Supporting Information

Rational Design of Coumarin-based Supramolecular Hydrogelators for Cell Imaging

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1. General Information

Chemical reagents and solvents were purchased from Aladdin and used without further purification. ^{1}H NMR and ^{13}C NMR were obtained on a Bruker Advance III 400 Instrument operating at 400 MHz. HRMS were recorded on a Water Q-Tof Mass Instrument.

2. Experimental Procedures

Gel test for G_1-G_6: Aqueous solution of G_1-G_6 was prepared in a glass vial by heating. The solution was left to cool to room temperature. Gel formation was judged by the "invert-vial" method.

Tgs (gel-to-sol transition temperature): The gel-to-sol transition temperatures (Tgs) were determined by using "dropping ball" method. A plastic ball (150 mg, 5mm in diameter) was placed on top of 1 ml hydrogels G_1-G_6 prepared in a glass vial. The vials were subsequently immersed in a thermostated oil bath. The temperature of the heating block was increased by 5 ℃ per minute and the Tgs was defined as the temperature at which the plastic ball reached the bottom of the vial. The experiments were repeated five times and took the average.

Rheology measurements: The rheological properties of hydrogels G_1-G_6 were measured with a Rotary Rheometer (Gemini HRnano). The dynamic frequency sweep measurements were performed using a sinusoidal shear strain of constant peak amplitude (1%) over a range of frequencies (0.01-10Hz) at 25℃. The dynamic time sweep measurements were performed using a sinusoidal shear strain of constant peak

amplitude (0.01%) and constant frequency (1Hz) at 25℃ over 60 minutes.

SEM: Samples were prepared by depositing dilute solutions (approximately 0.4 mg/ml) of G_1-G_6 on silicon wafer, freeze dried overnight, and sprayed with a thin gold layer. SEM images were taken on a FEI QUANTA 250 microscope.

TEM: Samples were prepared by placing three drops of dilute solution (approximately 0.4 mg/ml) of G_1-G_6 onto the copper omentum and dried overnight in the air. TEM images were taken on an analytical transmission electron microscope (JEM-2010).

Fluorescence microscope: Samples were prepared by placing the flocculent hydrogels of G_1-G_6 on a glass slide, washed with deionized water three times, and then freeze dried overnight. Fluorescence images were taken on a Olympus IX73 microscope and a two photon microscope (A1RMP, Nikon, Tokyo, Japan).

UV-Vis absorption: The solution (1×10^{-5} mol/L) and hydrogel state (4×10^{-2} mol/L) of G_1 were tested using instrument of Lambda 20 from Perkin Elmer, Inc., USA, respectively.

Fluorescence spectra: The solution (1×10^{-5} mol/L) and hydrogel state (4×10^{-2} mol/L) of G_1 were tested using LS 50B from Perkin Elmer, Inc., USA, respectively.

Single crystal X-ray diffraction: Crystal G_1 suitable for X-ray diffraction was obtained by slow evaporation of methanol/chloroform (v/v 1:1) solution at room temperature. Single-crystal data were collected on a Bruker SMART Apex II CCD-based X-ray diffractometer with Mo-Kαradiation (λ = 0.71073 Å) at 293 K. The empirical absorption correction was applied by using the SADABS program (G. M. Sheldrick, SADABS, program for empirical absorption correction of area detector data;

University of Göttingen, Göttingen, Germany, 1996). The structure was solved using direct method, and refined by full-matrix least-squares on F^2 (G. M. Sheldrick, SHELXTL97, program for crystal structure refinement, University of Göttingen, Germany, 1997).

***X-ray powder diffraction (XRD)*:** Xerogel and crystal of G_1 were tested using a D8 Advance instrument from Bruker-AXS Company.

2D Cell culture: 100μl of flocculent hydrogels were added into one well of 24 well plates. The plates were dried in a vacuum oven under 37℃. Then Mouse NIH/3T3 fibroblasts were seeded to one well of 24 well plate coated with nanofibrous xerogels. Cells were cultured in DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were maintained in 5% CO_2-95% air at 37℃.

Cell viability: 100μl of flocculent hydrogels from G_1-G_6 were added into a well of 96 well plates, respectively. The plates were dried in a vacuum oven under 37℃. Cells in 200μl culture medium were seeded in a 96 well plate. After cells adhered to the fibers, cells were further cultured for 24 h. 20μl of cell counting kit-8 (CCK-8) was added to each well. The plated cells were incubated at dark for 2 h and the absorbance at 450 nm was then determined. Three parallel replicates were prepared and the entire process was repeated three times.

3. Additional Experimental Data and Figures

3.1 Gel tests of G_1-G_6

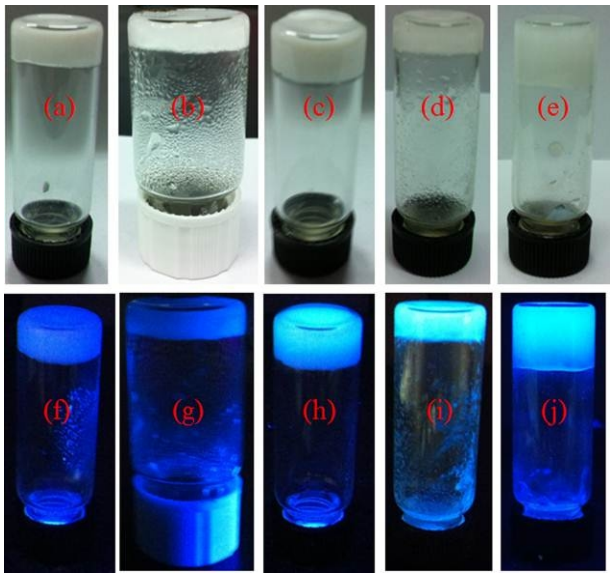


Fig.S1 Photographs of the assembled hydrogels from (a) **G_1**; (b) **G_2**; (c) **G_3**; (d) **G_5**; (e) **G_6** before UV irradiation and (f) **G_1**; (g) **G_2**; (h) **G_3**; (i) **G_5**; (j) **G_6** after UV irradiation.

Table S1 Gel-to-sol transition temperature (**Tgs**) and critical gelator concentration (**CGC**) of hydrogels G_1-G_6.

	G_1	**G_2**	**G_3**	**G_4**	**G_5**	**G_6**
Tgs (℃)	82	85	92	87	95	93
CGC(mg/ml)	9.5	3.6	10.4	2.8	12.5	4.7

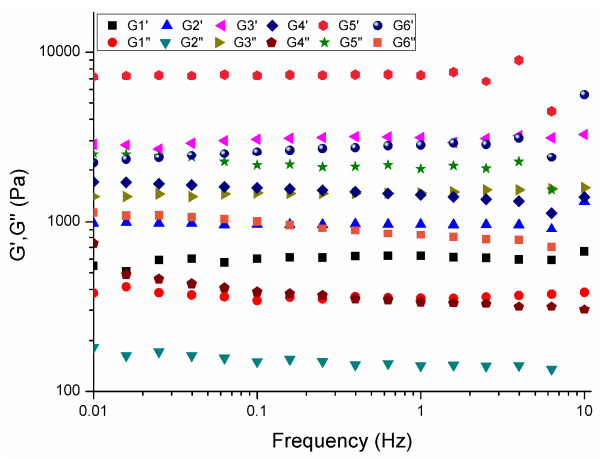


Fig.S2 Rheological measurement of frequency sweep at a strain of 1% for hydrogels assembled from **G_1-G_6**.

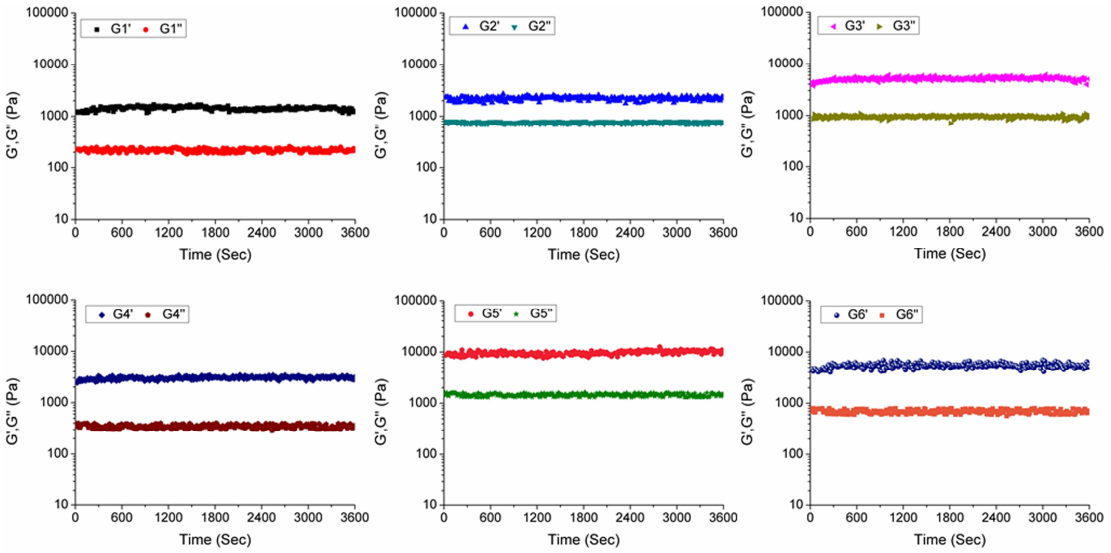


Fig.S3 Rheological measurement of time sweep at a frequency 1 rads^{-1} and a strain 0.01% for hydrogels assembled from **G_1-G_6**.

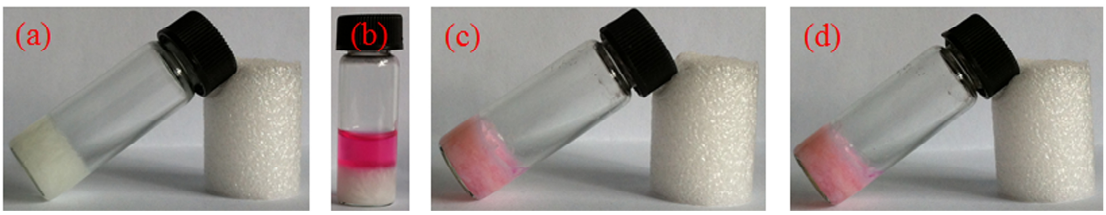


Fig.S4 Photographic images of the free-standing hydrogel G_1 in PBS before (a) and 0 min (b), 1 hour (c), and 7 days (d) after the addition of an equal volume of DMEM on top of the hydrogel. The lifetime of hydrogels G_2-G_6 was also tested by using the same methods and was also at least 7 days.

