Electronic Supplementary Material (ESI) for Analyst.

Photophysical properties and imaging application of a new polarity-sensitive fluorescent probe

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Instruments and Reagents

All the materials/solvents for synthesis/spectra measurement are analytical/spectroscopic grade, and they were used as received without further purification. NMR spectra were recorded on a Bruker NMR spectrometer (400MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). UV-Vis absorption spectra were recorded on a UV-2450 spectrophotometer. Fluorescence spectra were recorded with F-380A fluorospectrometer. All pH values were measured on PHSJ-4A pH meter. The confocal fluorescence imaging was performed on Leica TCS SP5 Confocal Laser Scanning Microscope. The fluorescence quantum yields were determined with rhodamine B as the standard (Φ=0.73).

Calculation of Δf values of Dio-DMSO binary systems

The dielectric constants (ε) and refractive indices (n) of the pure solvents as well as the calculation process of Δf values for Dio-DMSO binary systems were obtained according to the previous literature.[1-7]

The mixed solvents parameter ε_{mix} and n_{mix} were estimated from eqs 1 and 2,

$$\varepsilon_{mix} = f_a\varepsilon_a + f_b\varepsilon_b \qquad (1)$$

$$n_{mix}^2 = f_a n_a^2 + f_b n_b^2 \qquad (2)$$

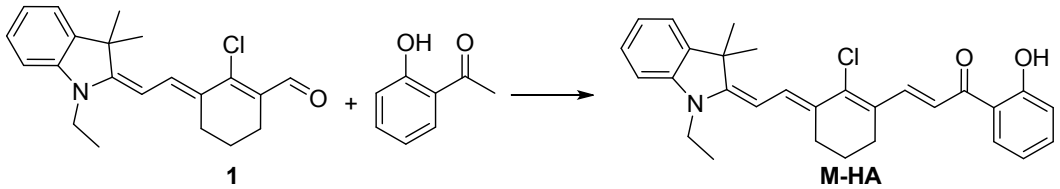
where the subscripts a and b represent the two different pure solvents and $f_{a,b}$ is the volume fraction of each solvent. The polarities of the pure and mixed solvents could be estimated using the definition of the Δf from

the Lippert-Mataga equation:

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

Synthesis of probe M-HA

Probe M-HA was facilely synthesized by only one step with moderate yield (Scheme S1). The compound 1 has been reported in the previous literature.[8]



Scheme S1 The synthesis of M-HA

Synthesis procedure: compound 1 (0.34 g, 1.0 mmol) and 2′-hydroxyacetophenone (0.272 g, 2.0 mmol) were dissolved in 20 mL methanol, then 15 % KOH aqueous solution 5.0 mL was added. The mixture was refluxed for about 10 h under N_2 atmosphere. After cooled to the room temperature, M-HA was obtained by column chromatography with eluent dichloromethane and methanol (20:1, V/V). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 1.275 (t, J=7.2 Hz, 3H), 1.656 (s, 6H), 1.874-1.930 (m, 2H), 2.604 (t, J=6.0 Hz, 4H), 3.745 (q, J=7.2 Hz, 2H), 5.534 (d, J=12.8 Hz, 1H), 6.696 (d, J=8.0 Hz, 1H), 6.924 (q, J=7.6 Hz, 2H), 7.016 (d, J=8.0 Hz, 1H), 7.093 (d, J=14.8 Hz, 1H), 7.189-7.221 (m, 2H), 7.456 (t, J=7.6 Hz, 1H), 7.706 (d, J=12.8 Hz, 1H), 7.874 (d, J=8.0 Hz, 1H), 8.507 (d, J=14.8 Hz, 1H), 13.215 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$)

δ(ppm): 11.20, 21.32, 26.26, 27.37, 28.24, 29.73, 37.08, 46.43, 92.88, 106.52, 117.43, 118.55, 120.57, 121.87, 124.32, 126.95, 127.85, 129.29, 130.16, 135.68, 139.44, 142.47, 143.76, 160.26, 163.52, 193.39. HRMS m/z calcd. for $C_{29}H_{30}ClNO_2$ [M+H$^+$]: 460.2043, found 460.1972.

