Supplementary Information

Macrocyclic enforcement of Twist in a Secondary Amide: Reactivity and Influence on Photoisomerisation

Contents

1. **Materials and methods**

Ortho-phenylenediamine, 2,6-Pyridinedicarboxylic acid, TBAOH were purchased from Sigma-Aldrich Chemical Company. Potassium tertiary butoxide, thionyl chloride, Lithium hexamethyldisilazine (LiHMDS), Triethylamine and Lawesson's reagent were purchased from Spectrochem. These chemicals were used as received. Spectroscopy-grade solvents purchased from commercial sources were used for spectroscopic analyses. Solvents (DMF, DCM, Ethanol, and Methanol) were dried and purified using literature procedures.

Nuclear Magnetic Resonance (NMR): Nuclear Magnetic Resonance (NMR) studies were performed on Bruker Ultra Shield (500 MHz) spectrometer with tetramethylsilane (TMS) as the internal standard.

High-resolution mass spectrometry (HRMS): The HRMS data of the compound was recorded using Bruker MicrOTOF-Q-II mass spectrophotometer in electrospray ionization (ESI) mode.

FT-IR analysis: FT-IR spectra of the solid cDP2 powder was recorded on a Perkin-Elmer spectrum two FT-IR spectrometer (UATR Two) using ATR method.

UV-Vis studies: Photo-isomerization of cDP2 was studied using UV-Vis spectroscopy. In all experiments 20 μM of cDP2 was used and measurements were carried out on an Analytikjena SPECORD 210 plus spectrometer.

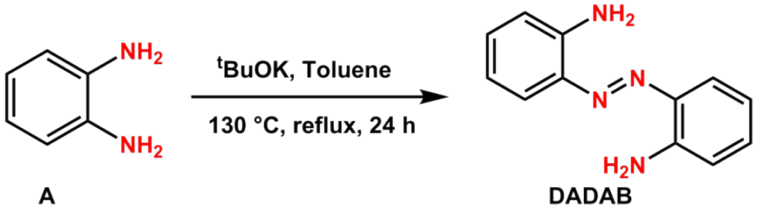
Single crystal X-ray diffraction (SC-XRD): Bright yellow plate shape crystals of cyclo-DiazoP2 (cDP2) were grown from THF under ambient conditions. Diffractable crystals were obtained after 10 to 15 days. Single-crystal X-ray diffraction data were recorded using graphite monochromatic Mo Kα ($\lambda = 0.71073$ Å). The data were collected at 100 K with an exposure time of 8 s per frame and at the crystal-to-detector distance of 6 cm. The data collection, integration, unit cell measurements, scaling, and absorption corrections were done using Bruker Smart Apex II software. The intensity data are processed by the Bruker SAINT Program suite. The crystal structure was refined by the full-matrix least-squares method using SHELXL97 present in the program suite WinGX (version 2014.1).27.

DFT calculations

The molecular structure optimization of the compounds was performed through DFT calculations at B3LYP level combined with 6-311 G (d,p) basis sets using Gaussian 09 program[1].

2. Synthetic procedures and chemical characterizations

2.1 Synthesis of DADAB and its characterization



Scheme S1. Synthesis of DADAB

o-phenylenediamine **(A)** (1 g, 9.2 mol) and potassium tert-butoxide (1.5 g,13.4 mol) was dissolved in 100 ml toluene in a round bottom flask. The solution was refluxed for 24 h at 130 °C. The progress of the reaction was monitored through TLC. The dark red coloured reaction mixture was cooled to 0 °C and passed through solid Na_2SO_4. The filtrate was evaporated under vacuum and purified by column chromatography (20% v/v Ethyl acetate in Hexane). The purified product was obtained as dark red crystalline solids in 60% yield.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ 7.60 (d, J = 8.15 Hz, 2H), 7.10 (t, J = 7.64 Hz, 2H), 6.74-6.67 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ = 143.13, 137.75, 131.38, 124.26, 117.66, 117.06

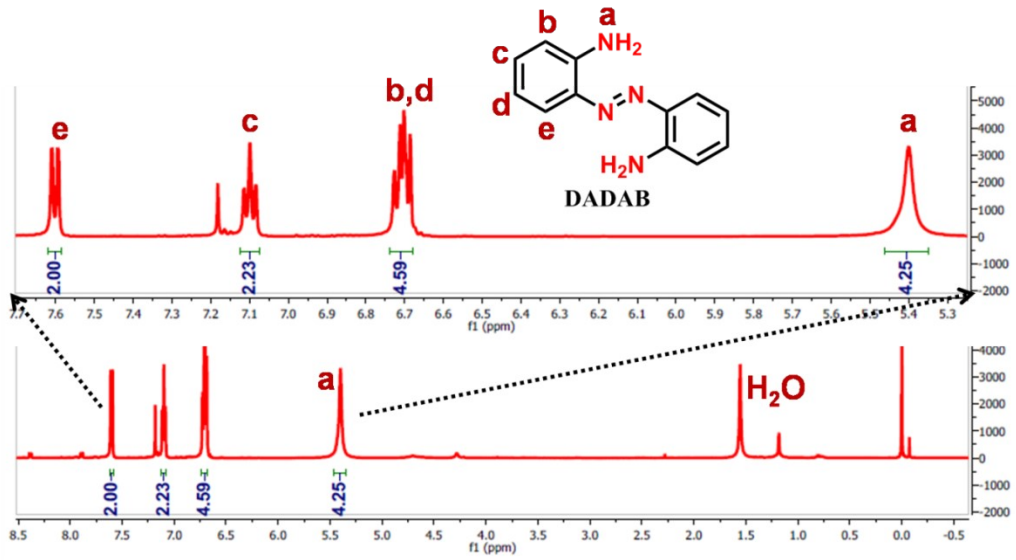


Figure S1. ^{1}H NMR (500 MHz, 298 K) of DADAB in $CDCl_3$.

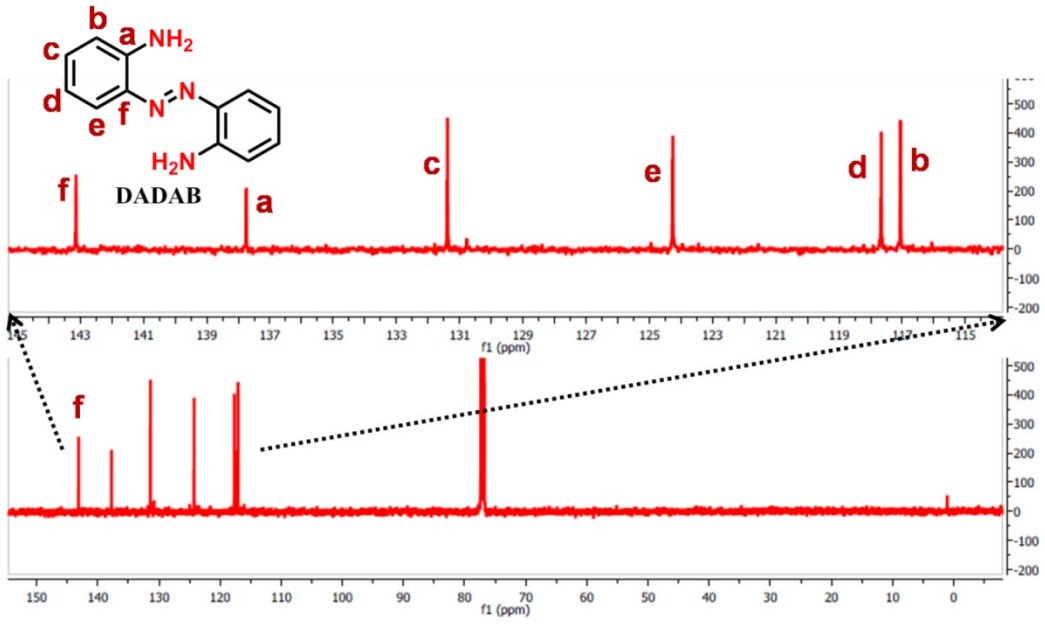


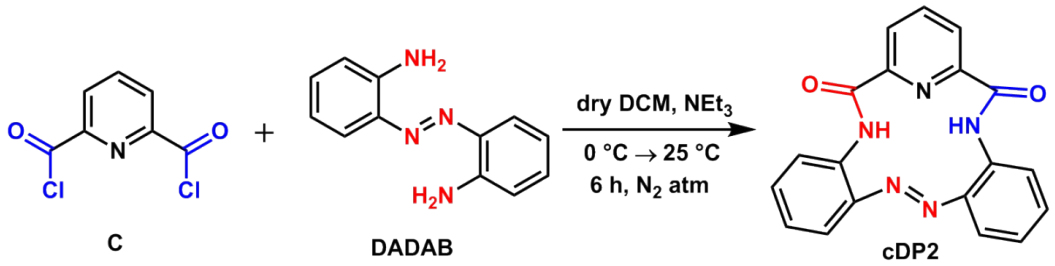
Figure S2. ^{13}C NMR (125 MHz) of DADAB in $CDCl_3$.

