Supplimentary Material for

Title: Bioinspired Nanophotosensitizers: Synthesis and Characterization of Porphyrin-Noble Metal Nanoparticle Conjugates

Authors: Jayeeta Bhaumik,[1,] Gitanjali Gogia,[2] Seema Kirar,[1] Lekshmi Vijay,[2] Neeraj S. Thakur,[1] Uttam C. Banerjee[1] and Joydev K. Laha[2,*]*

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SI-1. Materials and Methods

All the reagents were obtained from commercial sources (Sigma Aldrich, Spectrochem) and were used as received. All the solvents used for reaction were purified and dried according to standard methods prior to use. Reactions were monitored by thin layer chromatography (TLC), which was performed with 0.2 mm Merck pre-coated silica gel 60 F_{254} aluminium sheets. TLC plates were visualized with UV light. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 400 MHz and 100 MHz NMR spectrometer respectively, using $CDCl_3$ or DMSO-d_6. Chemical shifts are given in ppm and *J* values are given in Hz. ^{13}C NMR spectra were fully decoupled and were referenced to the middle peak of the solvent $CDCl_3$ at 77.00 ppm. Splitting patteren were designated as s, singlet; bs, broad siglet; d, doublet; dd, doublet of doublet; dt, doublet of triplet; t, triplet; m, multiplet. Mass spectra were recorded on GCMS-QP 5000 (shimadzu) [For EI] mass spectrometer. Compounds were routinely checked for their purity on the silica gel GF-254 and visualized under UV at wavelength 254 nm. Evaporation of solvents was performed at reduced pressure using a Buchi rotary evaporator. UV-visible spectroscopy was performed on Lab India absorption spectrophotometer. HPLC data were collected from a Waters HPLC equipped with a Photodiode array detector 2998, a e2695 Separations module, and a symmetry RPC18 column with dimensions of 250 mm*4.6 mm (model WAT054275) at a flow rate of 0.3 mL/min. Isocratic solvent system was run with 90% acetonitrile–10% H_2O–0.1% TFA solvent system.

SI-2. FTIR spectra of porphyrin nanoconjugates.

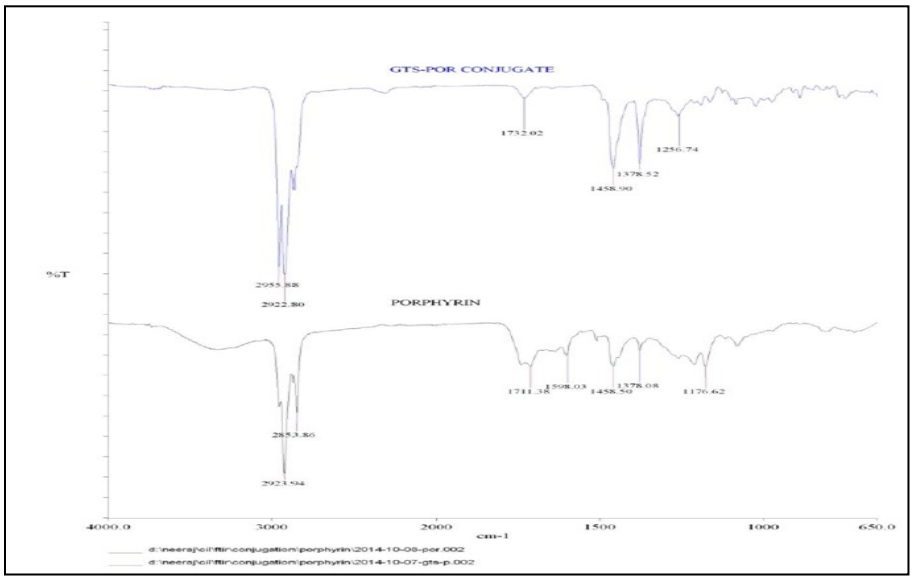


Figure S1. IR spectra of *C. sinensis* mediated synthesized AgNP-porphyrin conjugate and porphyrin **16**, overlay.