Spectra measurement

The probe M-HA was dissolved in various common solvents to obtain the stock solution (1.0 mM). The solvents used in our work contain toluene (Tol), 1,4-dioxane (Dio), ethyl acetate (EA), chloroform (Chl), dichloromethane (DCM), acetone (Ace), N,N-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), ethyl alcohol (EtOH), methanol (MeOH) and water. The test solution of the probe M-HA (10 μM) was diluted from the probe stock solution with corresponding solvents. For the binary mixed solvents, two different solvents were mixed directly by appropriate volume ratio. The resulting solution was detected immediately with UV-Vis and fluorescence spectrophotometer.

Computational details

Geometry optimizations and electronic-structure calculations for the Enol and Keto forms of M-HA in the ground state (S_0) were performed using density functional theory (DFT) method, and those in the excited state (S_1) were studied using the time-dependent DFT (TD-DFT) approach. The solvent environment was mimicked by the polarizable continuum model (PCM) implemented in the B.01 version of the

Gaussian 16 package.[9] The PBE0 functional together with 6-31G(d) basis set was adopted following Lin *et al*.[10,11] Under the harmonic oscillator approximation, the Huang-Rhys (HR) factor of each mode is defined as $HR_j = \frac{D_j^2 \omega_j}{2\mathrm{h}}$, and D_j represents the displacement along the mode *j* between two electronic states. The HR factors were calculated using the MOMAP program.[12]

Cell culture and and MTT assay

HepG2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum. The cells were seeded in confocal culture dishes and then incubated for 24 h at 37 °C under a humidified atmosphere containing 5 % CO_2 and 95 % air. For cytotoxicity assay, about 10^4 HepG2 cells were seeded into a 96-well plate and incubated with a series of concentrations of the probe M-HA (10^{-4}-10^{-8} μM). After 24 h, 20 μL of MTT (5.0 mg/ml in PBS) was added and incubated for another 4 h. Subsequently, the culture medium was removed, and 150 μL DMSO was added into the dishes to dissolve the formazan crystal product. The plate was shaken for 5 min, and then absorbance at 570 nm was measured by the microplate reader.

Fluorescence imaging in live cells

The HepG2 cells were seeded into a glass-bottom culture dishes up to the density of about 1×10^4 cells/well. Before the fluorescence imaging experiments, the HepG2 cells were washed with PBS (pH=7.4) three

times, then further incubated with the probe M-HA (10 μM) or corresponding organelles dyes for 30 min under normal culture conditions. Afterwards, the cells were imaged through a Leica TCS SP5 confocal microscopy. The fluorescence emission of M-HA was collected at 700-800 nm upon excitation at 543 nm, and the fluorescence emission of organelles dyes was collected at 500-550 nm upon excitation at 488 nm (for mitochondria dye Mito-Tracker Green), 500-550 nm upon excitation at 488 nm (for lysosomes dye Lyso-Tracker Green), 580-620 nm upon excitation at 543 nm (for endoplasmic reticulum dye ER-Tracker Red), 580-620 nm upon excitation at 543 nm (for Golgi apparatus dye Golgi-Tracker Red), respectively.

Solvent	[a]*E*N T	[b]Δf	[c]λ_{abs} (nm)	[c]λ_{em} (nm)	[c]λ_{em} (cm^{-1})	Stokes Shift (nm)	[c]$\Delta\nu$ (cm^{-1})	[c]ε (10^4 $M^{-1}\cdot cm^{-1}$)	[c]Φ_f
Tol	0.099	0.0132	550	660	15151.5	110	3030	2.30	0.0112
Dio	0.164	0.0223	549	680	14705.9	131	3509	2.09	0.0151
EA	0.228	0.1998	550	700	14285.7	150	3896	2.32	0.0224
Chl	0.259	0.1479	566	728	13736.3	162	3932	2.41	0.0413
DCM	0.309	0.2172	565	736	13587	171	4112	2.40	0.0645
Ace	0.355	0.285	562	738	13550	176	4243	2.83	0.117
DMF	0.386	0.2742	576	753	13280.2	177	4080	2.32	0.145
DMSO	0.444	0.265	577	756	13227.5	179	4104	2.22	0.187
EtOH	0.654	0.2886	556	757	13210	201	4776	2.65	0.127
MeOH	0.762	0.3102	559	767	13037.8	208	4851	2.46	0.181
Water	1.00	0.32	578	-[d]	-[d]	-[d]	-[d]	1.32	<0.001

Table S1 Photophysical properties of M-HA in different solvents

[a]Normalized solvent polarity, *E*N T;
[b]Orientation polarizability, Δf;
[c]Maximum absorption wavelength, λ_{abs}; maximum emission wavelength, λ_{em}; Stokes shifts, $\Delta\nu$ (cm^{-1}); molar absorption coefficient, ε; fluorescence quantum yield, Φ_f.
[d]No emission in water.

