Supporting Information

Rapid Targeting and Imaging of Mitochondria by Carbon Dots Using an Amino Acid-Based Amphiphile as the Carrier

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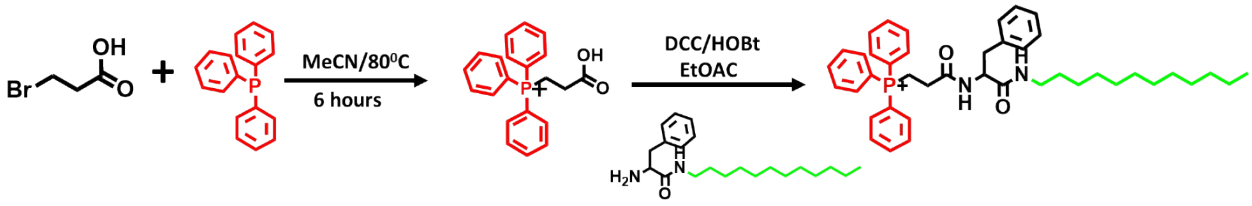


Fig.S1: The schematicpresentation of the synthetic route of the P.

Quantum yields (QYs) measurements of the C-dot:

The QYs of C-dot in the water relative method using quinin sulphate in 0.5 M H_2S0_4 solution (QY = 40 % in water) as a reference

sample. The QYs were calculated according to the below equation.

$$\phi_a = \phi_b \times \frac{A_b}{I_b} \times \frac{I_a}{A_a} \times \frac{\eta_a^{\,2}}{\eta_b^{\,2}}$$

Where ϕ is the QY, I is the emission intensity, A is the optical density, and η is the refractive index of the solvent. The subscripts of "b" and "a" refer to the reference and the C-dots, respectively. The absorbance values of all samples were kept under 0.5 at the excitation wavelength (365nm) to minimize re-absorption effects."

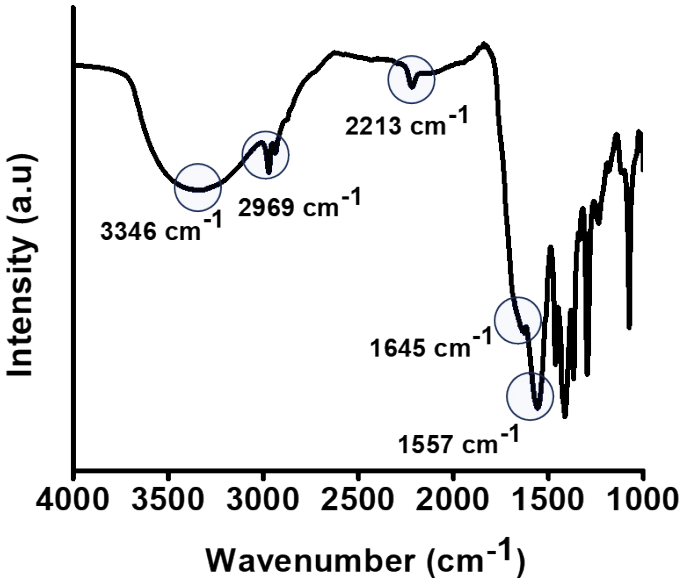


Fig.S2: FT-IR spectrum of the C-dots.