3.2 Additional SEM, TEM and Fluorescent images

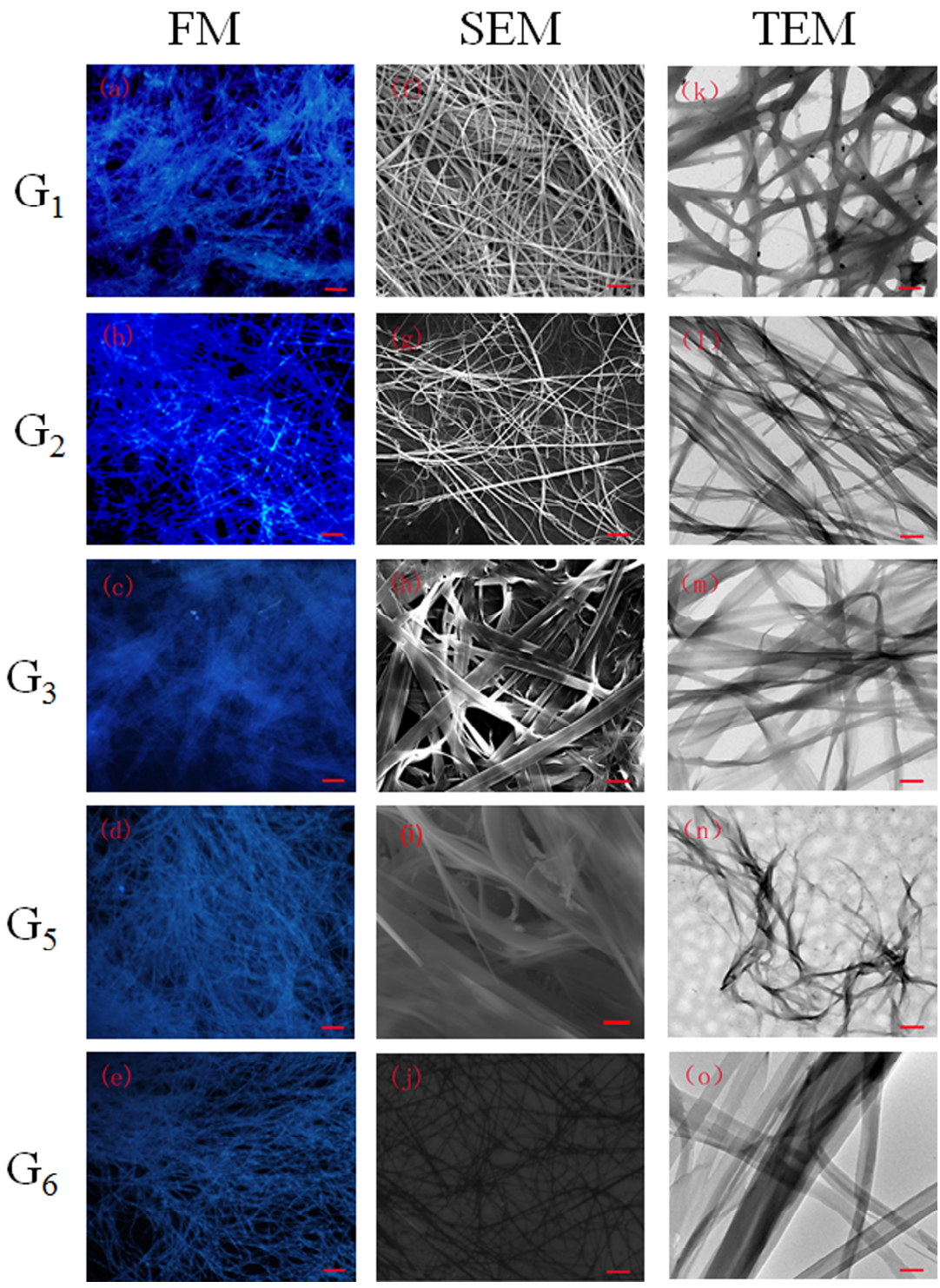


Fig.S5 Fluorescent images of xerogels assembled from: (a) **G1**, (b) **G2**, (c) **G3**, (d) **G5**, (e) **G6**, respectively. Scale bar = 100 μm. SEM images of xerogels from: (f) **G1**, (g) **G2**, (h) **G3**, (i) **G5**, (j) **G6**, respectively. Scale bar = 5 μm. TEM images of xerogels from: (k) **G1**, (l) **G2**, (m) **G3**, (n) **G5**, (o) **G6**, respectively. Scale bar = 200 nm.

3.3 Crystal and experimental data of **G_1**

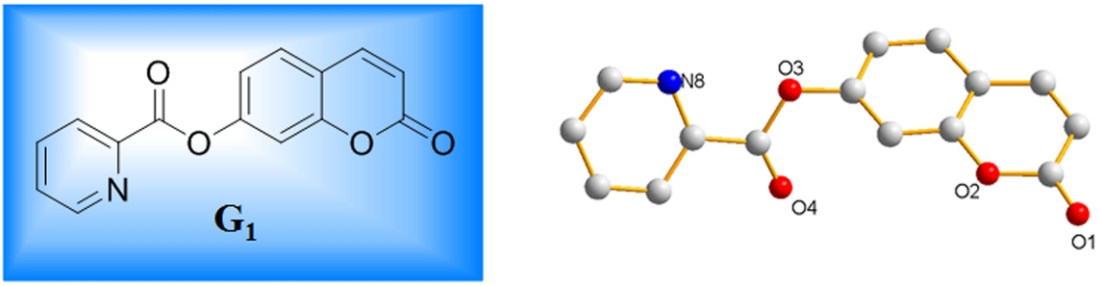


Fig.S6 Molecular structure of **G_1** in the crystal.

Table S2A Crystal data and structure refinement for **G_1**.

Empirical formula	C_{15} H_9 N O_4
Formula weight	267.23
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, $Pna2_1$
Unit cell dimensions	a = 11.595(4) Å α = 90°
	b = 3.8440(14) Å β = 90°
	c = 26.917(8) Å γ = 90°
Volume	1199.7(7) $Å^3$
Z, Calculated density	4, 1.480 Mg/m^3
Absorption coefficient	0.109 mm^{-1}
F(000)	552
Theta range for data collection	3.027 to 30.121°
Limiting indices	-14<=h<=16, -5<=k<=5, -23<=l<=37
Reflections collected / unique	7992 / 2716 [R(int) = 0.0703]
Completeness to theta = 26.000	99.7 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2716 / 1 / 181
Goodness-of-fit on F^2	0.848
Final R indices [I>2sigma(I)]	R1 = 0.0460, wR2 = 0.1143
R indices (all data)	R1 = 0.1180, wR2 = 0.1623
Absolute structure parameter	-0.1(10)
Extinction coefficient	n/a
Largest diff. peak and hole	0.180 and -0.212 e. $Å^{-3}$

Table S2B Bond lengths [Å] and angles [deg] for **G_1**.

O(3)-C(7)	1.394(5)
O(3)-C(10)	1.360(5)
C(5)-O(2)	1.379(6)

C(5)-C(6)	1.375(6)
C(5)-C(4)	1.387(7)
O(2)-C(1)	1.384(6)
C(7)-C(6)	1.378(6)
C(7)-C(8)	1.378(6)
C(4)-C(3)	1.437(7)
C(4)-C(9)	1.396(7)
C(8)-C(9)	1.370(7)
N(8)-C(11)	1.332(6)
N(8)-C(15)	1.336(6)
C(3)-C(2)	1.336(8)
C(12)-C(11)	1.384(7)
C(12)-C(13)	1.377(8)
O(4)-C(10)	1.189(5)
C(10)-C(11)	1.483(7)
C(1)-C(2)	1.438(8)
C(1)-O(1)	1.204(7)
C(15)-C(14)	1.378(8)
C(13)-C(14)	1.364(8)
C(10)-O(3)-C(7)	117.7(3)
O(2)-C(5)-C(4)	121.3(4)
C(6)-C(5)-O(2)	116.2(4)
C(6)-C(5)-C(4)	122.5(4)
C(5)-O(2)-C(1)	122.0(4)
C(6)-C(7)-O(3)	120.9(4)
C(6)-C(7)-C(8)	122.1(4)
C(8)-C(7)-O(3)	116.9(4)
C(5)-C(6)-C(7)	117.6(4)
C(5)-C(4)-C(3)	117.5(4)
C(5)-C(4)-C(9)	117.7(4)
C(9)-C(4)-C(3)	124.7(4)
C(9)-C(8)-C(7)	119.1(4)
C(11)-N(8)-C(15)	116.7(4)
C(2)-C(3)-C(4)	120.4(5)
C(13)-C(12)-C(11)	118.1(5)
C(8)-C(9)-C(4)	121.0(4)
O(3)-C(10)-C(11)	112.6(4)
O(4)-C(10)-O(3)	122.8(4)
O(4)-C(10)-C(11)	124.5(4)
N(8)-C(11)-C(12)	123.5(5)
N(8)-C(11)-C(10)	118.8(4)
C(12)-C(11)-C(10)	117.7(4)
O(2)-C(1)-C(2)	116.3(5)
O(1)-C(1)-O(2)	116.6(5)

	O(1)-C(1)-C(2)	127.2(5)
	C(3)-C(2)-C(1)	122.4(5)
	N(8)-C(15)-C(14)	123.8(5)
	C(14)-C(13)-C(12)	119.6(5)
	C(13)-C(14)-C(15)	118.3(5)

Table S2C Geometrical parameters of hydrogen bonds in crystal **G_1**.

D-H···A	D-H(Å)	H···A(Å)	D···A(Å)	D-H···A(deg)	Symmetry-for-A
C8-H8…O4	0.930	2.349	3.163	146.11	x+1/2, -y+5/2, z
C14-H14…O1	0.930	2.550	3.273	134.89	-x+1/2,y-1/2,z+1/2

Table S2D Short Ring-Interactions with Cg-Cg Distances in crystal **G_1**.