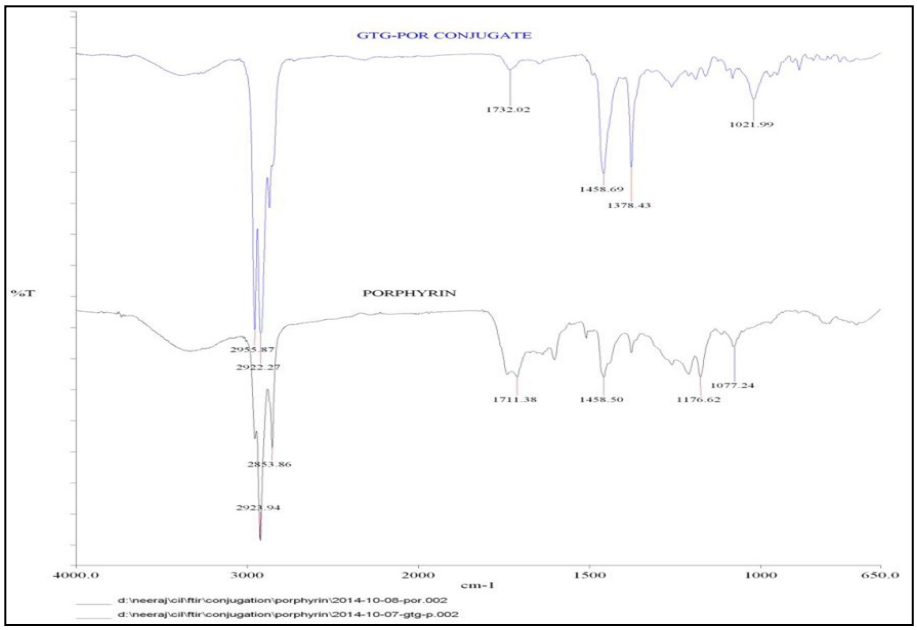


Figure S2. IR spectra of *C. sinensis* mediated synthesized AuNPs-porphyrin conjugate and porphyrin **16**, overlay.

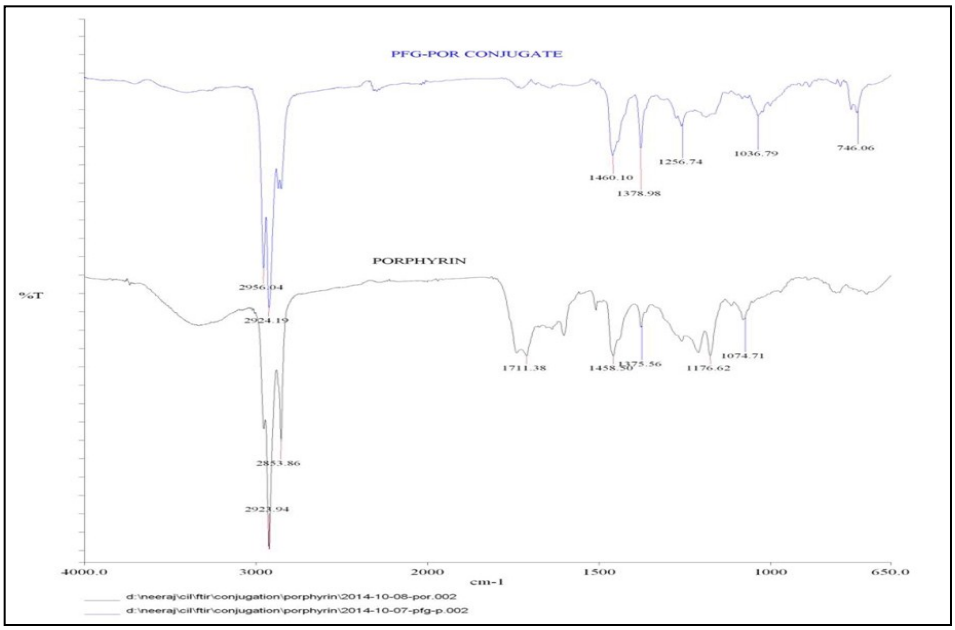


Figure S3. IR spectra of *P. fulgens* mediated synthesized AuNPs-porphyrin conjugate and porphyrin **16**, overlay.

SI-3. Estimation of porphyrin loading on nanoparticles

Drug loading calculation by absorption spectroscopy

Loading of porphyrin derivative **16** on the surface of various nanoparticles was calculated to quantify the extent of conjugation. First, the standard calibration curve of the porphyrin derivative was prepared, linear regression equations and R^2 values were also determined (Figure S4). The absorbance of supernatant solution of detached porphyrin (after treatment with NaOH solution to raise the pH) was taken at 411 nm. Concentration of the porphyrin derivative in the supernatant solution was calculated using linear regression equation. Finally, from the initial concentration of porphyrin, the percentage of the conjugated porphyrin on the surface of nanoparticles was calculated as ~12%, 10% and 12% for CS-AgNPs, CS-AuNPs and PF-AuNPs respectively (Table S1).

Drug Loading by HPLC method

Pyridyl porphyrin (**16**) loading efficiency on the surface of PF-AuNP, CS-AuNP and CS-AgNP were studied by the quantitative HPLC analysis of the supernatant solutions (after the treatment of nanoconjugates with NaOH to raise the pH) after the centrifugation at 12000g for 60 min of the reaction mixtures containing pyridyl porphyrin conjugated nanoparticles. Waters-e2695 (USA) HPLC systems controlled by Empower pro® software, equipped with auto sampler. Analytical C18 column (Phenomenex Luna 5u C18, 250 × 4.6 mm) was used for quantification.

Pyridyl porphyrin detection was done at 30 °C temperature using waters 2998 photodiode array detector at 350-550 nm. Pyridyl porphyrin (10 µL) was eluted isocratically at a flow rate of 0.5 mL/min using buffer A (acetonitrile) and buffer B (water containing 0.1% trifluoroacetic acid) as the mobile phase at the concentration ratio of 90:10 (v/v) respectively. The percent conjugation on the surface of the nanoparticles was determined by using following equation.

