Electronic Supplementary Material (ESI) for ChemComm.

Charge-Transfer Interactions for the Fabrication of Multifunctional Viral Nanoparticles

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1. Materials

Pyrene acid, 2,2'-((oxybis(ethane-2,1-diyl))bis(oxy))diethanol, N-(3-dimethylamino propyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP), *p*-toluenesulfonyl chloride, sodium azide (NaN_3), 3-aminophenylacetylene, copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium ascorbate (NaAsc), Poly(2-hydroxyethyl methacrylate) (Phema, MW 20000 Da), 3,5-dinitrobenzoyl chloride, ethylene glycol, 4,4'-bipyridine, triethylamine, methyl iodide, 1-bromotetradecane and other reagents were local commercial products and used as received. All organic solvents were dried and distilled before used.

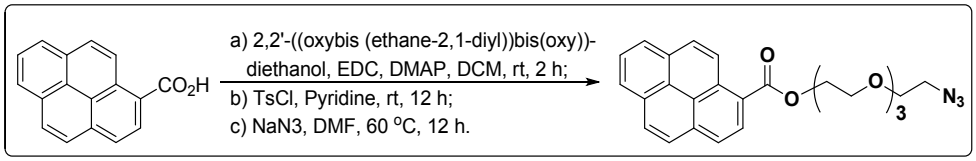
2. Measurements

NMR spectra were characterized on a Varian Mercury 300/400 apparatus spectrometer using $CDCl_3$ or DMSO-d_6 as solvent; UV-Vis spectra were acquired on Agilent Technologies 95-03 spectrometers; Fluorescence spectra were measured on Varian Cary Eclipse spectrometers, for the Job's plot experiments: a guest molecule with various concentrations was added into **TMV (wt)-PYR** aqueous (2 mL, 0.125 mg/mL). The molar ratio of **TMV (wt)-PYR** subunits {x = [**TMV(wt)-PYR**]/ ([**TMV(wt)-PYR**] + [guest molecule])} varies from 0 to 1. After incubating for 10 min, the changes of the fluorescence intensity (ΔI) of each sample at 417 nm (the fluorescence intensity of **TMV(wt)-PYR** subtracts the one of CT-complex (**TMV(wt)-PYR**/guest molecule) were recorded when the molar ratio is changed; ESI-MS spectra were recorded using a Micromass QTOF apparatus; MALDI-TOF MS analysis was performed using a Bruker Ultraflex I TOF/TOF mass spectrometer, for samle preparation: the virus (24 μL) was denatured by adding guanidine hydrochloride (6 μL, 6 M), and then the denatured proteins were spotted on MTP 384 massive target plate using Millipore Zip-TipsμC18 tips which can remove the excess salts and assist the binding of protein to the sinapic acid matrix; Transmission electron microscopy (TEM) measurements were carried out on a Hitachi H8000 electron microscope operating at an acceleration voltage of 120 kV, for sample preparation: samples

were prepared by drop-casting the aqueous solution on the carbon-coated copper grid, and then were negatively stained with a uranyl acetate solution; Size Exclusion Chromatography (SEC) analysis was performed on an AKTA explorer (GE Biotech) instrument using Sephadex G-25 column and Superose-6 size-exclusion column, for the analysis procedure: 100 uL viruses sample (conc. 1 mg/mL) were injected into FPLC with 10 mM Kphos as the buffer (pH=7.8) at the flow rate 0.4 mL/min, and the siginal was monitored at the absorbance of 260 nm; Dynamic Light Scattering (DLS) was performed by Malvern Zetasizer Nano-ZS; Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared in house with 12% acrylamide separating gel (30:1 acrylamide to bisacrylamide) with 5% acrylamide stacking gel.

3. Synthesis of PYR-Azide and DNB derivatives

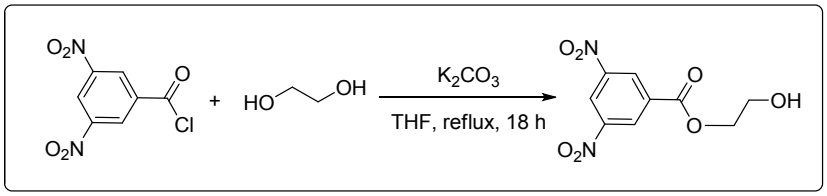
3.1 Synthesis of PYR-Azide



A solution of pyrene acid (0.5 g, 2.03 mmol), EDC (1.16 g, 6.09 mmol) and DMAP (0.74 g, 6.09 mmol) in 5 mL anhydrous DCM was added dropwise to the solution of 2,2'-((oxybis (ethane-2,1-diyl))bis(oxy))diethanol in 5 mL dry DCM. After the addition, the mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and re-dissolved by ethyl acetate. The organic layer was washed by water, brine, dried with sodium sulfate and filtered, followed by the removal of the solvents under vacuum. Then the crude product and *p*-toluensulfonyl chloride (0.20 g, 1.05 mmol) were added in 2 mL pyridine, and the mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and re-dissolved by DCM. The organic layer was washed by water, brine, dried with sodium sulfate and filtered, followed by the removal of the solvent under vacuum to afford a brown liquid. The brown liquid and sodium azide (0.23 g, 3.38 mmol) were dissolved in 4 mL DMF, and the mixture was stirred at 60 ^{o}C for 12 h. After that, ethyl acetate

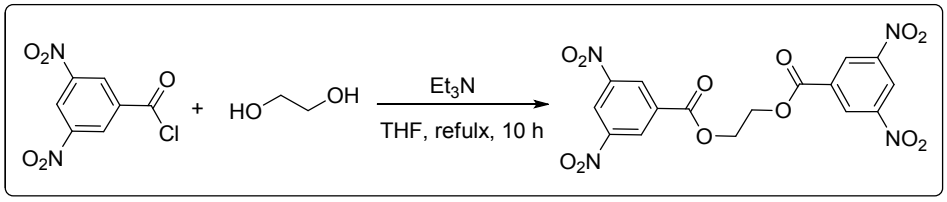
was added to the reaction solution, washed by saturated NH_4Cl solution, and the organic layer was dried with sodium sulfate, filtered, followed by the removal of the solvent under reduced pressure. The residue was purified using silica gel column chromatography with (v(DCM): v(methanol) = 50:1) to afford Pyrene-Azide, the brown liquid, 95 mg. ESI-MS (+): m/z 448 $[M + H]^+$, 465 $[M+NH_4]^+$; HRMS (ESI): m/z calcd for $C_{25}H_{25}N_3O_5$: 447.1794; found: 447.1787; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 9.27 (1H, d, *J* = 9.30 Hz, pyrene-H), 8.66 (1H, d, *J* = 8.1 Hz, pyrene-H), 8.15 (7H, m, pyrene-H), 4.67 (2H, t, *J* = 4.5 Hz, CH_2), 3.97 (2H, t, *J* = 5.1 Hz, CH_2), 3.69 (10H, m, CH_2), 3.31 (2H, t, *J* = 5.1 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 168.0, 134.4, 131.2, 131.1, 130.4, 129.7, 129.5, 128.6, 128.3, 127.2, 126.4, 126.4, 126.3, 125.0, 124.9, 124.2, 123.6, 70.9, 70.8, 70.8, 70.1, 69.4, 64.4, 50.7.

3.2 Synthesis of DNB



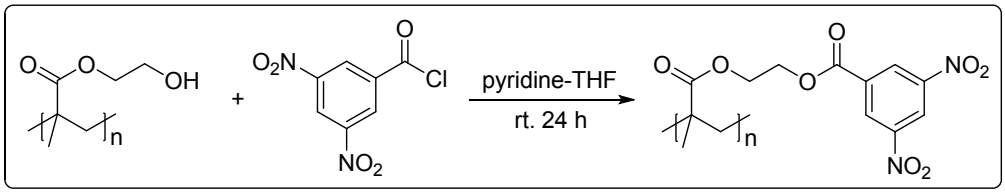
The synthesis of DNB was carried out as ref[1] to give a white powder. EI-MS (+): m/z = 257 $[M+H]^+$; ^1H NMR (300 MHz, DMSO, ppm): δ 9.04 (1H, s, ph-H), 8.97 (2H, s, ph-H), 5.09 (1H, s, OH), 4.40 (2H, m, $phCO_2CH_2$), 3.75 (2H, m, CH_2); ^{13}C NMR (75 MHz, DMSO, ppm): δ 163.1, 148.8, 133.2, 129.4, 122.9, 68.7, 59.2.