2.2 Synthesis of pyridine-2,6-dicarbonyl dichloride

Scheme S2. Synthesis of pyridine-2,6-dicarbonyl dichloride **(C)**.

25 ml round bottom flask was charged with (500 mg, 3 mmol) pyridine-2,6-dicarboxylic acid. 2 drops of DMF was added as a catalyst, followed by 4 ml of thionyl chloride under N_2 atmosphere. The reaction mixture was refluxed for 12 h at 90 °C. Completion of reaction was monitored by TLC. After completion solvent was evaporated under reduced pressure resulting in white solids and used in further reaction without further purification.

2.3 Synthesis of cycloDiazoP2 and characterisation



Scheme S3. Synthesis of cycloDiazoP2 (**cDP2**).

1 g of **C** (4.9 mmol) and 1g of DADAB (4.9 mmol) were separately dissolved in dry DCM (20 ml each) under constant N_2 purging. 20 µl Et_3N was to solution of DADAB and stirred for 30 min at 0 °C. Solution of DADAB was added dropwise to **C** over a period of 1 h at 0 °C followed by evolution of HCl (g). Then reaction mixture was stirred for another 5 h at 25 °C. Completion of reaction was monitored by TLC. The solvent was evaporated under vacuum and purified by column chromatography (30% v/v Ethyl acetate in Hexane). Pure product was isolated as yellow powder (1.3 g, 80% yield).

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ 13.70 (s, 2H), 8.42 (d, J = 7.6 Hz, 2H), 8.19 (d, J = 7.49, 2H), 8.10 (t, *J* = 7.08, 1H), 7.93 (dd, *J* = 6.88, 2H), 7.45 (dt, *J* = 6.25, 2H), 7.19 (dt, *J* = 6.73, 2H);^{13}C NMR (175 MHz, $CDCl_3$) δ 161.46, 147.06, 141.44, 140.49, 133.27, 124.51, 124.38, 124.14, 124.12, 119.62. ; HRMS (ESI): m/z calculated for $C_{19}H_{13}N_5O_2$ = 344.1142, Observed m/z = 344.1141.

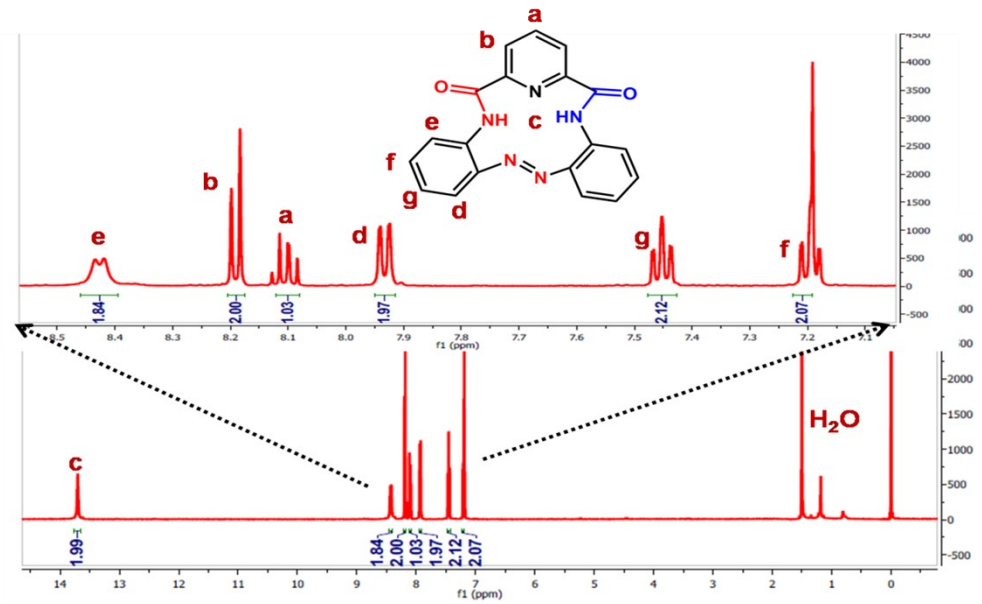


Figure S3. ^{1}H NMR (500 MHz, 298 K) of cDP2 in $CDCl_3$.

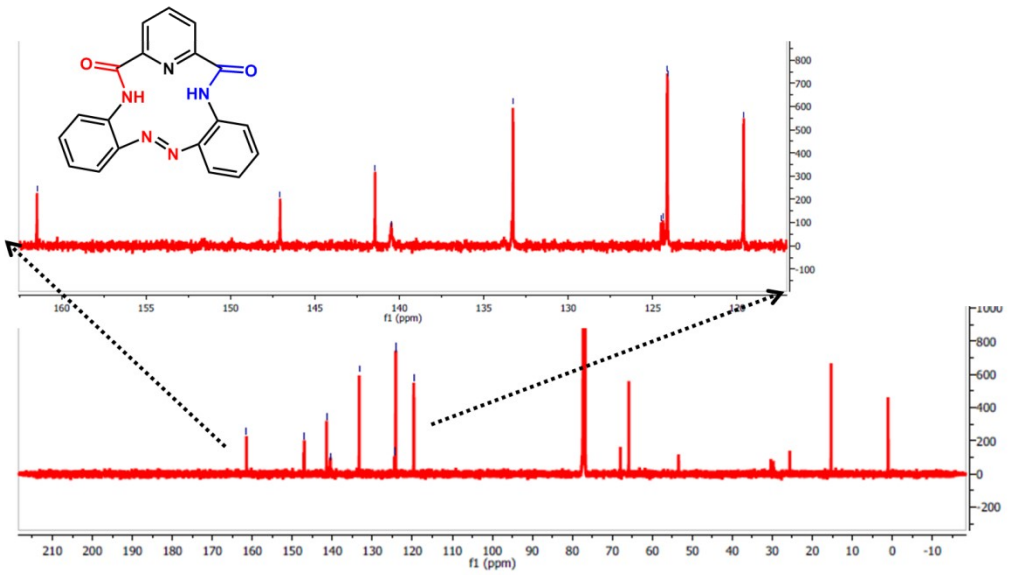


Figure S4. ^{13}C NMR (175 MHz, 298 K) of cDP2 in $CDCl_3$.

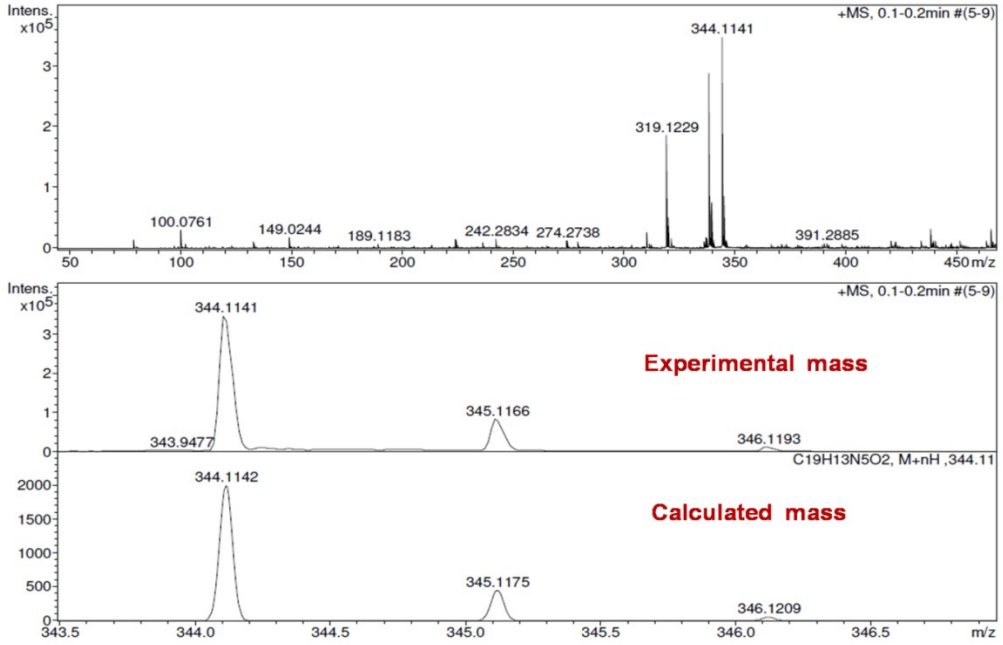


Figure S5. ESI-MS spectra of cDP2.