Analysis of Short Ring-Interactions with Cg-Cg Distances < 6.0 Angstrom		
Cg(I)	= Plane number I	
Cg-Cg	= Distance between ring Centroids (Ang.)	
Cg(I)		Cg-Cg
Cg(1)	[1] -> Cg(1)	3.844(3)
Cg(1)	[1] -> Cg(3)	4.523(3)
Cg(2)	[1] -> Cg(1)	5.929(4)
Cg(2)	[1] -> Cg(2)	3.844(3)
Cg(3)	[1] -> Cg(1)	4.546(3)
Cg(3)	[1] -> Cg(3)	3.844(3)

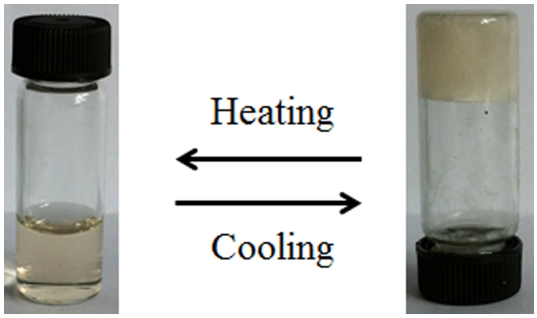


Fig.S7 Photographs of the reversible gel-sol transition of hydrogel G_1 triggered by temperature.

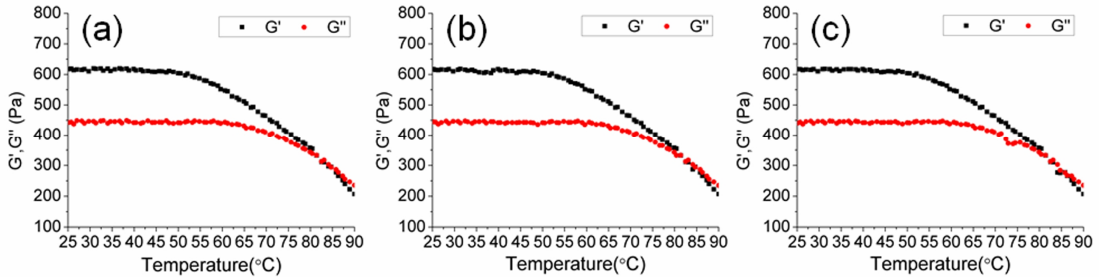


Fig.S8 Rheological measurement of reversible gel-sol transition of hydrogel G_1 triggered by temperature three times at a frequency 6 rads^{-1} and a strain 5%. (a), (b)

and (c): Heating after gel formation for the first time, second time and third time, respectively.

3.4 Concentration-dependent ^{1}H NMR of G_1

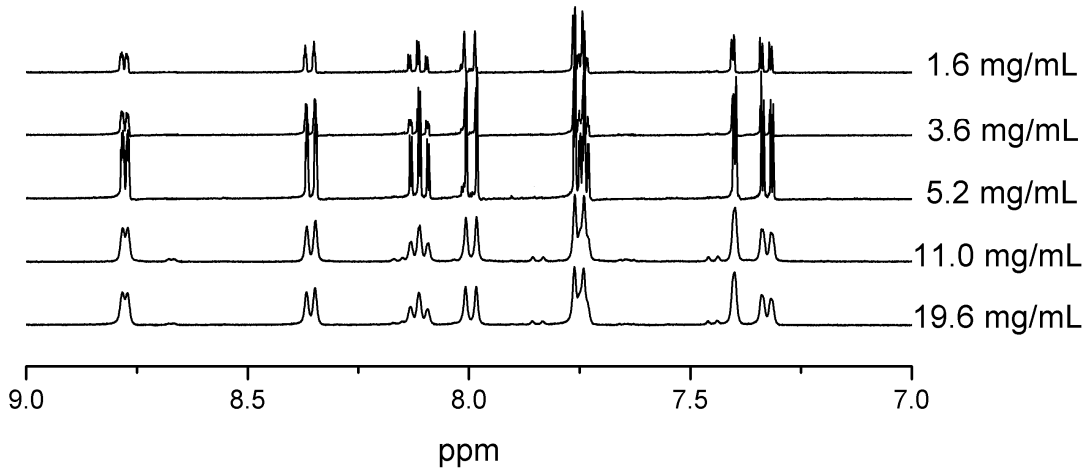


Fig. S9 Concentration-dependent ^{1}H NMR of G_1 in CH_3OD at 25℃.

3.5 UV absorption spectra of G_1

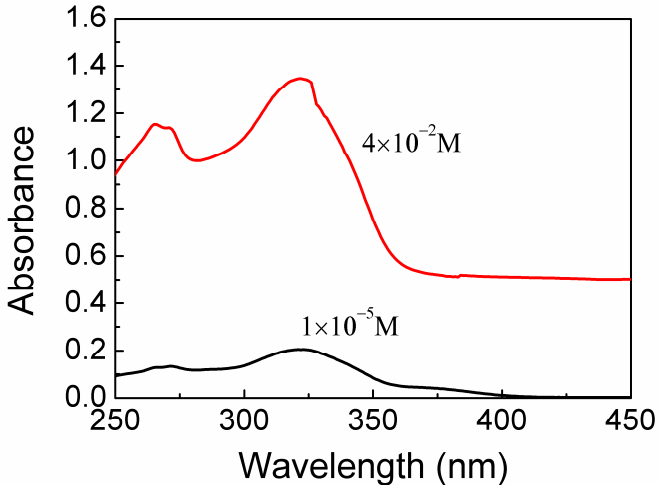
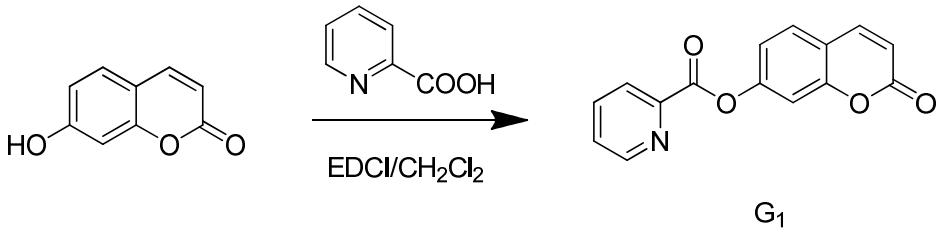


Fig. S10 UV absorption spectra of G_1 in the solution (1×10^{-5} mol/L) and hydrogel state (4×10^{-2} mol/L), λ_{max} = 322nm.

4. Synthesis and Characterizations

Scheme S1. Synthetic route to **G_1**



G_1

Synthesis of G_1: To a solution of 7-hydroxycoumarin (1.62 g, 10 mmol), 2-Picolinic Acid (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) was added EDCI (2.10 g, 11 mmol). The mixture was stirred for 2 hours at room temperature. The resulting solution was washed three times with water (5 ml), saturated $NaHCO_3$ solution (5 ml). Then the organic layer was collected and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give the product **G_1** as white powder (2.45 g, 91.76%). ^{1}H NMR (400MHz, $CDCl_3$) δ=8.87(d, J=4.0Hz, 1H), 8.29(d, J=7.6Hz, 1H), 7.95(t, J=8.0Hz, 1H), 7.72(d, J=9.6Hz, 1H), 7.60(t, J=4.0Hz, 1H), 7.55(d, J=8.4Hz, 1H), 7.3(s, 1H), 7.23(d, J=8.0Hz, 1H), 6.43(d, J=9.6Hz, 1H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.4, 160.4, 154.8, 153.5, 150.4, 146.9, 143.0, 137.6, 128.9, 128.0, 126.3, 118.7, 117.1, 116.4, 110.8. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0609.

G_2-G_6 were synthesized using the same procedure for **G_1**.

G_2:4-Methylumbelliferone (1.76 g, 10 mmol), 2-Picolinic Acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_2** as white powder (2.44 g, 85.01%). ^{1}H NMR (400MHz, $CDCl_3$) δ=8.87(d, J=4.0Hz, 1H), 8.30(d, J=8.0Hz, 1H), 7.95(t, J=2.0Hz, 1H), 7.68(d, J=8.0Hz, 1H), 7.60(t, J=4.0Hz, 1H), 7.30(d, J=2.0Hz, 1H), 7.25(s, 1H), 6.30(s, 1H), 2.46(d, J=1.2Hz, 3H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.4, 160.7, 154.3, 153.4, 152.2, 150.4, 146.9, 137.6, 128.0, 126.3, 125.7, 118.4, 114.8, 110.9, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0771.

G_3:7-hydroxycoumarin (1.62 g, 10 mmol), Nicotinic acid(1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_3** as

white powder (2.35 g, 88.01%). ^{1}H NMR (400MHz, $CDCl_3$) δ=9.40(d, J=1.6Hz, 1H), 8.89(dd, J1=4.8Hz J2=1.6Hz, 1H), 8.49(dt, J1=8.0Hz J2=1.6Hz, 1H), 7.73(d, J=9.6Hz, 1H), 7.57(d, J=8.4Hz, 1H), 7.51-7.54(m, 1H), 7.27(d, J=2.4Hz, 1H), 7.20(dd, J1=8.4Hz J2=2.4Hz, 1H), 6.44(d, J=9.6Hz, 1H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.5, 160.4, 154.9, 154.6, 153.1, 151.6, 143.0, 137.9, 129.0, 125.1, 123.8, 118.5, 117.2, 116.5, 110.7. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0610.

