By substituting $[HG]$ of (4) to (3), F_{obs} is given by function of H_0, G_0 and K_d, thus the scattered graph can be fitted with respect to G_0, to estimate value of the only unknown constant, K_d.

B. Approximation of excited state population in given molar extinction coefficients

For a solution of *x* and *y* without absorption interference, assume that the molar extinction coefficients and concentrations of *x*, *y* are $\varepsilon_x, \varepsilon_y$ and c_x, c_y, respectively.

If the solution is partitioned by 2n (n ∈), with half of partitions banishes *x* to the others, and reversely to *y*, and if two sets of partitions appeared alternatingly, then we calculate the absorbance with following definitions;

$$da_x = \varepsilon_x \cdot 2 \cdot c_x \cdot \frac{l}{2n} = \varepsilon_x \cdot c_x \cdot \frac{l}{n} \quad , \qquad da_y = \varepsilon_y \cdot c_y \cdot \frac{l}{n}$$

Then, $dA_j = either\ da_x\ (for\ j = 2k-1)\ or\ da_y (for\ j = 2k)$

By definition of absorbance and transmittance,

$$1 - dT_j = 1 - 10^{-dA_j}$$

Thus, relative transmitted intensity at the front of j[th] partition is:

$$1 \times \prod_{k=1}^{j-1} dT_k$$

With a definition of $q = 10^{-da_x - da_y} = 10^{-(\frac{\varepsilon_x c_x l}{n} + \frac{\varepsilon_y c_y l}{n})}$, the amount of light intensity by species *x* is :

$$\sum_{k=1}^{n} [(1 - 10^{-da_x}) \cdot \prod_{m=0}^{2(k-1)} dT_m] = (1 - 10^{-da_x}) \cdot [1 + q + q^2 + \cdots + q^{n-1}],$$

$$= (1 - 10^{-da_x}) \cdot \frac{1 - q^n}{1 - q}$$

By taking limit of *n* to infinity, we can write:

$$\lim_{n \to \infty} \frac{1 - 10^{-da_x}}{1 - 10^{-da_x - da_y}} \cdot (1 - q^n)$$

Applying L'Hopital's theorem, the value of limit becomes

$$= \frac{\varepsilon_x c_x}{\varepsilon_x c_x + \varepsilon_y c_y} \cdot (1 - T) = \frac{A_x}{A_x + A_y} \cdot (1 - T) \qquad (5)$$

C. Approximation of pseudo-first order kinetics.

For given catalyst C and substrate S, we can consider the following reaction kinetics.

$$\mathrm{C} + \mathrm{S} \rightleftharpoons_{k_{-1}}^{k_1} \mathrm{CS} \rightarrow^{k_2} \mathrm{C} + \mathrm{P}$$

We can find few relations between variables,

$$\mathrm{C}_0 = [C] + [CS] \qquad (6)$$

$$S_0 = [S] + [CS] + [P] \qquad (7)$$

With the constant defined;

$$K_{C,S} = \frac{[CS]}{[C] \cdot [S]}$$

Then, the rate of production of P is

$$\frac{d}{dt}[P] = k_2[CS] \qquad (8)$$

By assuming steady-state kinetics for [CS], we can use following equation;

$$\frac{d}{dt}[CS] = k_1[C][S] - (k_{-1} + k_2)[CS] \approx 0\ , Hence\ [CS] = \frac{k_1}{k_{-1} + k_2}[C] \cdot [S] \qquad (9)$$

From (6), (8), and the by defining $K_S = k_1 \,/\, (k_{-1} + k_2)$

$$[\mathrm{CS}] = K_S(C_0 - [CS]) \cdot [S]$$

$$[\mathrm{CS}] = \frac{K_S \cdot C_0[S]}{1 + K_S[S]} \qquad (10)$$

Thus equation (8) becomes as follow;

$$\frac{d}{dt}[P] = k_2 \cdot \frac{K_S}{1 + K_S[S]} C_0[S]$$

Since k_1, k_{-1} are involved in kinetic equilibrium we assume two conditions that;

$$k_1 \gg k_2 \text{ and } k_{-1} \gg k_2$$

Then the equation becomes;

$$\approx k_2 \cdot \frac{k_1}{k_{-1}} \cdot C_0[S] = k_{obs} \cdot C_0[S] \qquad (12)$$

Since we used steady-state approximation,
we can choose appropriate value of $s_0' = [S] + [P]$,

$$\frac{d}{dt}([S] + [P]) = 0, \frac{d}{dt}[S] = -\frac{d}{dt}[P]$$

The equation (12) becomes;

$$\frac{d}{dt}[S] = -k_{obs} \cdot C_0 \cdot [S] \qquad (13)$$

A solution of equation (13) is;

$$[S] = s_0' \cdot e^{-k_{obs} \cdot C_0 \cdot t}$$

$$[P] = s_0' \cdot (1 - e^{-k_{obs} \cdot C_0 \cdot t})$$

Thus, the fluorescence can be fitted with several constants;

$$\mathrm{F} = \alpha \cdot [\mathrm{S}] + \beta \cdot [P] = s_0^{'} \cdot \{\beta - (\beta - \alpha) \cdot e^{-k_{obs} \cdot C_0 \cdot t}\} \qquad (14)$$

Which gave k_{obs} of few tens, thus the approximations we used are valid.

D. Job's Plot Analysis

Job's plot analysis starts from the following equation.

$$F_{tot} = F(H) + F(HG) = \alpha \cdot [H] + \beta \cdot [HG]$$

By substituting $[H] = H_0 - [HG]$

$$F_{tot} = \alpha \cdot H_0 + (\beta - \alpha) \cdot [HG]$$

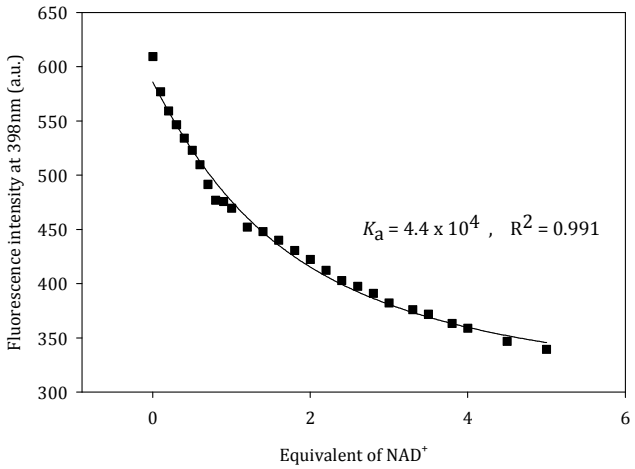
$$\Delta F = \alpha \cdot H_0 - F_{tot} = (\beta - \alpha) \cdot [HG] \qquad (15)$$

Thus, the corrected fluorescence ΔF is linearly correlated to $[HG]$.

At the peak point, binding stoichiometry is optimal to make $[HG]$, directly implies the binding stoichiometry.

2) NAD^+

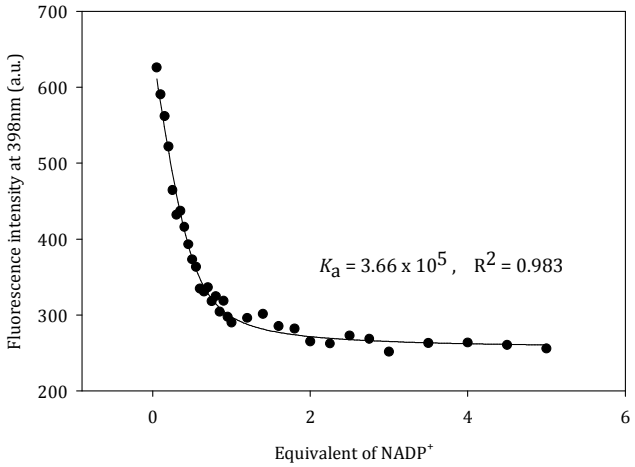
A. Figure S1. Fluorescence spectral change of **PyDPA** upon addition of NAD^+



Fluorescence at 398 nm was recorded where [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm, square: observed fluorescence, solid line: fitted curve. The fluorescence changes were fitted with equation (4) described above.

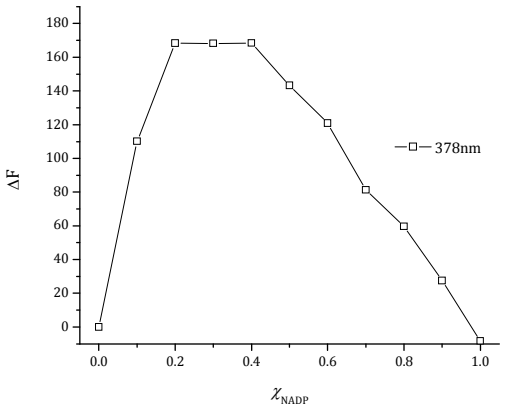
3) $NADP^+$

A. Figure S2. Fluorescence spectral change of **PyDPA** upon addition of $NADP^+$



Fluorescence at 398 nm was recorded where [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm, circle: observed fluorescence, solid line: fitted curve. The fluorescence changes were fitted with equation (4) described above. Binding constant was estimated using a twofold concentration of $NADP^+$.

B. Figure S3. Job's plot for $NADP^+$ and **PyDPA**.

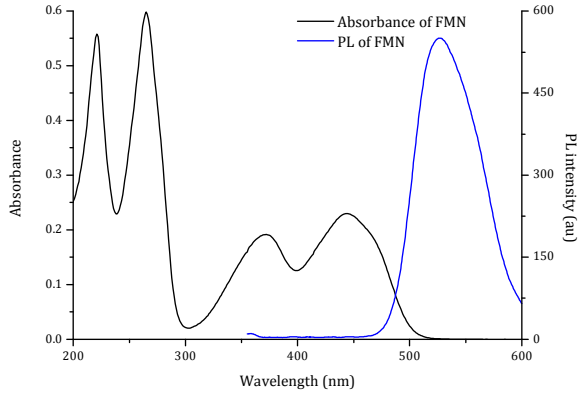


Fluorescence at 378 nm was recorded and corrected with standard equation (15) shown before. Here, [$NADP^+$] + [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm.