3.3 Synthesis of DDNB



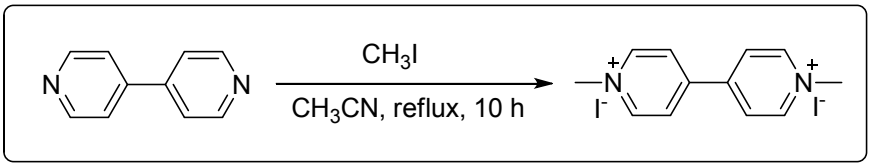
The synthesis of DNB was carried out as ref[1] to a white powder. EI-MS (+): m/z = 468 $[M+NH_4]^+$; ^1H NMR (DMSO, 300 MHz, ppm): δ 9.04 (2H, s, ph-H), 8.94 (4H, s, ph-H), 4.83 (4H, s, CO_2CH_2); ^{13}C NMR (DMSO, 75 MHz, ppm): δ 162.1 (2C, $ph\underline{C}O_2CH_2$), 148.1, 131.9, 128.4, 122.2 (12C, ph-C), 63.6 (2C, $phCO_2\underline{C}H_2$).

3.4 Synthesis of DNB-Polyhema



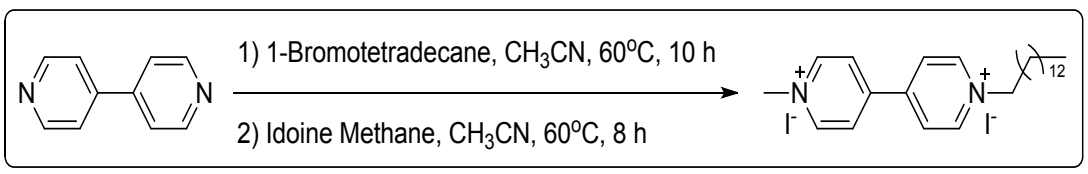
Poly(2-hydroxyethyl methacrylate) (polyhema, MW 20K, 0.13 g, 1.0 mmol -OH equiv.) and 3, 5-dinitrobenzoyl chloride (0.27 g, 1.2 mmol) were dissolved in 6 mL mixed solvent of THF-pyridine (2:1, volume ratio). After stirring at room temperature for 24 h, the solvent was removed under reduced pressure, and the crude mixture was re-dissolved in DCM. The organic layer was washed by water, brine, and dried with anhydrous sodium sulfate. The solution was concentrated under reduced pressure until the final volume reached 1 mL, and then was added dropwise to cold ethyl ether, affording a white solid, 120 mg, yield 37 %. ^{1}H NMR (DMSO, 300 MHz, ppm): δ 8.99 (1H, s, ph-H), 8.83 (2H, s, ph-H), 4.42 (2H, s, OCH_2), 4.09 (2H, s, OCH_2), 0.57-1.19 (5H, m).

3.5 Synthesis of MV



The synthesis was carried as ref[2] to give a red powder. EI-MS (+): m/z = 186 ; ^{1}H NMR (DMSO, 300 MHz, ppm): δ 9.31 (4H, d, *J* = 5.25 Hz, pyridinium-H), 8.79 (4H, d, *J* = 5.13 Hz, pyridinium-H), 4.45 (6H, s, CH_3); ^{13}C NMR (DMSO, 75 MHz, ppm): δ 148.0, 146.5, 126.0, 48.0.

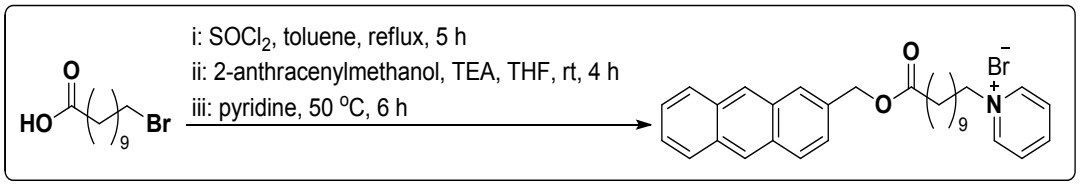
3.6 Synthesis of TV



The synthesis was carried as ref[3] to afford a red powder. ESI-MS (+): m/z = 368; ^{1}H NMR (DMSO, 300 MHz, ppm): δ 9.39 (2H, d, *J* = 9.0 Hz, pyridinium-H),): 9.29 (2H, d, *J*

= 6.0 Hz, pyridinium-H), 8.78 (4H, m, pyridinium-H), 4.69 (2H, t, *J* = 9.0 Hz, $NC\underline{H_2}$), 4.45 (3H, s, CH_3), 1.97 (2H, m, CH_2), 1.23-1.30 (22H, m, CH_2), 0.84 (3H, t, *J* = 6.0 Hz, CH3) ; ^{13}C NMR (DMSO, 75 MHz, ppm): δ 148.51, 148.17, 146.60, 145.73, 126.58, 126.13, 126.07, 60.89, 48.11, 31.29, 30.77, 29.05, 29.02, 28.95, 28.83, 28.17, 28.44, 25.10, 22.10, 13.98.

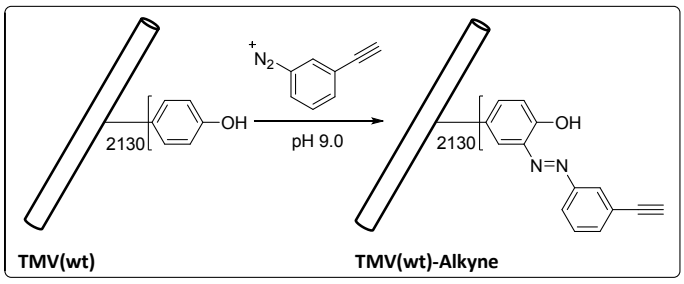
3.7 Synthesis of 2-AP



The synthesis of **2-AP** was carried out as ref [4]. MS-ESI (+): m/z = 454; ^1H NMR (300 MHz, DMSO, δppm): 9.11 (2H, d, *J* = 6.9 Hz, pyridinium-H), 8.60 (1H, t, *J* = 7.2 Hz, pyridinium-H), 8.57 (1H, s, anthracene-H), 8.56 (1H, s, anthracene-H), 8.15 (2H, dd, J_1 = 7.2 Hz, J_2 = 6.9 Hz, pyridinium-H), 8.07 (4H, m, anthracene-H), 7.50 (3H, m, anthracene -H), 5.28 (2H, s, OCH_2), 4.57 (2H, t, *J* = 7.5 Hz, NCH_2), 2.39 (2H, t, *J* = 7.2 Hz, CH_2CO), 1.84 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.19 (12H, m, CH_2); ^{13}C NMR (75 MHz, DMSO, δppm): 173.33 (C=O), 145.91, 145.16, 128.92 (5C, pyridinium-C), 133.86, 131.88, 131.81, 131.18,131.07, 128.51, 128.46, 127.06, 126.59, 126.42, 126.19, 126.16, 126.02 (14C, anthracene-C), 65.95, 61.12, 33.95, 31.15, 29.16, 29.11, 29.05, 28.85, 28.76, 25.78, 24.93.

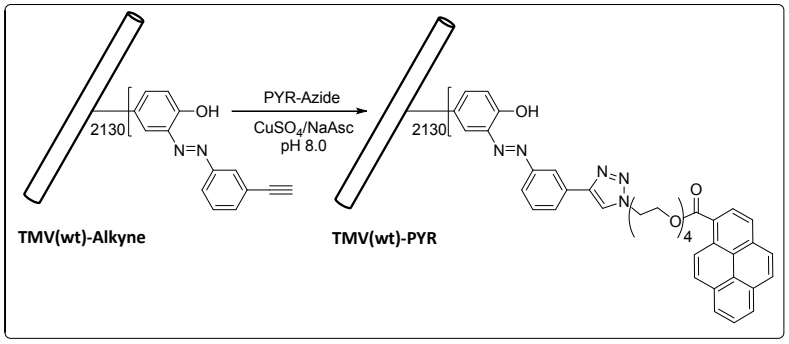
4. Preparation of TMV (wt)-Alkyne and TMV (wt)-PYR

4.1 TMV(wt)-Alkyne



An aqueous of sodium nitrite (50 µL, 207 mg/mL) was added to the premixed solutions of *p*-toluene sulfonic acid (800 µL, 0.3 mol/L) in water and 3-aminophenyl acetylene (150 µL, 78 mg/mL) in acetonitrile. The mixture was incubated at 0 ^{o}C for 1 h in dark place. After that, diazonium salt (608 µL, 12.9 mg/mL) was added to the solution of TMV (wt) (1.28 mL, 35 mg/mL) in borate buffer (13.10 mL, 0.1 M, pH = 9.0), and the mixture was incubated at 0 ^{o}C for 2 hours in a dark place. The modified virus sample was purified by 40% (w/w) sucrose gradient ultracentrifugation using a Beckman OptimaTM L90K Ultracentrifuge. After, the pellet was dissolved in Kphos buffer, and characterized by UV-Vis, Fluorescence spectrameter, MALDI-TOF MS, DLS, SDS-PAGE and TEM.

4.2 TMV (wt)-PYR



PYR-Azide (200 µL, 0.11 M) in DMSO and TMV (wt)-alkyne (636 µL, 25 mg/mL) were mixed with Tris.HCl buffer (3.08 mL, 10 mM, pH = 8.0), and then the aqueous of $CuSO_4$ (40 µL, 0.1 M) and NaASC (40 µL, 0.2 M) were added, followed by incubating at room temperature for 18 hours. The modified viruses were purified by 40% (w/w) sucrose gradient centrifugation using a Beckman OptimaTM L90K Ultracentrifuge. The pellet was dissolved in buffer and characterized by and characterized by UV-Vis, Fluorescence spectrameter, FPLC, MALDI-TOF MS, DLS, SDS-PAGE and TEM.

