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First preparation of a triterpenoid-based supramolecular hydrogel in physiological phosphate buffered saline

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Content

Experimental Section

Materials

Glycyrrhetinic acid (**GA**), 2-[2-(2-chloroethoxy)ethoxy]ethanol, 4-dimethylaminopyridine (DMAP), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and other reagents were obtained from commercial suppliers and used as received. All the solvents were dried and distilled before used.

Instruments and methods

^{1}H, ^{31}P, and ^{13}C NMR spectra were recorded on a Bruker AVANCE III HD 400 MHz spectrometer; Electrospray ionization mass spectrometry (ESI-MS) was performed on Bruker ESQUIRE-LC spectrometer; High-resolution mass spectrometry (HRMS) was acquired using a quadrupole time-of-flight (Q-ToF) instrument; Transmission electron microscopy (TEM) images were obtained on a JEOL JEM-1011 microscope operating at an accelerating voltage of 100 kV. The samples were prepared by drop-casting the sol on the carbon coated copper grid, dried in air, and then were negatively stained with a uranyl acetate solution; Rheological data were obtained using a TA-AR2000ex rheometer with a 8 mm diameter parallel plate geometry at 25 ^{o}C. UV-Vis spectra were recorded on Thermo Scientific Nanodrop 2000C. FTIR spectra were measured on a Vertex 70 spectrometer; X-ray diffraction (XRD) analysis was attained using a Rigaku D/max 2500v X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å), operating at a voltage of 45 kV and a current of 100 mA; Theoretical computation was carried out using Gaussian 09 AM1 method.

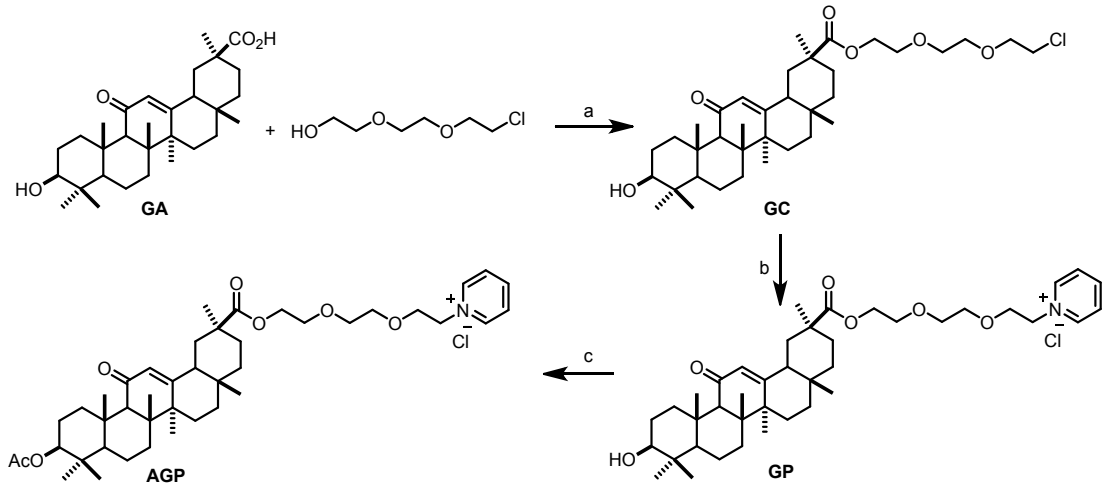
Cell Viability

The fibroblast cell line 3T3-L1 was provided by Norman Bethune Health Science Center of Jilin University and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 at 37 ◦C. The cells were seeded in 96-well culture plate with a seeding density of 1×10^5 cells per well. After overnight incubation, the previous media was replaced by fresh DMEM. Then, **GP** dissolved in DMSO at certain concentrations was added into the respective wells and incubated for 24 h. After that, CellTiter-Blue™ cell viability assay (Promega)[1] was performed for each well. The culture media in each well was replaced by 100 µL pre-warmed media containing 10% CellTiter-Blue™ reagent and incubated for 3 h at 37 ◦C and 5% CO_2. The fluorescence intensity was measured at 550/590 nm (Ex/Em) using Tecan Infinite M200 microplate reader. Cells treated with media containing DMSO only were considered 100% viable.

Cell Imaging

The 3T3-L1 cells were seeded in 12-well culture plate with a seeding density of 4×10^4 cells per well. After overnight incubation, the cells were treated with DMEM containing 70µM of **GP** and co-cultured at 37 ◦C. After 24 h, the treated cells were washed with PBS and fixed with 4% (w/v) paraformaldehyde for 15 min at room temperature. Then, the samples were stained with 1µg/mL FITC-phalloidin solution for 1 h, followed by DAPI staining of the cell nucleic acid at a concentration of 1 µg/mL for 15 min. After washing, the cells were imaged by a LSM 700 confocal laser scanning microscope imaging system (Carl Zeiss). The FITC was excited using a 488 nm laser, emitting 500-600 nm fluorescence. DAPI was excited with 405 nm laser, emitting 420-500 nm fluorescence.