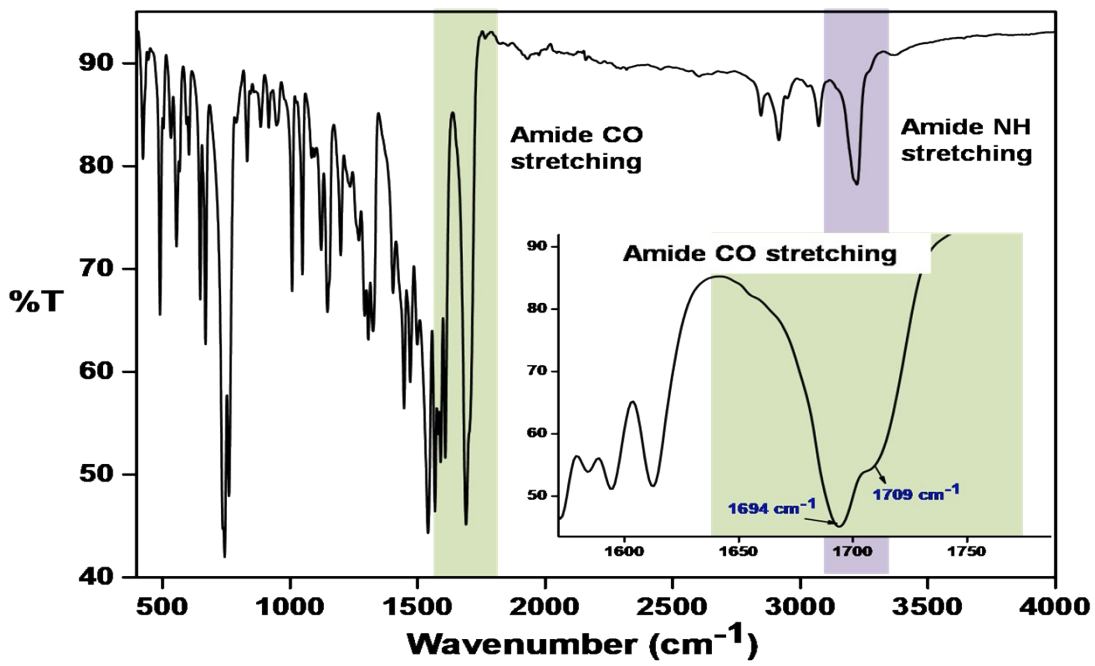
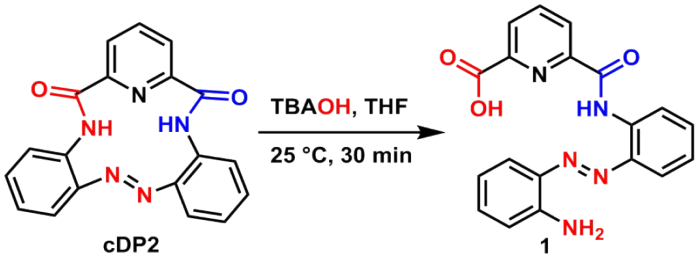


Figure S6. FTIR-spectrum of cDP2. Inset highlights the amide CO stretching band centered at 1694 cm^{-1} and exhibiting a shouldering band at 1709 cm^{-1}. The amide NH stretching was centered at 3229 cm^{-1}.

2.4 Synthesis of 1 and its characterization



Scheme S4. Base induced cleavage of cDP2 macrocycle.

1 eq cDP2 was dissolved in 10 ml THF. 10 eq TBAOH was added dropwise to it at 25 °C. The yellow coloured solution instantly turns red. The reaction mixture was further stirred for 30 min at 25 °C. Completion of reaction was confirmed by TLC. Then the solvent was evaporated under reduced pressure to get a dark red viscous liquid. The viscous liquid was redissolved in DCM and acidified with 1 N HCl and Neutralised by saturated Na_2HCO_3 solution. The aqueous layer was extracted with DCM. Combined organic layer was washed with brine and distilled water and evaporated under reduced pressure to get orange solids in 95% yield.

Base hydrolysis of the twisted amide can be carried out using similar procedure. 100 eq of NaOH dissolved in minimum amount of distilled water was added to 1 eq cDP2 in THF. The reaction mixture was heated at 70 °C for 6 h. After completion of reaction 1 N HCl was added to neutralise the reaction mixture. The aqueous layer was extracted with DCM. Combined organic layer was washed with brine and distilled water and evaporated under reduced pressure to get orange solids in similar yields as above procedure.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.28 (s, 1H), 8.77 (d, *J* = 8.2 Hz, 1H), 8.57 (d, *J* = 7.8 Hz, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 8.12 (t, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.85, 161.44, 149.87, 149.41, 148.11, 141.45, 140.44, 136.90, 136.20, 134.21, 131.63, 128.32, 125.72, 124.72, 120.64, 118.77, 117.46, 116.82, 116.65. HRMS (ESI): m/z + 1 calculated for $C_{19}H_{15}N_5O_3$ = 362.1278 g·mol^{-1}, Observed m/z + 1 = 362.1248 g·mol^{-1}.

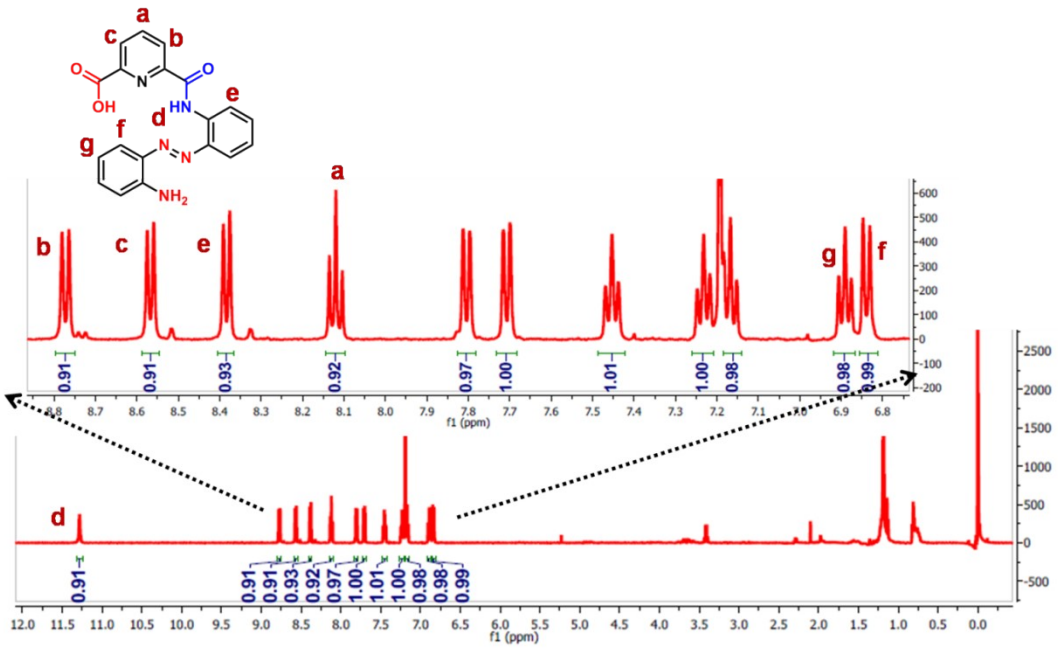


Figure S7. ^{1}H NMR (500 MHz, 298K) of **1** in $CDCl_3$.

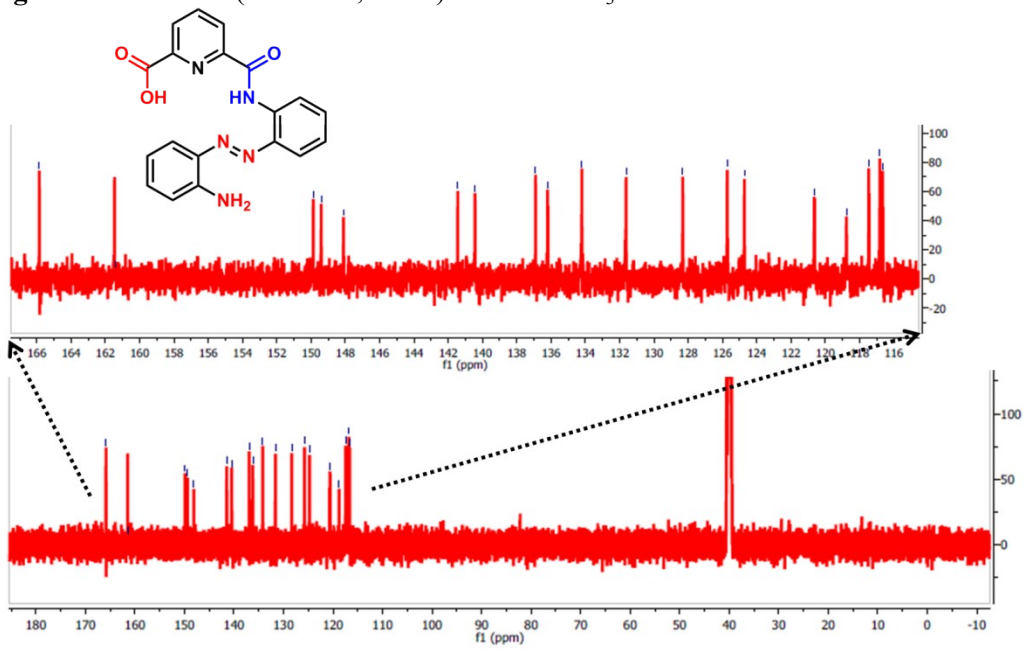


Figure S8. ^{13}C NMR (125 MHz, 298K) of **1** in DMSO-d_6 .