4) FMN

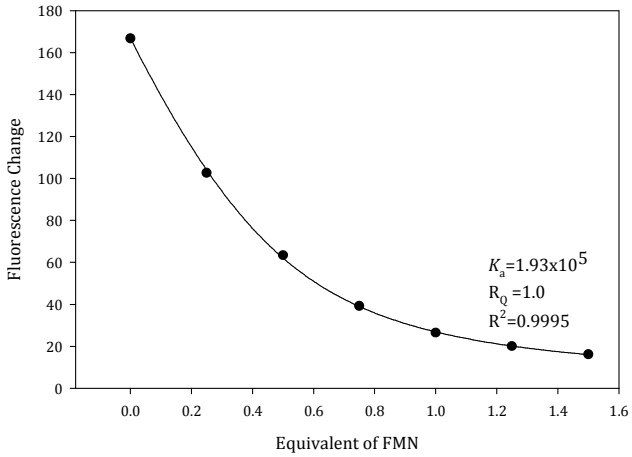
A. The extinction coefficients were obtained from their individual UV-vis spectra (11500, 6150 for pyrene and isoalloxazine, respectively), and binding constants were estimated to be $1.93 \times 10^5\ M^{-1}$ from its fitting with respect to a twofold concentration of FMN, shown in Figure S4.

B. Figure S4. Photophysical property of FMN



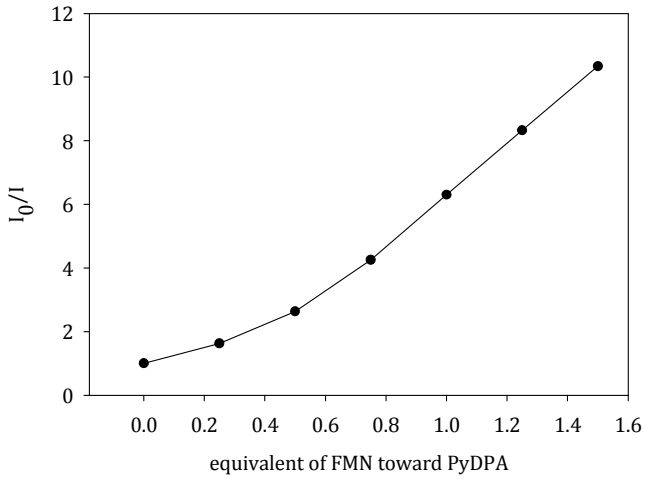
[FMN] = 20μM in HEPES buffer(1 mM, 1% DMSO, pH = 7.4). FMN shows its absorption in range of 300 ~ 500 nm and corresponding PL is observed in range of 450 ~ 650 nm. For photoluminescence measurement, λ_{ex} = 320 nm.

C. Figure S5. Fluorescence spectral change of **PyDPA** upon addition of FMN and its fitting result with respect to a twofold concentration of added FMN.



Fluorescence at 398 nm was recorded where [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm, circle: observed fluorescence, solid line: fitted curve. The fluorescence changes were fitted using equation (4) and (5) described above. A binding constant was estimated using a twofold concentration of $NADP^+$.

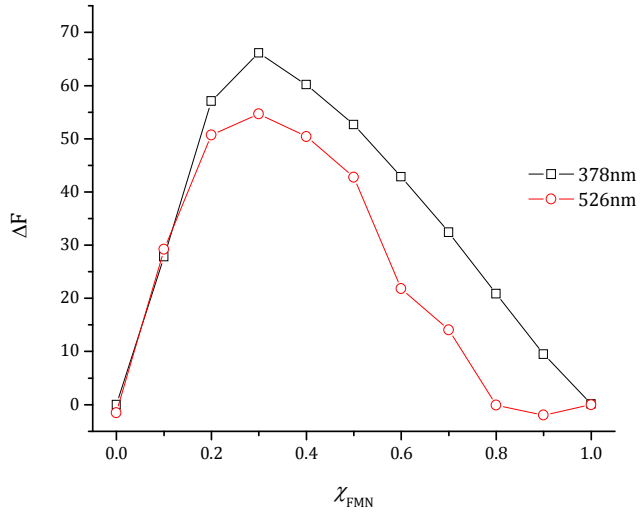
D. Figure S6. Stern-Volmer plot of **PyDPA** with respect to FMN concentration



Fluorescence at 398 nm was recorded where [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm, circle: calculated from observed value.

Two different linear correlations (0~0.5 and 0.75~1.5) were shown separately in the graph.

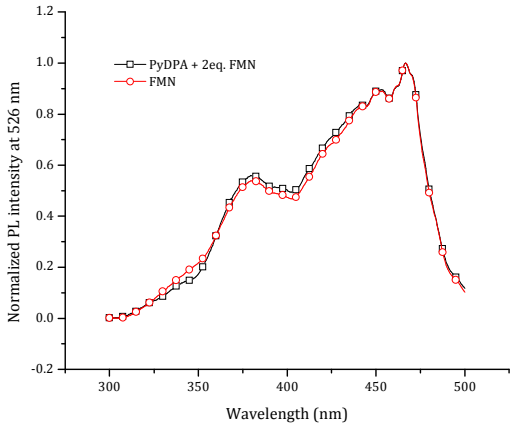
E. Figure S7. Job's plot for FMN and **PyDPA**



Fluorescence at 378 nm was recorded and corrected with standard equation (15) shown before.

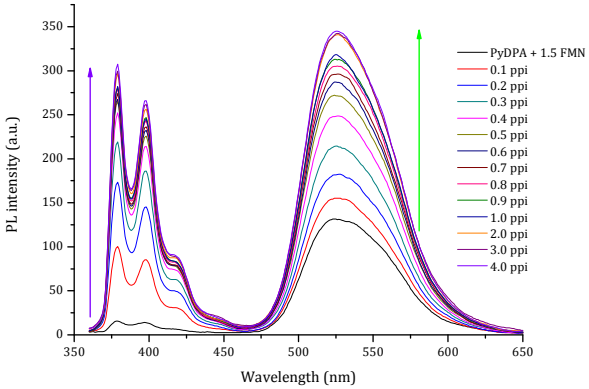
Here, [FMN] + [**PyDPA**] = 20 μM in 1 mM HEPES buffer (pH 7.40). excitation = 344 nm.

F. Figure S8. Normalized excitation spectra at 526 nm, either in the presence or absence of **PyDPA**



Fluorescence intensity of FMN at 526 nm was recorded with respect to excitation wavelength. The graph represents FMN intensity either with (black) or without (red) **PyDPA** where [FMN] = 20 μM in HEPES buffer (1 mM, pH 7.40).

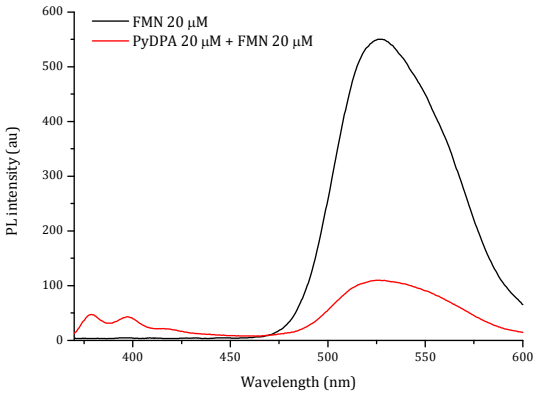
G. Figure S9. Pyrophosphate addition to 1:1.5 solution of **PyDPA** and FMN



Fluorescence intensity of **PyDPA**-FMN system was recorded with respect to pyrophosphate addition, where [**PyDPA**] = 20 μM and [FMN] = 30 μM in 1 mM HEPES buffer (pH 7.40) excitation = 344 nm. Pyrophosphate concentration is expressed as equiv.alents relative to **PyDPA**.

Fluorescence intensities of both pyrene and FMN are increased significantly with pyrophosphate concentration.

H. Figure S10. Fluorescence spectra of FMN with respect to the presence of **PyDPA**.

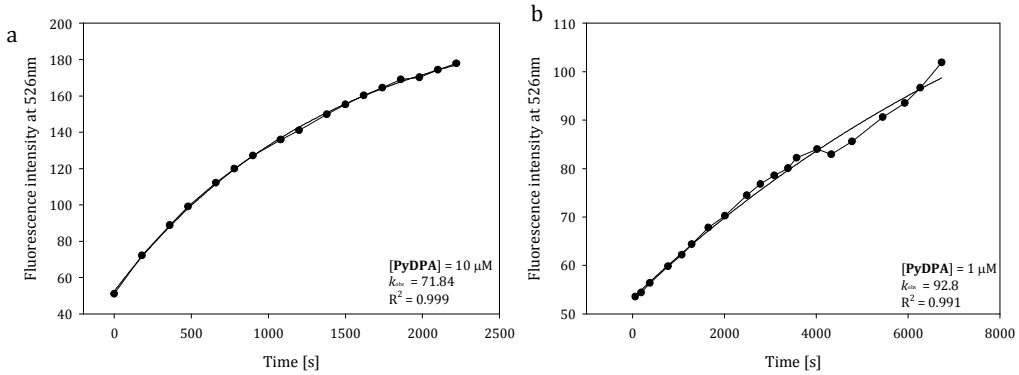


Fluorescence intensity of FMN was recorded in the absence (black solid line) or presence (red solid line) of **PyDPA**, where [FMN] = 20 μM in 1 mM HEPES buffer (pH 7.40), excitation = 320 nm.

The fluorescence of FMN is greatly decreased by **PyDPA** addition, which implies that the fluorescence of FMN was quenched by **PyDPA**.

5) FAD

A. Figure S11. Pseudo first-order kinetics of FAD self-lysis at different concentrations of PyDPA



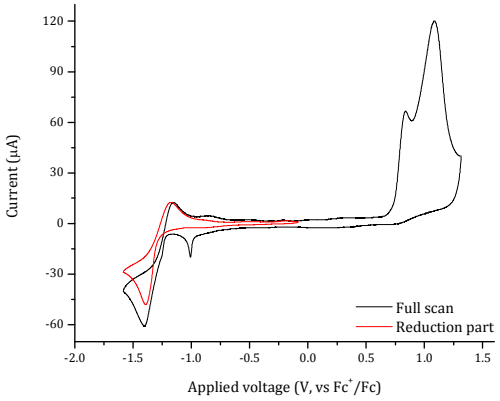
The fluorescence at 526 nm was recorded at [FAD] = 10 μM and [**PyDPA**] = either (a) 10 μM or (b) 1 μM in 1 mM HEPES buffer (pH 7.40) with excitation = 344 nm, circle: observed value, solid line: fitted result by equation (14) described above.

Electrochemical data

Cyclic voltammetry (CV) experiments were performed using a CH instruments 660 electrochemical analyzer, in the form of ~1 mM solution in 10 mM tetraethylammonium perchlorate as supporting electrolyte in acetonitrile. $Ag/Ag(NO_3)$ was employed for reference electrode, applied bias and corresponding current were measured. Finally collected data were corrected by ferrocenium/ferrocene redox pair.