Synthesis of GP and AGP



Scheme S1. Synthetic route of **GP** and **AGP**. Reagents and conditions: a) EDC, DMAP, DCM, rt, 4 h; b) Pyridine, DMF, 80 °C, 36 h; c) Ac_2O, DMAP, pyridine, rt, 24 h.

Glycyrrhetinic acid (**GA**, 5 g, 10.6 mmol), 4-dimethylaminopyridine (DMAP, 1.70 g, 13.8 mmol), and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 2.65 g, 13.8 mmol) were dissolved in dichloromethane (DCM, 50 mL), and then 2-[2-(2-Chloroethoxy)ethoxy]ethanol (1.95 mL, 13.8 mmol) was added. After the mixture was stirred at room temperature for 4 h, the reaction was quenched by adding dilute hydrogen chloride (1 M). The organic layer was washed by diluted hydrogen chloride, water, brine, and dried by anhydrous sodium sulfate. After removing all the solvents, a white powder **GC** was obtained. **GC** was re-dissolved in mixed solvents of pyridine (15 mL) and dimethylformamide (DMF, 1 mL), and then heated at 80 °C for 36 h. After that, the solvents were removed under the reduced pressure. The crude product was purified by column chromatography (V(CH_2Cl_2): V(CH_3OH) = 2:1) to afford a white solid, **GP** (1.93 g, yield 26%). ESI-MS (+): 664.7 $[M]^+$; HRMS (ESI): m/z calcd. for $C_{41}H_{62}NO_6$: 664.4577, found: 664.4579; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 9.34 (d, 2 × 1H, *J* = 3 Hz, pyridinium-H), 8.59 (t, 1H, *J* = 6 Hz, pyridinium-H), 8.13 (t, 2 × 1H, *J* = 6 Hz, pyridinium-H), 5.57 (s, 1H, 12-H), 5.13 (t, 2H, J_1 = 6 Hz, J_2 = 3 Hz, OCH_2), 4.31 (m, 1H, OCH_2), 4.13 (m, 1H, OCH_2), 4.06 (t, 2H, J_1 = 6 Hz, J_2 = 3 Hz, OCH_2), 3.55-3.65 (m, 3 × 2H, OCH_2), 3.24 (br, 1H, 3-H), 0.76, 0.77, 0.97, 1.08, 1.13, 1.34 (s, 7 × 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 200.57, 176.46, 169.99, 145.83, 145.59, 128.27, 128.19, 78.64, 70.15, 69.17, 63.20, 61.90, 54.90, 53.53, 48.54, 45.51, 44.13, 43.36, 41.22, 39.20, 37.69, 32.78, 31.90, 31.21, 28.69, 28.40, 28.20, 27.31, 26.54, 26.42, 23.44, 18.79, 17.52, 16.55, 15.77.

Compound **AGP** was synthesized according to ref. [2]. ESI-MS (+): 706 $[M]^+$; HRMS (ESI): m/z calcd. for $C_{43}H_{64}NO_7$: 706.4683, found: 706.4682; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 9.48 (d, 2 × 1H, *J* = 3 Hz, pyridinium-H), 8.47 (t, 1H, *J* = 6 Hz, pyridinium-H), 8.02 (s, broad, 2 × 1H, pyridinium-H), 5.51 (s, 1H, 12-H), 5.16 (m, 2H, OCH_2), 4.41 (m, 1H, OCH_2), 4.21 (m, 1H, OCH_2), 3.97-4.06 (m, 3H, OCH_2, 3-H), 3.47-3.53 (m, 6H, OCH_2), 1.96 (s, 3H, CH_3CO), 0.67, 0.78, 1.02, 1.05, 1.27 (s, 7 × 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 199.17, 175.32, 169.94, 168.77, 144.96, 144.37, 127.23, 126.69, 79.56, 69.34, 69.04, 68.42, 68.25, 62.02, 60.76, 60.21, 54.90, 53.99, 47.40, 44.43, 42.96, 42.24, 40.12, 37.81, 37.03, 36.65, 35.89, 31.66, 30.83, 30.14, 27.61, 27.28, 27.04, 25.46, 25.34, 22.54, 22.35, 20.30, 17.73, 15.68, 15.45.

Table S1 Gelation test results of **GP** in different solvents.

Entry	Solvent	State [a]	MGC [b]
1	H_2O	P	-
2	Kphos (1 mM, pH 7.4)	P	-
3	Kphos (10 mM, pH 7.4)	G	8.5
4	Kphos (100 mM, pH 7.4)	PG	-
5	Kphos (10 mM, pH 5.2)	P	-
6	Kphos (10 mM, pH 8.0)	G	9.3
7	PBS (1x, pH 7.4)	G	6.6
8	PBS (10x, pH 7.4)	P	-
9	NaCl (10 mM, 2.5)	P	-
10	NaCl (10 mM, 7.4)	P	-
11	NaCl (10 mM, 10.5)	P	-

[a] G = gel, P = precipitate, PG = partial gel. [b] MGC is the minimum gelator concentration (mg/mL) at which the gel formed.