5. Figure S1-S27

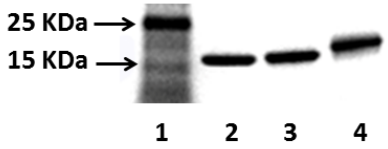


Figure S1. SDS-PAGE: lane 1, protein markers; lane 2, **TMV(wt)**; lane 3, **TMV(wt)-Alkyne**; lane 4, **TMV(wt)-PYR**.

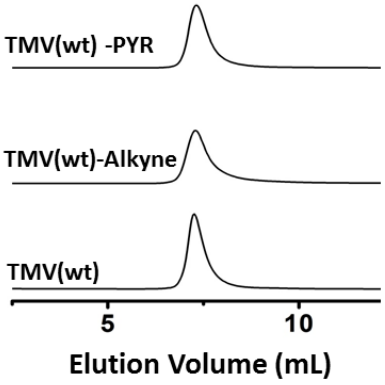


Figure S2. SEC diagram of **TMV(wt)**, **TMV(wt)-Alkyne**, and **TMV(wt)-PYR** monitored at 260 nm.

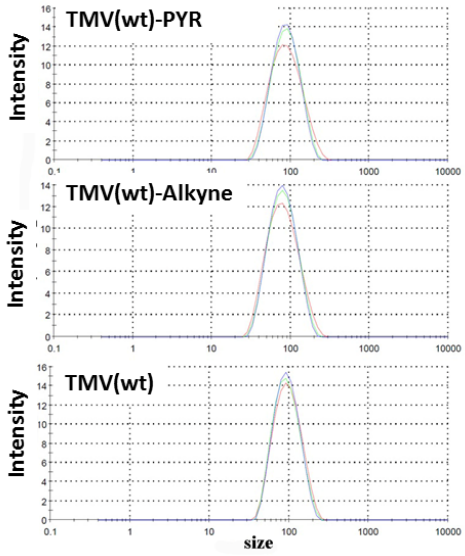


Figure S3. DLS of **TMV(wt)**, **TMV(wt)-Alkyne**, and **TMV(wt)-PYR**

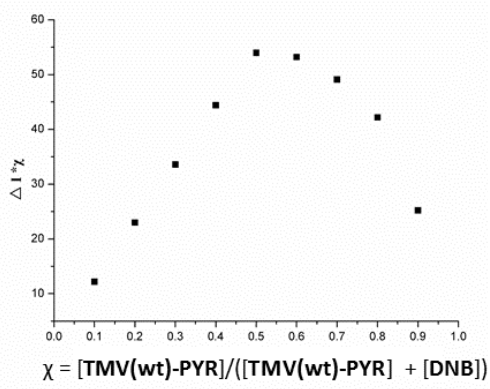


Figure S4. Job's plot showing 1:1 complex formation for **TMV(wt)-PYR/DNB**.

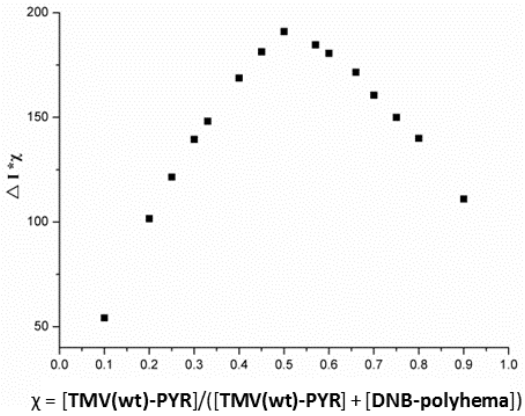


Figure S5. Job's plot showing 1:1 complex formation for **TMV(wt)-PYR/DNB-polyhema**.

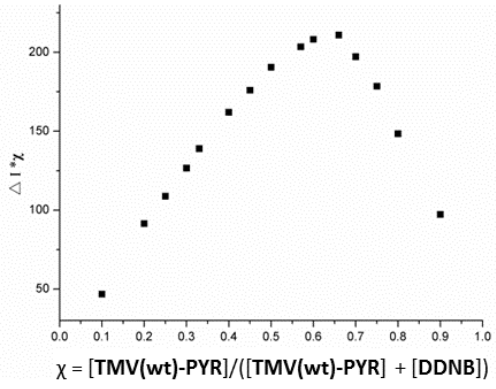


Figure S6. Job's plot showing 2:1 complex formation for **TMV(wt)-PYR/DDNB**.

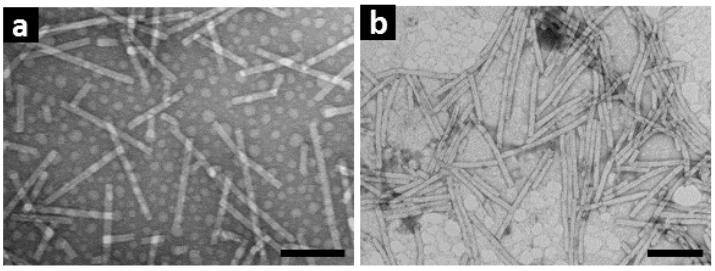


Figure S7. TEM image of uranyl acetate-stained (a) **TMV(wt)-PYR/DNB** (1:1, molar ratio), and (b) **TMV(wt)-PYR/MV/CB[8]** (1:1:1, molar ratio).The scale bar is 300 nm.

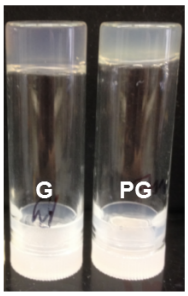


Figure S8. Photographies of **TMV(wt)-PYR** (left) and **TMV(wt)** (right)-based hydrogel with **2-AP**, respectively. For the hydrogel preparation: **TMV(wt)-PYR** (0.12 mg/mL) was premixed with **2-AP** (3 mg/mL) in Kphos buffer (pH=7.8), and then the mixture was heated to 45 ^{o}C. After cooling to room temperature, the hydrogel formed.