Table S2 The comparison of M-HA with some representative probes

Near-infrared emission	Molar absorption coefficient ($M^{-1}\cdot cm^{-1}$)	Stokes shift (nm)	Application	Ref
No	No mentioned	~39-72	Solvents and proteins detection	1
No	~6000-9000	~97	Solvents and proteins detection	2
No	~18400	59-167	Solvents and proteins detection	3
No	~3000	42-83	Live cells imaging	5
No	~3000-30000	~120	Live cells imaging	6
No	4430-11680	10-34	Solvents detection	14

Yes	13200-28300	110-208	Live cells imaging	This work

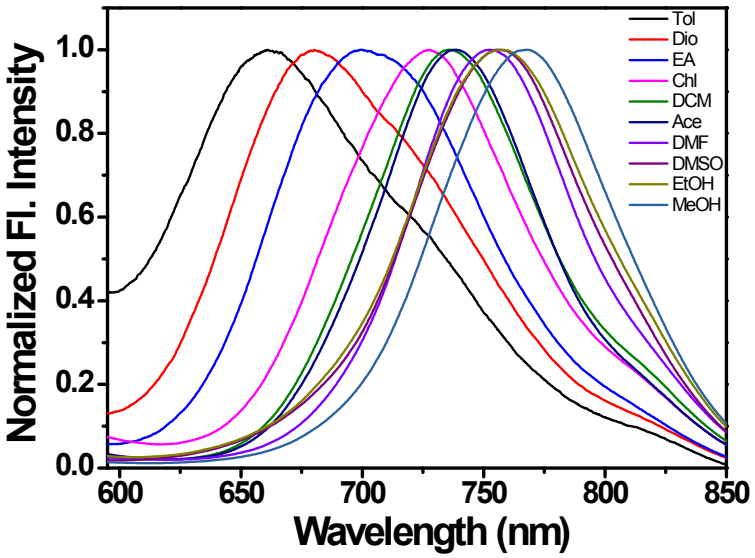


Figure S1 The normalized fluorescence spectra of M-HA (10 μM) in solvents with different polarity.