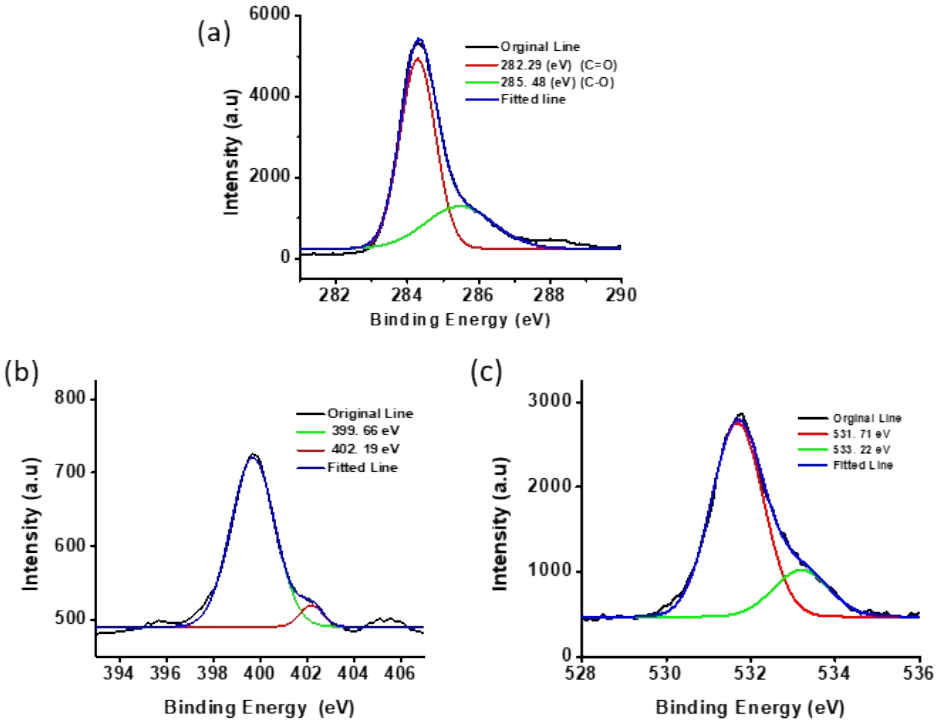


Fig.S3: The XPS spectra of the C-dots (a) C1S (b) N1S (c) O1S.

System	$\tau_1[\alpha_1]$	$\tau_1[\alpha_1]$	Average life time (ns)
0.05 mg/mL C-dot	5.9(3.29)	11.85(96.71)	11.47
0.05 mg/mL C-dot + P_1(10 wt%)	2.32(3.06)	12.23(96.94)	10.82
0.5 mg/mL C-dot	5.53(5.69)	11.23(94.31)	10.61
0.5 mg/mL C-dot + P_1(10 wt%)	5.6(5.2)	11.41(94.8)	10.83

Fig.S4: The TCSPC life time and related parameters of the C-dots with the addition of P with varying percentages.

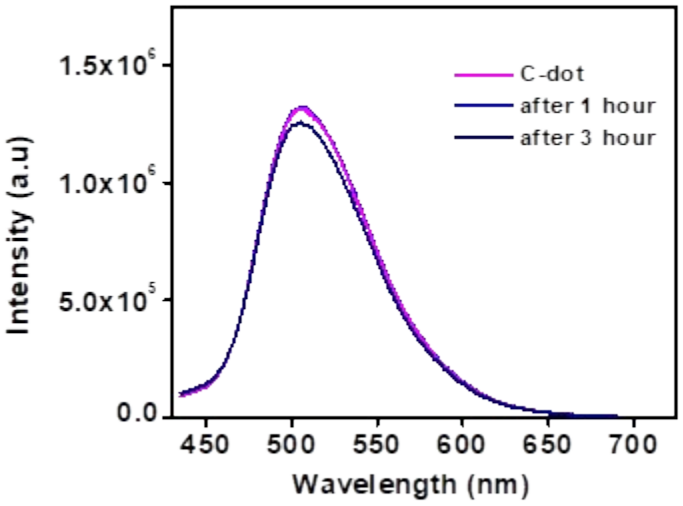


Fig.S5: The change of the emission of the C-dots with time after exposing is under the photo radiative condition (day broad light)

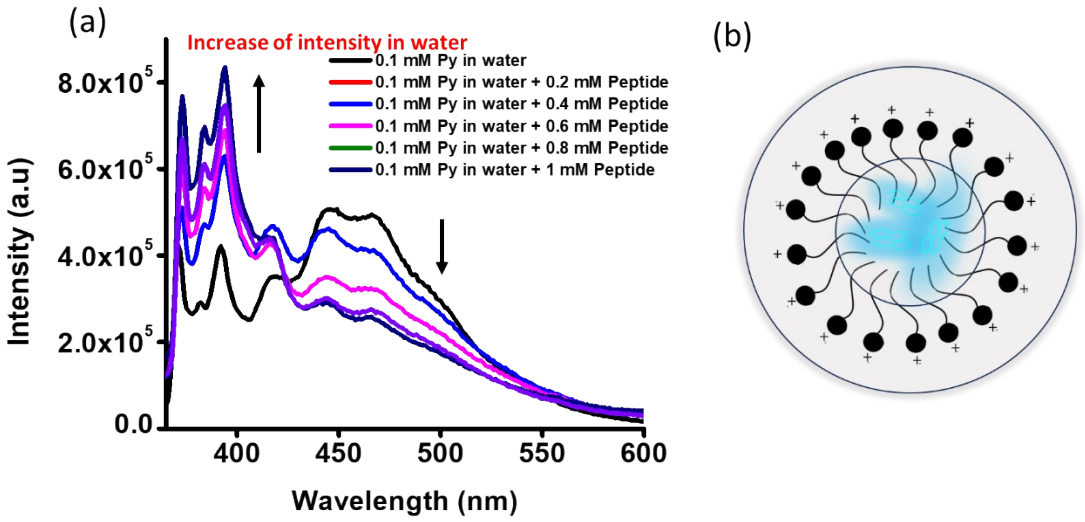


Fig.S6: (a) The change in the emission spectra of the pyrene in water with the gradual addition of P. Showing the increase in intensity of Pyrene; (b) probable aggregation of P and the presence of the pyrene moieties into the hydrophobic pocket of the aggregate.