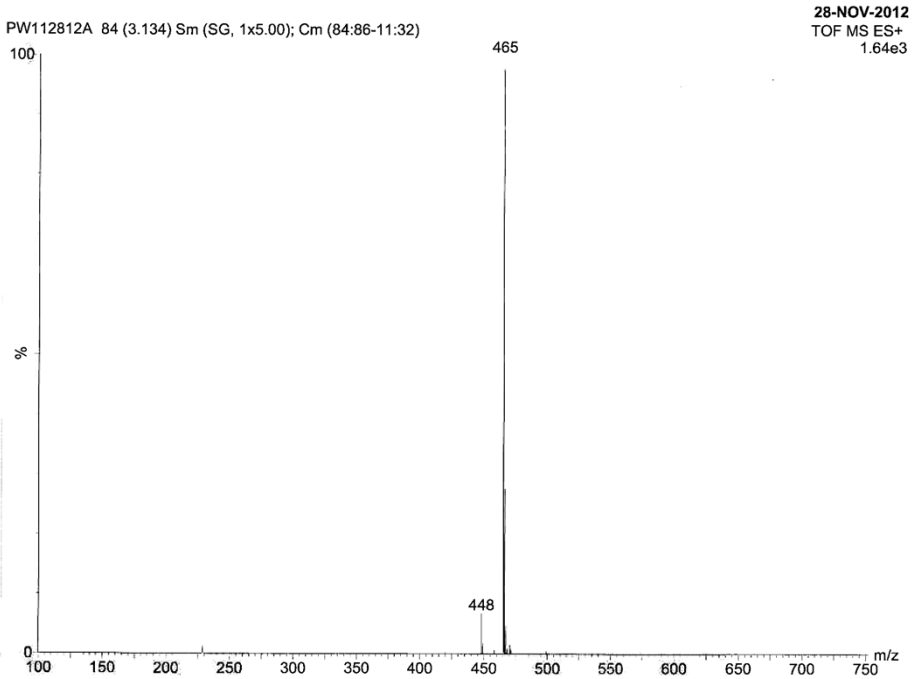


Figure S9. ESI-MS (+) Spectrum of **PYR-Azide**

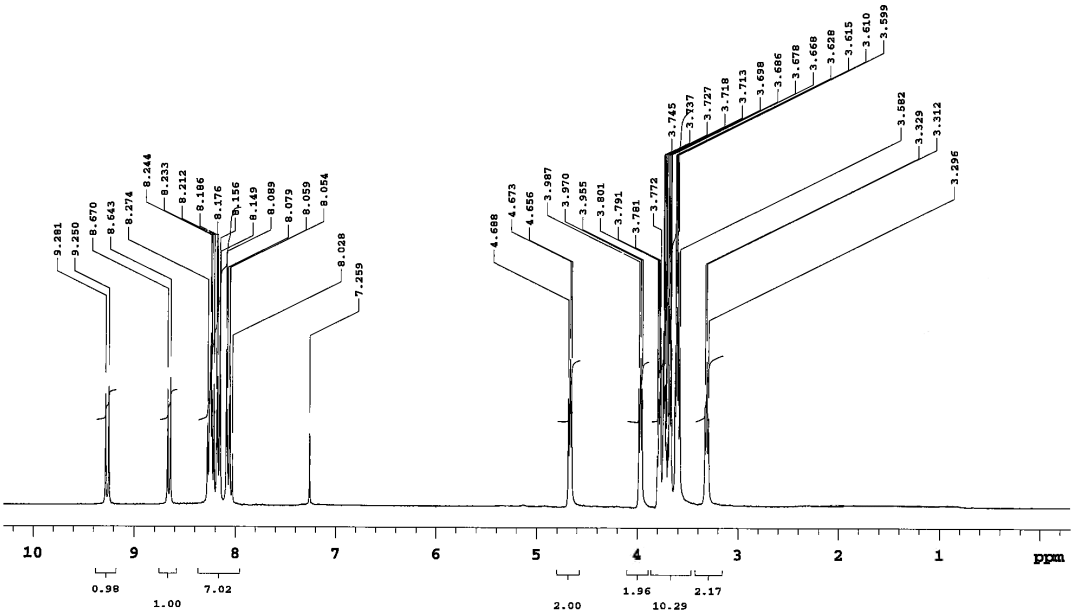


Figure S10. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of **PYR-Azide**

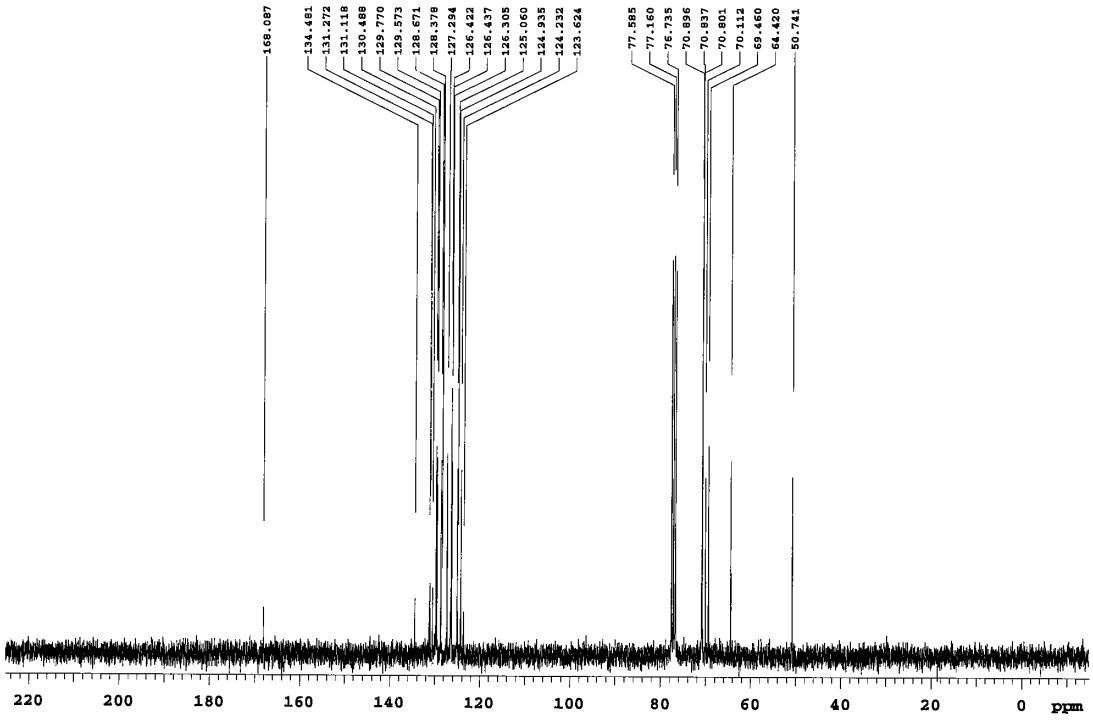


Figure S11. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of **PYR-Azide**

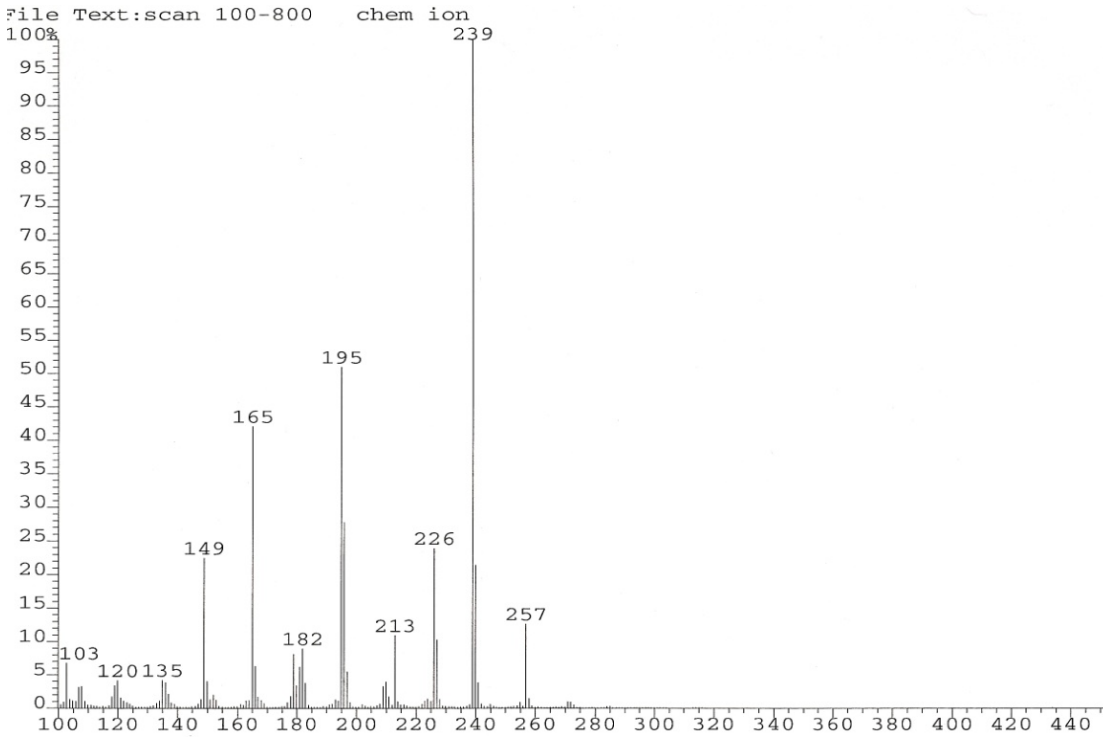


Figure S12. EI-MS (+) Spectrum of **DNB**