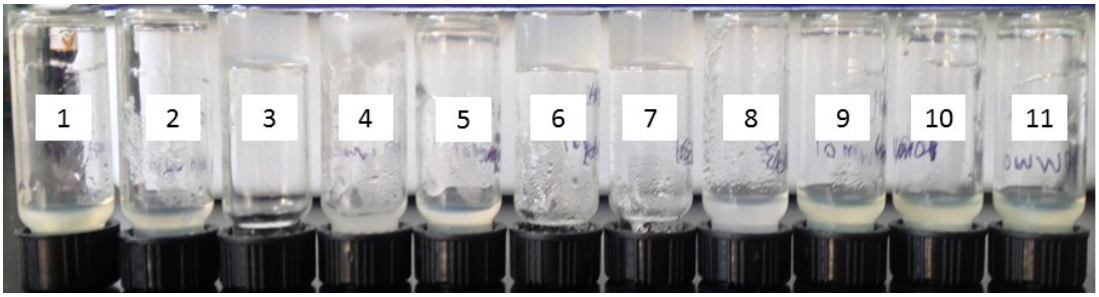


Fig. S1 Photograph of the gelation test results of **GP** in different solvents (the numbers correspond to the ones in Table S1).

The Gibbs free energy change (ΔG) during the gel-to-sol transition can be expressed as:

$$\Delta G = -RT\ln K = \Delta H - T\Delta S$$

That is, $\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$, where *K* is the equilibrium constant of the thermo-reversible gel-to-sol transition. For one component gel, $K = \frac{[Gelator]}{[Gel]}$, assuming unit activity of the gel and taking concentration of the solution to be equal to the dissolved concentration of the gelator, the equilibrium constant can be expressed as: $K = [Gelator]$. According to the above, a plot of ln*K* versus 1/T can allow us to calculate the thermodynamic parameters.

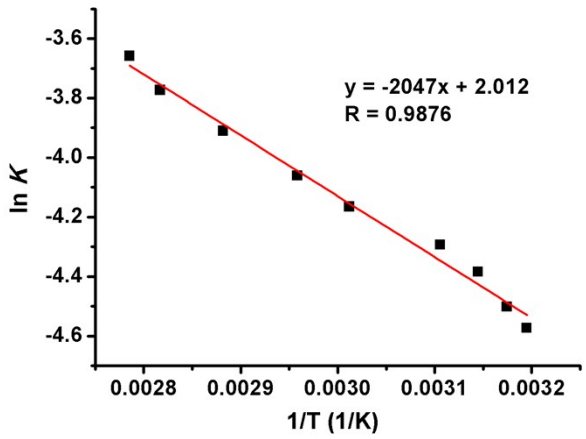


Fig. S2 Plot of ln*K versus* 1/T for the hydrogel of **GP**.

y = -2047x + 2.012; ΔH/R = 2047 K, ΔH = 17.0 kJ/mol; ΔS/R = 2.012, ΔS = 16.7 J/(mol·K); T = 298 K, ΔG = ΔH – TΔS = 12.0 kJ/mol.

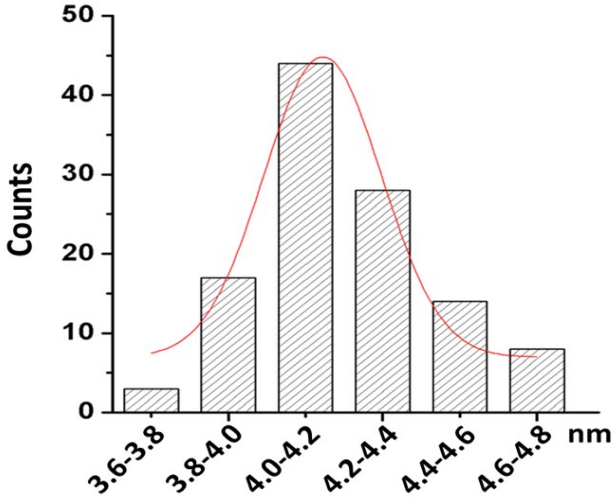


Fig. S3 Size distribution in fiber diameter of hydrogel **GP** in PBS (10 mM, pH 7.4)

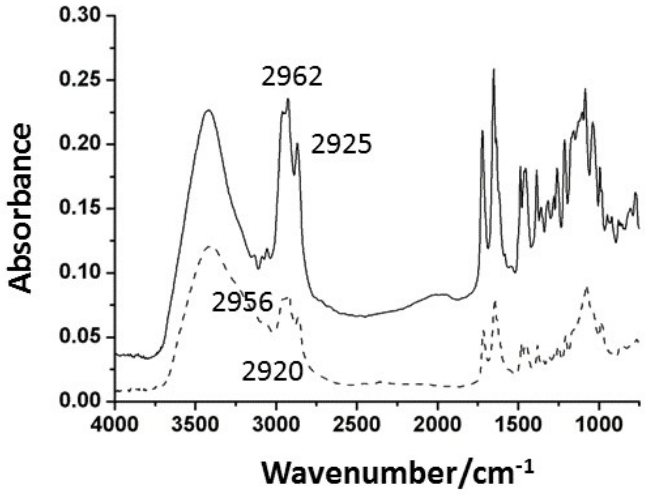


Fig. S4 FTIR spectra of a powder sample (solid line) and xerogel (dash line) of **GP**.