Conjugation (%) = [(Total dye added – Dye in the supernatent)/Total dye added] x 100

The percent loading on the surface of nanoparticles was determined using the following equation;

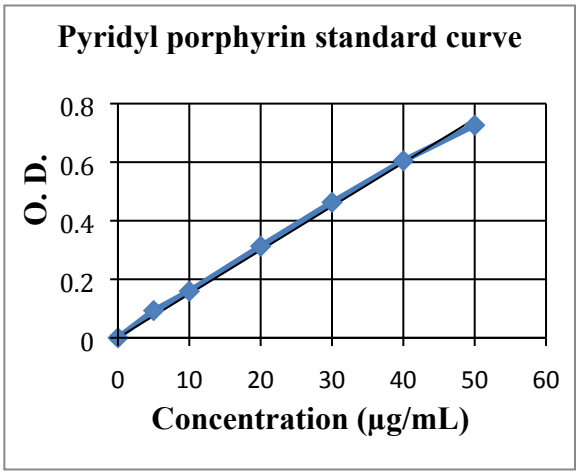
$Loading\ (\%) = Conj\ (\%) - Conj\ (\%)_{blank}$

Where *conj (%)* denotes percent conjugation of pyridyl porphyrin in both nanoparticle solution and nanoparticle blank solution, $conj\ (\%)_{blank}$ denotes the conjugation of pyridyl porphyrin in nanoparticle blank solution.

The standard calibration curve of pyridyl porphyrin was drawn and liner line equation was obtained using HPLC runs at different concentrations of pyridyl porphyrin.

Standard calibration curve of porphyrin 16 to calculate loading on nanoparticles

(a)



(b)

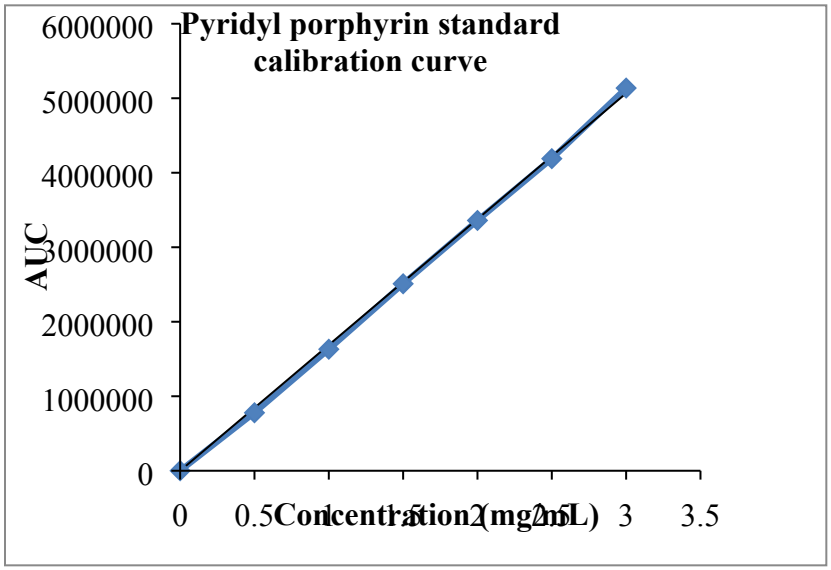


Figure S4. Standard calibration curve for drug loading of pyridyl porphyrin **16**: (a) using absorption spectroscopy; (b) using HPLC method

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Table S1. Determination of loading of porphyrin on the surface of various gold and silver nanoparticles using absorption spectroscopy.

S. No.	**Parameters**	Porphyrin (y=0.0150x, R^2=0.9970)		
1	**Nanoparticle**	**CS-AgNP**	**CS-AuNP**	**PF-AuNP**
2	Abs[a]	0.044	0.037	0.044
3	$[C_o]$[b]	2.9 µg/mL	2.4 µg/mL	3.0 µg/mL
4	$[C_i]$[c]	25 µg/mL	25 µg/mL	25 µg/mL
5	L_d (%)[d]	12%	10%	12%

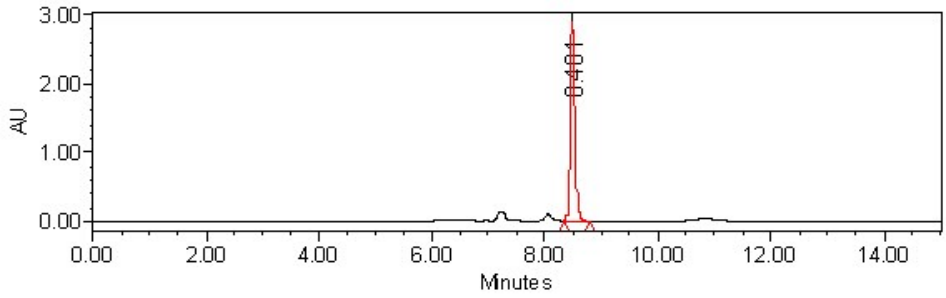
a= absorbance of supernatant solution containing porphyrin after NaOH treatment and centrifugation; b= concentration of porphyrin in the supernatant solution, calculated using linear regression equation y = mx+c; c= initial concentration of porphyrin used in the conjugation reaction.; d= percent loading of porphyrin on the surface of nanoparticles. This was calculated using the following equation: $L_d\,(\%) = \{[C_o]/[C_i]\} \times 100$

Table S2. Data for the calibration curve obtained by HPLC method (AUC = area under the curve).