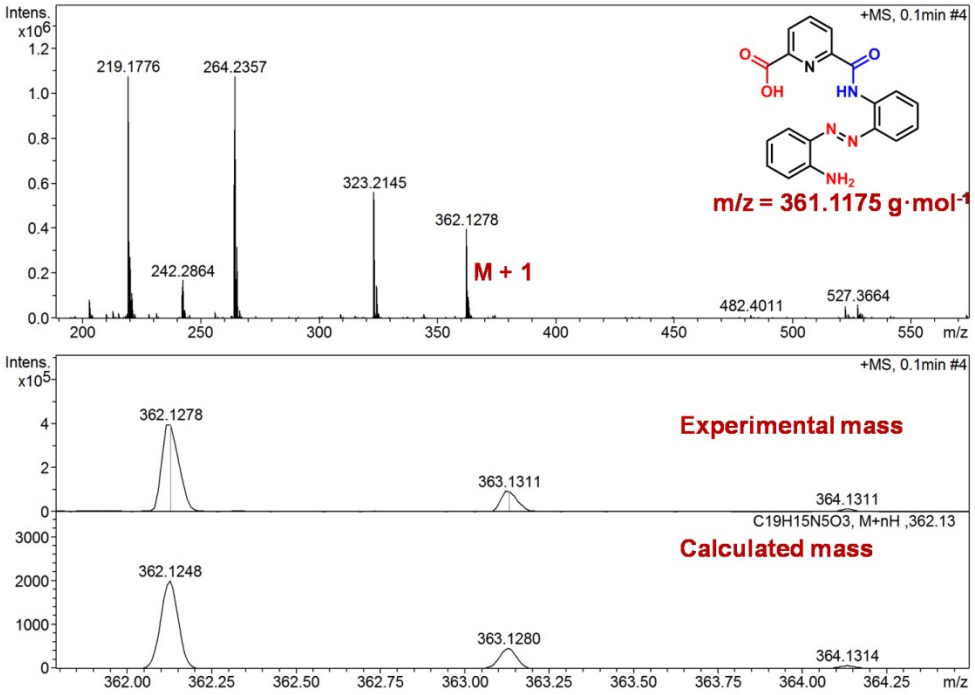
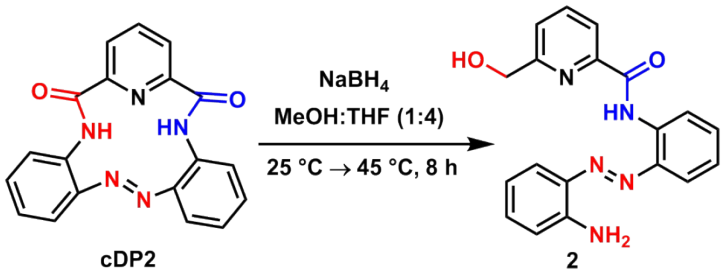


Figure S9. ESI-MS of **1**.

2.5 Synthesis of 2 and characterization



Scheme S5. Reduction of cDP2 using sodium borohydride to generate alcohol **2**.

50 mg of cDP2 was dissolved in 8 ml of THF in a 25 ml round bottom flask. 10 eq of was dissolved in 2 ml of methanol and added to cDP2 solution at 25 °C. Then the reaction mixture was heated at 45 °C for 8 h. Completion of reaction was indicated by colour change from yellow to red and by TLC. The solvent was evaporated under reduced pressure and purified by column chromatography (30% Ethyl acetate: Hexane). Product 2 was isolated as red crystalline solid in 95% yield.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ 11.67 (s, 1H), 8.77 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.89 – 7.79 (m, 2H), 7.69 (d, J = 8.1 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.11 (t, J = 7.7 Hz, 1H), 6.80 (dd, J = 14.7, 7.5 Hz, 2H), 5.34 (s, 2H), 4.81 (s, J = 6.1 Hz, 2H), 3.01 (t, J = 6.1 Hz, 1H).; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ 161.84, 158.90, 149.67, 144.60, 141.03, 138.43, 138.27, 136.13, 133.13, 131.82, 123.90, 123.82, 122.71, 121.50, 120.25, 118.21, 117.51, 116.13, , 65.08

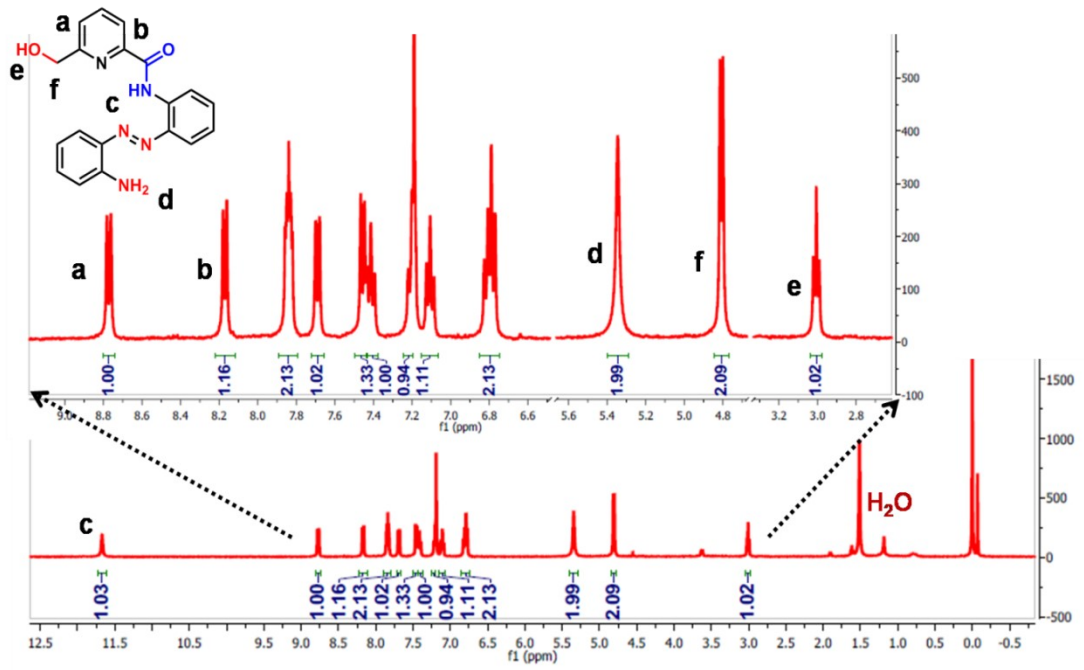


Figure S10. ^{1}H NMR (500 MHz, 298 K) of **2** in $CDCl_3$.

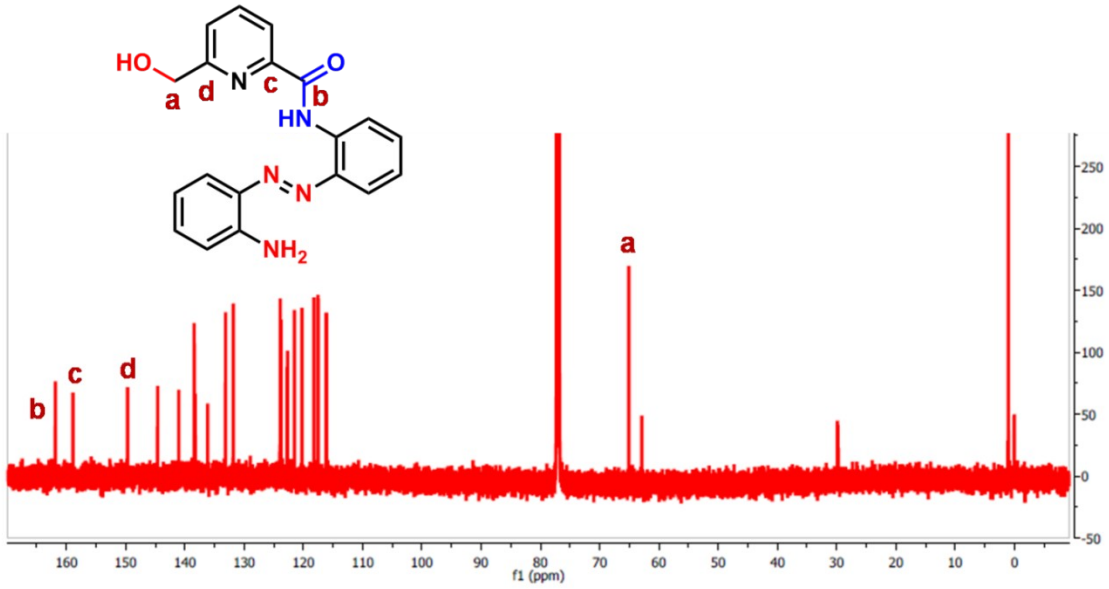
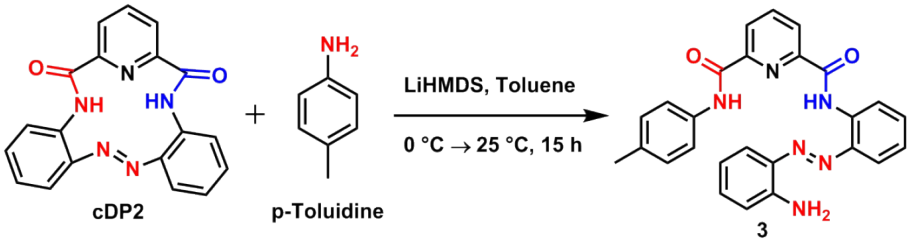


Figure S11. ^{13}C NMR (125 MHz, 298 K) of **2** in $CDCl_3$.