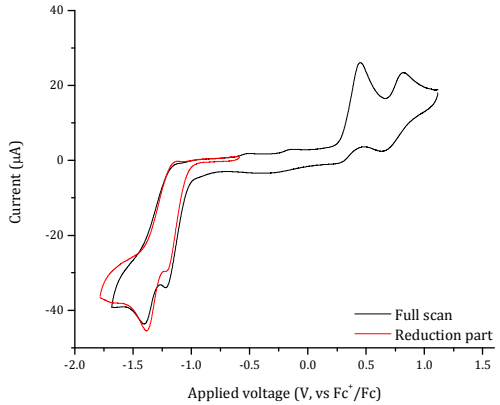
1) CV

A. Figure S12. CV data for **5**



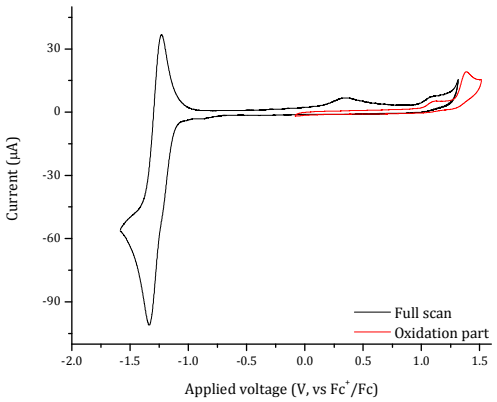
Reduction potential was calculated to be 1.197 V vs Fc/Fc^+, from the equation $\frac{V_{pc}+V_{pa}}{2}$. The peak at -.0.8 V vs Fc/Fc^+ were assigned to be reduction of dissolved oxygen in acetonitrile.

B. Figure S13. CV data for **BnN**



The first reduction potential was calculated to be 0.97 V vs Fc/Fc^+, obtained from onset point of the graph of reduction part.

C. Figure S14. CV data for **MEI**



Reduction potential was calculated to be 1.201 V vs Fc^{+}/Fc pair, from the equation $\frac{V_{pc}+V_{pa}}{2}$.

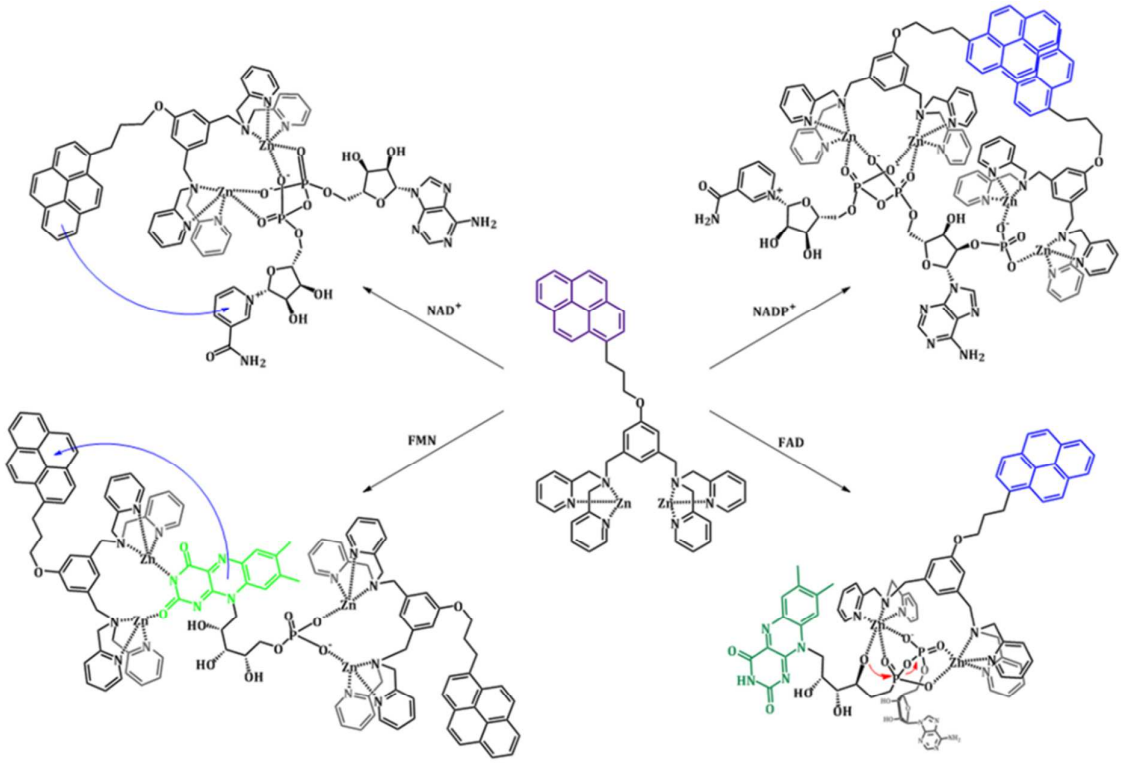
2) Table S1. Electrochemical potentials of compounds [a]

	5	**BnN**	**MEI**
LUMO	1.197V (-3.519V) [b]	0.97V (-3.75V) [b]	1.201V (-3.515V) [b]
HOMO	-2.153V (-6.869V) [d]	-0.81V (-5.53V) [b] -3.45V (-8.17V) [d]	-1.20 V (-5.916V) [b] -1.32 V (-6.12V) [d]
E_{00} [c]	3.35eV (370nm)	4.42eV (280nm)	2.52eV (493nm)

[a]Energy levels relative to Fc/Fc^{+} pair and corresponding value relative to vacuum level (in parentheses) are shown. [b]Obtained from CV. [c]Obtained from either cross point of normalized UV-Vis absorption spectrum and normalized PL intensity (**5** and **MEI**) or UV-Vis absorption spectrum onset point (**BnN**). [d]Obtained from E_{00}, optical transition energy.