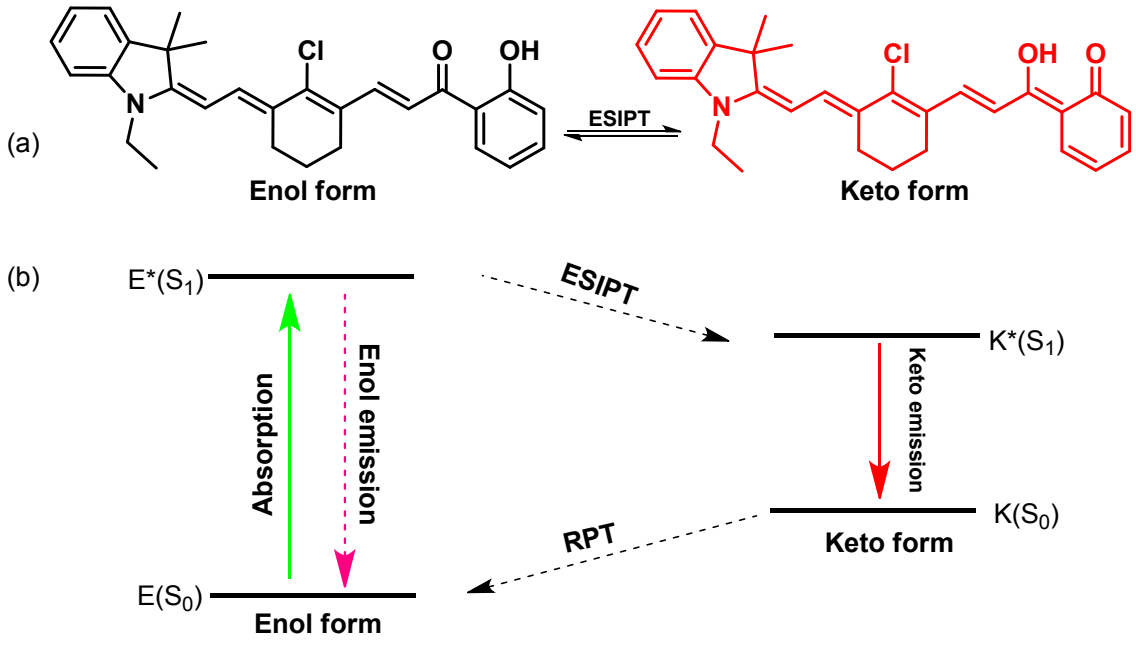


Figure S2 (a) The structures of M-HA before and after ESIPT. (b) The energy level diagram of M-HA.

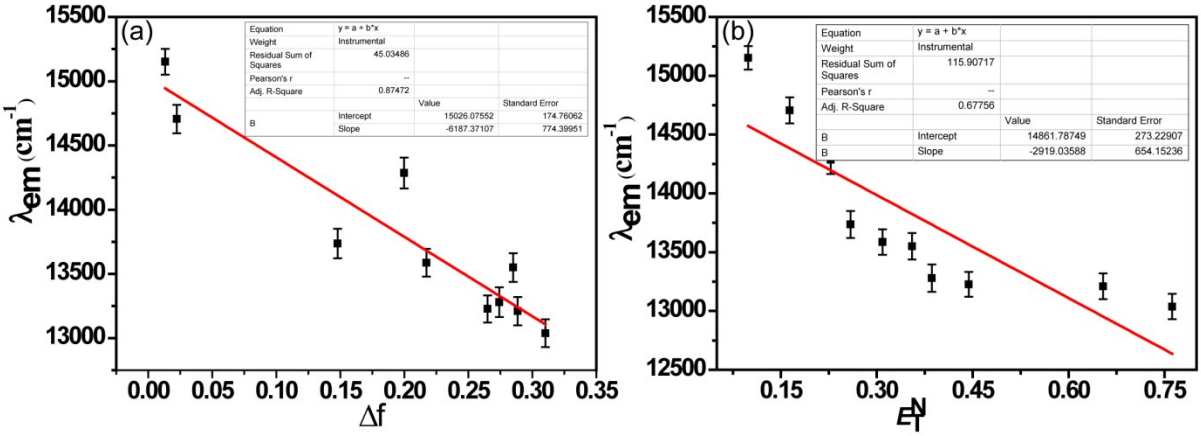


Figure S3 The linear relationship of emission wavelength λ_{em} (cm^{-1}) of M-HA vs. solvent parameters Δf and EN T values of the solvents.

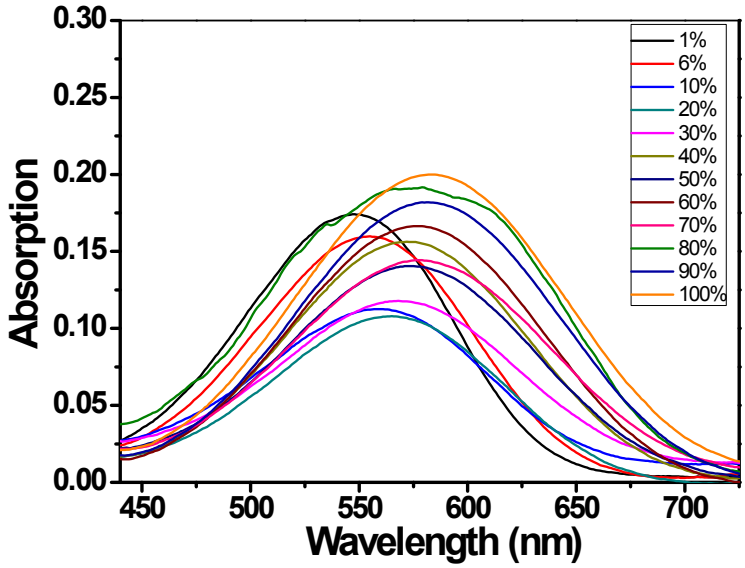


Figure S4 The absorption spectra of M-HA (10 μM) in Dio-DMSO mixtures. The volume fraction of DMSO is ranging from 1% to 100%.