2.6 Synthesis of 3 and characterization



Scheme S6. Transamidation of cDP2 using *para*-toluidine.

100 mg of cDP2 (0.29 mmol) was dissolved in 50 ml of toluene in a 100 ml round bottom flask and N_2 was purged into it. 45 mg *p*-Toluidine (0.43 mmol) was dissolved in 10 ml toluene and mixed under N_2. Then, LiHMDS (5eq) was added dropwise to the reaction mixture under N_2 atmosphere at 0 °C. Reaction mixture was stirred for 1 h in 0 °C then left at 25 °C for 14 h. The completion of reaction was monitored through TLC and crude product was purified through column chromatography (20% Ethyl acetate: Hexane). Pure product was isolated as reddish-brown powder in 30% yield.
^{1}H NMR (500 MHz, $CDCl_3$): δ 11.32 (s, 1H), 9.42 (s, 1H), 8.74 (d, *J* = 8.38 Hz, 1H), 8.47 (dd, *J* = 7.89 Hz, 3.13 Hz, 2H), 8.10 (t, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 8.1 Hz, 1H), 7.47 (dd, *J* = 17.4, 8.5 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 6.88 (d, *J* = 8.1 Hz, 2H), 6.84 (t, *J* = 7.36 Hz ,1H), 6.41 (d, *J* = 8.2 Hz, 1H), 6.32 (t, *J* = 7.6 Hz, 1H), 5.41 (s, 2H), 2.22 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.27, 160.94, 149.42, 143.78, 141.35, 139.59, 137.82, 135.33, 134.37, 134.10, 133.28, 132.97, 131.67, 129.35, 125.71, 125.65, 124.46, 124.14, 120.74, 120.12, 118.27, 117.26, 117.11, 20.91

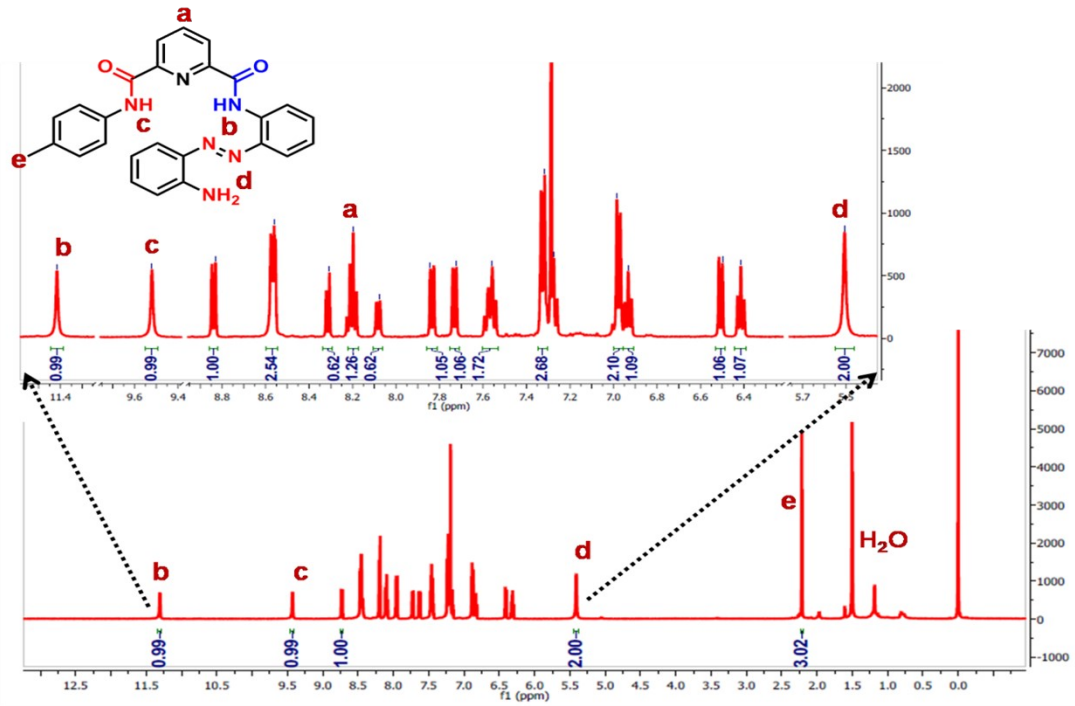


Figure S12. ^{1}H NMR (500 MHz, 298 K) of 3 in $CDCl_3$.

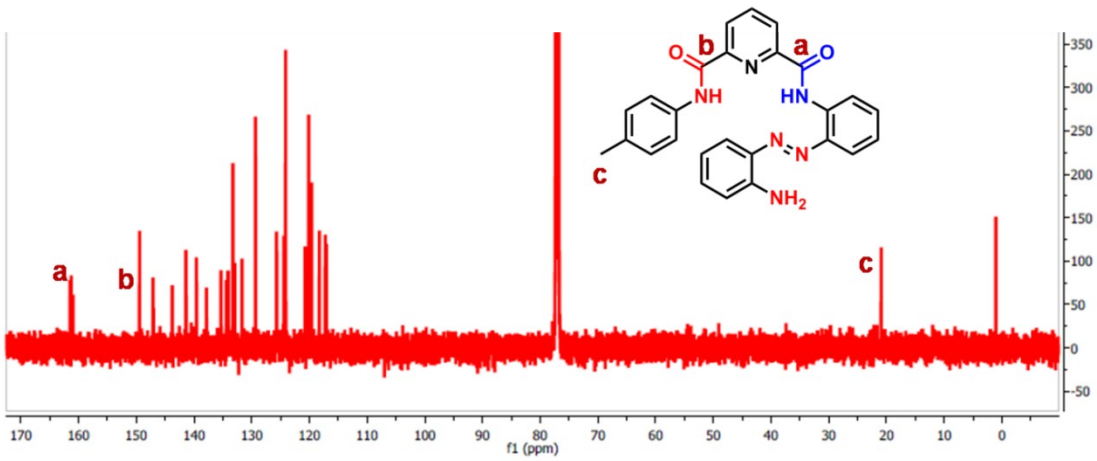


Figure S13. ^{13}C NMR (125 MHz, 298 K) of **3** in $CDCl_3$.

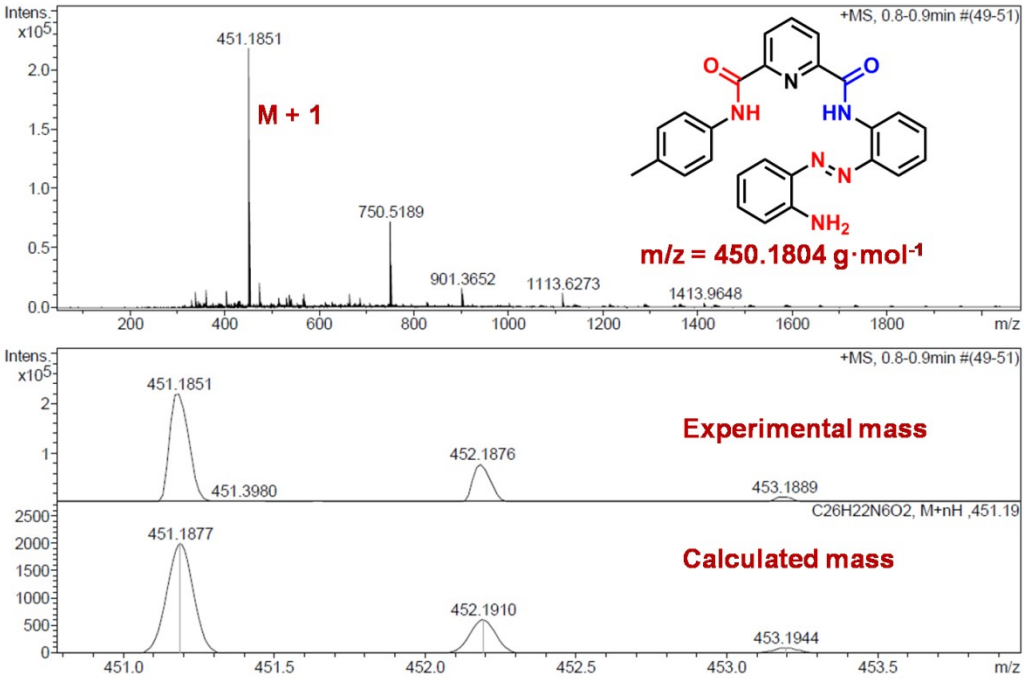
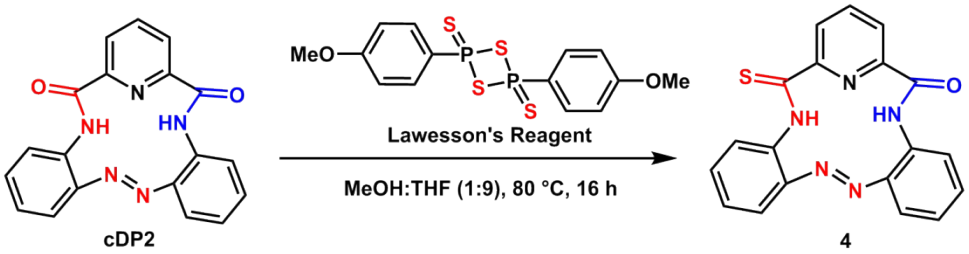


Figure S14. ESI-MS of **3**.