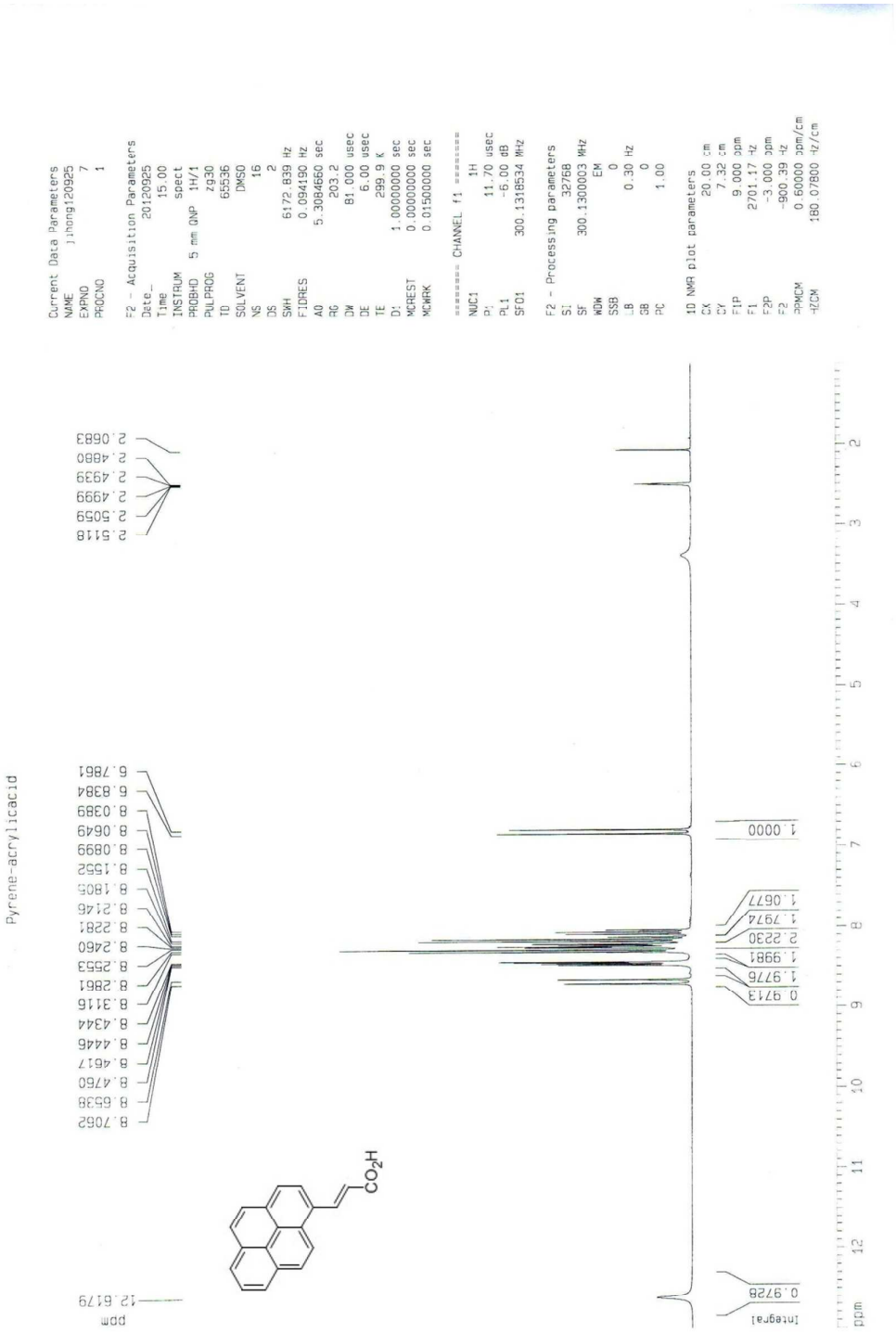
Plausible Binding Modes

Figure S15. Plausible binding modes of **PyDPA**

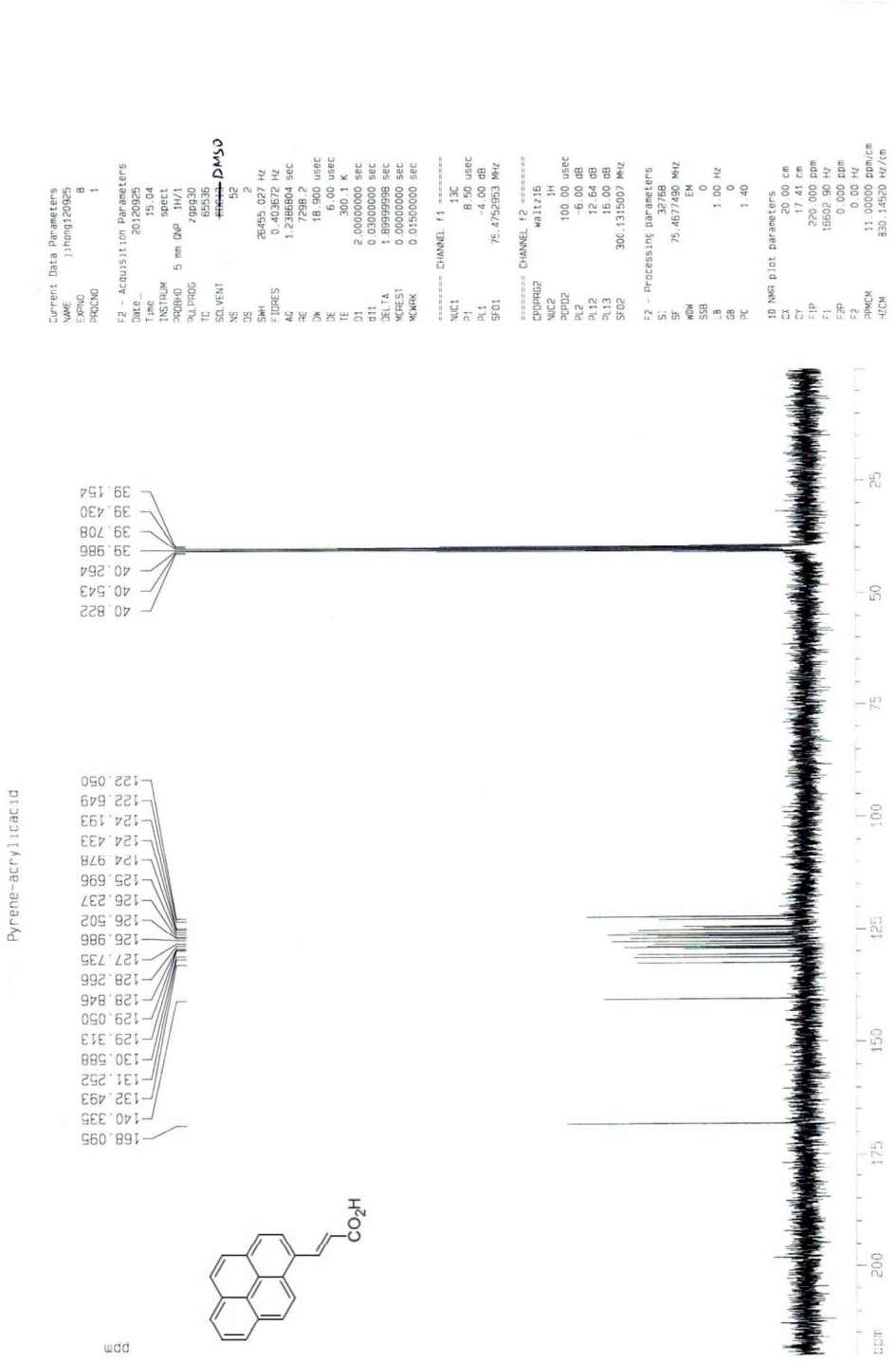


Copies of NMR spectra

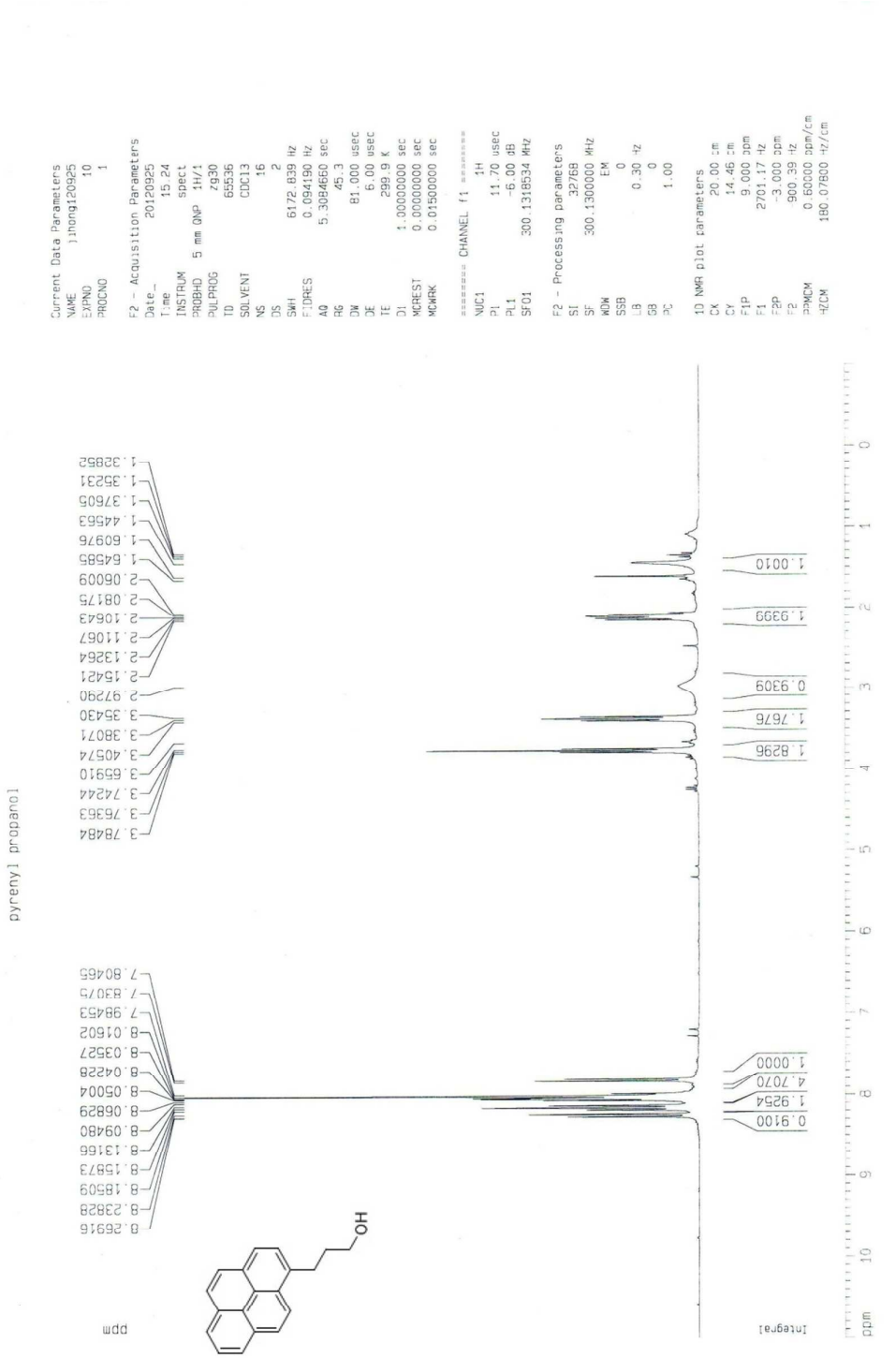
1) Figure S16. ^{1}H NMR spectrum of **3**



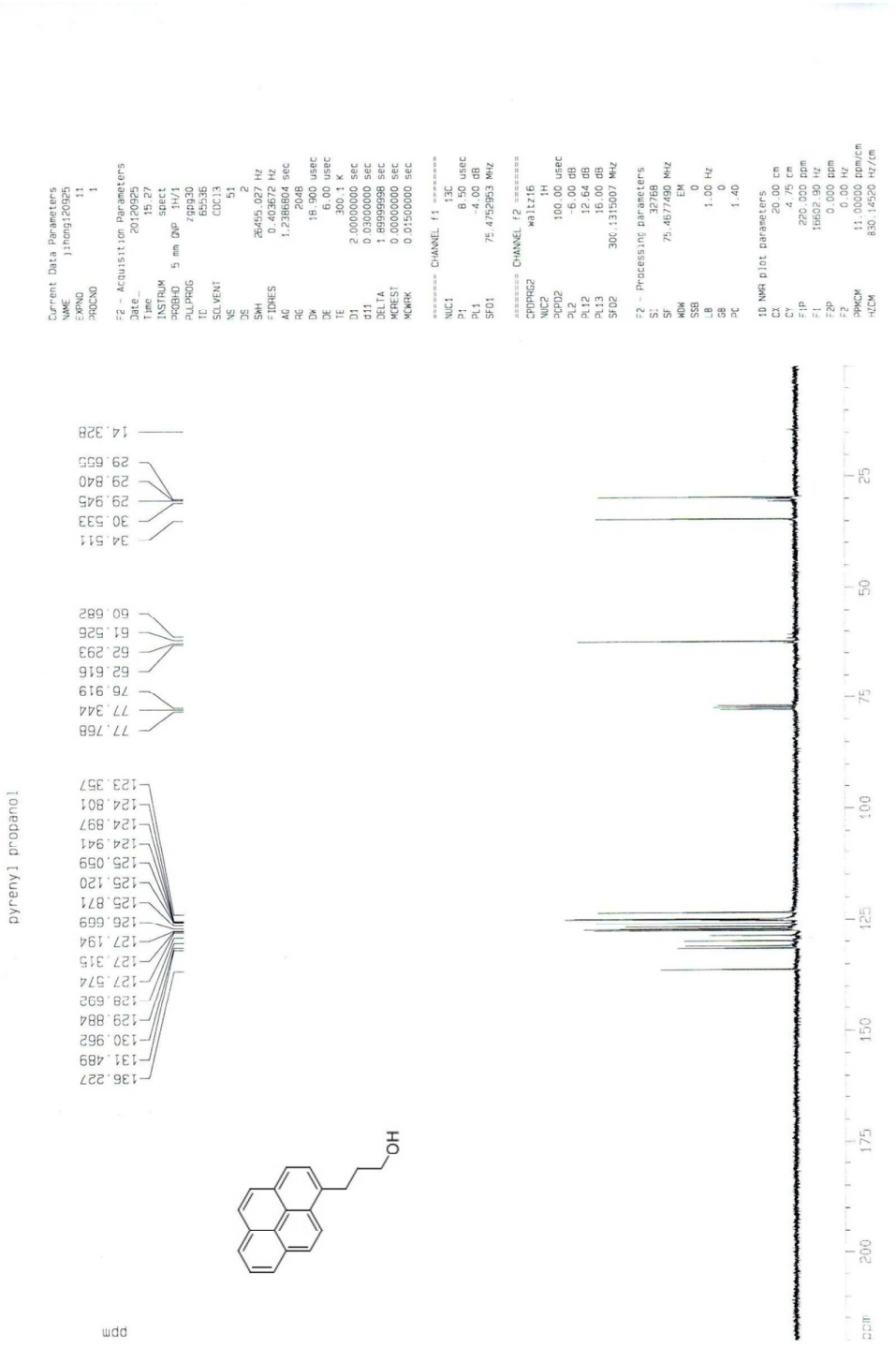
2) Figure S17. ^{13}C NMR spectrum of **3**