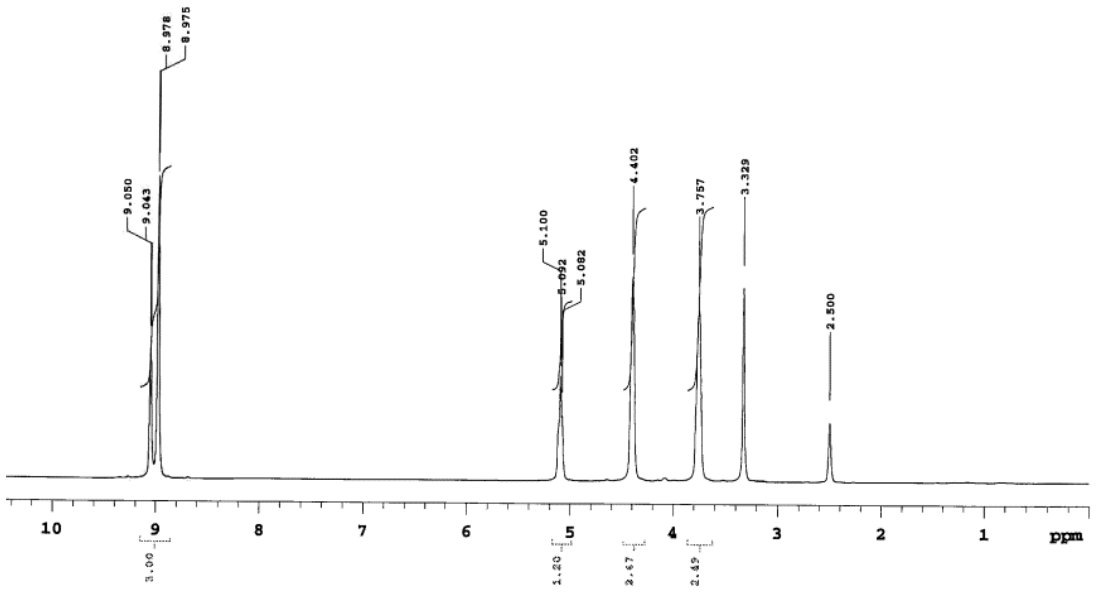


Figure S13. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **DNB**

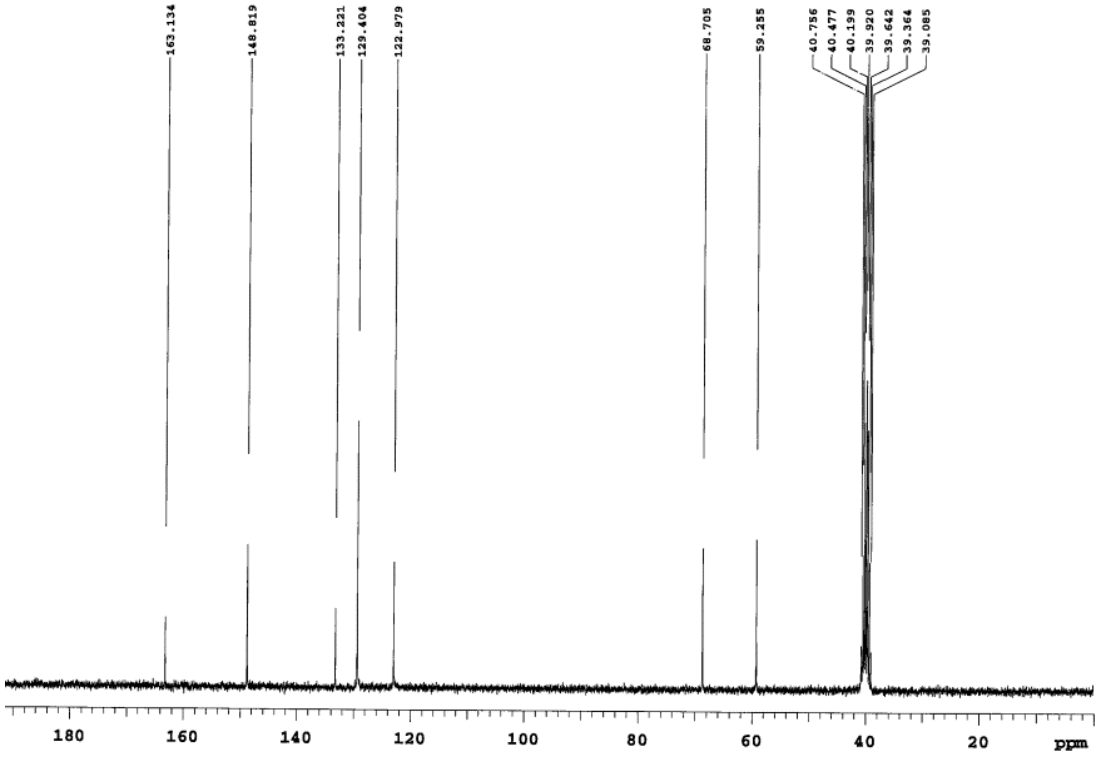


Figure S14. ^{13}C NMR Spectrum (DMSO, 75 MHz) of **DNB**

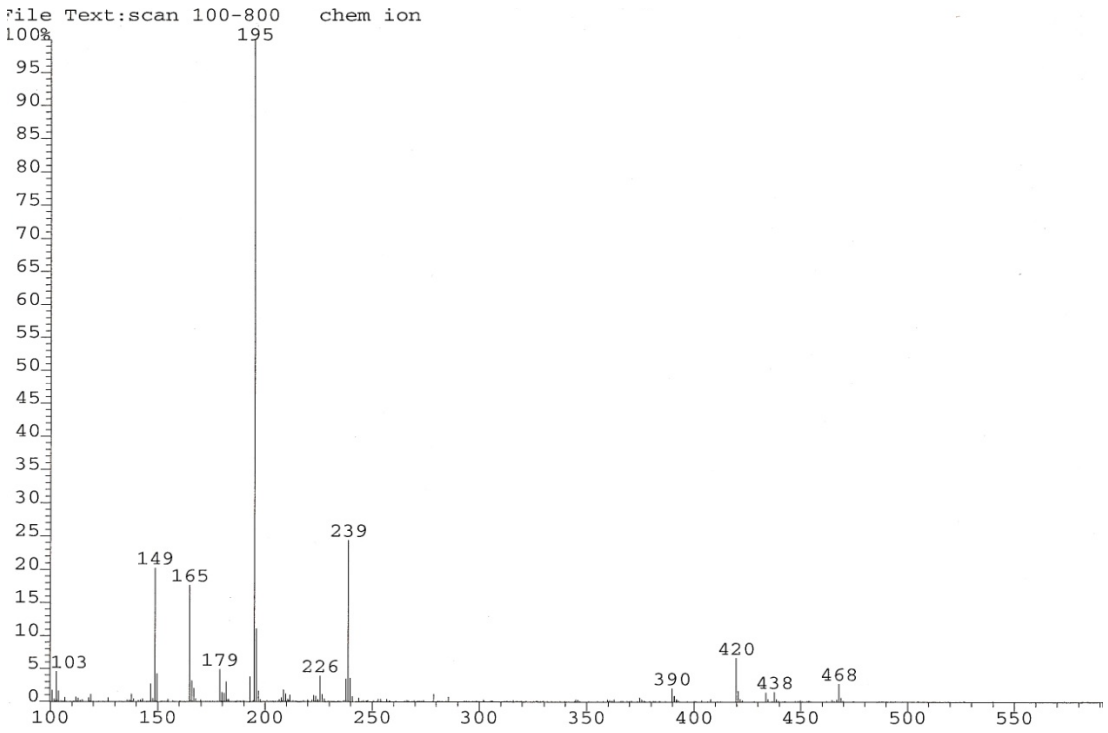


Figure S15. EI-MS (+) Spectrum of **DDNB**

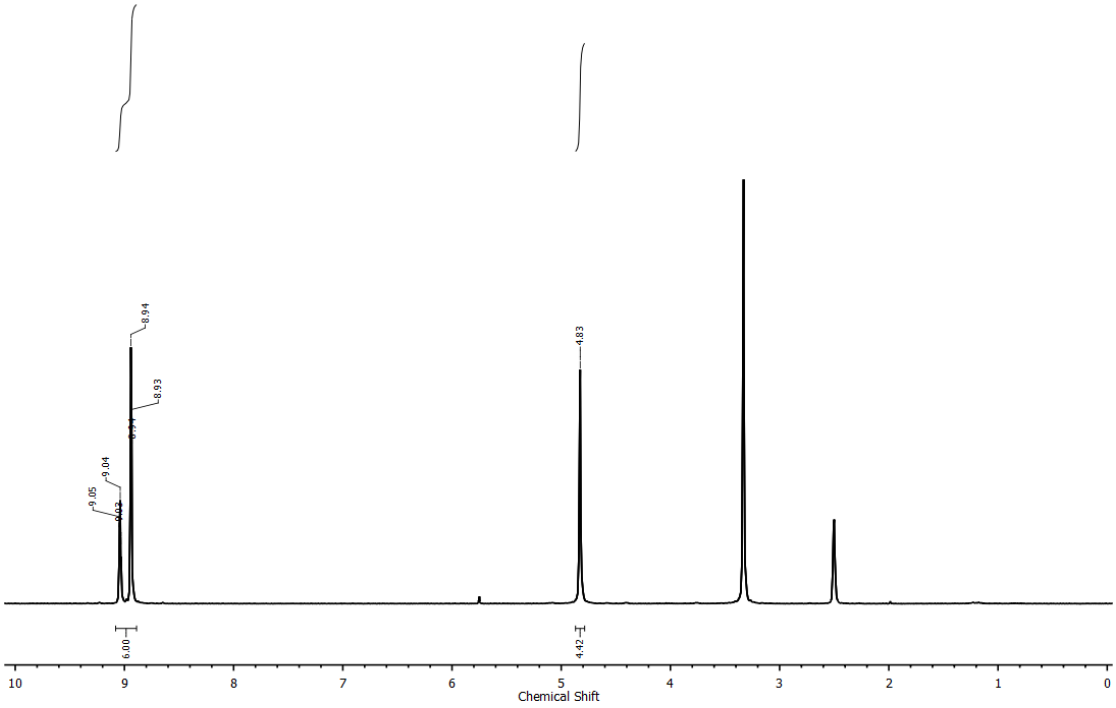


Figure S16. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **DDNB**