S. No	Porphyin conc (mg/mL)	AUC1	AUC2	AUC3	Average AUC
1	0	0	0	0	0
2	0.5	783000	775197	777236	778478
3	1	1599627	1609181	1684620	1631143
4	1.5	2514194	2523393	2491137	2509575
5	2	3348018	3374111	3356851	3359660
6	2.5	4175253	4198105	4194205	4189188
7	3	5067527	5105233	5237346	5136702

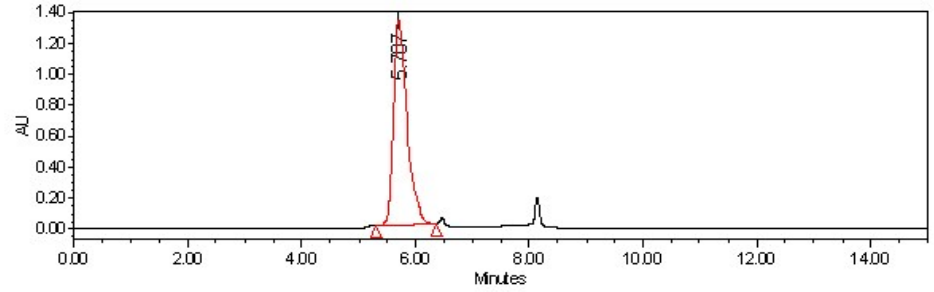
SI-4. HPLC chromatogram of selected porphyrins

HPLC chromatogram of porphyrin 14



HPLC t_R = 8.40 min (using a isocratic of 90% acetonitrile–10% H_2O–0.1% TFA ran over 40 min): purity of compound **14** >95%

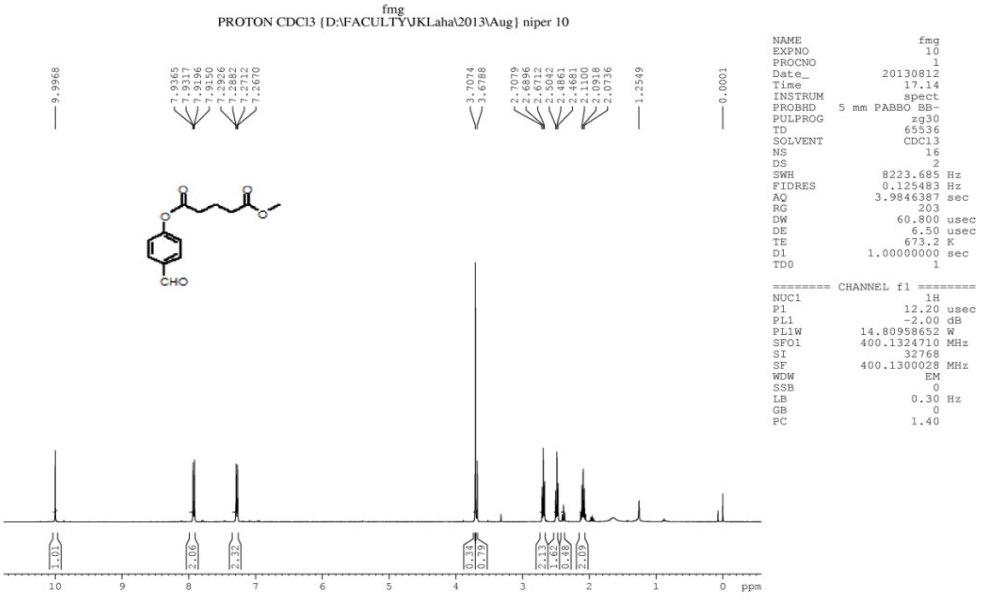
HPLC chromatogram of porphyrin 16



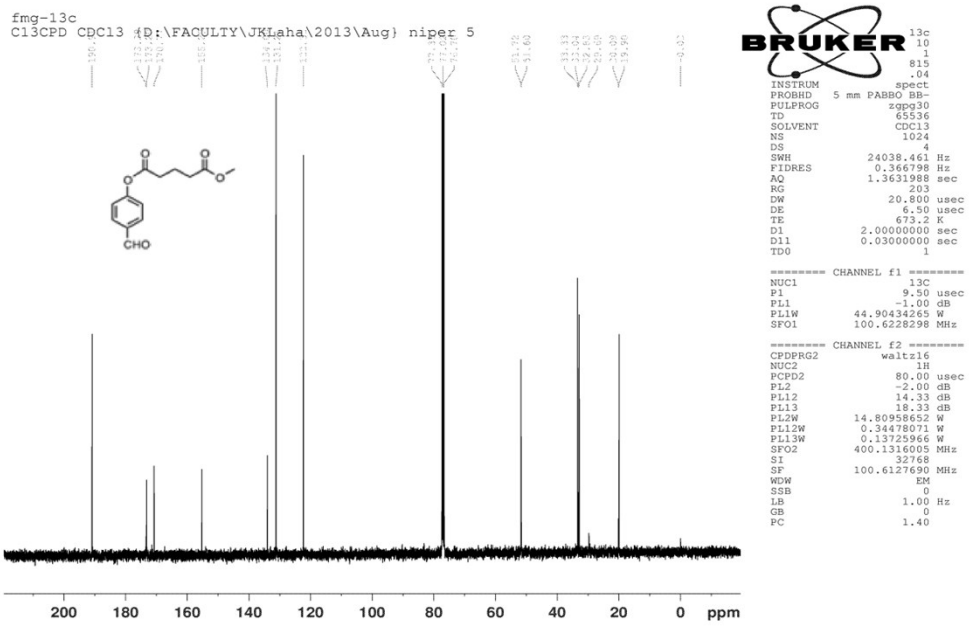
HPLC t_R = 5.71 min (using a isocratic of 90% acetonitrile–10% H_2O–0.1% TFA ran over 40 min): purity of compound **16** >95%