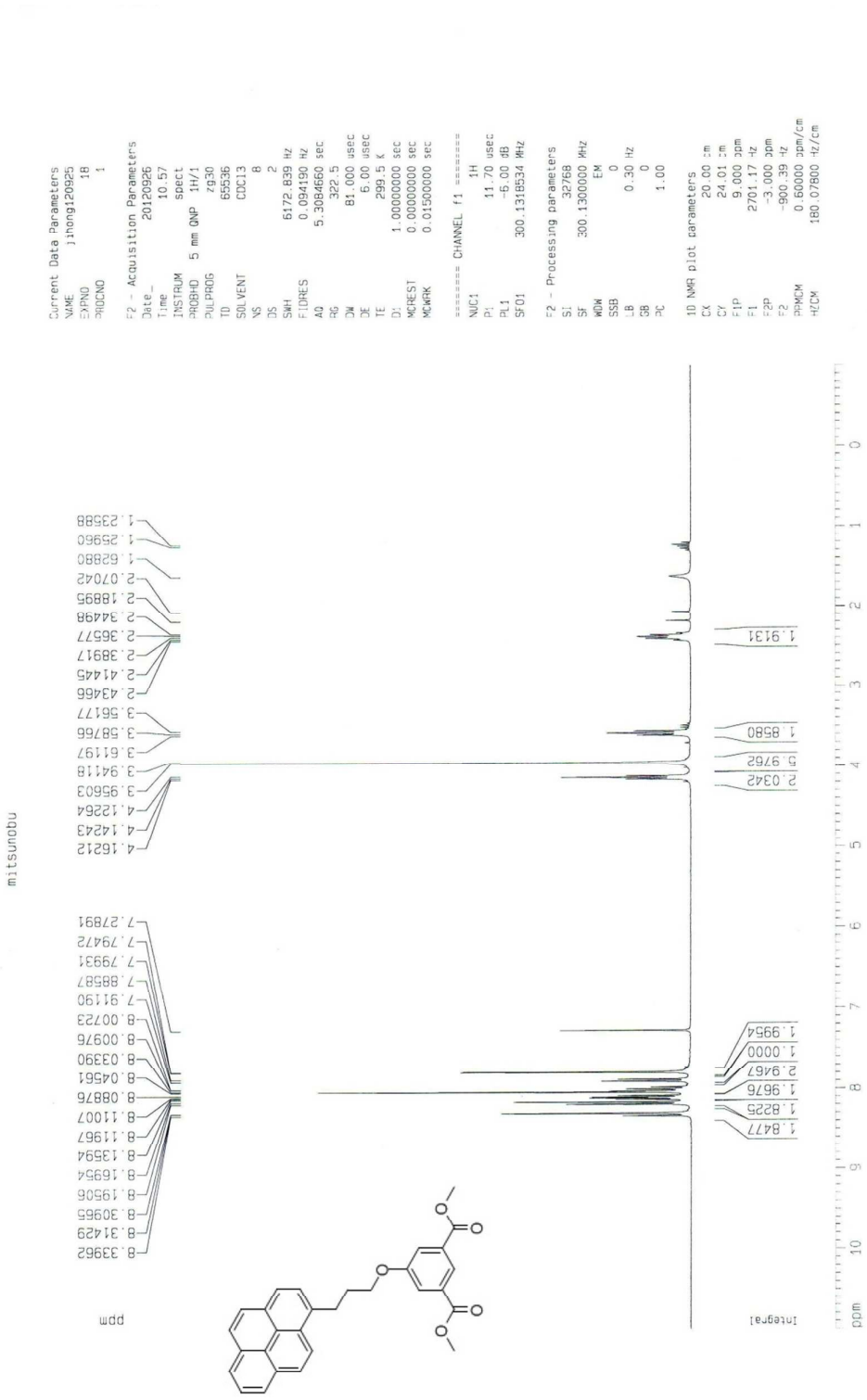
3) Figure S18. ^{1}H NMR spectrum of **4**



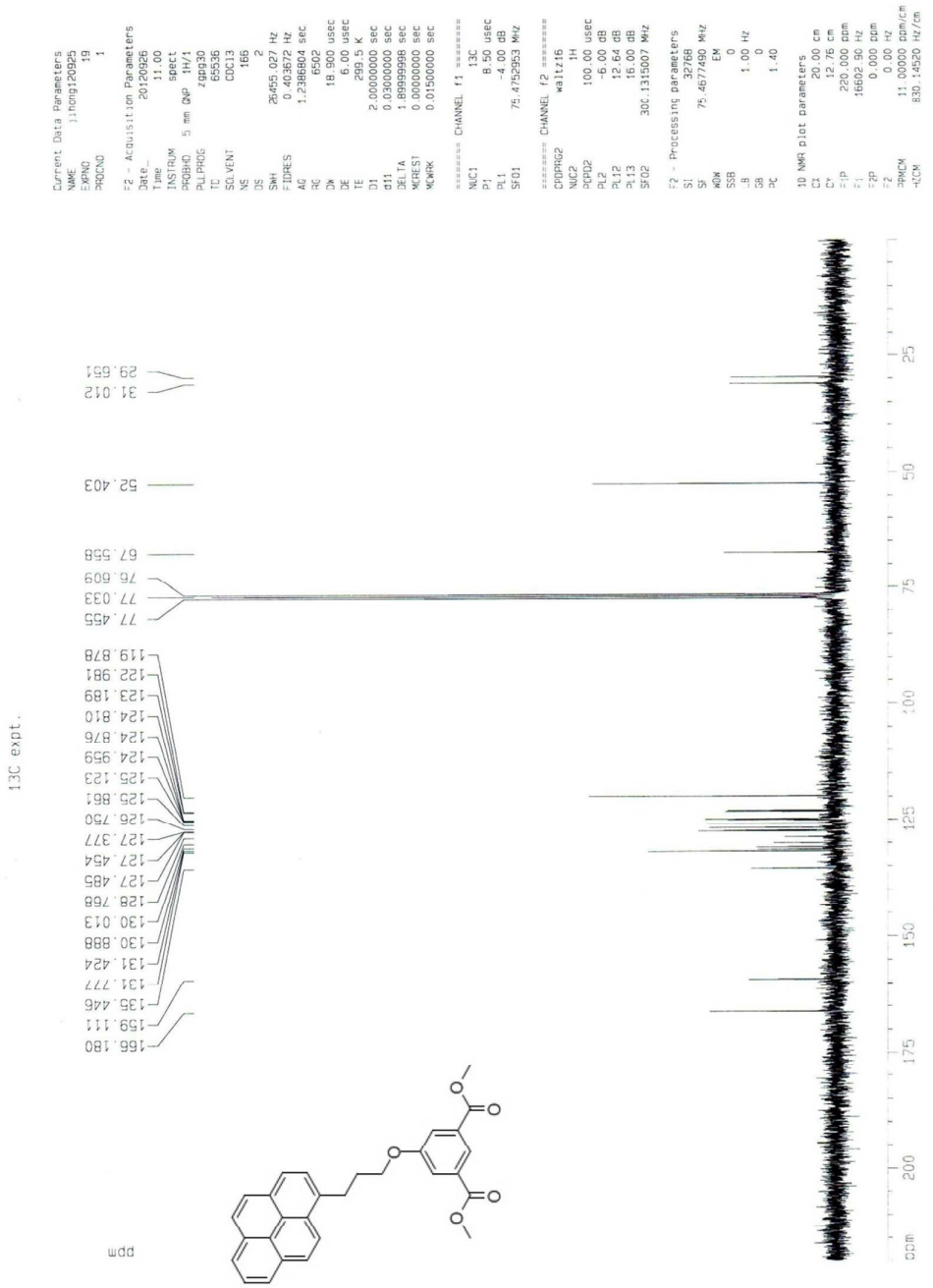
4) Figure S19. ^{13}C NMR spectrum of **4**



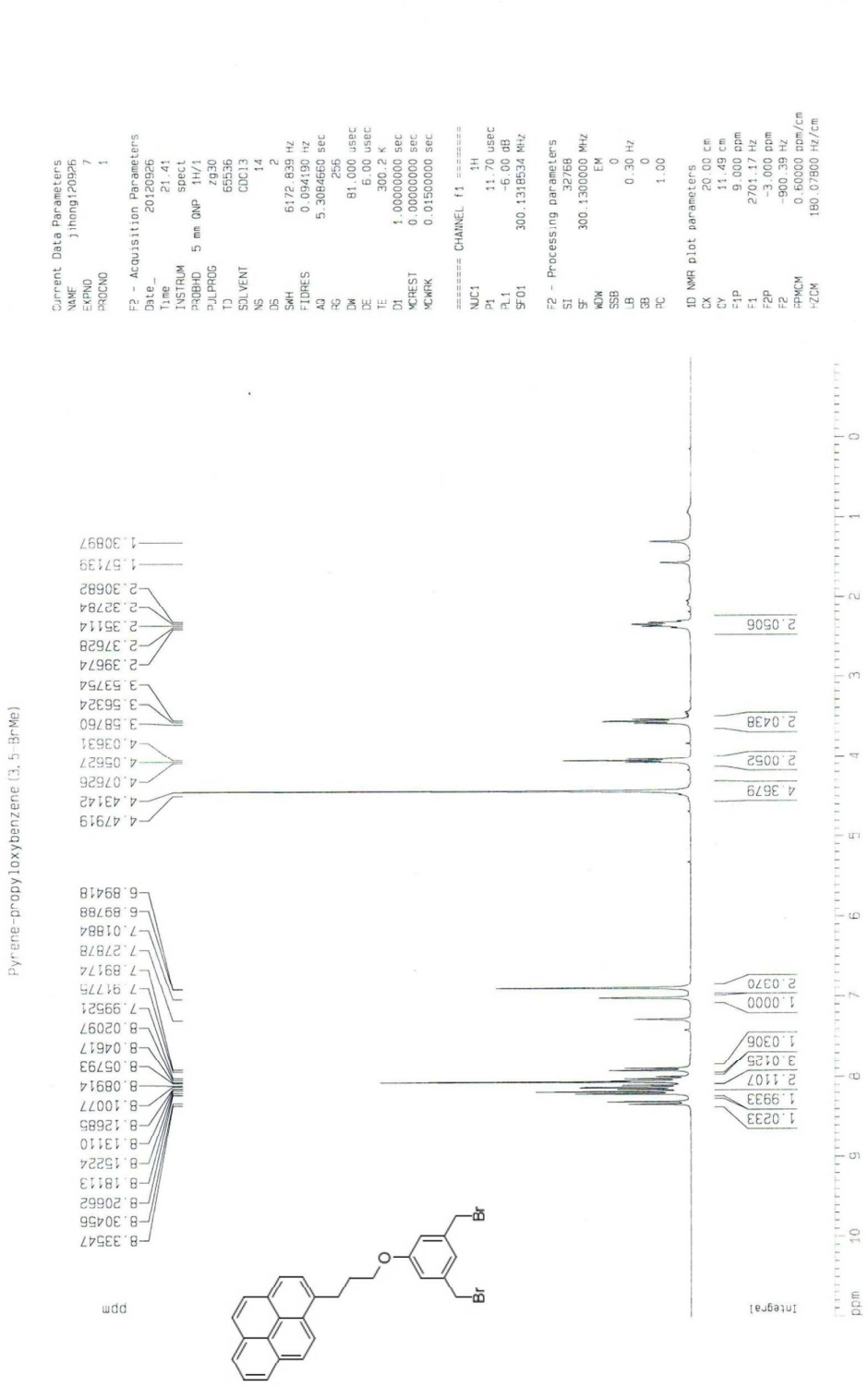
5) Figure S20. ^{1}H NMR spectrum of **5**