2.7 Synthesis of 4 and its characterization



Scheme S7. Reaction of **cDP2** with Lawesson's reagent to yield the thioamide **4**.

cDP2 (100 mg, 0.29 mmol) was dissolved in 45 ml of THF then 5 ml of Methanol was added to it in a 100 ml round bottom flask. Lawesson's Reagent (522 mg, 1.45 mmol) was added to the reaction mixture at 25 °C. Reaction mixture was then refluxed at 80 °C for 16 h. Yellow colored initial solution turned orange after completion of reaction checked through TLC. The reaction mixture was filtered while hot to remove any unreacted Lawesson's reagent. The filtrate was evaporated under reduced pressure and product was purified by column chromatography ($CHCl_3$). The pure product was isolated as orange solids in 80 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 15.69 (s, 1H), 13.90 (s, 1H), 9.09 (d, J = 8.2 Hz, 1H), 8.80 (d, J = 8.3 Hz, 1H), 8.49 (d, J = 7.7 Hz, 1H), 8.19 (d, J = 7.5 Hz, 1H), 8.08 (dd, J = 7.4, 5.5 Hz, 2H), 7.86 (d, J = 8.3 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.31 – 7.22 (m, 2H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 182.54, 161.82, 148.68, 144.69, 141.94, 141.15, 138.81, 137.00, 133.56, 133.17, 130.56, 125.82, 124.99, 124.19, 123.81, 119.30, 118.85, 115.28.

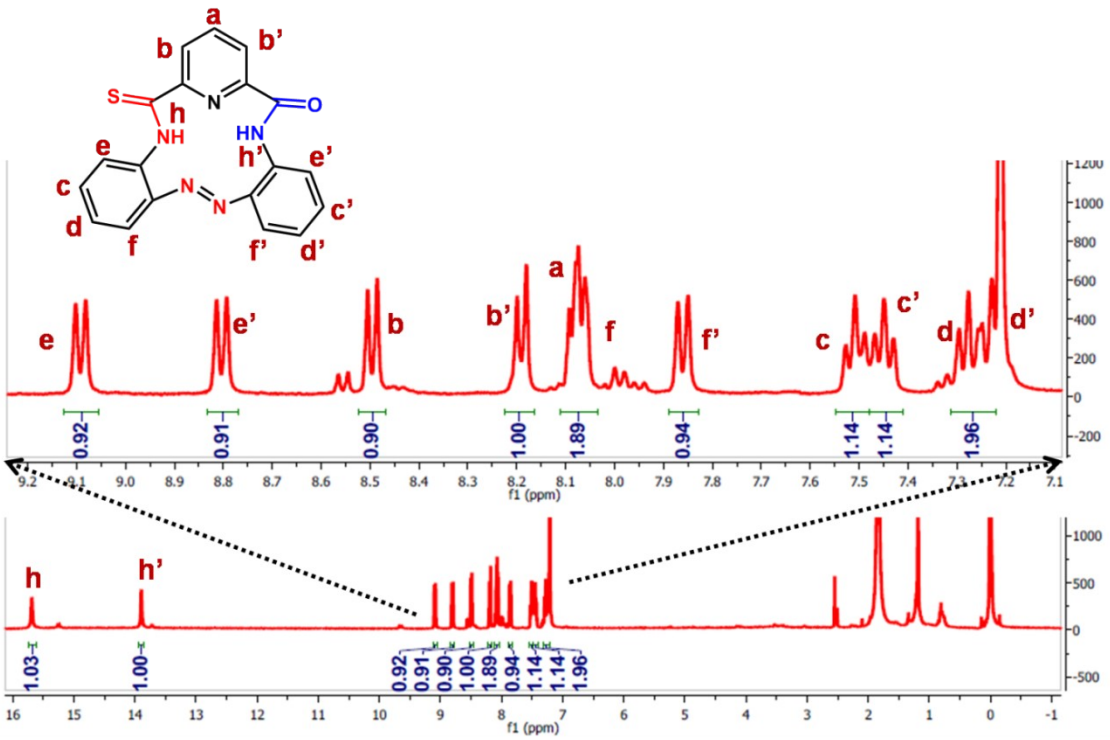


Figure S15. ^{1}H NMR (400 MHz, 298 K) of **4** in $CDCl_3$.

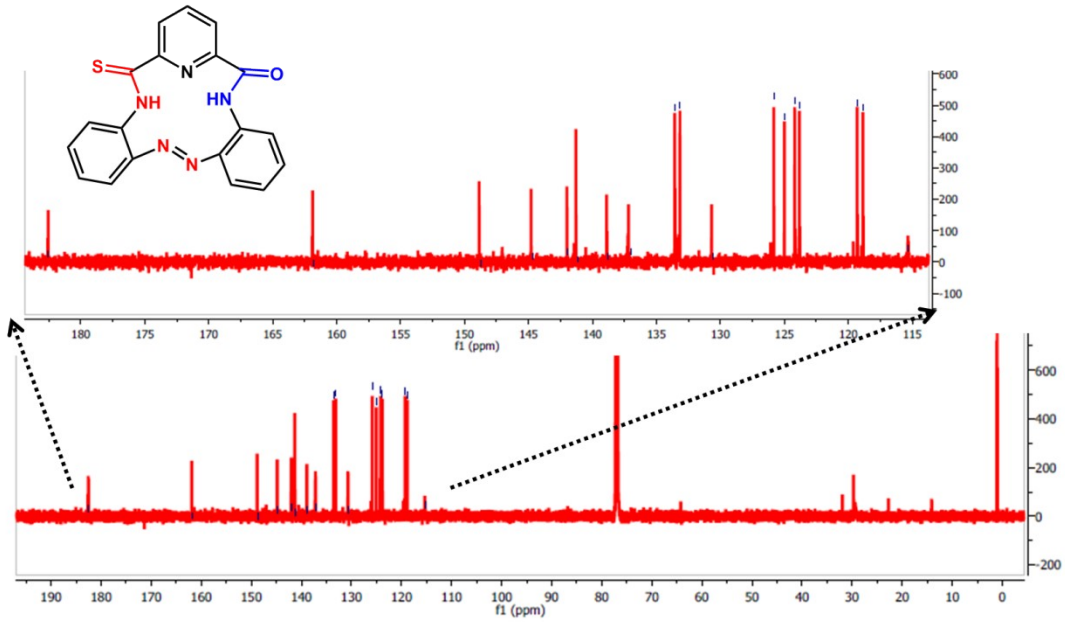
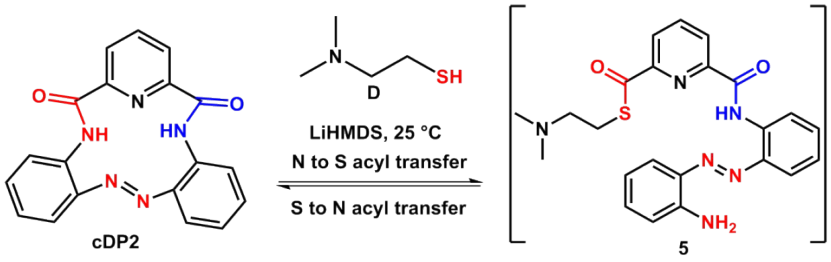


Figure S16. ^{13}C NMR (175 MHz, 298 K) of **4** in $CDCl_3$.

2.8 Synthesis of 5 and its characterization



Scheme S7. Formation of **5** (not isolated) by the reaction of cDP2 and 2-(dimethylamino)ethane-1-thiol in THF using LiHMDS.

cDP2 (100 mg, 0.29 mmol) and 2-(dimethylamino)ethane-1-thiol 'D' (61 mg, 0.58 mmol) was dissolved in 50 ml THF in a 100 ml round bottom flask. LiHMDS (1.45 mmol) was diluted in 5 ml THF and slowly added to the reaction mixture at 25 °C. The colour of reaction mixture changes from yellow to dark. As the reaction progresses the colour again turned to yellow in 15 minutes. Addition of another 1.45 mmol of LiHMDS again returned the reaction mixture to red. ESI-MS of the red solution hints at the formation of product 5. After confirming completion of reaction by TLC, solvent was evaporated in reduced pressure to get viscous red mass. However, purification through solvent extraction or column chromatography yielded cDP2 in more than 90% yield.