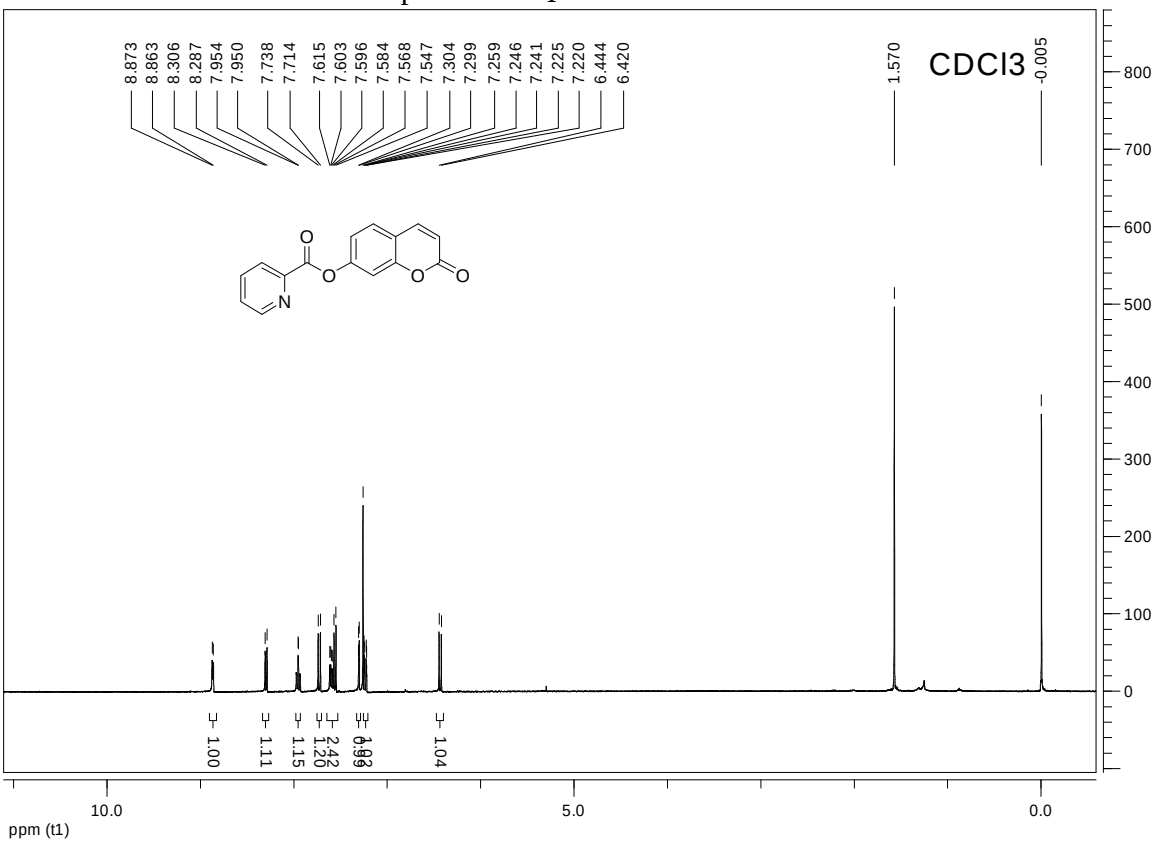
G_4: 4-Methylumbelliferone (1.76 g, 10 mmol), Nicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_4** as white powder (2.51 g, 89.32%). ^{1}H NMR (400MHz, $CDCl_3$) δ=9.4(s, 1H), 8.89(dd, J_1=4.8Hz J_2=1.6Hz, 1H), 8.46(d, J=8.0Hz, 1H), 7.68(d, J=8.8Hz, 1H), 7.48-7.51(m, 1H), 7.27(d, J=2.0Hz, 1H), 7.22(dd, J_1=8.8Hz J_2=2.4Hz, 1H), 6.31(d, J=1.2Hz, 1H), 2.47(d, J=1.2Hz, 3H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.5, 160.5, 154.6, 154.4, 153.0, 152.0, 151.6, 137.9, 125.8, 125.1, 123.7, 118.4, 118.2, 115.0, 110.7, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0755.

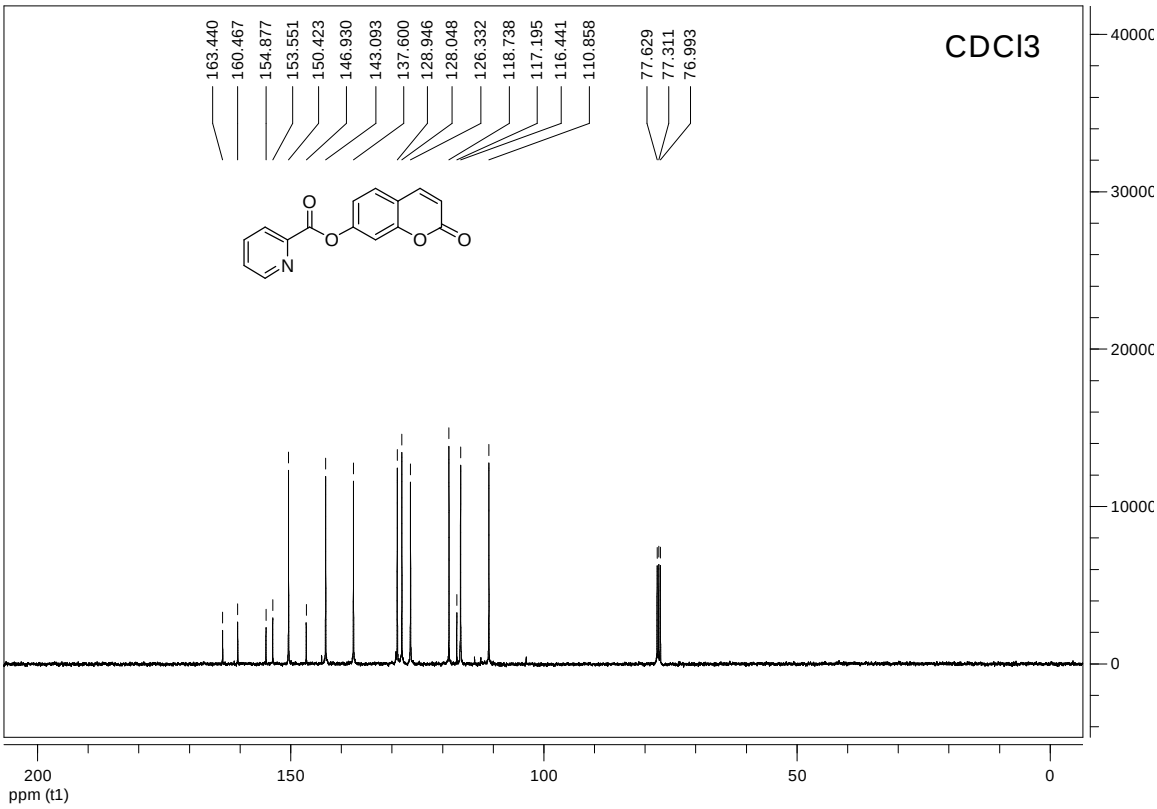
G_5: 7-hydroxycoumarin (1.62 g, 10 mmol), Isonicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_5** as white powder (2.28 g, 85.39%). ^{1}H NMR (400MHz, $CDCl_3$) δ=8.89(d, J=6.0Hz, 2H), 8.01(d, J=6.0Hz, 2H), 7.73(d, J=9.6Hz, 1H), 7.57(d, J=8.4Hz, 1H), 7.27(d, J=2.4Hz, 1H), 7.20(dd, J_1=8.4Hz J_2=2.4Hz, 1H), 6.44(d, J=9.6Hz, 1H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.4, 160.4, 154.9, 153.0, 151.2, 143.0, 136.2, 129.0, 123.4, 118.4, 117.3, 116.7, 110.7. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0607.

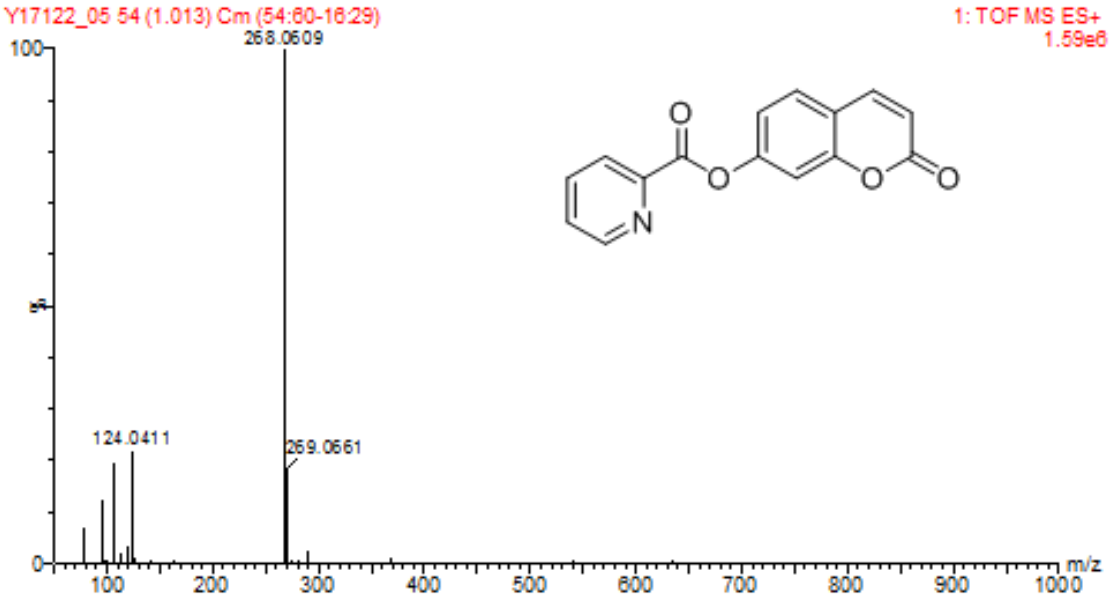
G_6: 4-Methylumbelliferone (1.76 g, 10 mmol), Isonicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_6** as white powder (2.60 g, 90.59%). ^{1}H NMR (400MHz, $CDCl_3$) δ=8.89(d, J=6.0Hz, 2H), 8.01(d, J=6.0Hz, 2H), 7.68(d, J=8.8Hz, 1H), 7.26(d, J=2.4Hz, 1H), 7.20(dd, J_1=8.8Hz J_2=2.4Hz, 1H), 6.31(d, J=1.2Hz, 1H), 2.47(d, J=1.2Hz, 3H). ^{13}C NMR (400MHz, $CDCl_3$) δ=163.4, 160.5, 154.4, 152.9, 152.0, 151.2, 136.2, 125.9, 123.4, 118.5, 118.0, 115.0, 110.6, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0764.