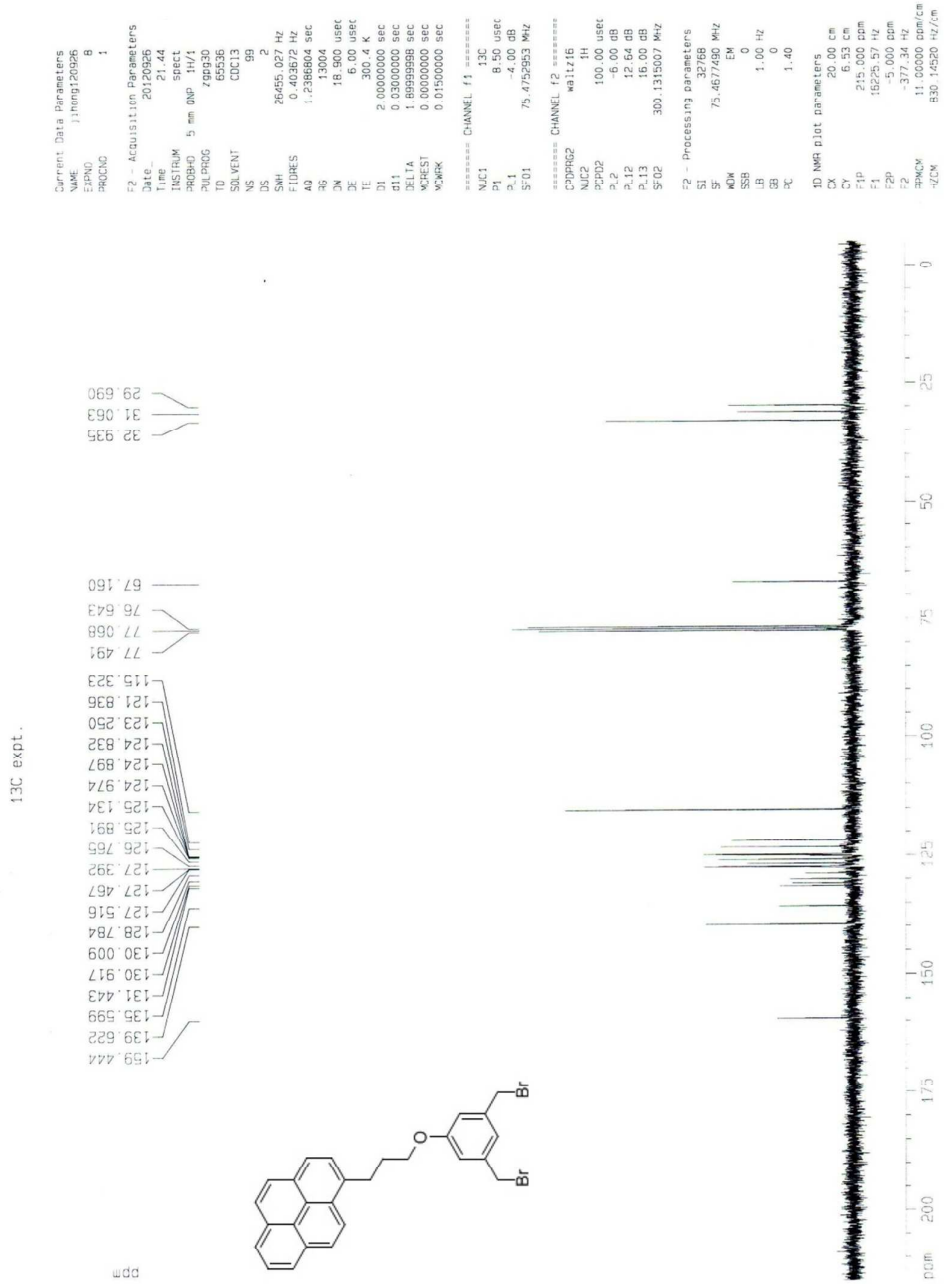
6) Figure S21. ^{13}C NMR spectrum of **5**



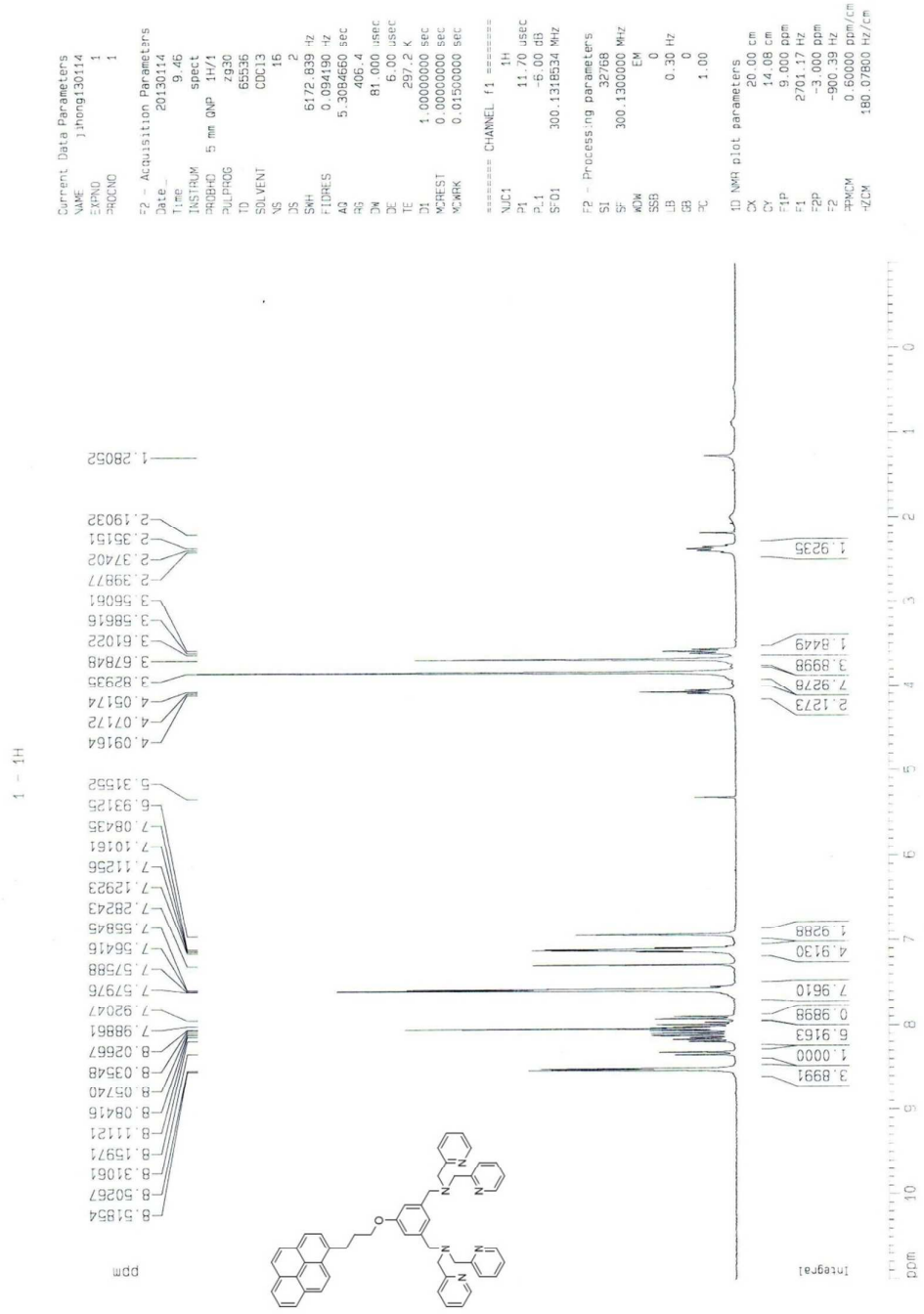
7) Figure S22. ^{1}H NMR spectrum of **6**



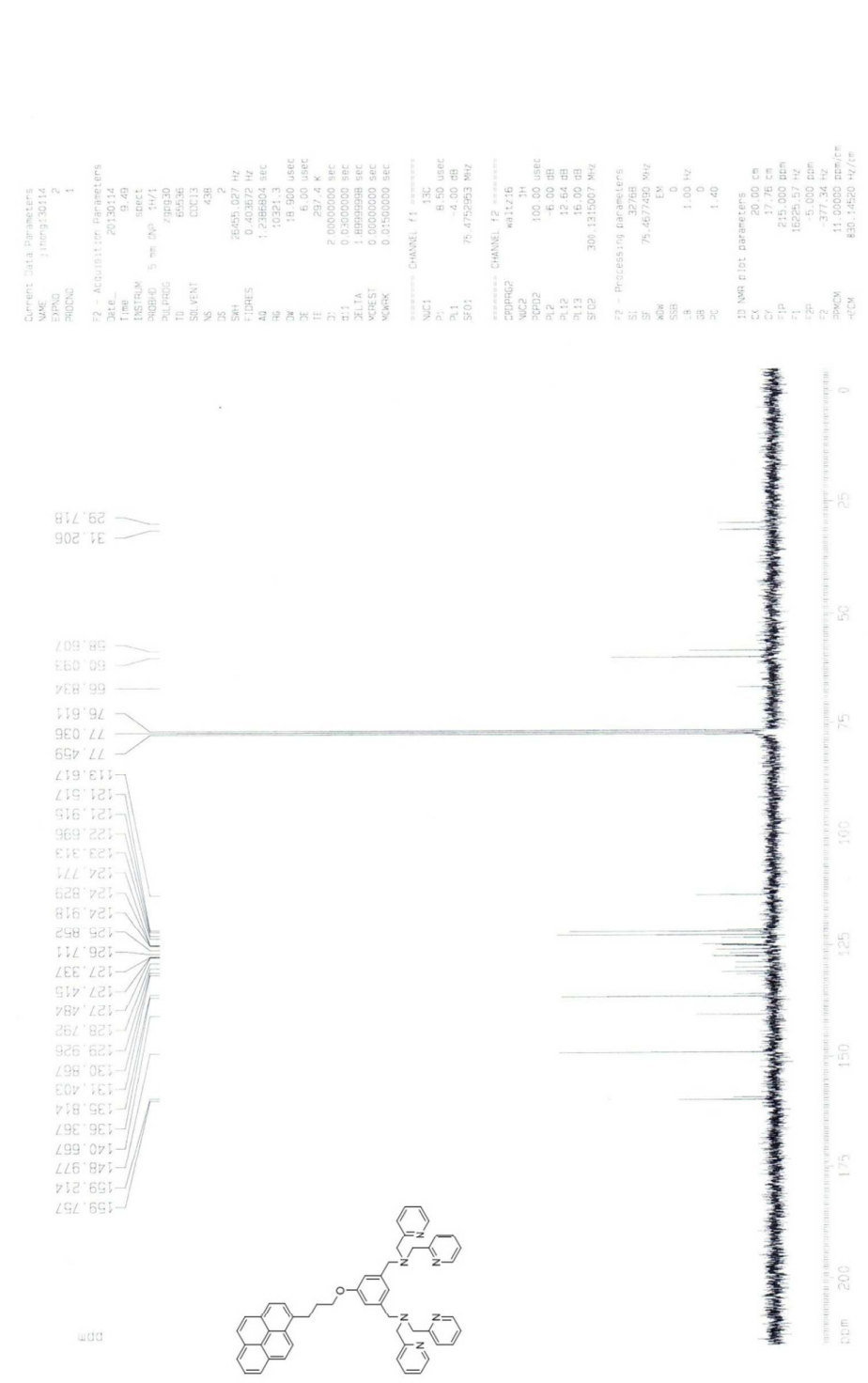
8) Figure S23. ^{13}C NMR spectrum of **6**