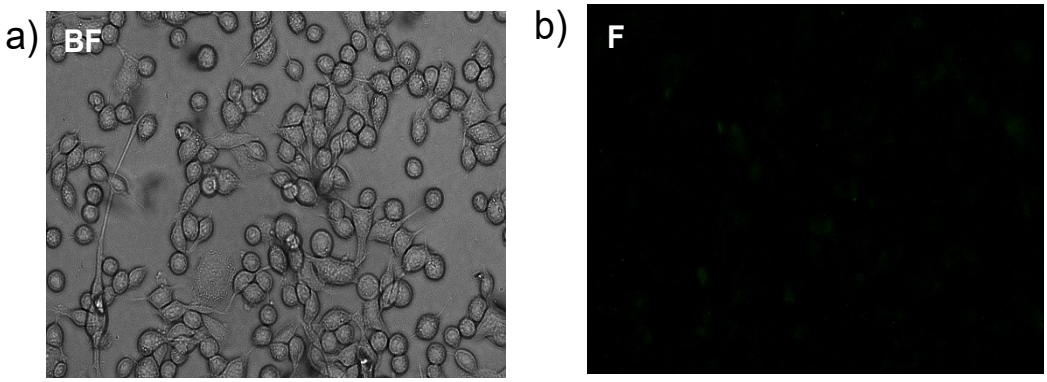


Fig.S7: The photograph of the KB cellsafter incubating them with C-dots (50 µg/1ml) (a) in bright field (b) under fluorescence microscope.

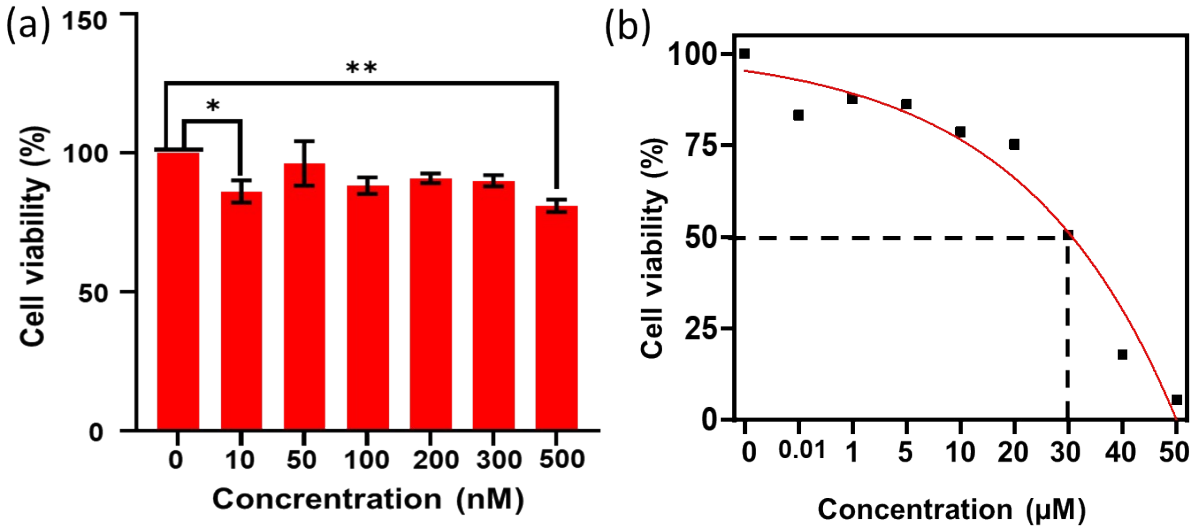


Fig S8. (a) MTT-based cytotoxicity assay of CDTP (50μg c-dot in 1ml+ 0.2 mM P) in KB cell. Typically, KB cells are incubated with different doses of CDTP for 24 hours. Next, cells are washed with 7.4 PBS buffer and MTT-based cytotoxicity assay is performed ass 100% viability of control cell without any sample treatment. Error bar represents mean ±S.D.; asterisks denote significant differences between the values (*P <0.05,**P < 0.01); Dunnett's multiple comparison test. (b) IC_{50} value is approximately 30 μM of P in CDTP.