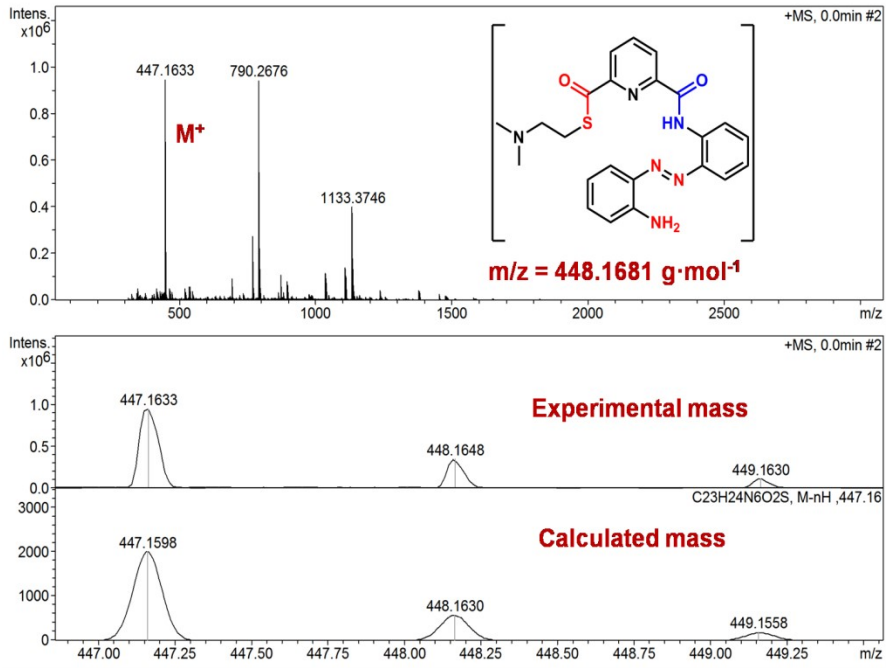


Figure S17. ESI-MS of **5**.

3. The *trans* to *cis* photo-isomerization of DADAB

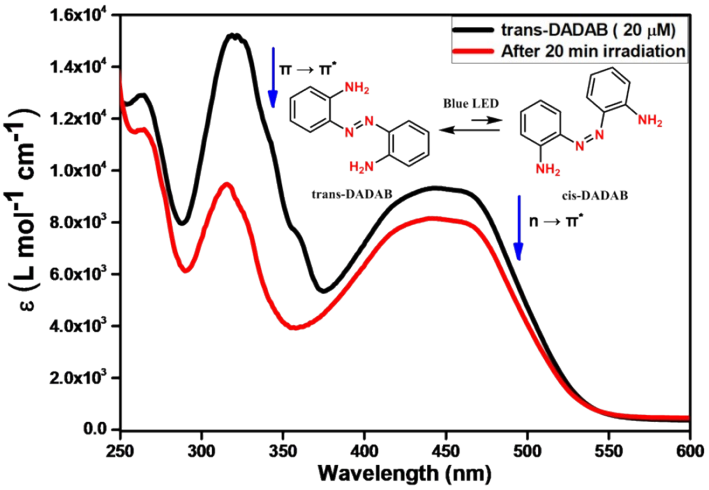


Figure S18. The UV-vis changes during the photo-isomerization of DADAB (20 μM in $CHCl_3$ at 298 K) before (black profile) and after irradiation with blue LED (red profile) to get *cis*-DADAB.

4. The *trans* to *cis* photo-isomerization of cDP2

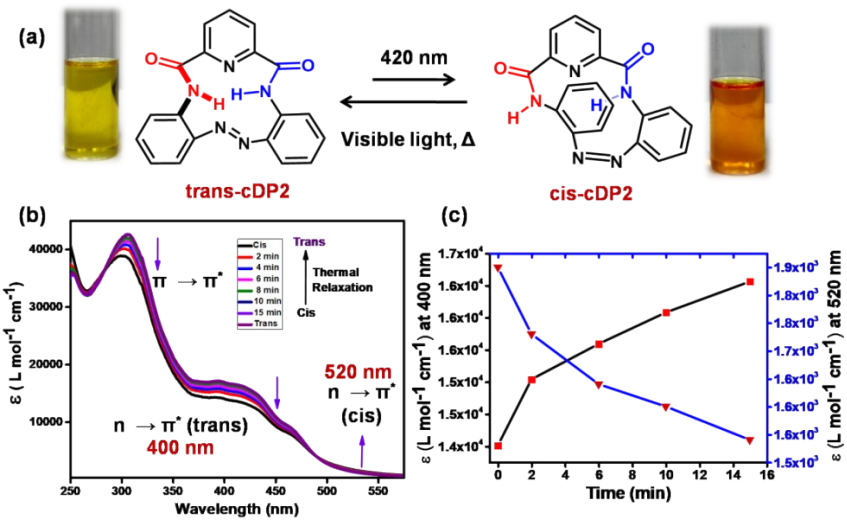


Figure S19. (a) Photo-isomerization of cDP2. Inset show colour change before and after irradiation. (b) Thermal relation of *cis*-cDP2 after 15 min irradiation in $CHCl_3$. The isomerisation was followed through UV-Vis spectroscopy after every 2 min. *Cis*-cDP2 converts into *trans*-cDP2 at 298 K in dark in 30 min. (c) n to π^* transition from *cis*-cDP2 at 520 nm decreases with thermal relaxation under dark while the n to π^* transition from *trans*-cDP2 at 400 nm increases with as the *cis*-isomer converts back to *cis*-cDP2.

5. The *trans* to *cis* photo-isomerization of cDP2 through ^{1}H NMR

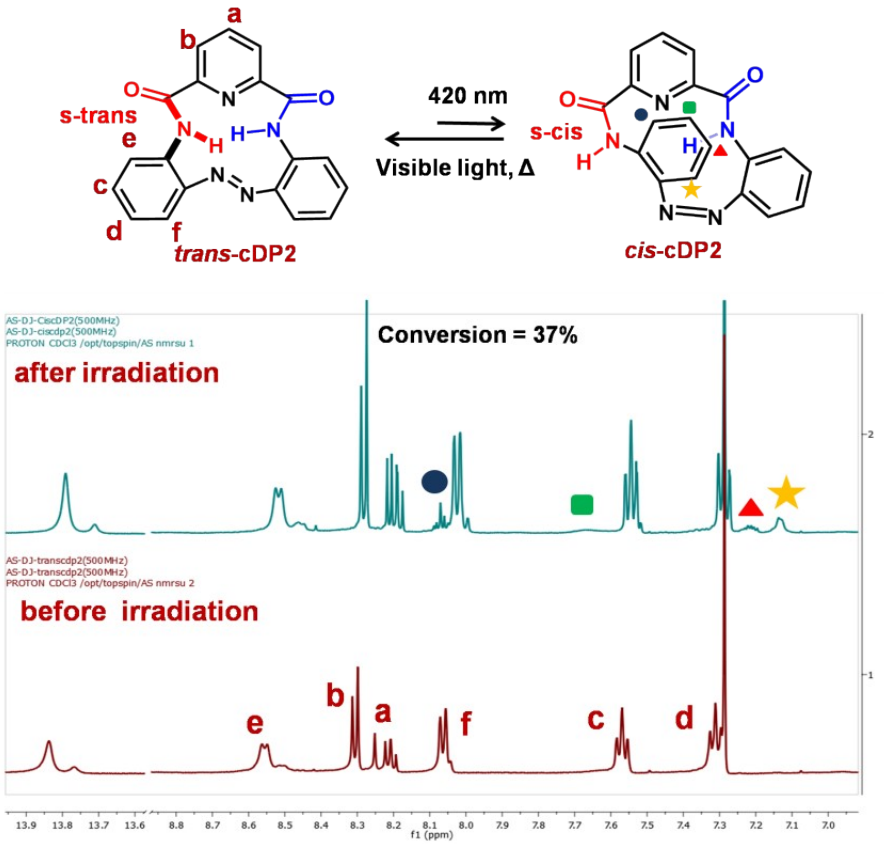


Figure S20: Changes in the ^{1}H NMR (500 MHz, 298 K) in $CDCl_3$ before and after irradiation with blue LED for 30 min.