Fig. S5 Photograph of the gelation test of **AGP** in PBS (6.6 mg/mL).

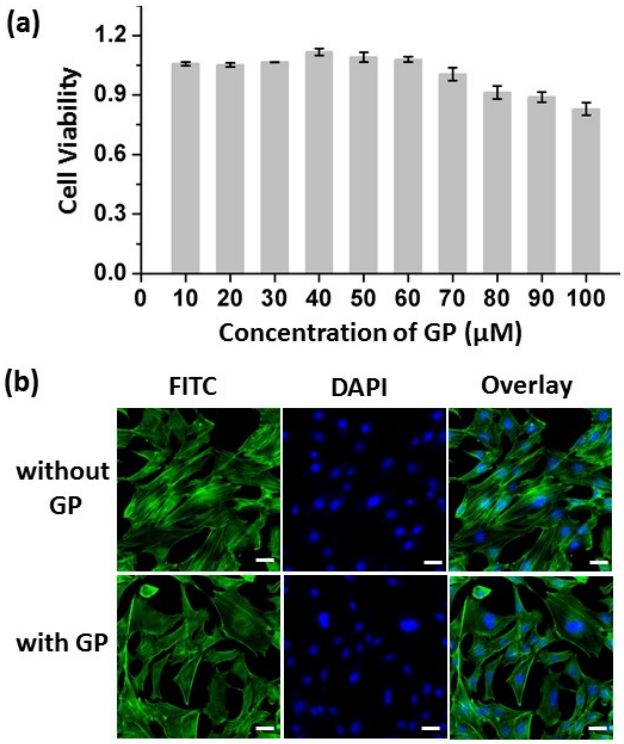


Fig. S6 (a) 3T3-L1 fibroblast cells viability of **GP** and (b) 3T3-L1 cell morphologies after the treatment with **GP** (70 μM) for 24 h. (green color, FITC-phalloidin stained actin cytoskeleton; blue color, DAPI stained cell nucleic acid.)

Table S2 The loading capacity of doxorubicin in terms of quantity by the hydrogel.

Entry	Conc. of doxorubicin (mg/mL)	State [a]
1	0.2	G
2	0.5	G
3	1.0	G
4	4.0	G
5	8.0	G
6	9.0	PG

[a] G = gel, PG = partial gel. The concentration of **GP** hydrogel in PBS (pH 7.4) is 10 mM.

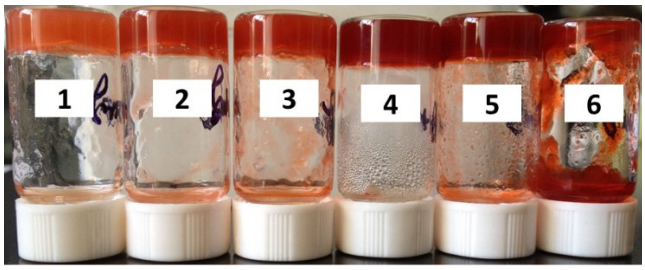


Fig. S7 Photograph of the loading capacity test of doxorubicin by **GP** hydrogel.