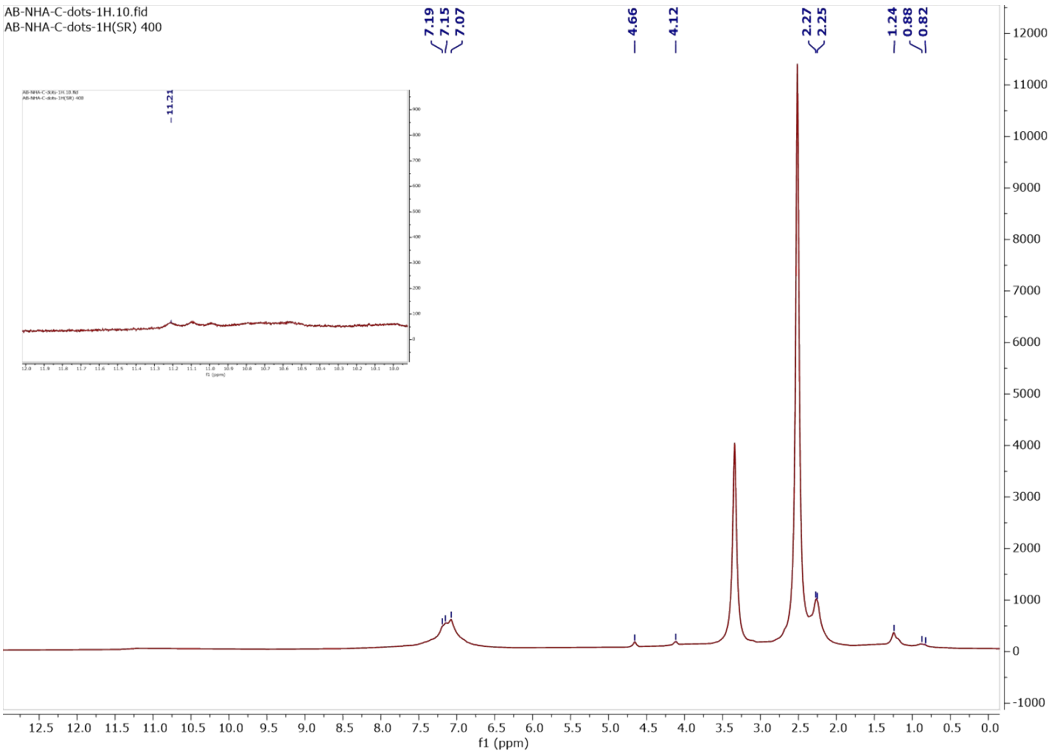


Fig. S8: The ^{1}H-NMR of the C-dots (DMSO-D_6)

Synthesis procedure of P

Synthesis of the TPP-C_2-COOH: 10 millimole 1-bromo propionic acid and the 12 millimole triphenyl phosphine has been mixed and taken in a clean dried round bottom flax. Then the mixture was completely dissolved in the dry MeCN and refluxed for the 6 hours at the 80oC. Then the reaction mixture was cooled down and the MeCN was removed by the rotary evaporator. The while sticky material was dried purified by the column chromatography in 0.5 % mixture of MeOH/$CHCl_3$. The purified compound was confirmed by the HRMS and NMR spectroscopy which has been given in the supporting information.

Synthesis of Boc-Phe-OH: 3.3 g (20 mmol) of L-Phenylalanine was taken in a 250mL round bottomed flask and 20 mL 0.5(N) NaOH and equal volume of 1,4-dioxane were added to it to dissolve the amino acid completely. 4.60g (21.1 mmol) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture, cooled in an ice bath and stirred for 10 hours at room

temperature. Then the solution volume was reduced to one third in a rotary evaporator. The resulting mixture was acidified with saturated $KHSO_4$ solution and the aqueous layer was extracted with ethyl acetate (3 x 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain the white solid product.

Synthesis of the Boc-Phe-C_{12}: 7.78 g (18 mmol) of Boc-Phe-OH was dissolved in 12mL dry N, N-dimethyl formamide (DMF) and cooled in an ice bath. 80mL of Ethyl acetate was added to it. It was followed by addition of 2.43g (18.00mmol) of 1-hydroxybenzotriazolemonohydrate (HOBt.H2O) and 3.70 g of dodecylamine (20mmol). 4.12 g (20mmol) of N,N-dicylohexylcarbodiimide (DCC) was added after all the reagents are properly dissolved. The reaction mixture was stirred for 24 hours in room temperature (27°C). The reaction mixture was diluted with ethyl acetate and filtered to separate N, N- dicyclohexyl urea (DCU). The ethyl acetate layer was washed with 1(N) HCl (3 x 30 mL), brine (2 x 30 mL), saturated sodium carbonate solution (2 x 30 mL) and brine (2 x 30 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography using chloroform/methanol (97:3) as eluent to obtain the pure white product.