5. ^{1}H and ^{13}C NMR and HRMS spectra of compounds G_1-G_6

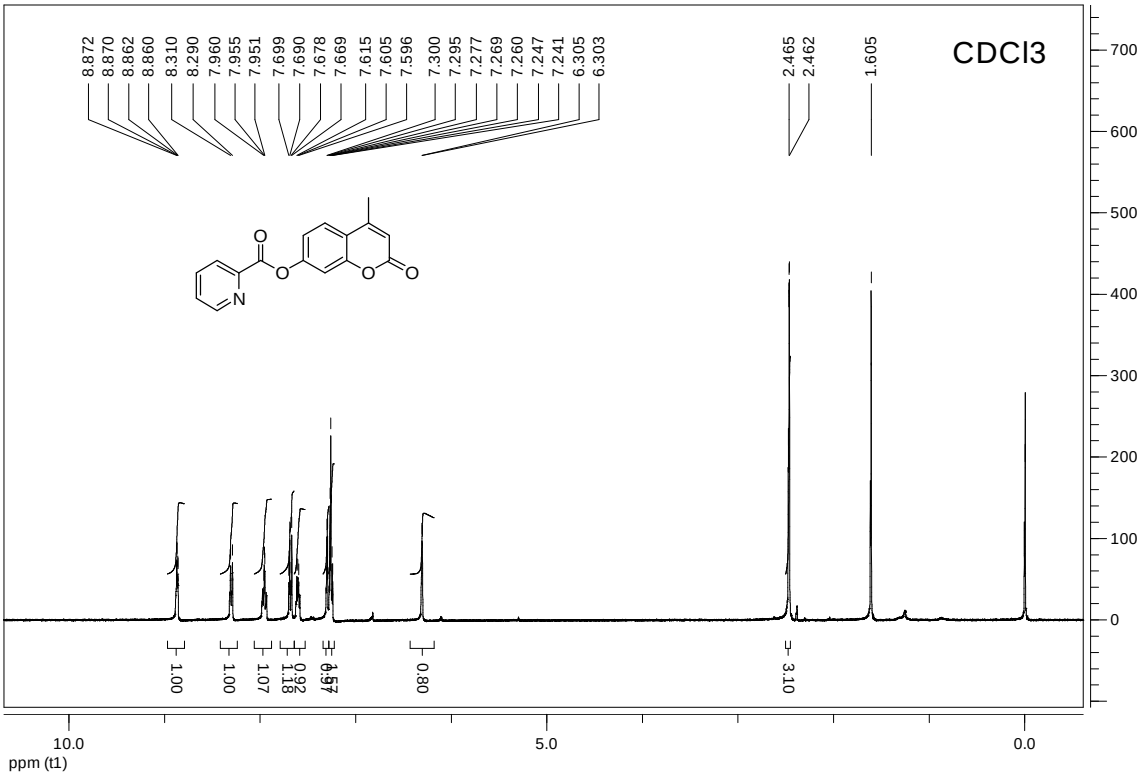
^{1}H and ^{13}C NMR and HRMS Spectra of **G_1**

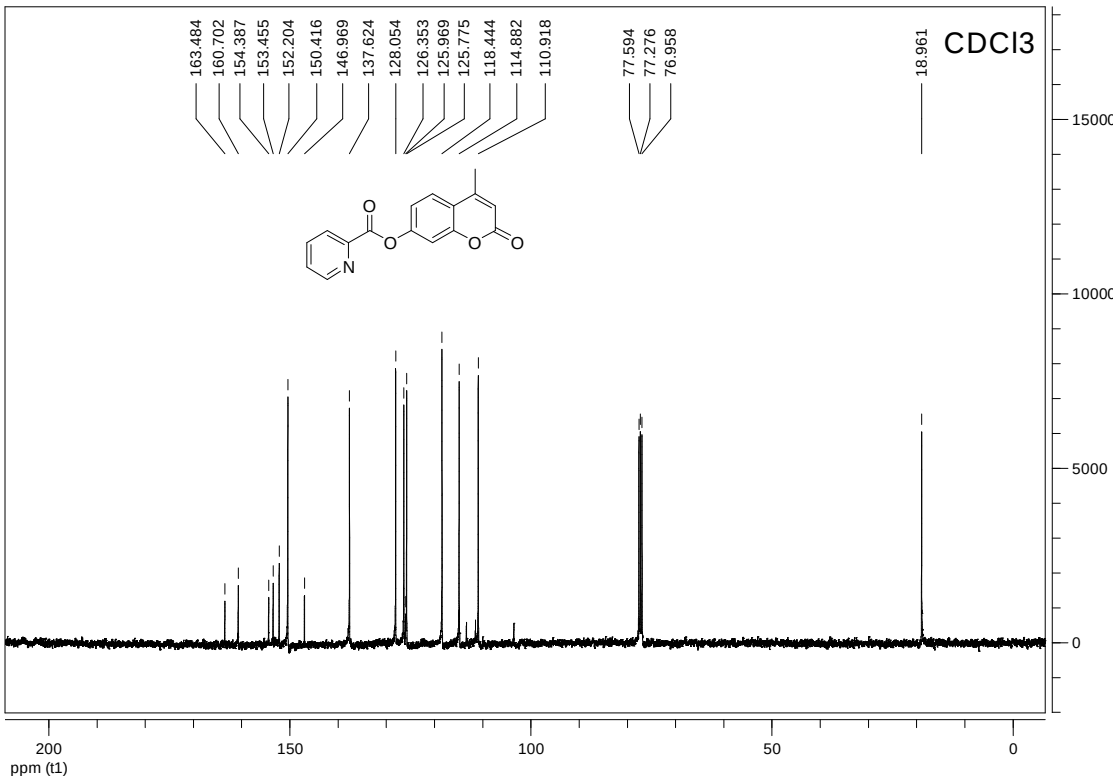


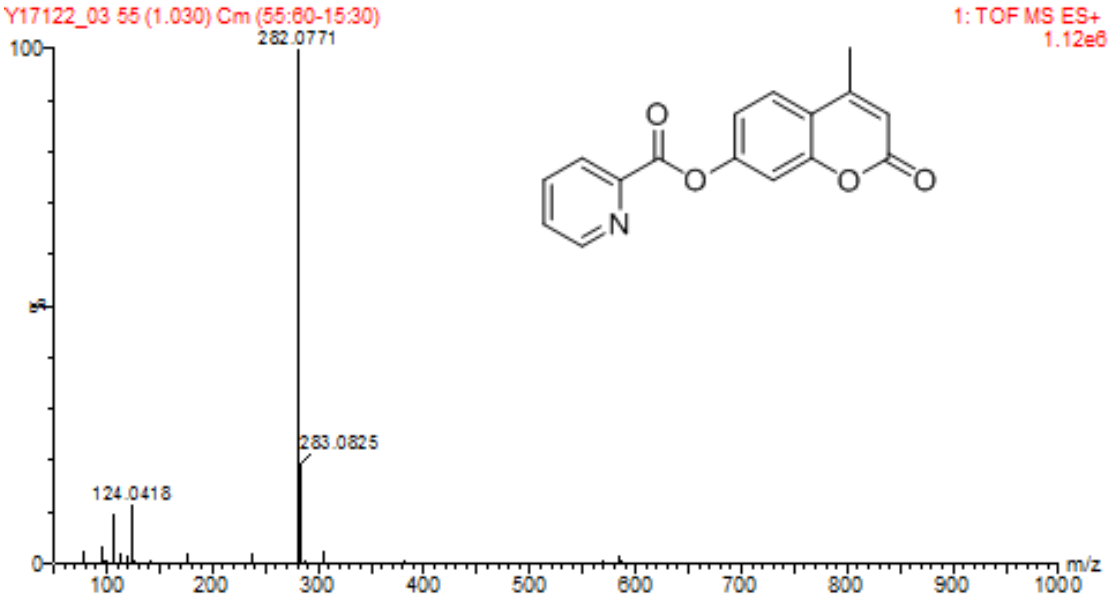




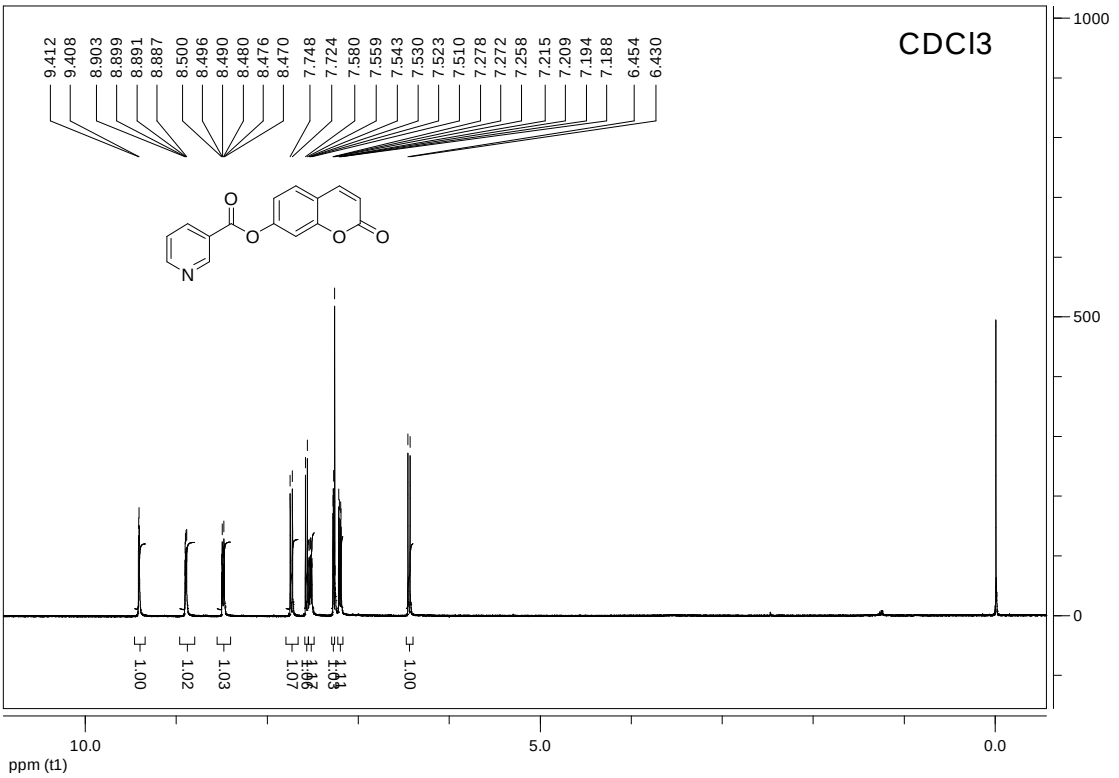
^{1}H and ^{13}C NMR and HRMS Spectra of **G_2**