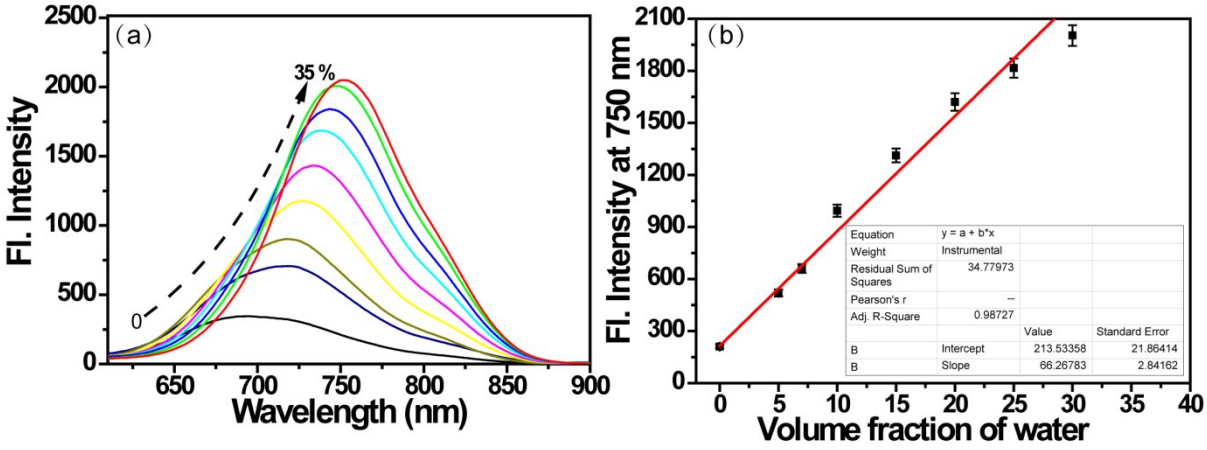


Figure S5 (a) The fluorescence spectra of M-HA (10 μM) in Dio-water mixtures (water from 1% to 35%). (b) The excellent linear relationship between fluorescence intensity of M-HA at 750 nm and different volume fraction of water (0-30%).

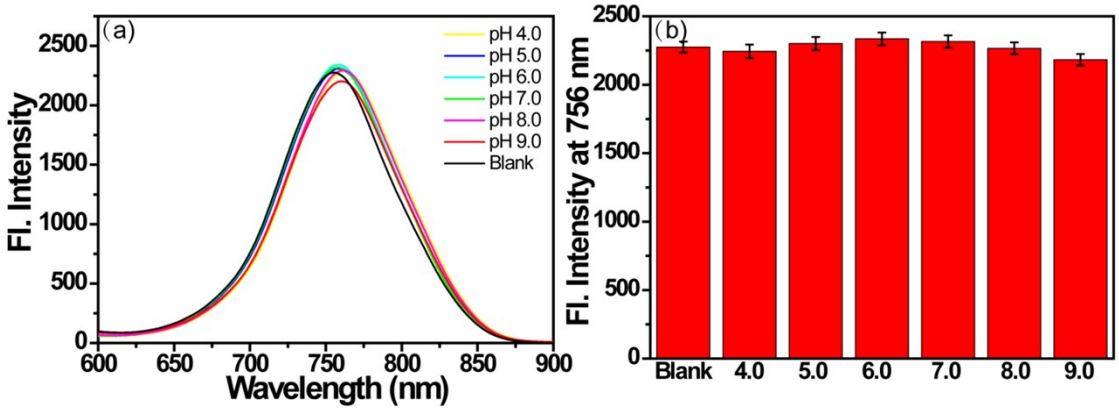


Figure S6 (a) The fluorescence spectra of M-HA (10 μM) within DMSO-water binary systems (90:10, V/V) under different pH values. (b) The quantitative fluorescence intensity of M-HA at 756 nm within DMSO-water binary systems (90:10, V/V) under different pH values.

