

Supporting Information

A biocompatible porous Mg-gallate Metal Organic Framework as a promising antioxidant carrier

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1. Synthesis

Gallic acid (H_4gal), potassium hydroxide and magnesium chloride were obtained commercially and used without any further purification.

1.1 Exploratory synthesis

Exploratory syntheses were performed using the hydrothermal high-throughput setup developed by Stock *et al.*,^{ref} which combines miniaturization (volume of the reactor ~ 1.5 mL), parallelization (reaction performed with 24 reactors in parallel) and automation of the analysis (use of high throughput Bruker D8 Advance X-ray diffractometer), allowing both the fast identification of new crystalline phases and the determination of phase diagrams.¹ Among the parameters explored, temperature, heating time and concentration of the reactants did not play a significant role. Although $Mg(OH)_2$ could be used as the magnesium precursor, it sometimes led to residual unreacted $Mg(OH)_2$ being present in the final product; for this reason it was eventually replaced with $MgCl_2$. As shown in Figure S1, the key parameters influencing the nature of the resulting product were found to be the pH and the ligand to metal ratio. Two hybrid crystalline phases were identified.

$Mg(H_2gal) \cdot 2H_2O$ is isolated in pure form over various $H_4gal/Mg(II)$ ratios (1:2 – 2:1). However the KOH/H_4gal ratio needs to be around 2:1, which corresponds to pH range 7-8. The second magnesium phase requires more basic conditions with a KOH/H_4gal ratio = 4-5 which corresponds to pH ~ 10 and the most repeatable synthesis conditions for isolating phase 2 in pure form use a $H_4gal/Mg(II)$ ratio of 1:2. Optimized hydrothermal syntheses of both phases are summarized in Table S1.

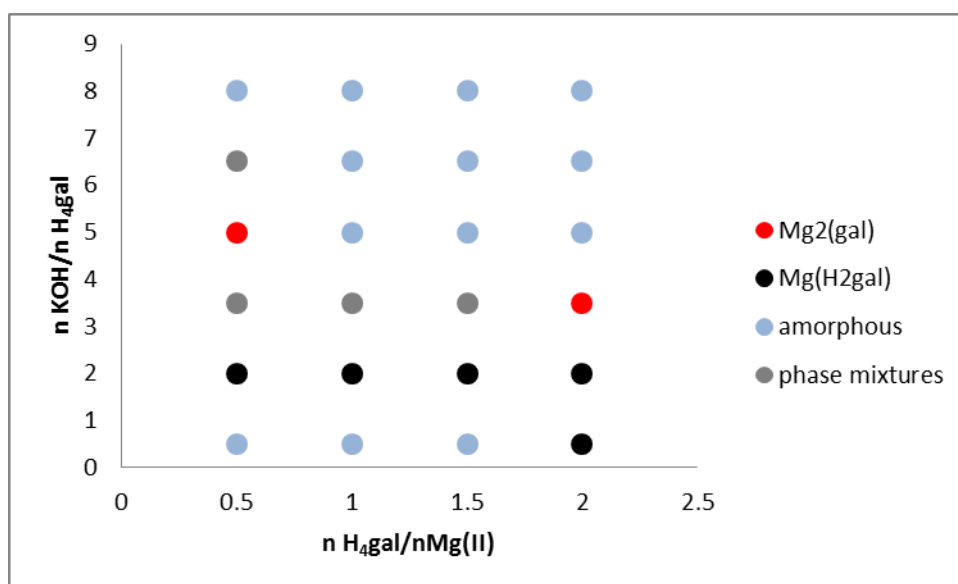


Figure S1. Phase diagram of the $\text{H}_4\text{gal}/\text{Mg}(\text{II})$ system, extracted from a high-throughput experiment carried out at 120 °C for 24 h. $V_{\text{H}_2\text{O}} = 1\text{ mL}$, $[\text{Mg}(\text{II})] = 0.2\text{ mol L}^{-1}$, $\text{Mg}(\text{II})$ precursor = MgCl_2 .

Table S1. Optimized hydrothermal syntheses of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ and $\text{Mg}_2(\text{gal}) \cdot x\text{H}_2\text{O}$.

	$\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$	$\text{Mg}_2(\text{gal}) \cdot x\text{H}_2\text{O}$
Mg(II) precursor	MgCl_2	MgCl_2
$[\text{Mg}(\text{II})]$ ($\text{mmol} \cdot \text{mL}^{-1}$)	0.2	0.2
$n\text{H}_4\text{gal}/n\text{Mg}(\text{II})$	2	0.5
$n\text{KOH}/n\text{H}_4\text{gal}$	1.25	5
initial pH	7-8	10
final pH	8	12
temperature (°C)	120	120
heating time (h)	24	24

1.2 Round bottom flask synthesis of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$

10 g of MgCl_2 , 38 g of H_4gal and 500 mL of water were placed in a 1 L round bottom flask, and stirred under reflux. A 10 M aqueous solution of KOH was added to adjust the pH to 8, and the mixture was heated for 24 hour. The light grey solid was recovered by filtration, washed with water and dried in air. Yield $\sim 77\%$.