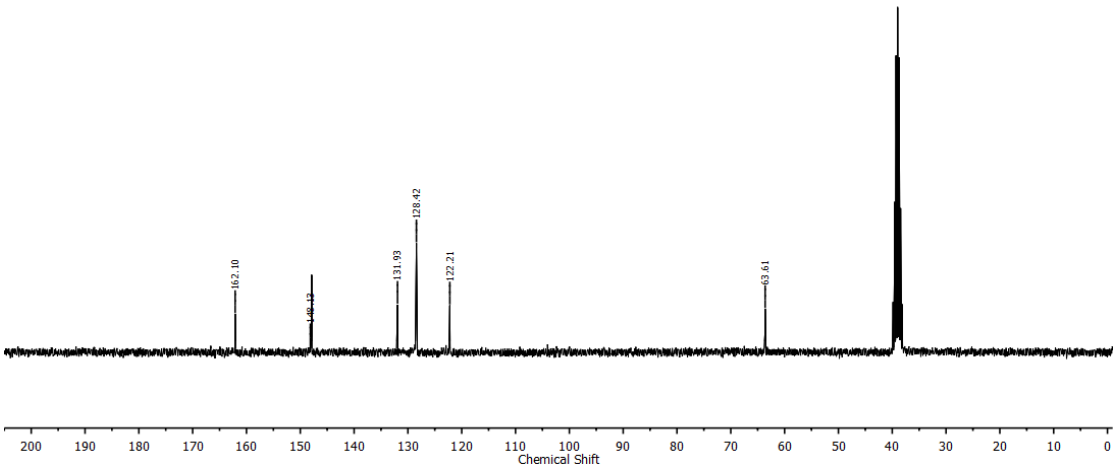


Figure S17. ^{13}C NMR Spectrum (DMSO, 75 MHz) of **DDNB**

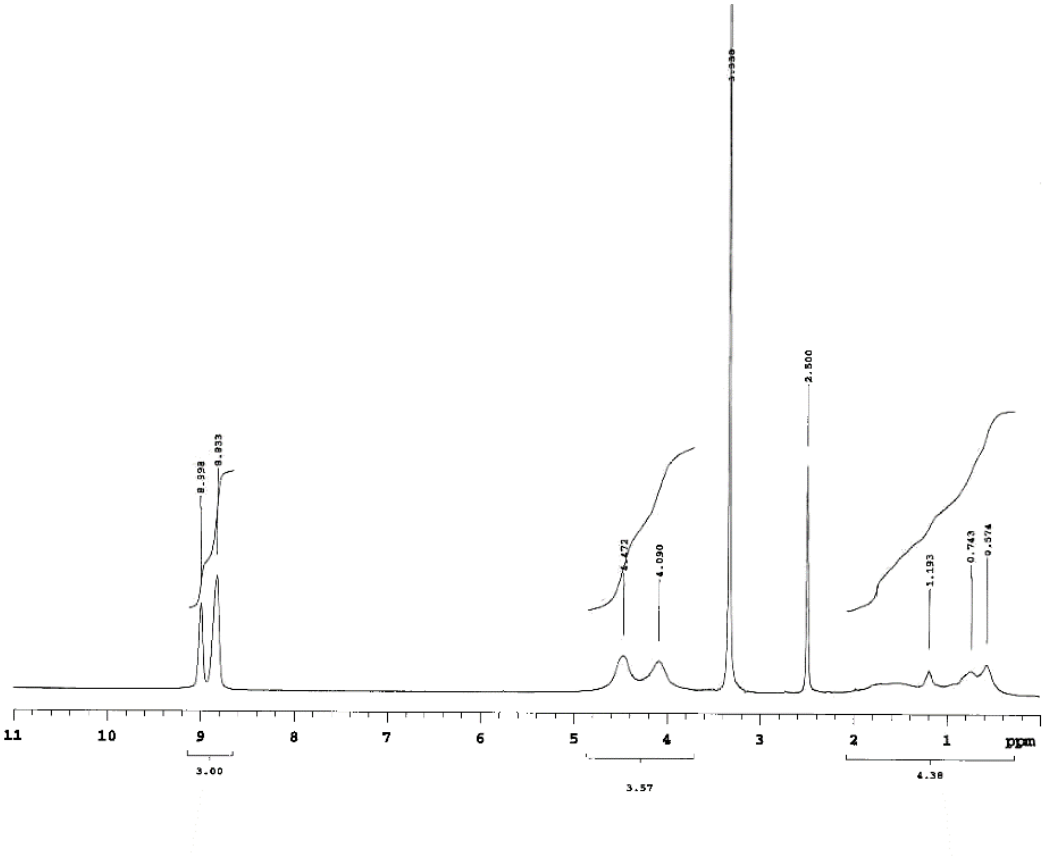


Figure S18. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **DNB-Polyhema**

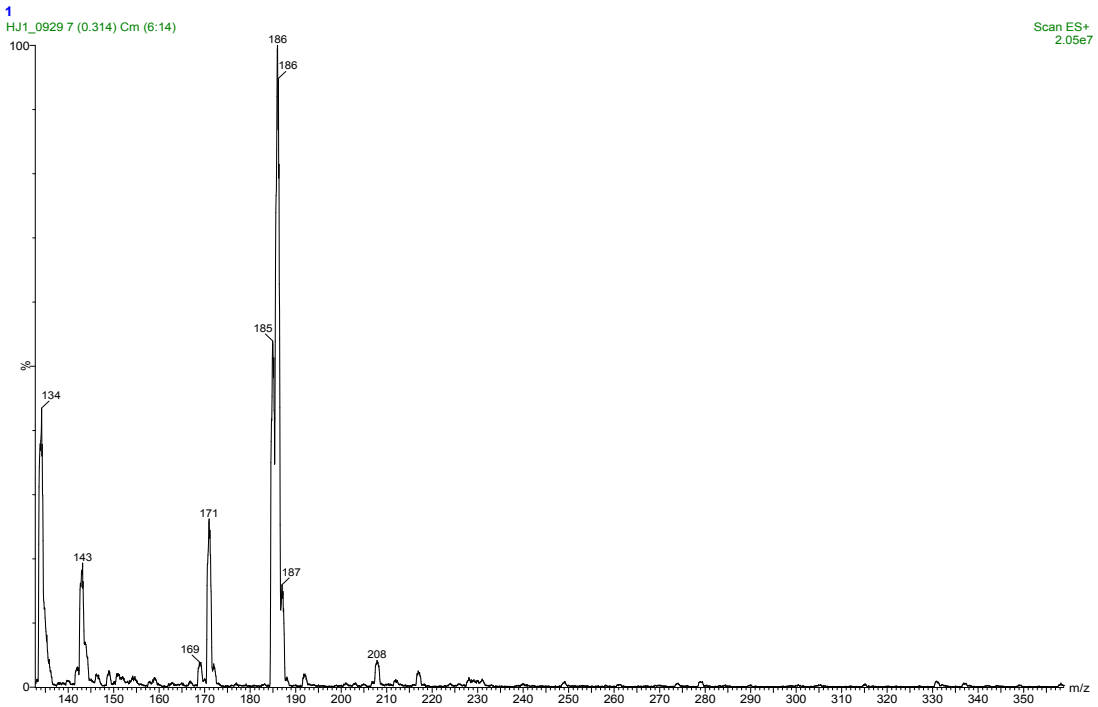


Figure S19. EI-MS (+) Spectrum of **MV**

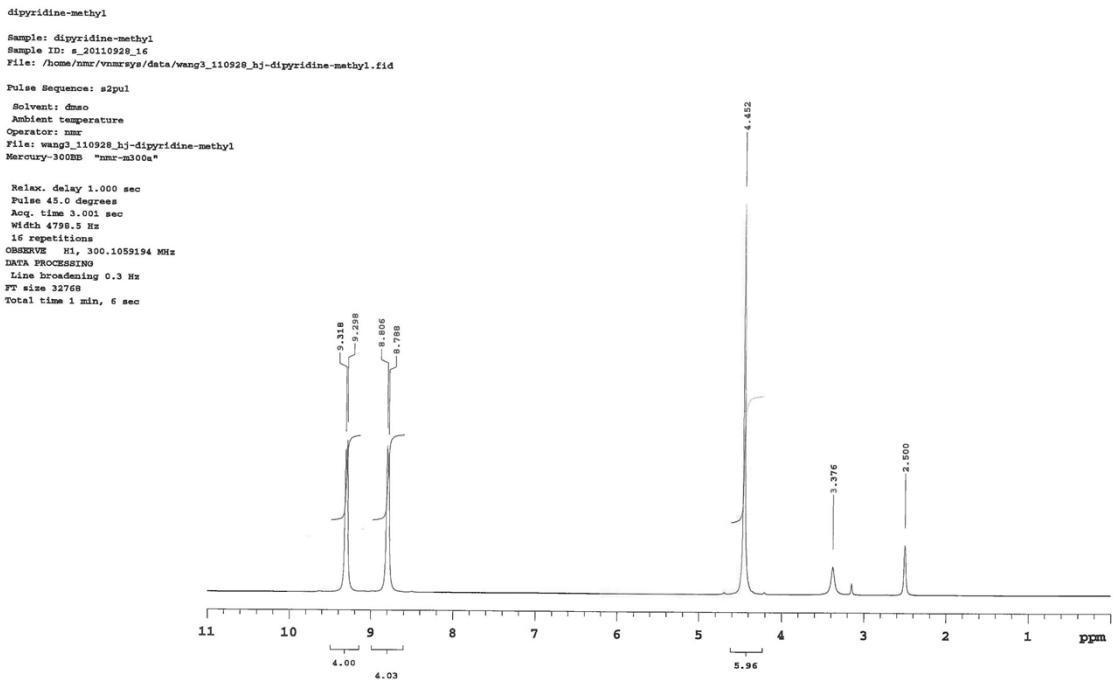