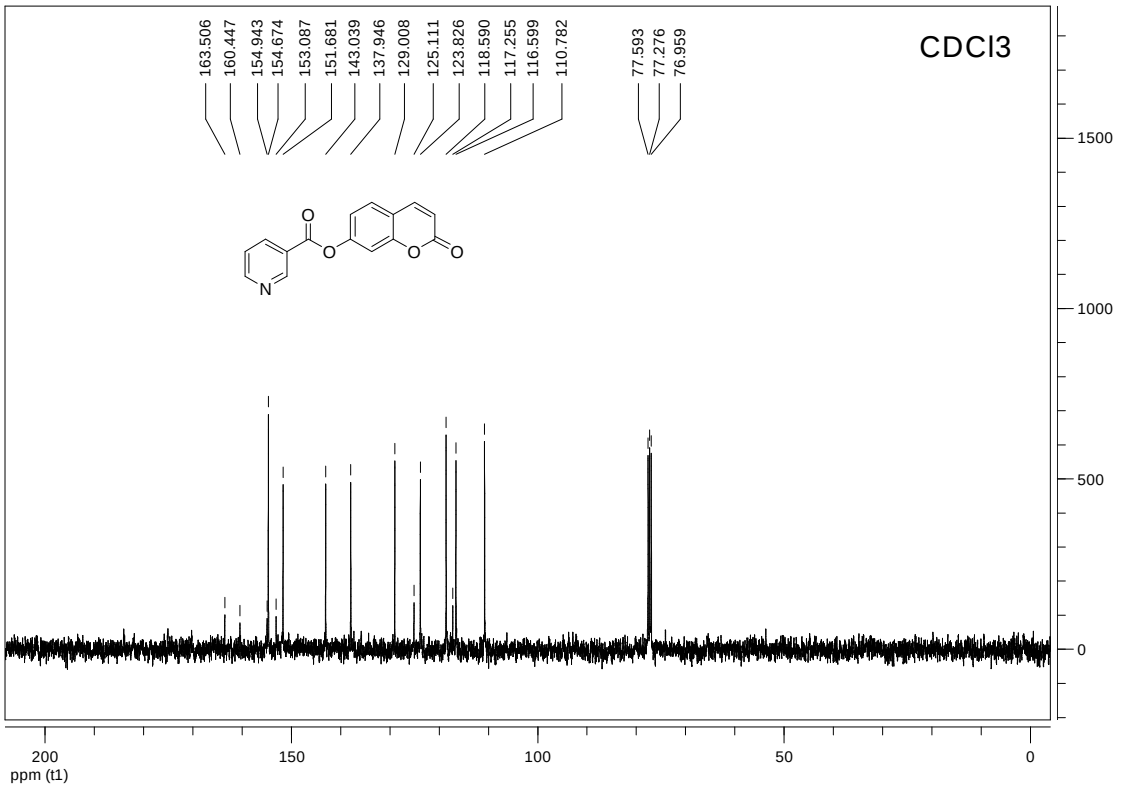


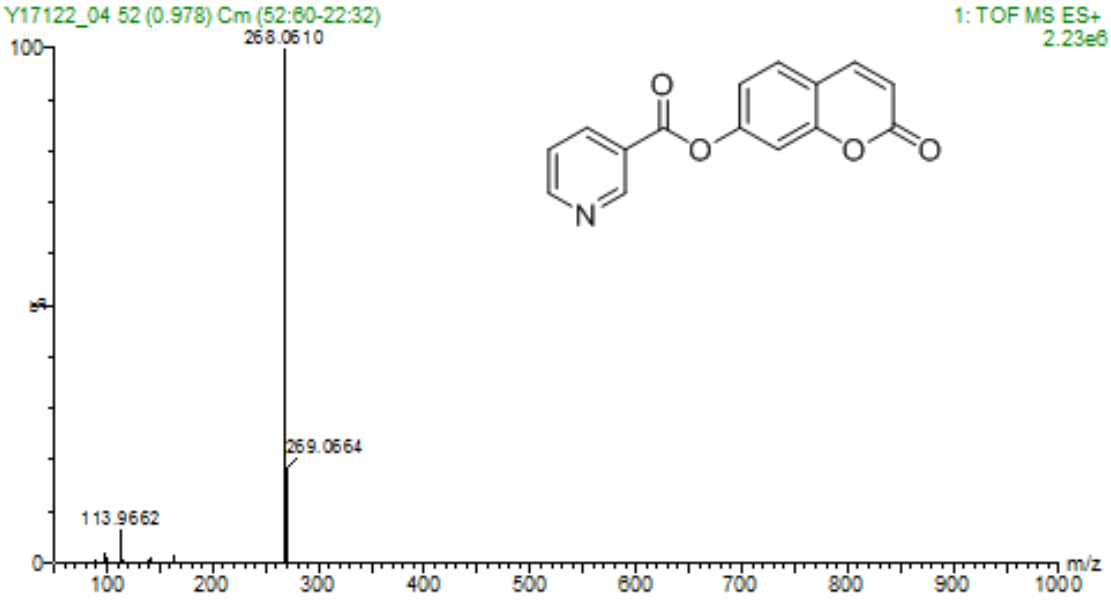

163.484
160.702
154.387
153.455
152.204
150.416
146.969
137.624
128.054
126.353
125.969
125.775
118.444
114.882
110.918
77.594
77.276
76.958
18.961
CDCl3
O
O
O
O
N
15000
10000
5000
0
200
150
100
50
0
ppm (t1)


Y17122_03 55 (1.030) Cm (55:60-15:30)
1: TOF MS ES+
1.12e6
282.0771
283.0825
124.0418
100
%
0
100
200
300
400
500
600
700
800
900
1000
m/z
O
O
O
O
N

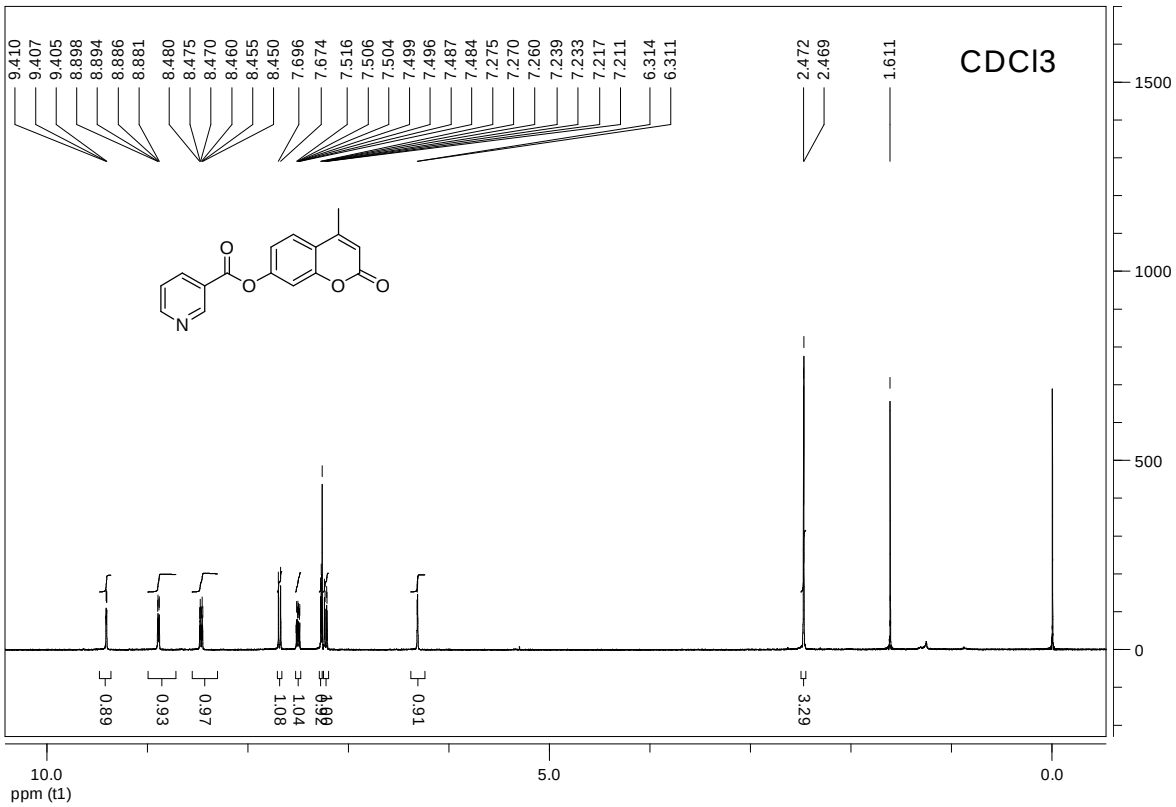
^{1}H and ^{13}C NMR and HRMS Spectra of **G_3**