SI-5. NMR spectra of selected compounds

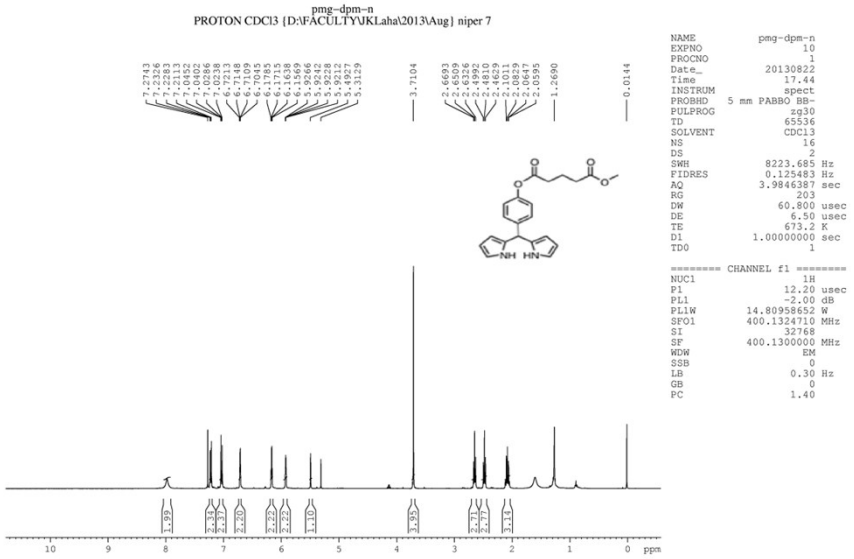
^{1}H NMR spectra of compound 2



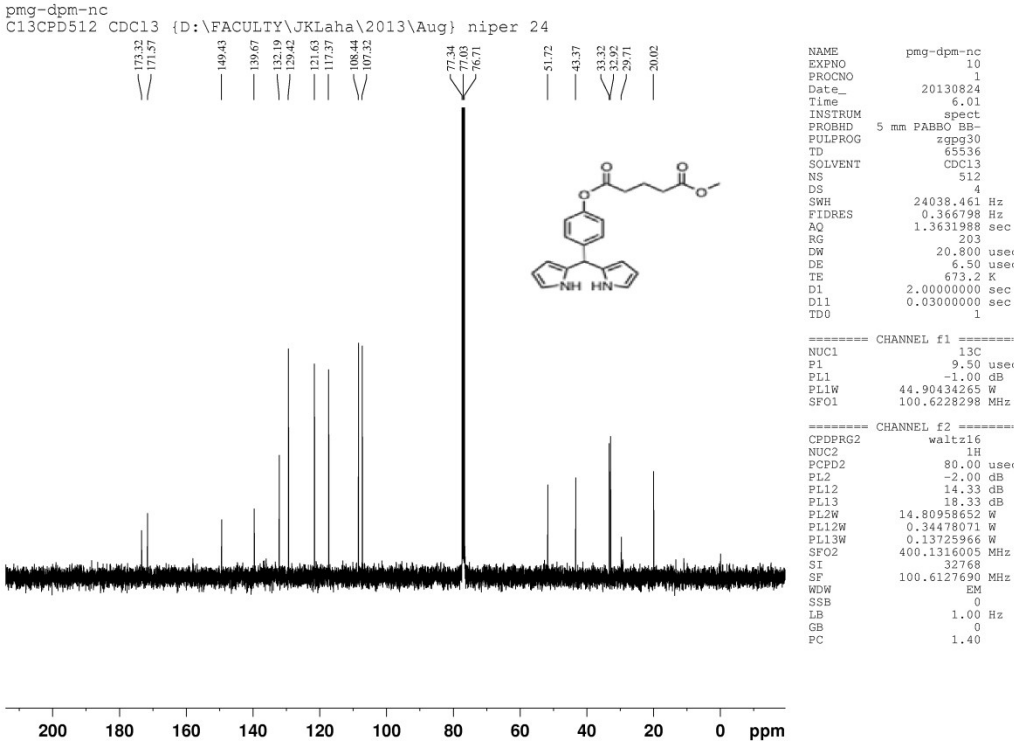
^{13}C NMR spectra of compound 2