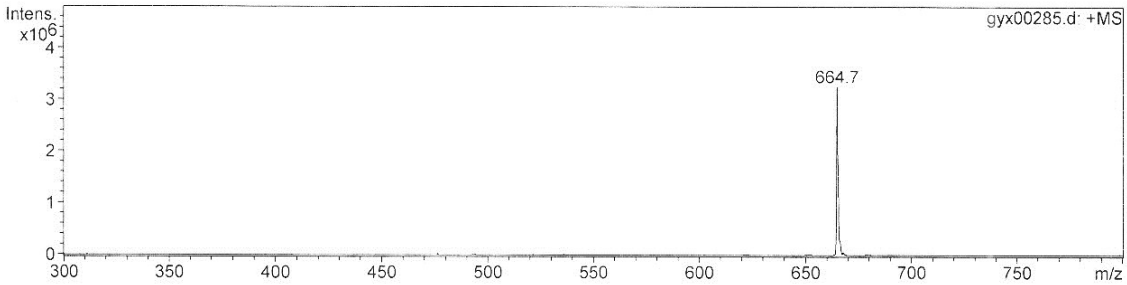


Fig. S8 ESI-MS (+) spectrum of compound **GP**

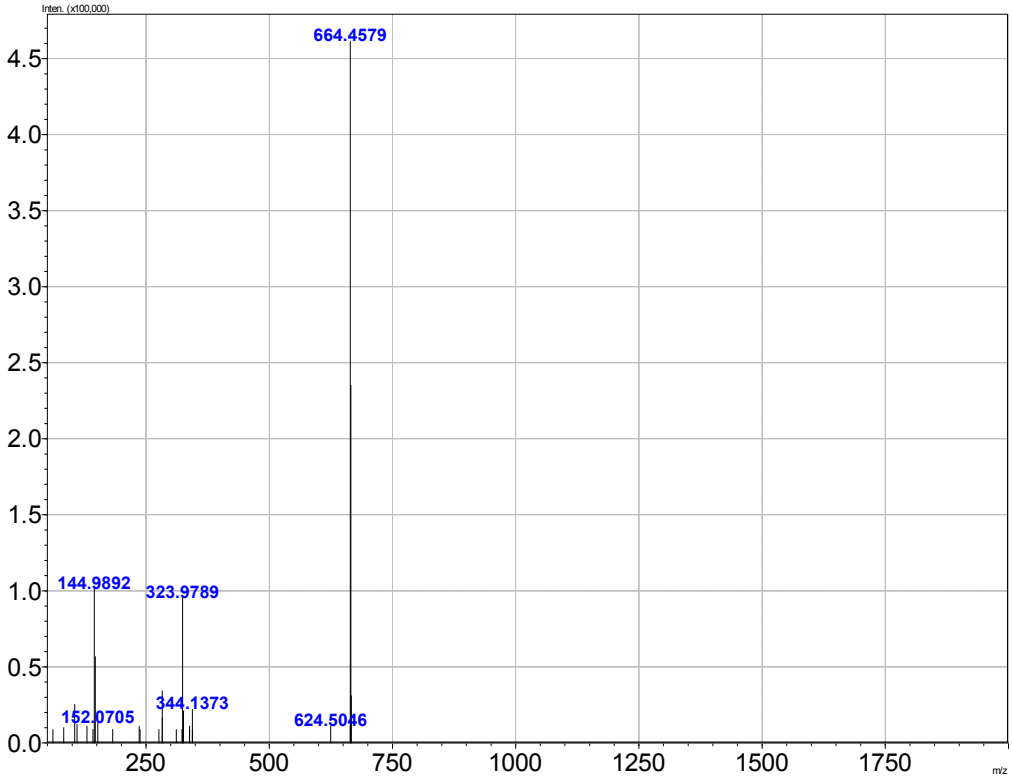


Fig. S9 High resolution ESI-MS (+) spectrum of compound **GP**

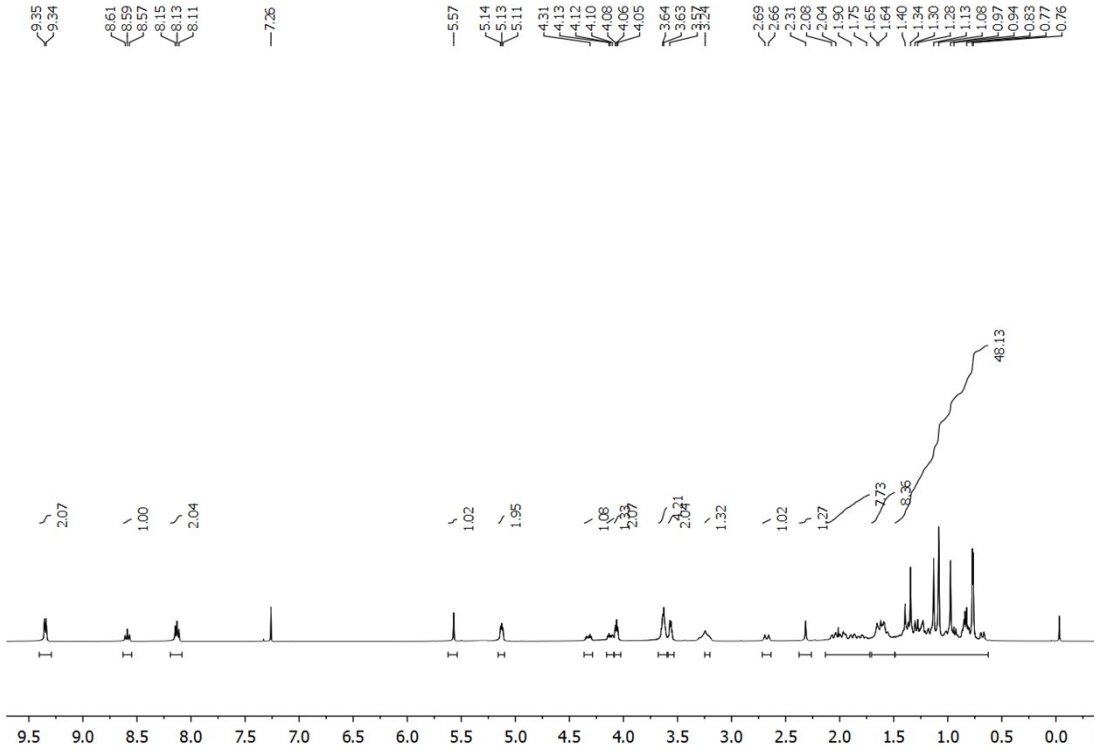


Fig. S10 ^{1}H NMR spectrum of compound **GP** (300 MHz, $CDCl_3$)