Figure S20. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **MV**

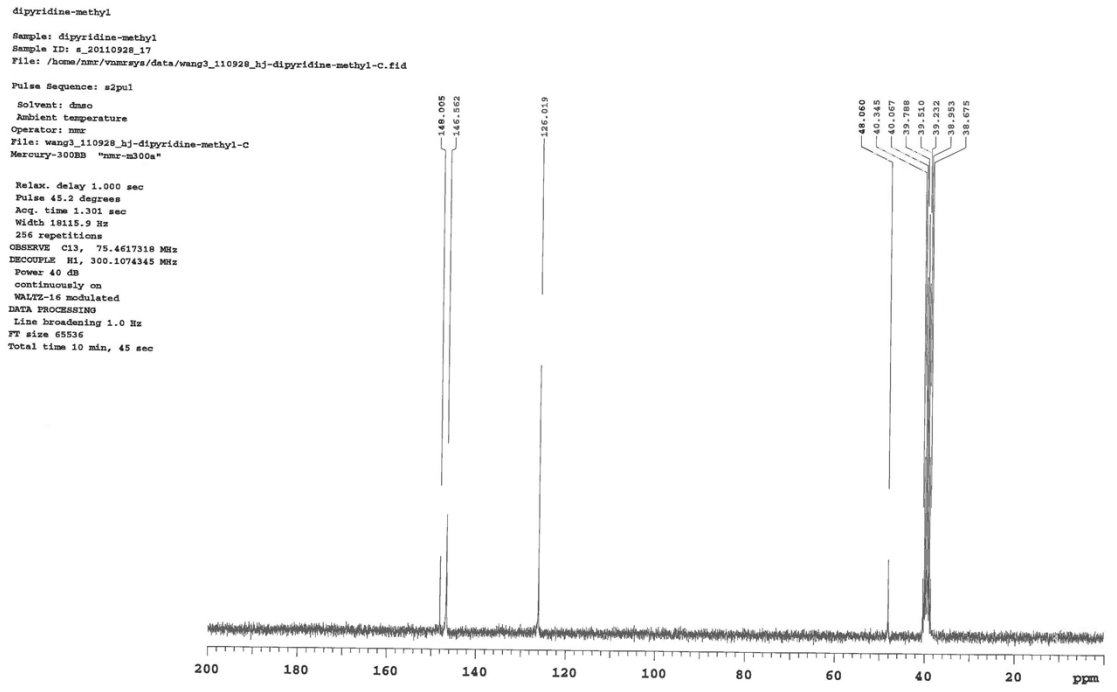


Figure S21. ^{13}C NMR Spectrum (DMSO, 75 MHz) of **MV**

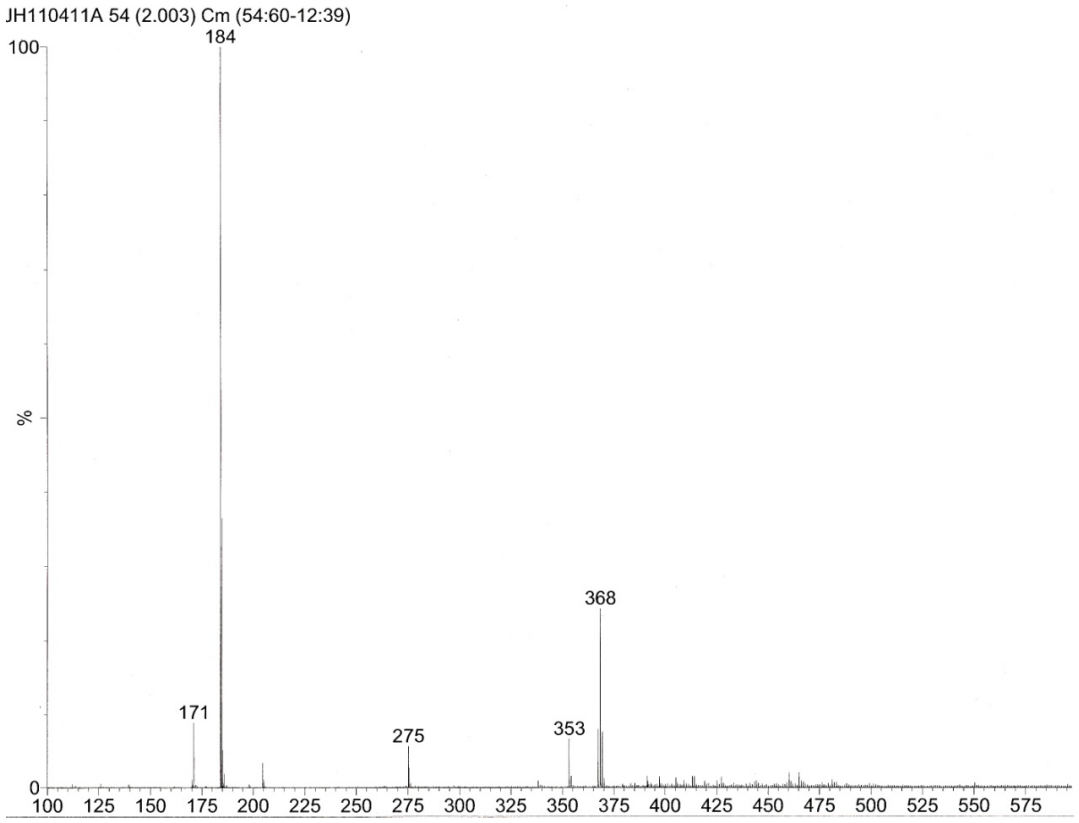


Figure S22. ESI-MS (+) Spectrum of **TV**

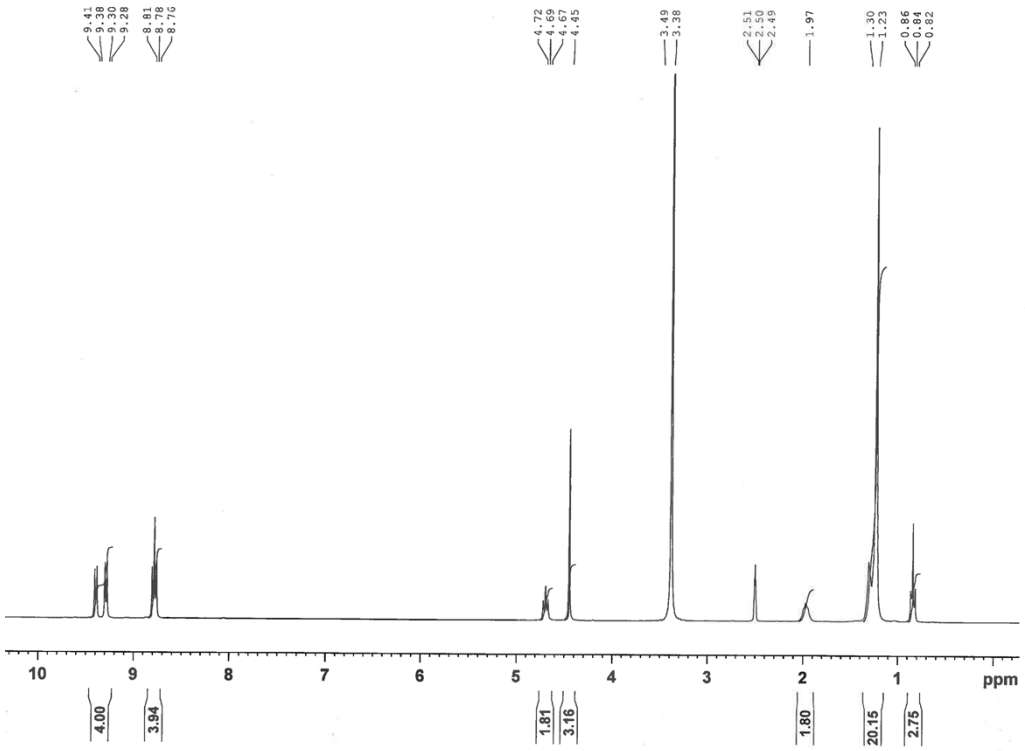


Figure S23. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **TV**

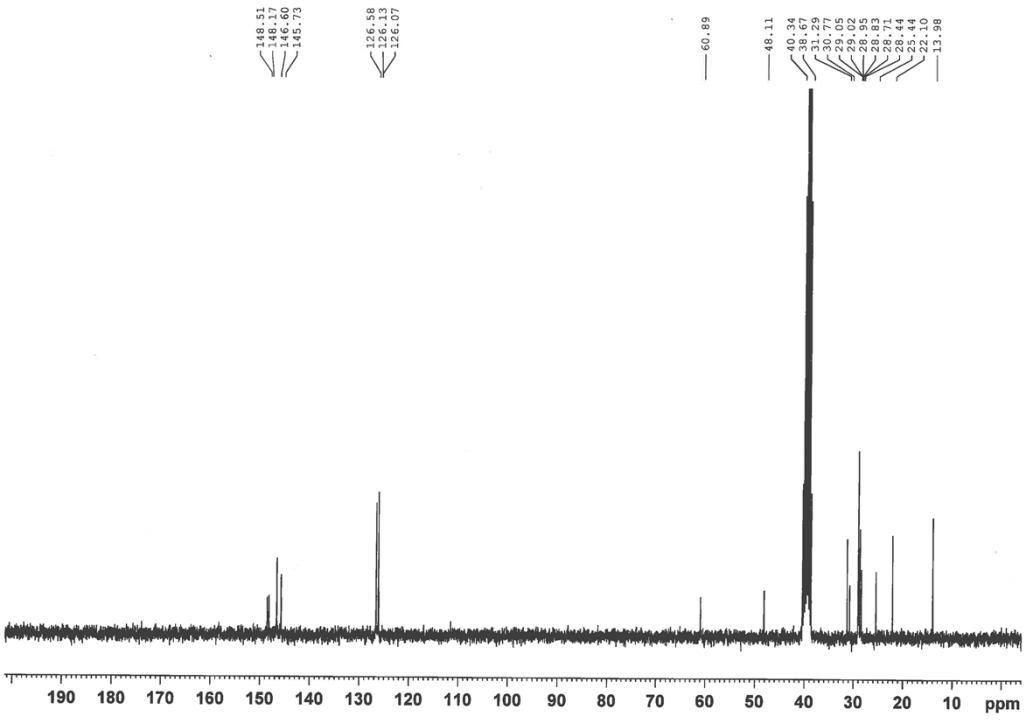