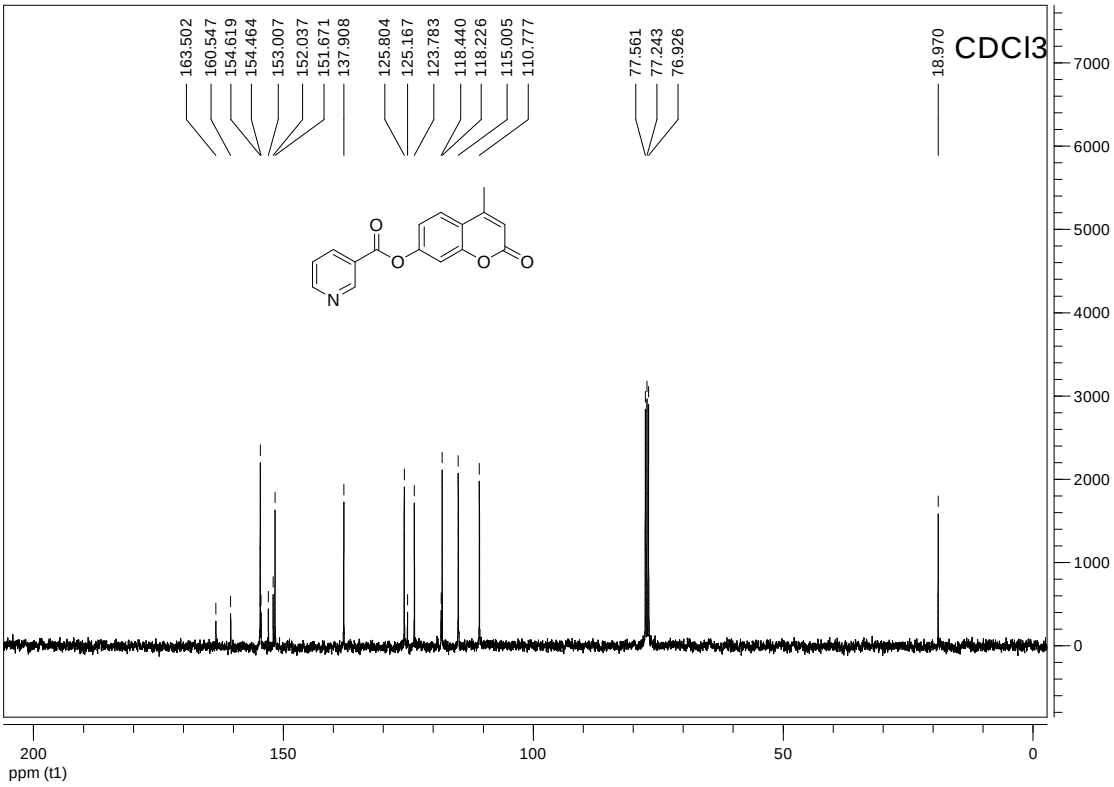


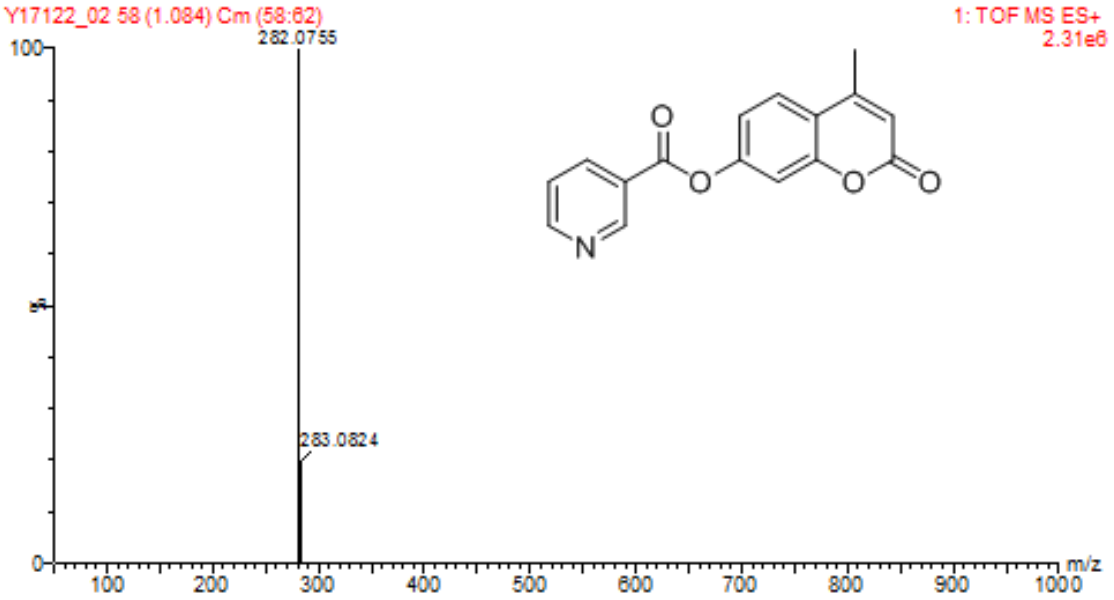




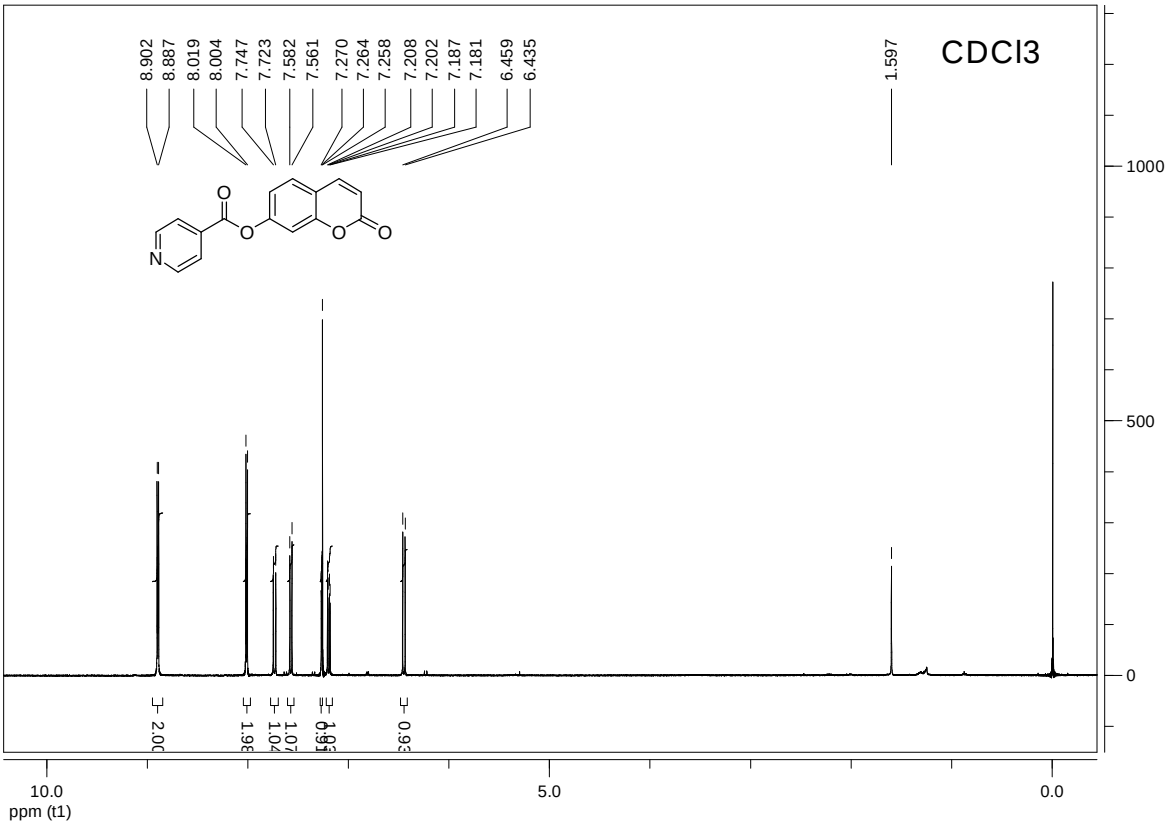
^{1}H and ^{13}C NMR and HRMS Spectra of **G_4**

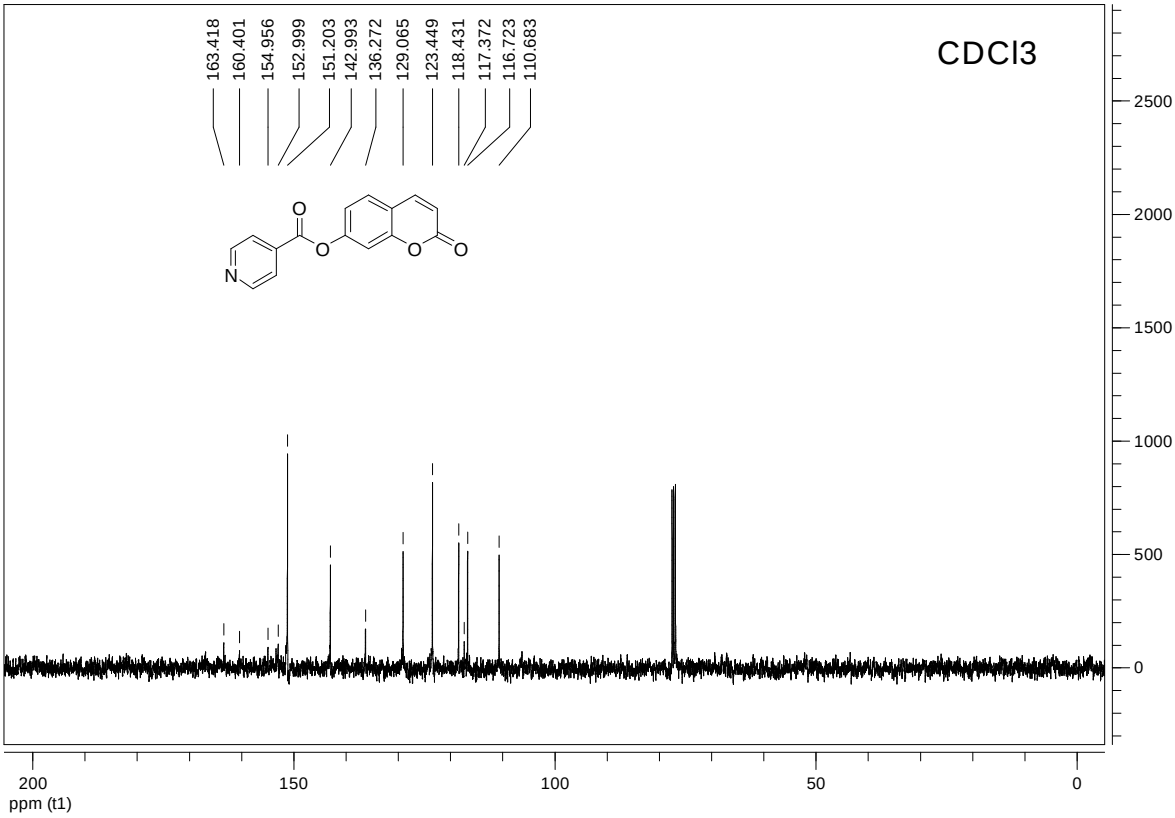


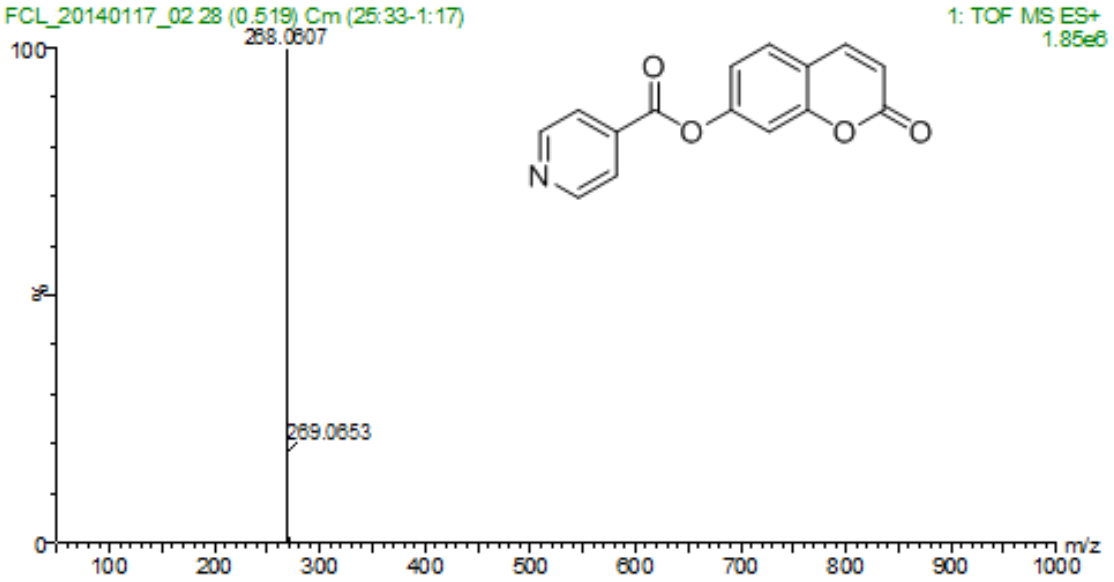

163.502
160.547
154.619
154.464
153.007
152.037
151.671
137.908
125.804
125.167
123.783
118.440
118.226
115.005
110.777
77.561
77.243
76.926
18.970
CDCl3
7000
6000
5000
4000
3000
2000
1000
0
200
150
100
50
0
ppm (t1)