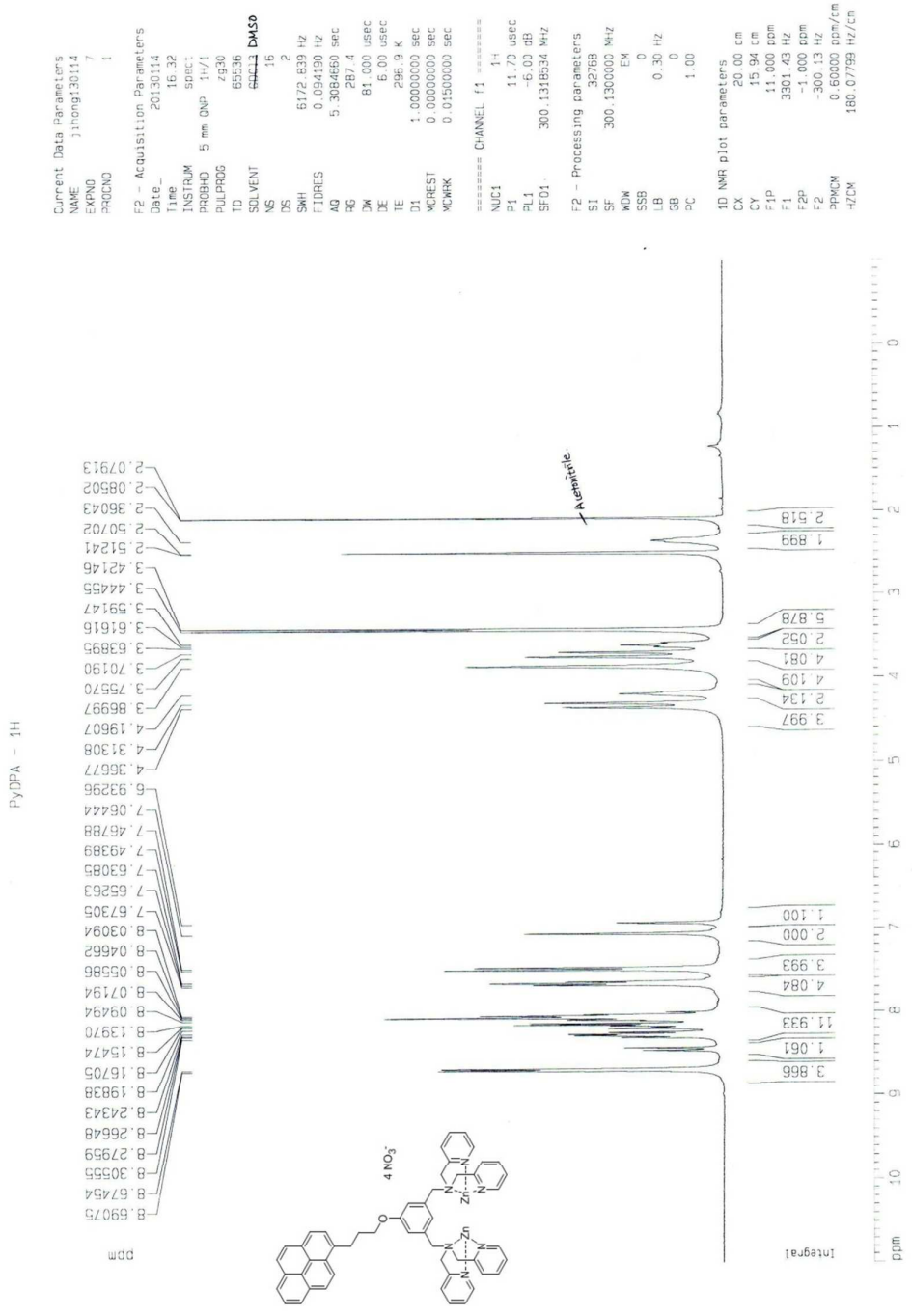
9) Figure S24. ^{1}H NMR spectrum of **1**



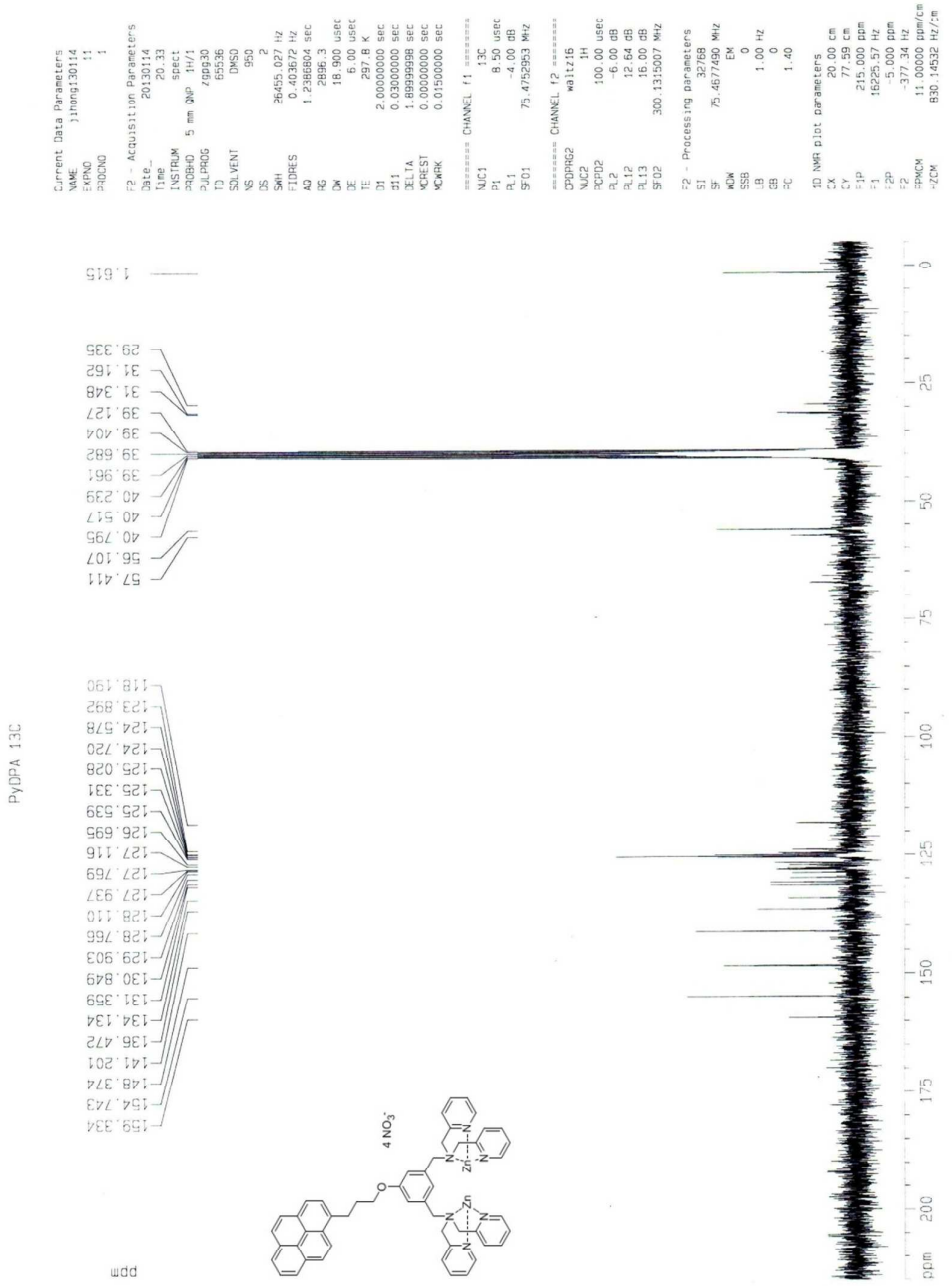
10) Figure S25. ^{13}C NMR spectrum of **1**