Synthesis of the NH_2-Phe-C_{12}: To 5.82g (14.55mmol) of Boc-Phe-C12, 15 mL of formic acid was added to remove the Boc protection. The reaction was monitored using TLC. After 8 hours, formic acid was removed under vacuum using a liquid nitrogen trap. 10 mL of water is added, and the pH was balanced to 8-8.5 using Na_2CO_3. Subsequent addition of ethyl acetate precipitates out the beige crude product. The filtrate was dried and further purified using basic alumina column, chloroform and methanol (9:1) as eluent.

Synthesis of the TPP-Phe-C_{12} (P): 3.32 gm,10 millimole of NH_2-Phe-C12 was dissolved in 10mL dry N,N-dimethyl formamide (DMF) and cooled in an ice bath. 80mL of Ethyl acetate was added to it. It was followed by addition of 1.62 g (12 mmol) of 1-hydroxybenzotriazolemonohydrate (HOBt.H_2O) and 3.36 g of TPP-C2-COOH (10 mmol). 2.47 g (12mmol) of N,N-dicylohexyl carbodiimide (DCC) was added after all the reagents are properly dissolved. The reaction mixture was stirred for 24 hours in room temperature (27°C). The reaction mixture was diluted with ethyl acetate and filtered to separate N,N- dicyclohexyl urea (DCU). The ethyl acetate layer was washed with 1(N) HCl (3 x 30 mL), brine (2 x 30 mL), saturated sodium carbonate solution (2 x 30 mL) and brine (2 x 30 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography using chloroform/methanol (97:3) as eluent to obtain the pure white product. The synthetic route of this synthesis has been given in the supporting information (FigS1). All these compounds are characterised using HR-MS and NMR spectroscopy which has been provided in the supporting information.