Y17122_02 58 (1.084) Cm (58:62)
1: TOF MS ES+
2.31e6
282.0755
283.0824
100
%
0
100
200
300
400
500
600
700
800
900
1000
m/z

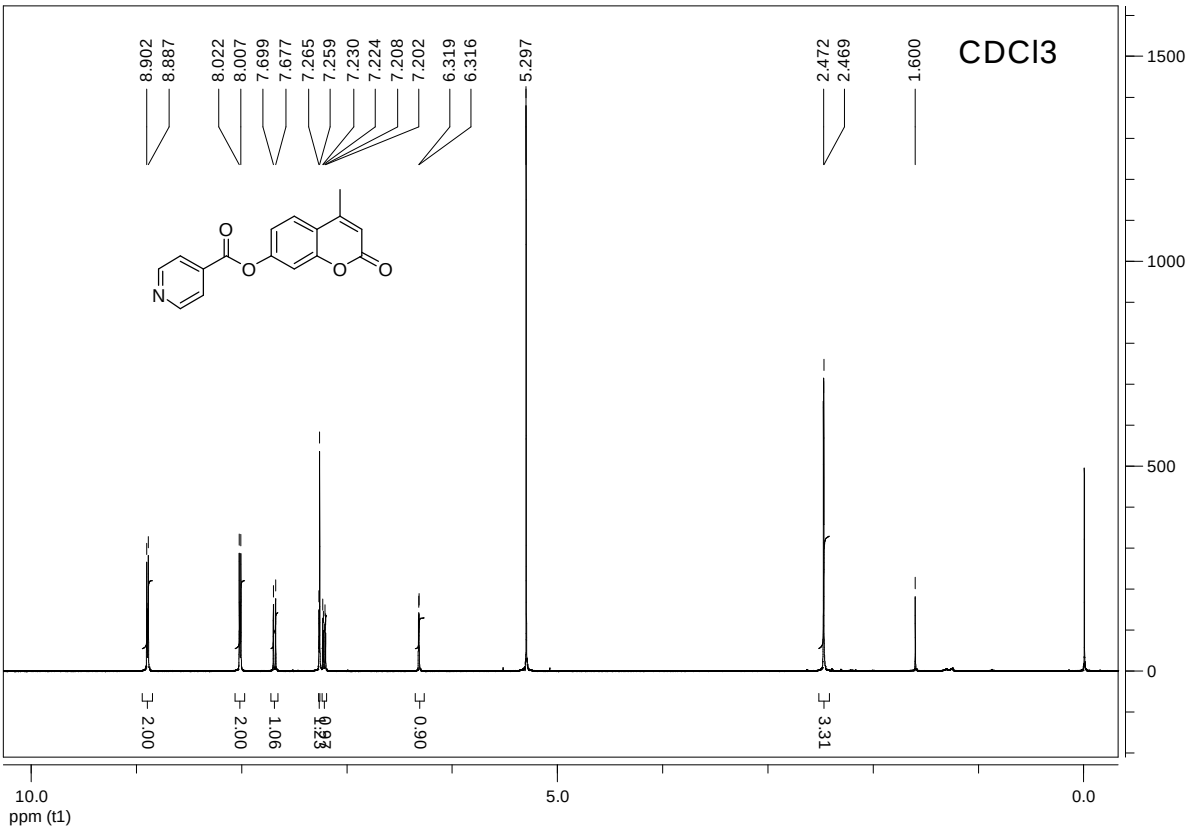
^{1}H and ^{13}C NMR and HRMS Spectra of **G_5**

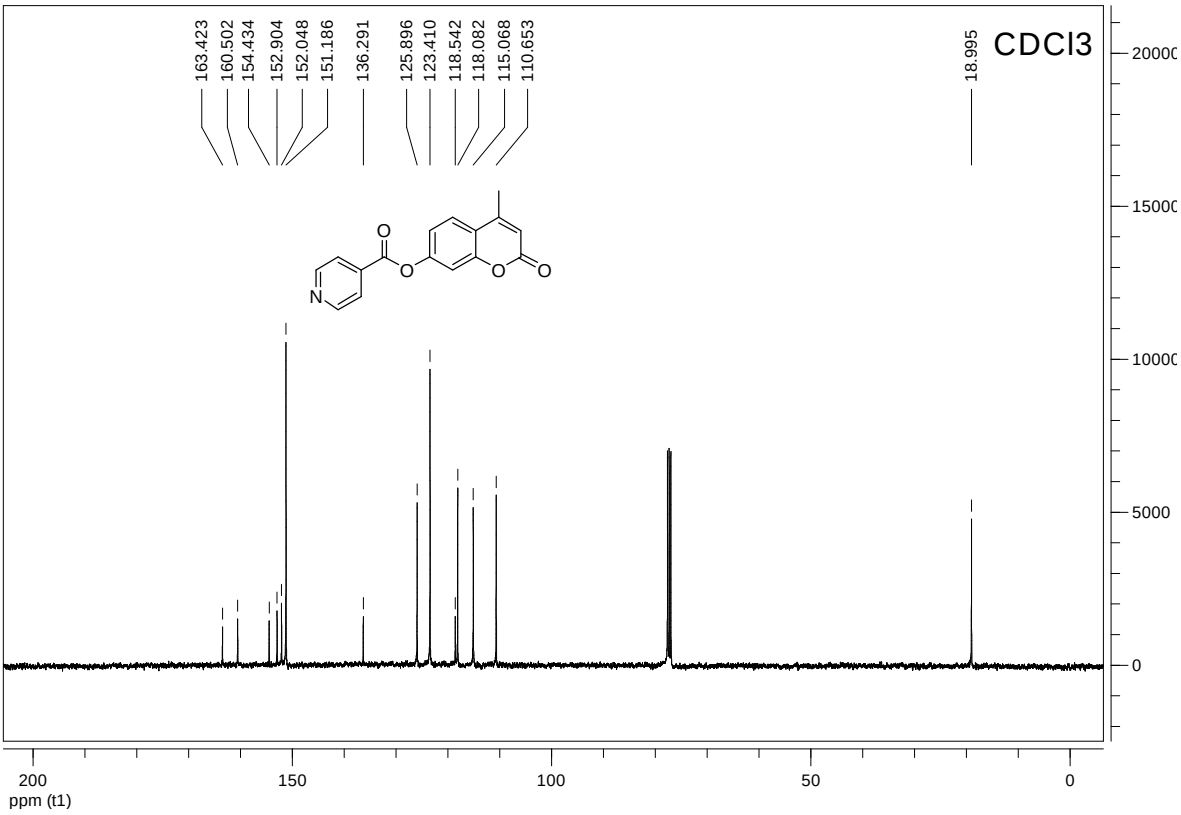


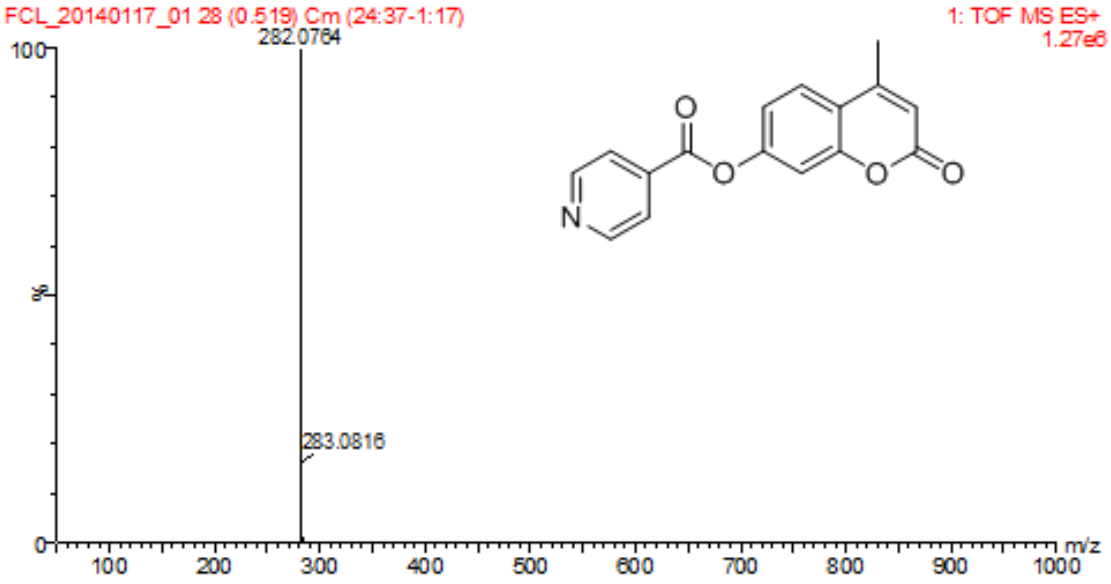




^{1}H and ^{13}C NMR and HRMS Spectra of **G_6**




163.423
160.502
154.434
152.904
152.048
151.186
136.291
125.896
123.410
118.542
118.082
115.068
110.653
18.995
CDCl3
O
O
O
O
N
200
150
100
50
0
ppm (t1)
20000
15000
10000
5000
0


FCL_20140117_01 28 (0.519) Cm (24:37-1:17)
1: TOF MS ES+
1.27e6
282.0764
283.0816
100
%
0
100
200
300
400
500
600
700
800
900
1000
m/z
O
O
O
O
N