Figure S24. ^{13}C NMR Spectrum (DMSO, 75 MHz) of **TV**

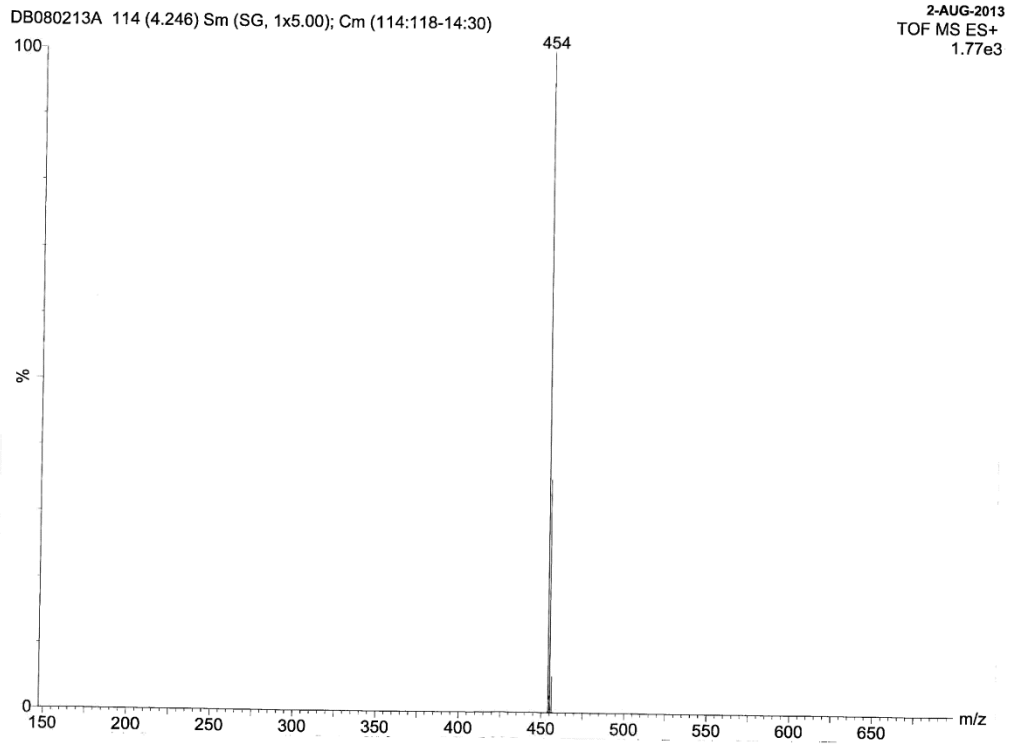


Figure S25. ESI-MS (+) Spectrum of **2-AP**

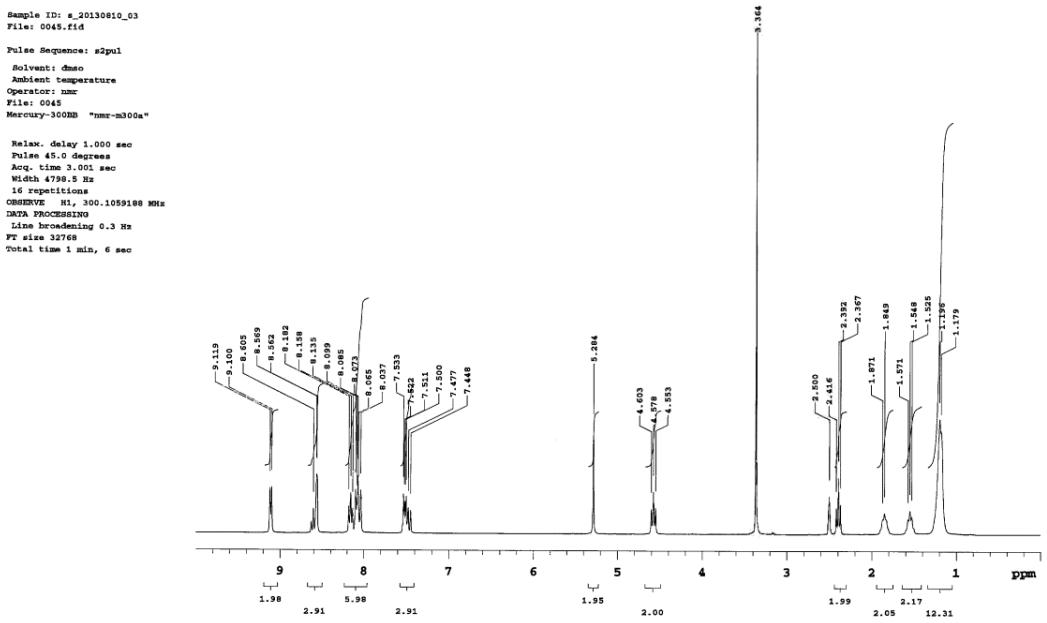


Figure S26. ^{1}H NMR Spectrum (DMSO, 300 MHz) of **2-AP**

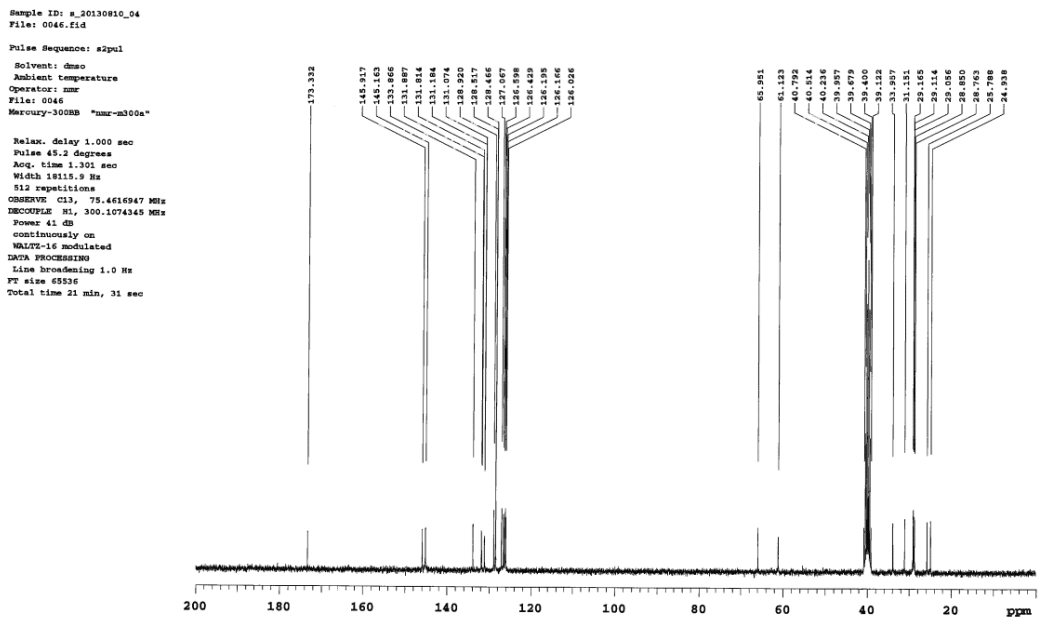


Figure S27. ^{13}C NMR Spectrum (DMSO, 75 MHz) of **2-AP**

6. References

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