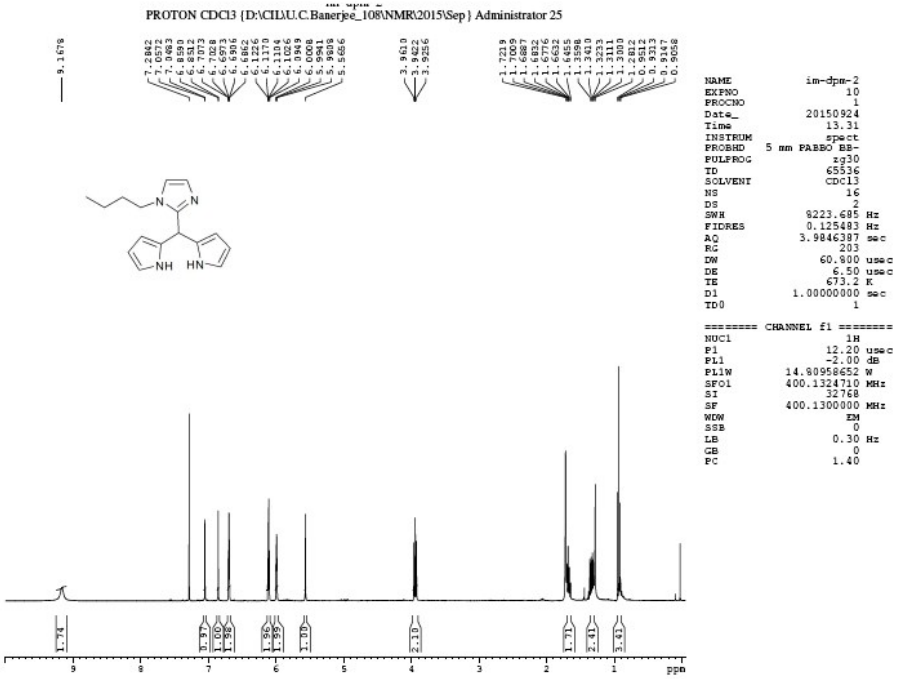
^{1}H NMR spectra of compound 6



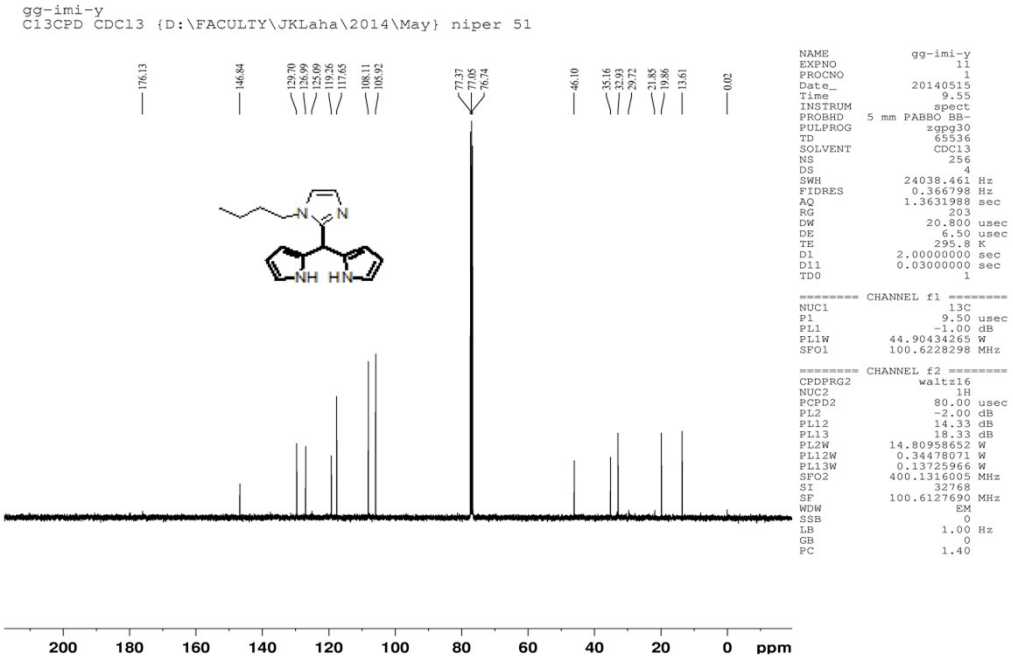
^{13}C NMR spectra of compound 6



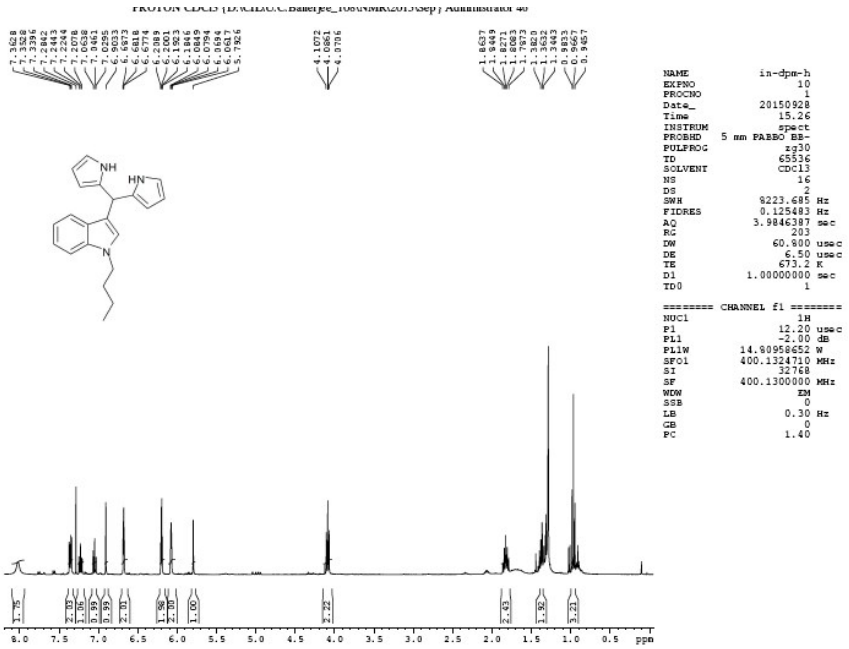
^{1}H NMR spectra of compound 8