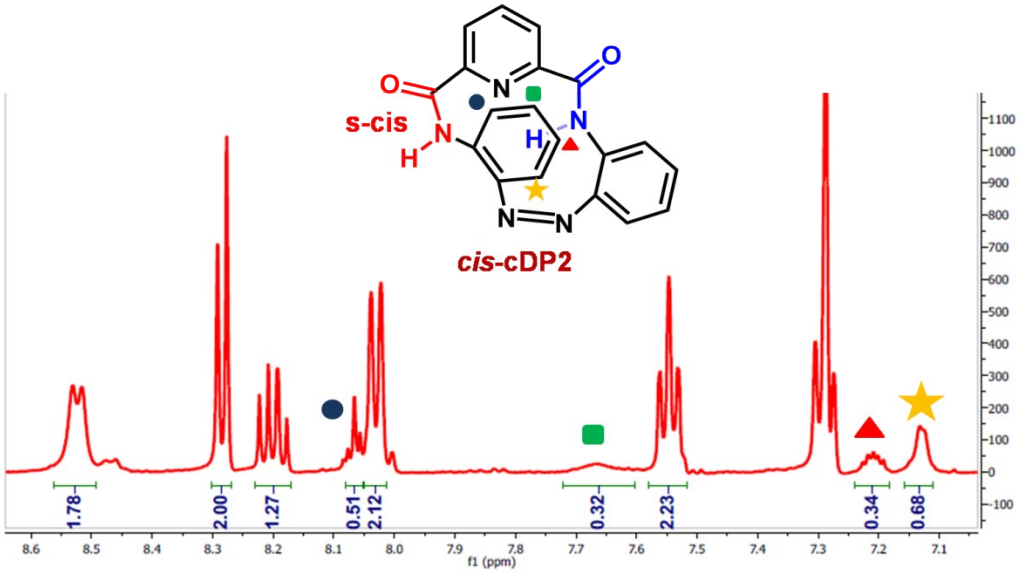


Figure S21: Enlarged ^{1}H NMR (500 MHz, 298 K) of cDP2 after photo-irradiation by blue LED

6. SC-XRD data

Identification code	**CCDC-2202808**	**CCDC-2202809**
Empirical formula	C19 H13 N5 O2	C19 H17 N5 O2
Formula weight	343.34	347.38
Temperature/K	140	140
Crystal system	Monoclinic	Monoclinic
Space group	P 21/n	P 21/c
a/Å	7.04	10.33
b/Å	18.39	18.09
c/Å	12.11	8.82
α/°	90	90
β/°	104.35	97.01
γ/°	90	90
Volume/Å^3	1520.78	1638.12
Z	4	4
ρcalc g/cm^3	1.500	1.408
μ/mm^{-1}	0.102	0.096
F(000)	712.0	728.0
Radiation	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	2.813 to 26.370	2.28 to 30.09
Reflections collected	10881	32357
Goodness-of-fit on F2	1.058	1.056
Final R indexes [all data]	R_1 = 0.0397, wR_2 = 0.1039	R_1 = 0.0779, wR_2 = 0.1523

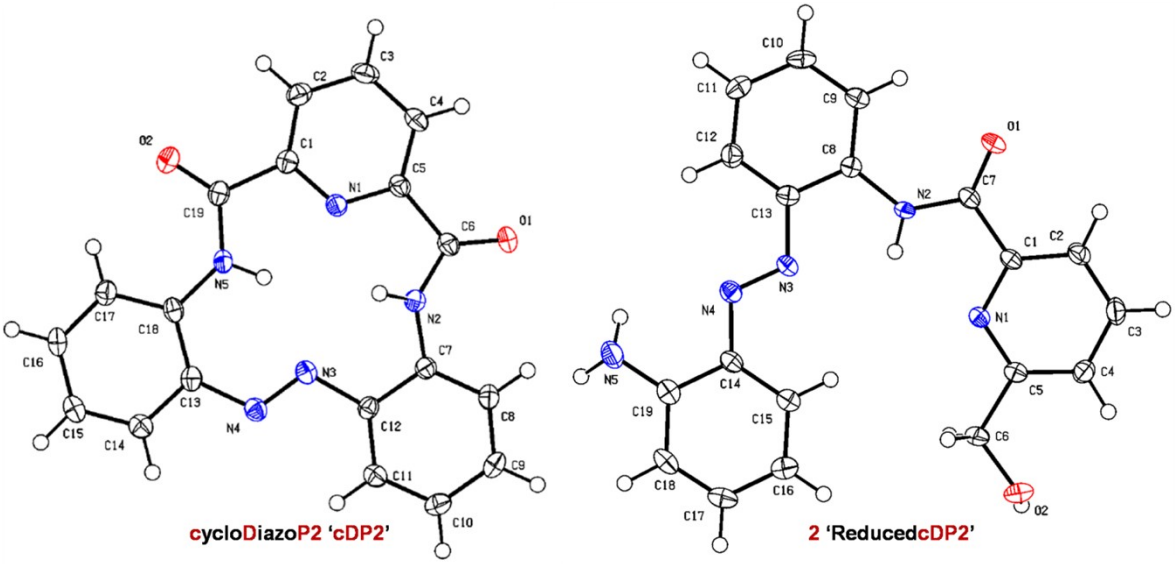


Figure S22. ORTEP diagrams of cDP2 and **2**.

7. DFT optimized structure of cis-cDP2

Structural optimization of cis-cDP2

Basis set = B3LYP

Level of theory = 6-311 +g (d,p)

Charge = 0

Multiplicity = 1

Symbol	X	Y	Z
C1	-1.26165	1.986717	0.116581
C2	-2.23086	2.900142	0.524741
C3	-3.56917	2.520231	0.40408
C4	-3.89796	1.259728	-0.0948
C5	-2.85139	0.408798	-0.45524
N6	-1.58445	0.787034	-0.35985
H7	-4.35477	3.205679	0.700778
H8	-1.93623	3.864496	0.918218
H9	-4.92484	0.937246	-0.2096
C10	-3.05927	-0.9623	-1.05591
O11	-4.04363	-1.21552	-1.72852
N12	-2.02076	-1.85325	-0.88869

H13	-2.06706	-2.65662	-1.50356
C14	0.233856	2.234666	0.223634
O15	0.703776	3.21729	0.771455
N16	0.91684	1.192327	-0.34506
H17	0.26982	0.512539	-0.73229
C18	-1.10473	-1.92722	0.204625
C19	-1.60347	-2.08323	1.497616
C20	0.295559	-1.87551	0.011598
C21	-0.74544	-2.21877	2.586325
H22	-2.67725	-2.1219	1.639624
C23	1.149292	-2.05023	1.107692
C24	0.632875	-2.22304	2.386958
H25	-1.155	-2.34878	3.581201
H26	2.219327	-2.06073	0.952236
H27	1.306923	-2.36241	3.223904
C28	2.267366	0.846561	-0.32415
C29	2.658274	-0.37711	-0.92283
C30	3.245743	1.6687	0.251229
C31	4.015646	-0.70756	-0.98028
C32	4.5815	1.283488	0.243178
H33	2.935745	2.602752	0.697846
C34	4.972977	0.093687	-0.37098
H35	4.298904	-1.61105	-1.50801
H36	5.321324	1.929284	0.701677
H37	6.017057	-0.19434	-0.39979
N38	1.795092	-1.22393	-1.71584
N39	0.763266	-1.81316	-1.3377

Item	Value	Threshold	Converged?
Maximum Force	0.000025	0.00045	YES
RMS Force	0.000004	0.0003	YES
Maximum Displacement	0.000558	0.0018	YES
RMS Displacement	0.000142	0.0012	YES

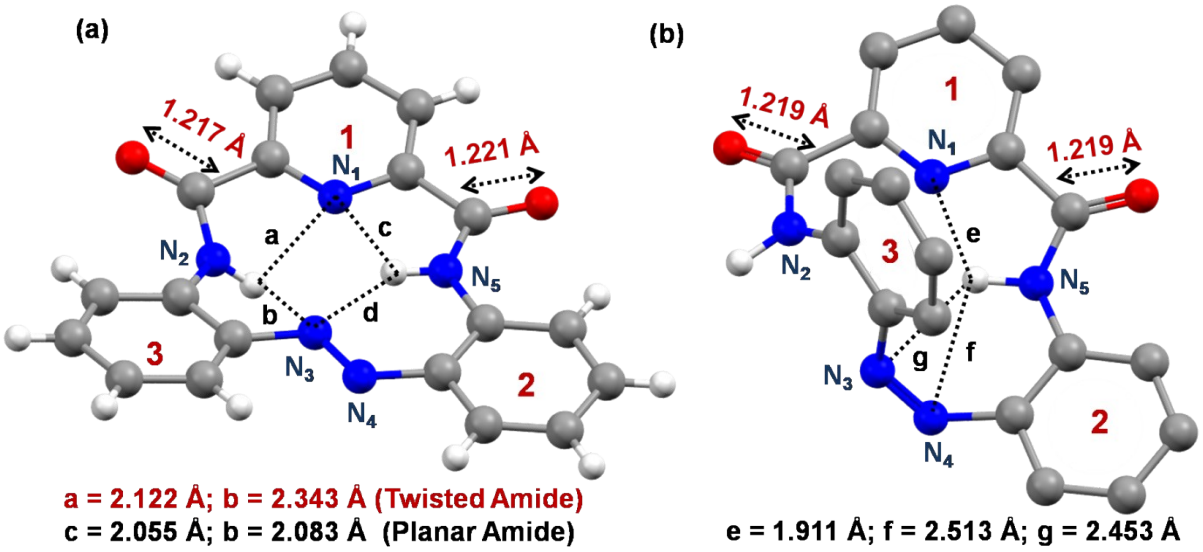


Figure S23. Comparison between *trans*-cDP2 and *cis*-cDP2. (a) SC-XRD structure of *trans*-cDP2. Amide H-bond length are mentioned below the structure. (b) DFT optimised structure of cis-cDP2 (B3LYP/6-311 G (d,p)). The twisted amide rotates from s-trans conformation to s-*cis* conformation after photo-irradiation where as planar amide remains unaffected. The s-cis amide formed after rotation is planar in nature. The phenyl ring 3 folds around diazo bond to give a folded book like structure.

8. References

1. Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, **2013**.