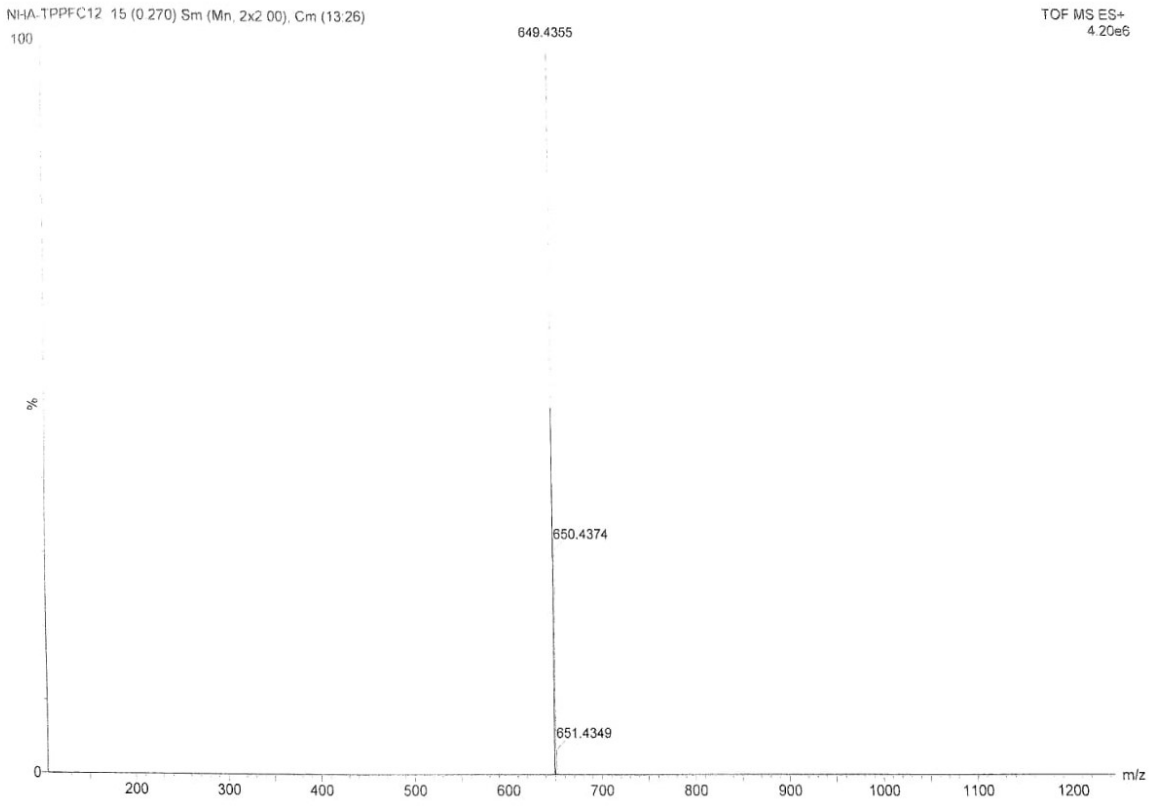
NMR and HRMS of the P

^{1}H-NMR (DMSO-D_6): δ 8.37-8.35 (d, NH), δ 8.04-8.02 (t, NH), δ 7.91-7.75 (m,15H of phosphine group), δ 7.23-7.16 (m,5H, Phe), δ 4.43-4.37 (m, 1H, α H), δ 3.72-3.63 (m, 2H, β H), δ 3.06-3.01 (m,2H), δ 2.95-2.85(m,2H), δ 2.71-2.66 (m,2H), δ1.33-1.27 (m, 20H), δ 0.85-0.82 (t,3H).

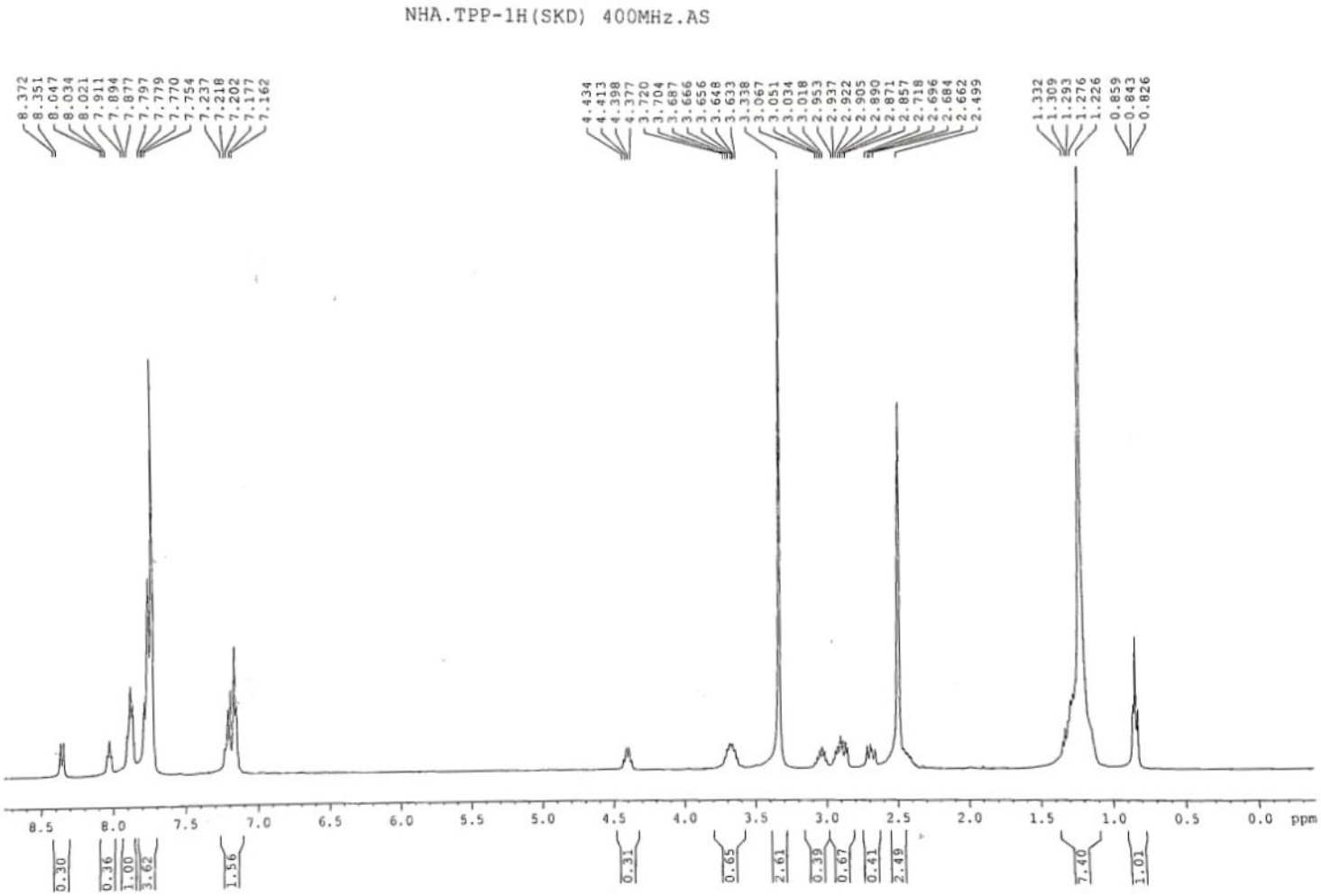
^{13}C-NMR (DMSO-D_6):
170.81,168.88,168.72,138.11,135.39,135.36,134.15,134.05,130.73,130.60,129.61,128.46,126.73, 119.22,118.36,54.82,31.75,29.91,29.52,29.47,29.43,29.37,29.19,29.17,27.83,26.76,22,55,17.68,1 7.15,14.41.