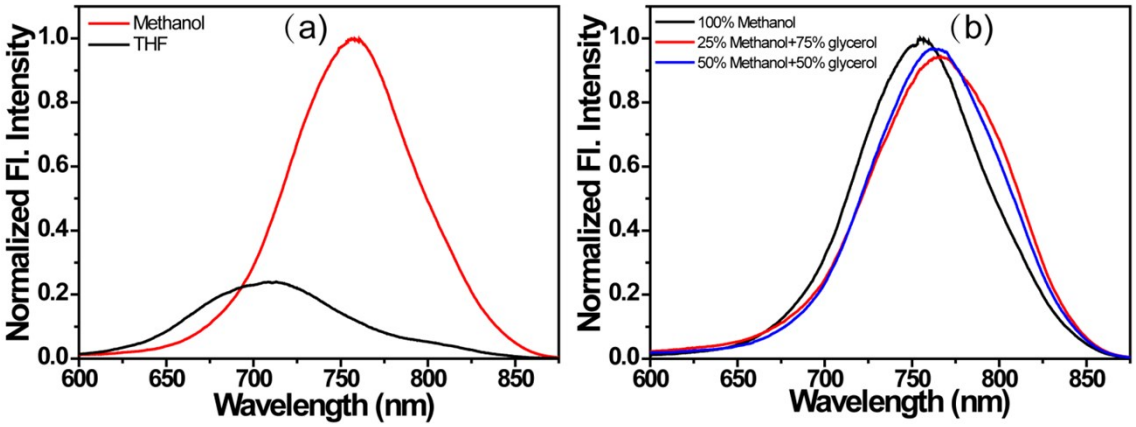


Figure S7 (a) The fluorescence spectra of M-HA (10 μM) in methanol and tetrahydrofuran (THF). (b) The fluorescence spectra of M-HA in methanol-glycerol system under different viscosity (the proportions of glycerol are ranging from 0 to 75%). THF and methanol have almost the same viscosity (0.53 cP vs 0.60 cP) but different polarity (ε=7.6 vs 32.63). It's obvious that the fluorescence intensity of M-HA displayed huge difference in them. Meanwhile, the fluorescence intensity of M-HA changed little with increasing viscosity when added different proportions of glycerol to methanol.

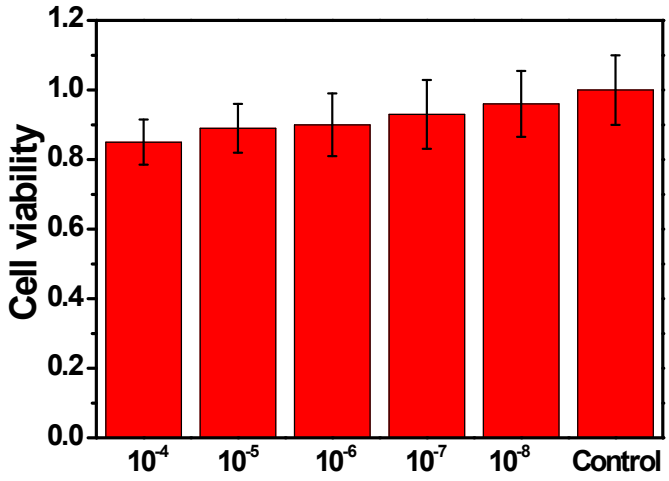


Figure S8 The cell viability of HepG2 cells incubated with different concentrations of M-HA. The cells were seeded at about $5x10^4$ cells/well on a 96-well plate. The cells were treated with media containing M-HA (10^{-4}-10^{-8} M) for 10 h, and MTT assay was then performed. The data are based on the average and show the standard deviation (n=5). The IC50 value was calculated to be 0.17 mM.