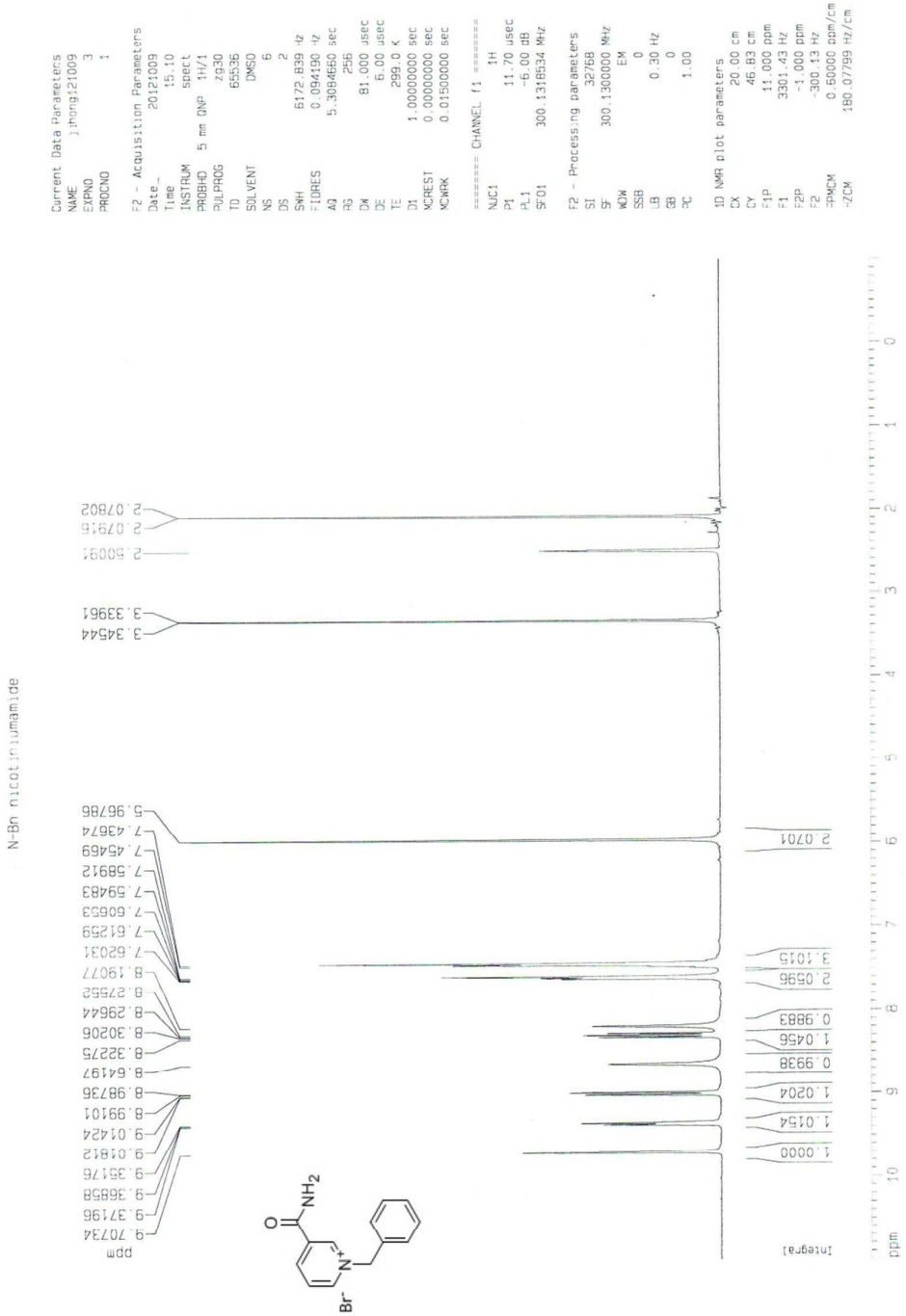
11) Figure S26. ^{1}H NMR spectrum of **PyDPA**



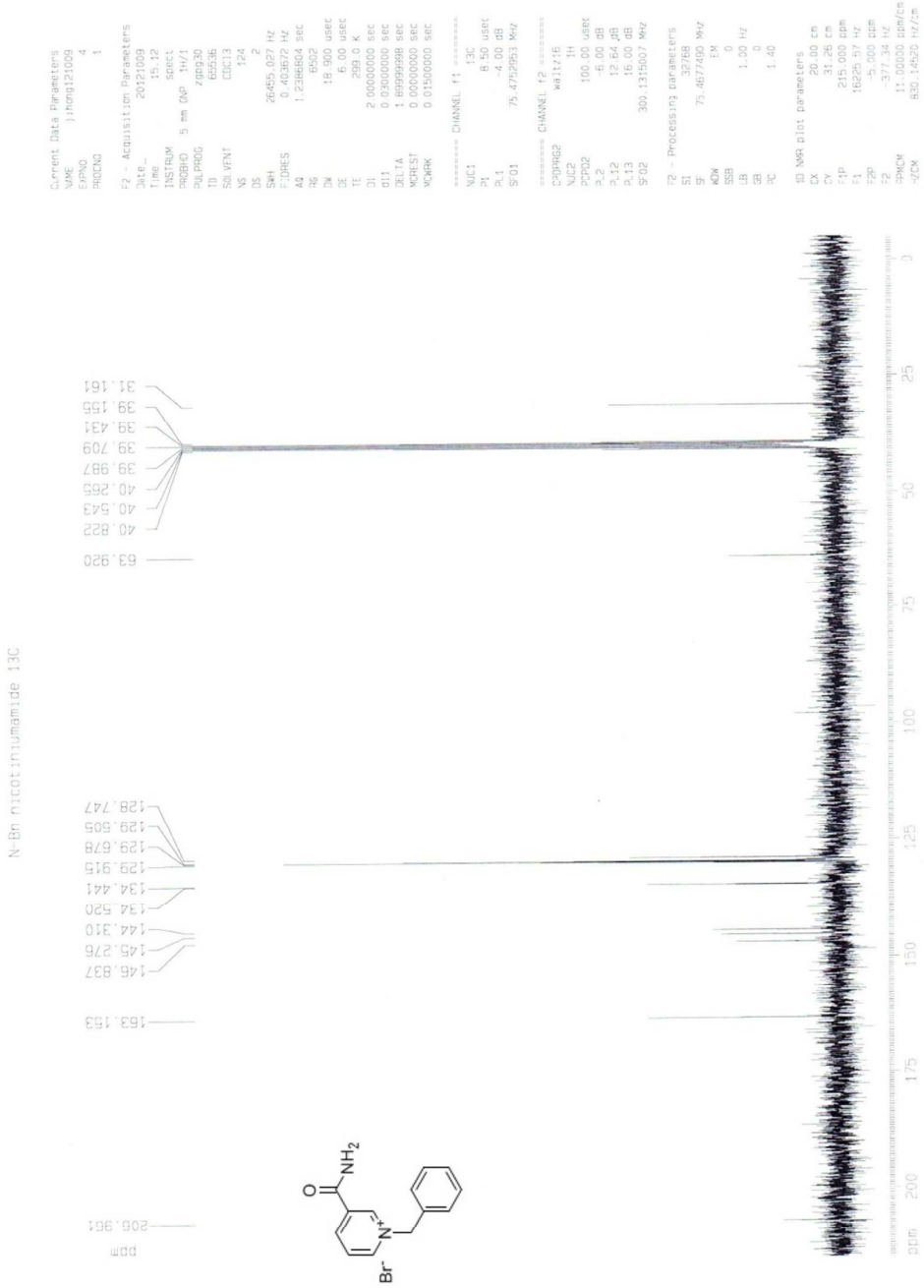
12) Figure S27. ^{13}C NMR spectrum of **PyDPA**



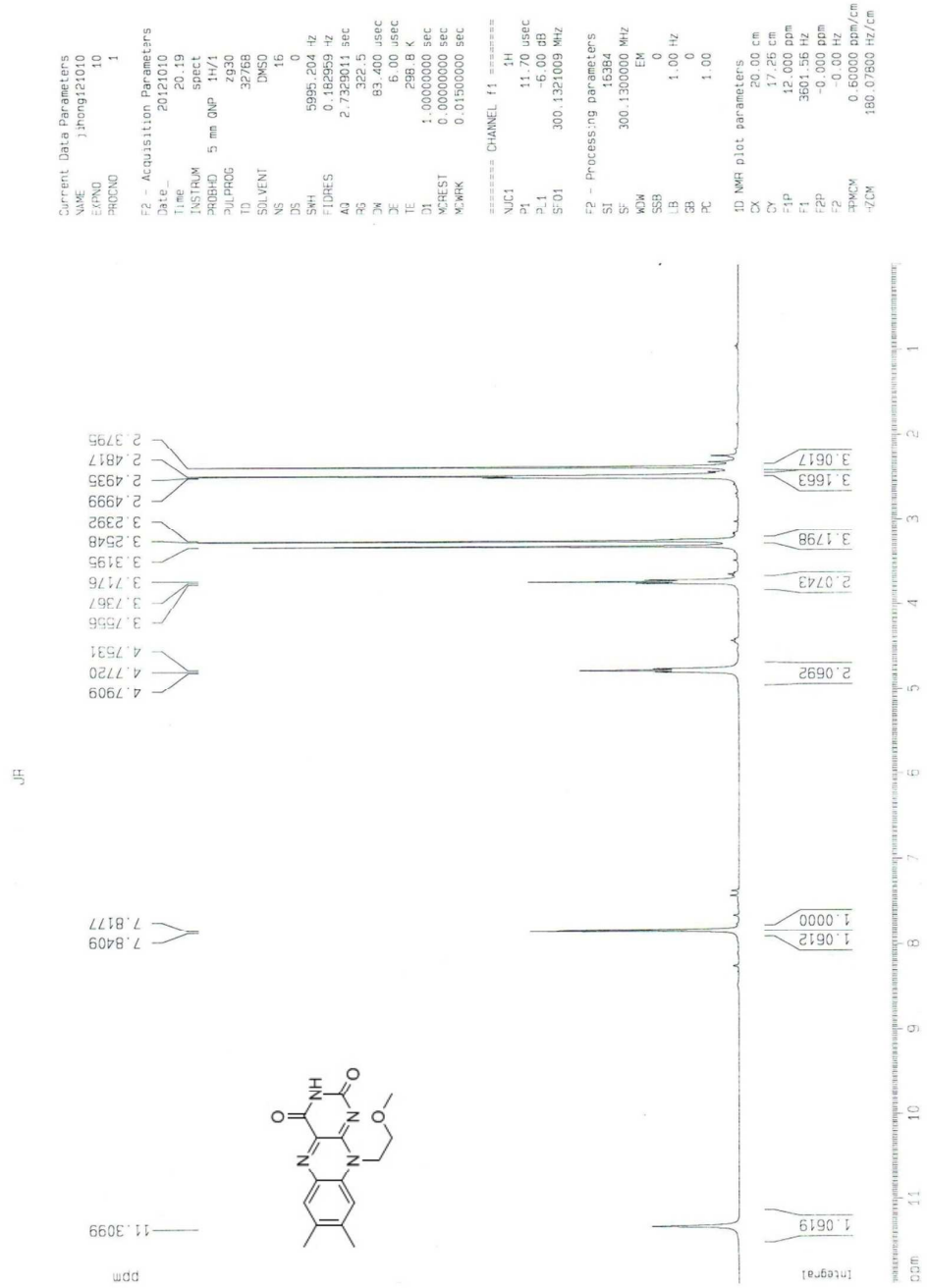
13) Figure S28. ^{1}H NMR spectrum of **BnN**



14) Figure S29. ^{13}C NMR spectrum of **BnN**



15) Figure S30. ^{1}H NMR spectrum of **MEI**



16) Figure S31. ^{13}C NMR spectrum of **MEI**