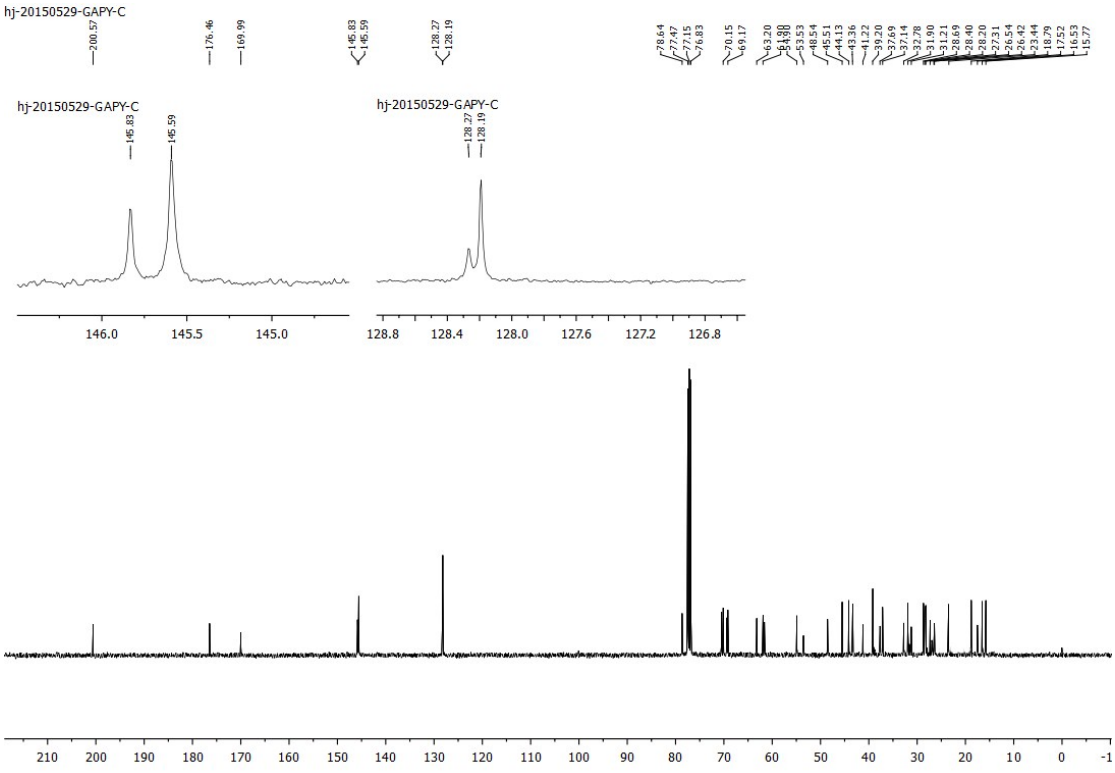


Fig. S11 ^{13}C NMR spectrum of compound **GP** (75 MHz, $CDCl_3$)