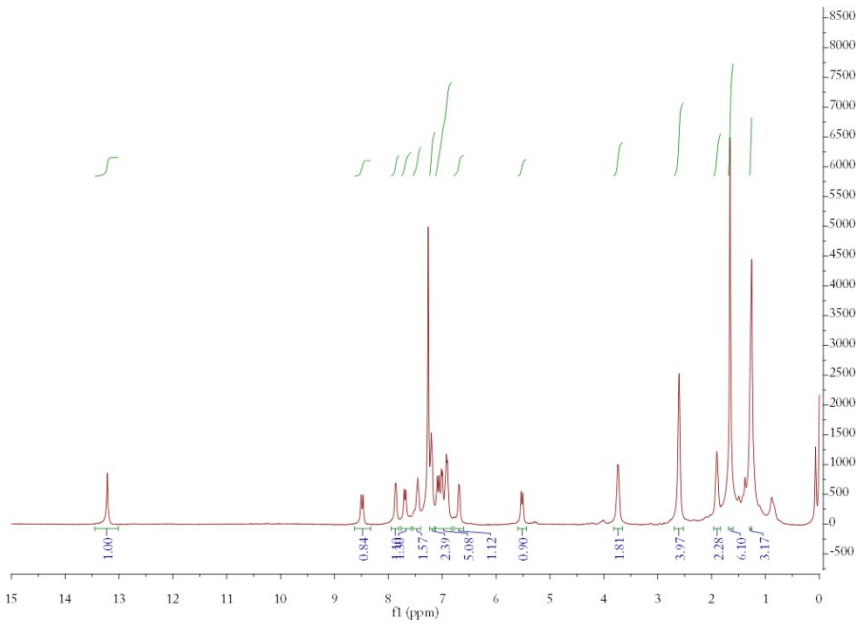


Figure S9 The ^{1}H NMR spectrum of M-HA.

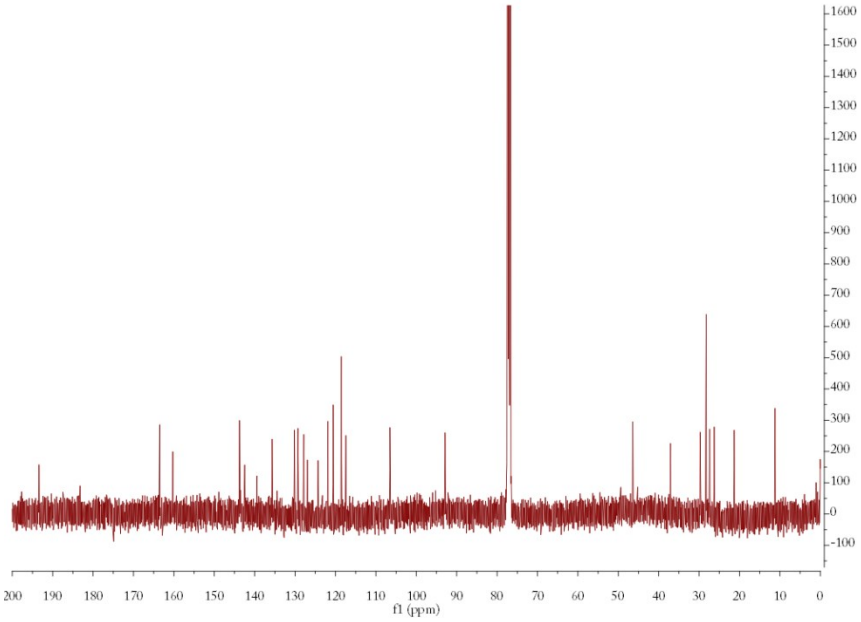


Figure S10 The ^{13}C NMR spectrum of M-HA.

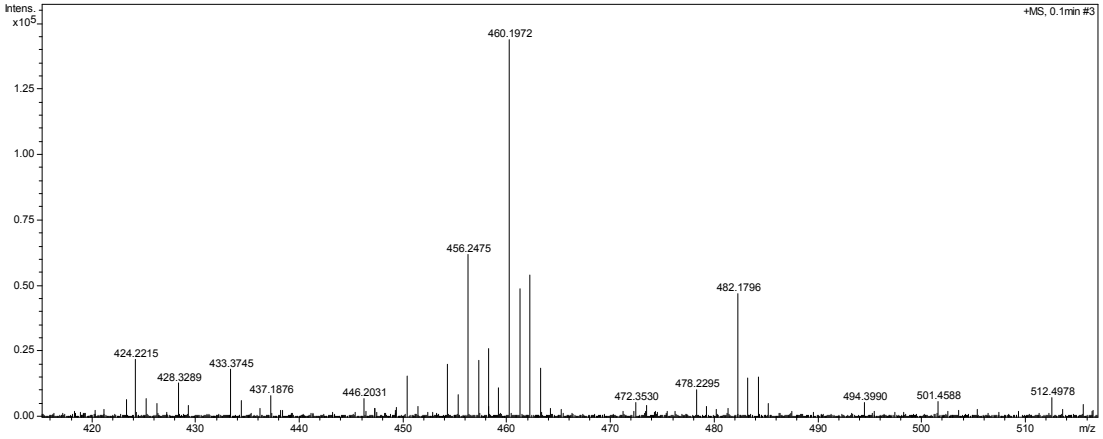


Figure S11 The MS spectrum of M-HA.

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