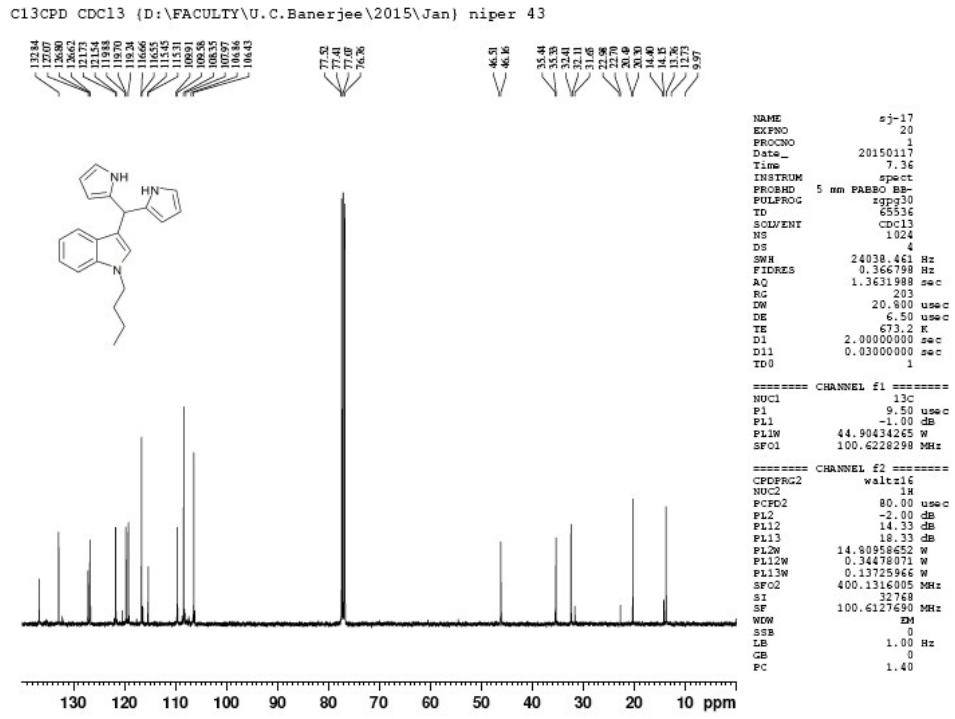
^{13}C NMR spectra of compound 8



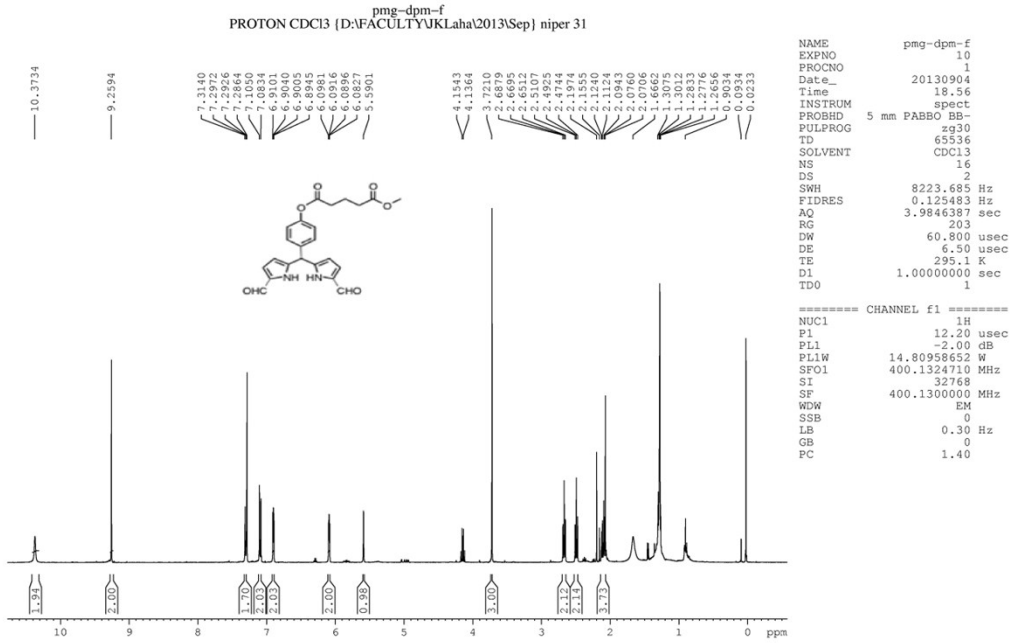
^{1}H NMR spectra of compound 9



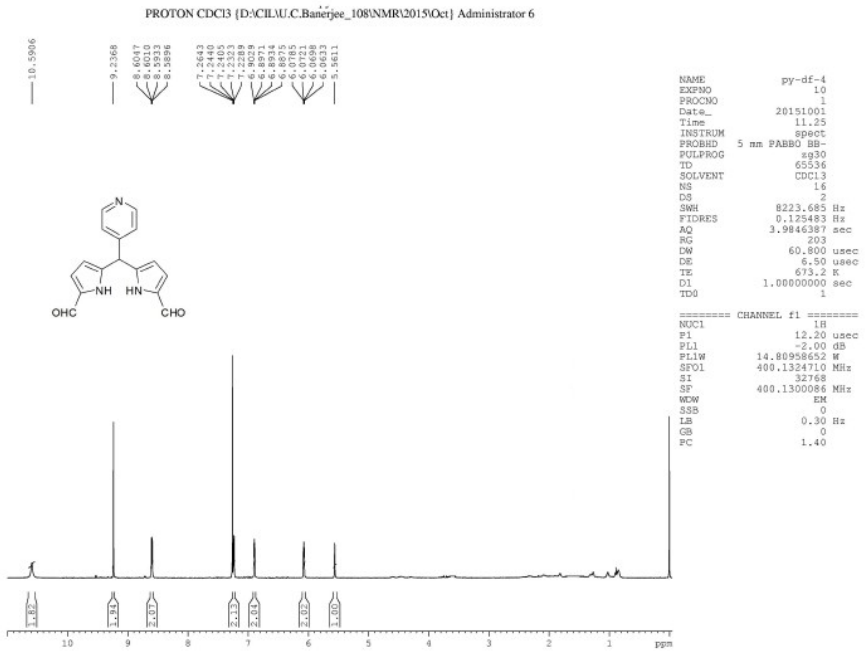