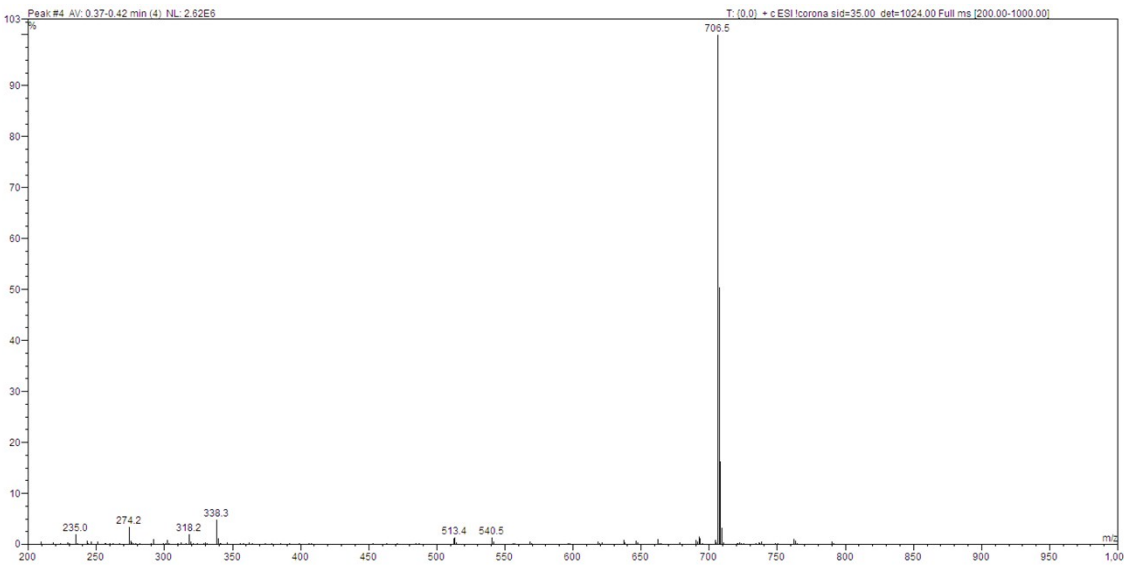


Fig. S12 ESI-MS (+) spectrum of compound **AGP**

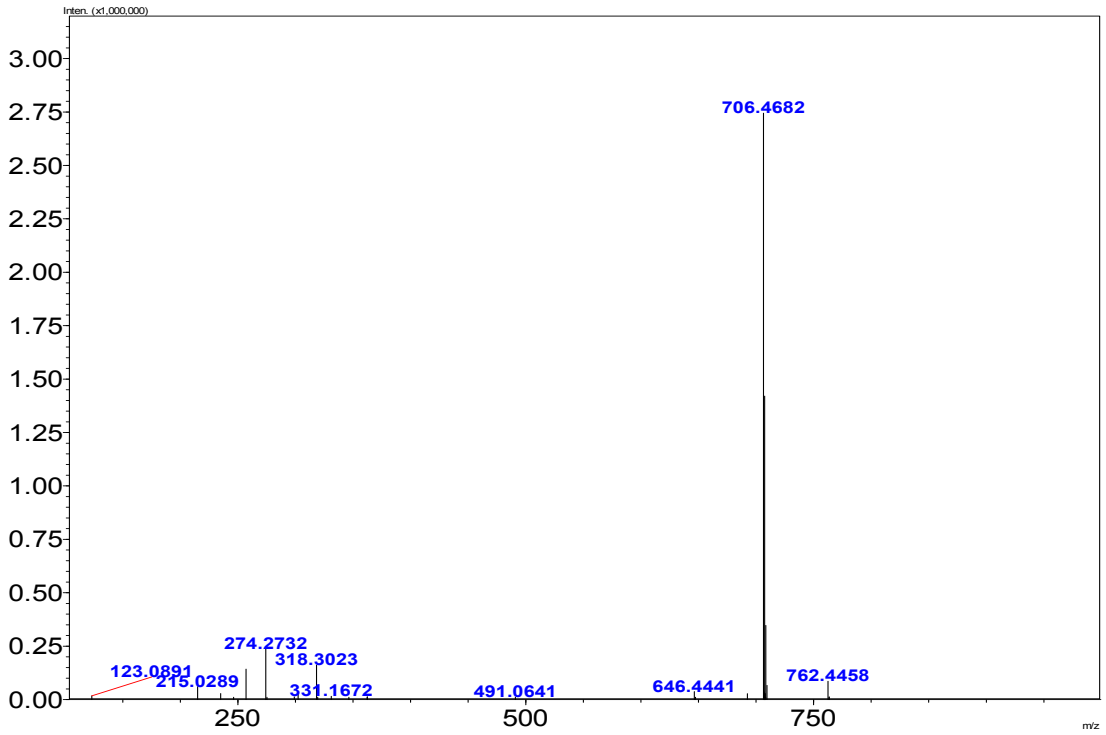


Fig. S13 High resolution ESI-MS (+) spectrum of compound **AGP**

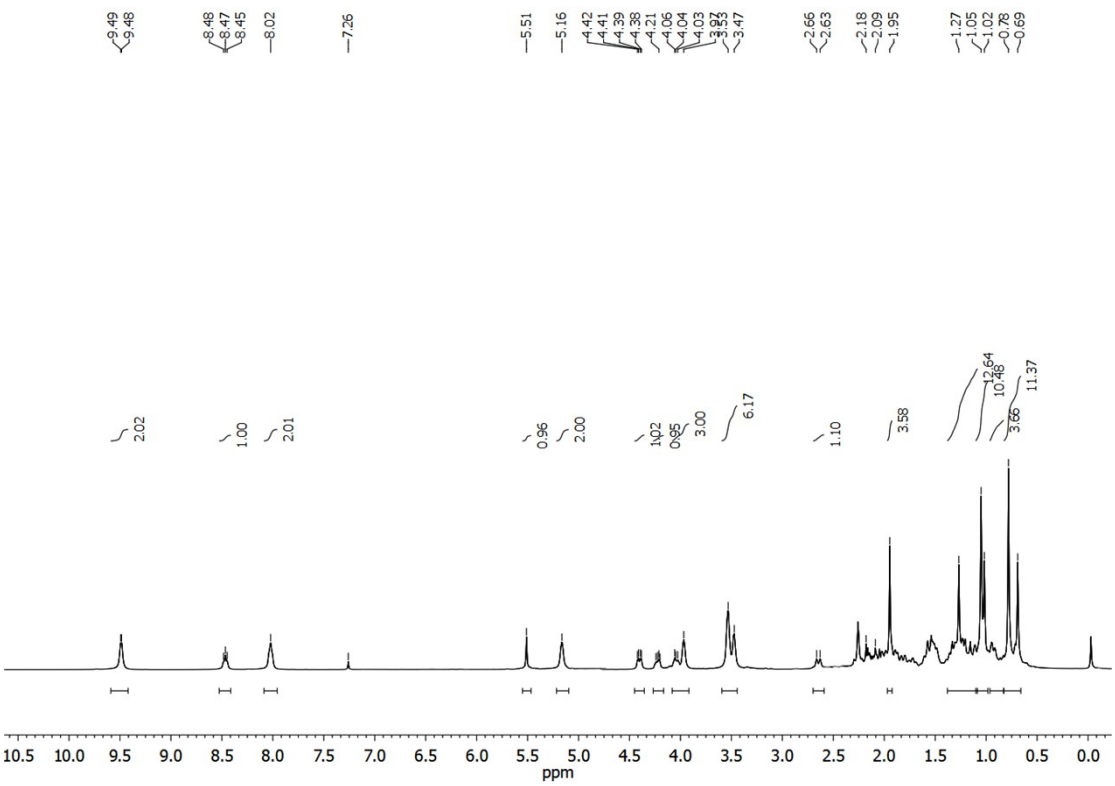


Fig. S14 ^{1}H NMR spectrum of compound **AGP** (300 MHz, $CDCl_3$)