^{31}P-NMR (DMSO-D_6): δ 26.92 (S,1P)

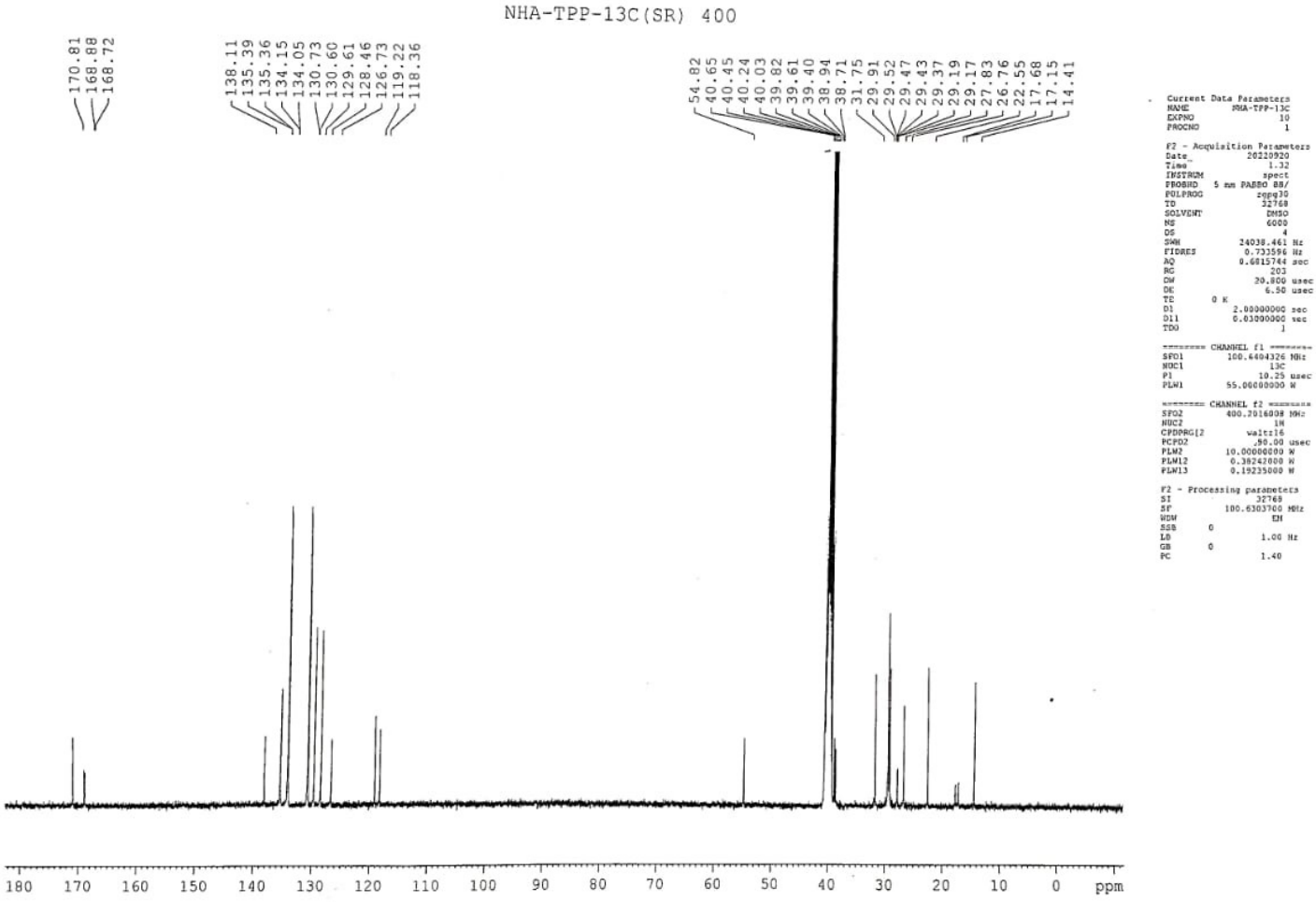
HRMS: Found, m/z 649 .43 $[M]^+$ calculated: 649.36