^{13}C NMR spectra of compound 9



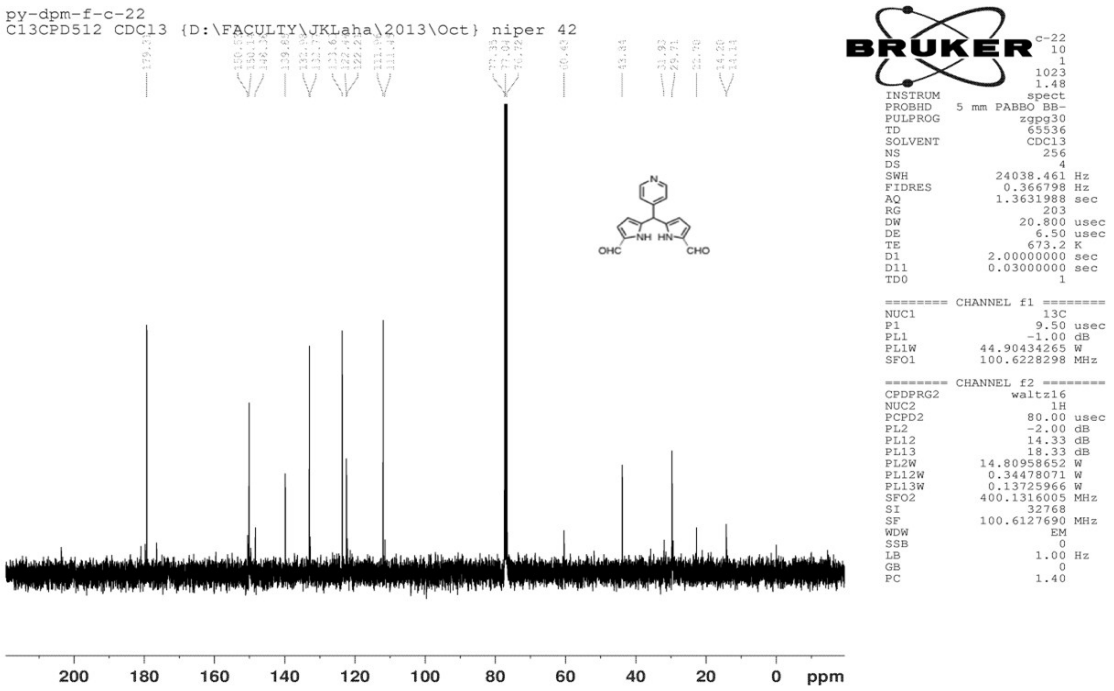
^{1}H NMR spectra of compound 11



^{1}H NMR spectra of compound 12



^{13}C NMR spectra of compound 12



^{1}H NMR spectra of compound 14