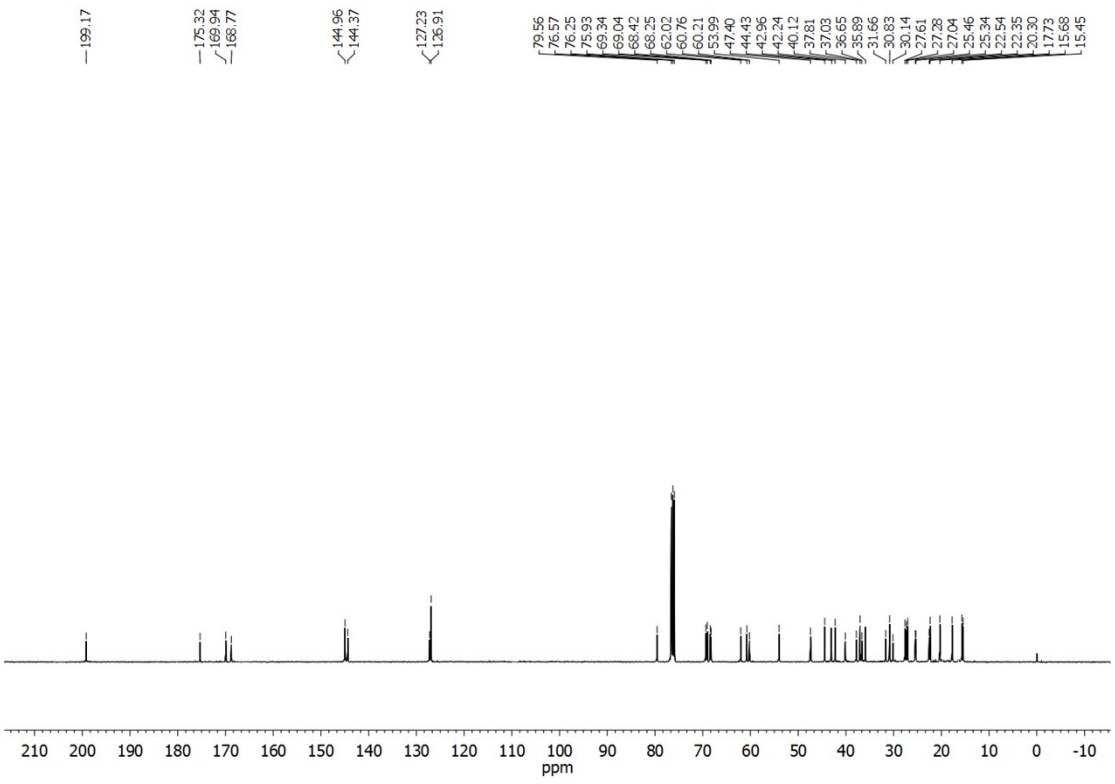


Fig. S15 ^{13}C NMR spectrum of compound **AGP** (75 MHz, $CDCl_3$)

References

1. K. Bigl, A. Schmitt, I. Meiners, G. Münch, T. Arendt, *Toxicol. In Vitro*, 2007, **21**, 962.
2. J. Hu, M. Zhang, Y. Ju, *Soft Matter*, 2009, **5**, 4971.