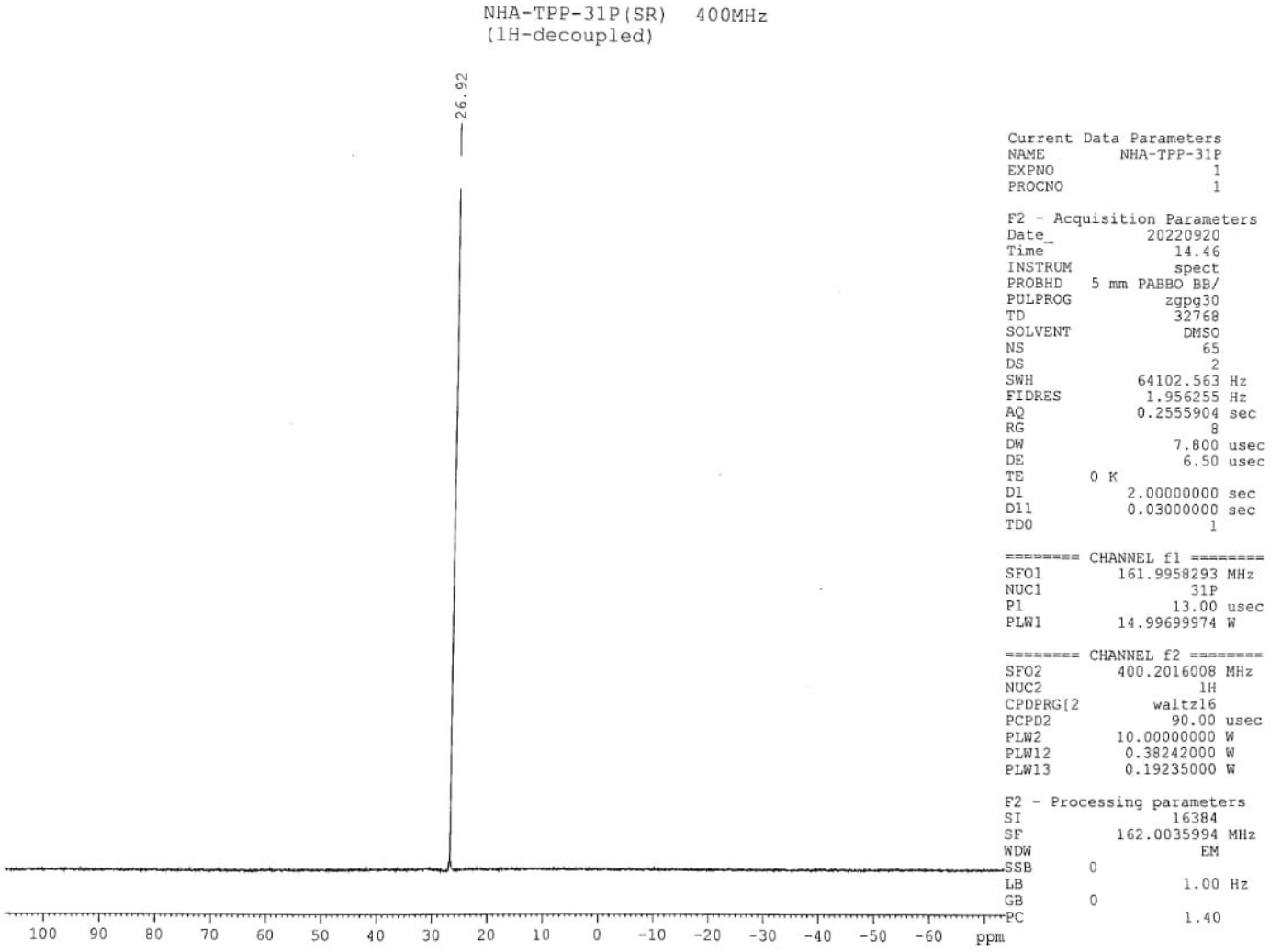
HRMS(high resolution mass spectrum) of P.



^{1}H-NMR spectrum of Pin $(CD_3)_2$ SO.



^{13}C-NMR spectrum of P in $(CD_3)_2$ SO.



^{31}P-NMR spectrum of P in $(CD_3)_2$ SO.