2. Material characterization

2.1 Powder X-ray diffraction

Routine powder X-Ray diffraction patterns were collected at 293 K on a Siemens D5000 Diffractometer working in the $(\theta-2\theta)$ mode by using $\text{CuK}\alpha$ radiation. Typical XRD diagrams of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ and $\text{Mg}_2(\text{gal})\cdot x\text{H}_2\text{O}$ are shown Figure S2.

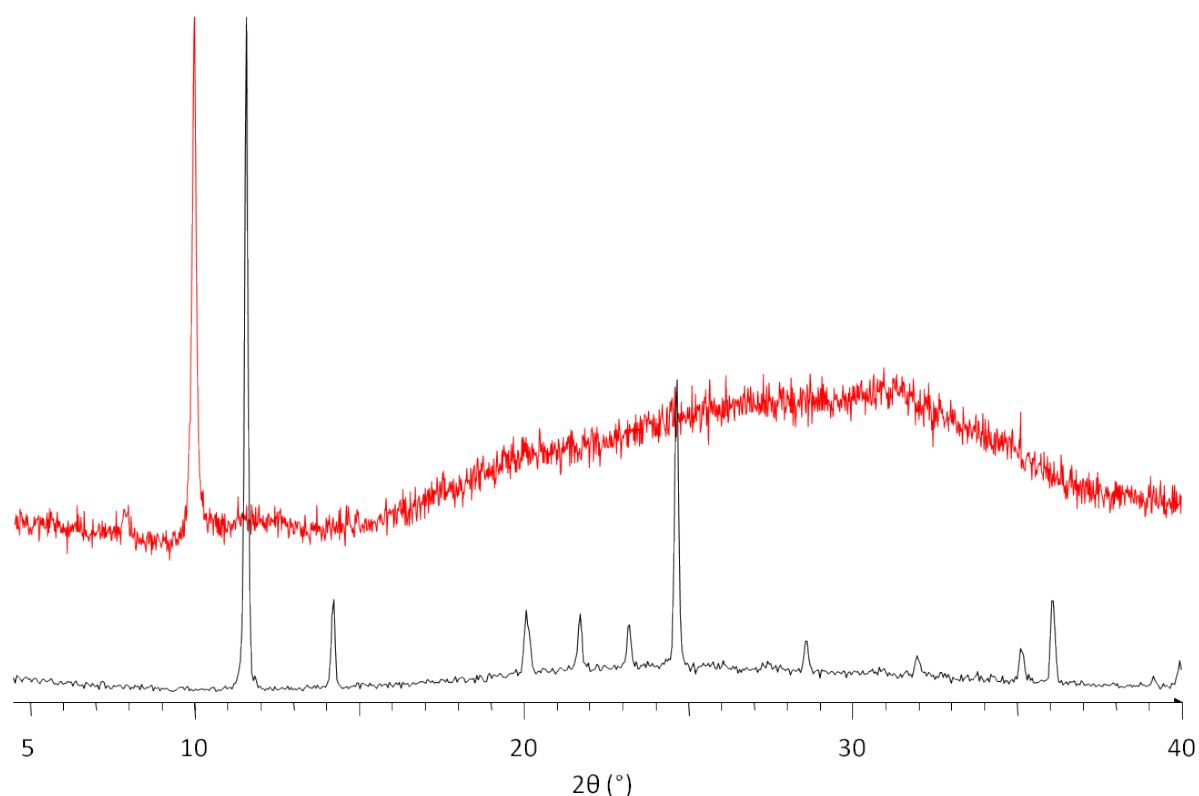


Figure S2. Comparison of the PXRD diagrams of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ (bottom, black) and $\text{Mg}_2(\text{gal})\cdot x\text{H}_2\text{O}$ (top, red).

High resolution powder X-ray diffraction data of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ were measured at room temperature using a Bruker D8 Advance diffractometer with a Debye-Scherrer geometry, in the 2θ range $10-80^\circ$. The D8 system is equipped with a $\text{Ge}(111)$ monochromator producing $\text{Cu } K\alpha_1$ radiation ($\lambda = 1.540598 \text{ \AA}$) and a LynxEye detector.

Extractions from the peak positions, pattern indexing as well as Rietveld refinement were carried out with the TOPAS program.² The structure of the Ni analogue³ was used as a starting point of the Rietveld refinement. the final Rietveld plot (Figure S3) corresponds to satisfactory model indicator and profile factors ($R_{\text{Bragg}} = 0.016$, $R_p = 0.031$, $R_{\text{WP}} = 0.044$). It involves the following parameters: 20 atomic coordinates (H atoms were fixed during the refinement and soft restrains were maintained on distances and angles of the organic moiety), 1 overall thermal factor, 1 scale factor, 1 zero-point, 2 cell parameters, 10 background parameters and 5 ones to model the evolution of asymmetric and anisotropic diffraction lines shape.

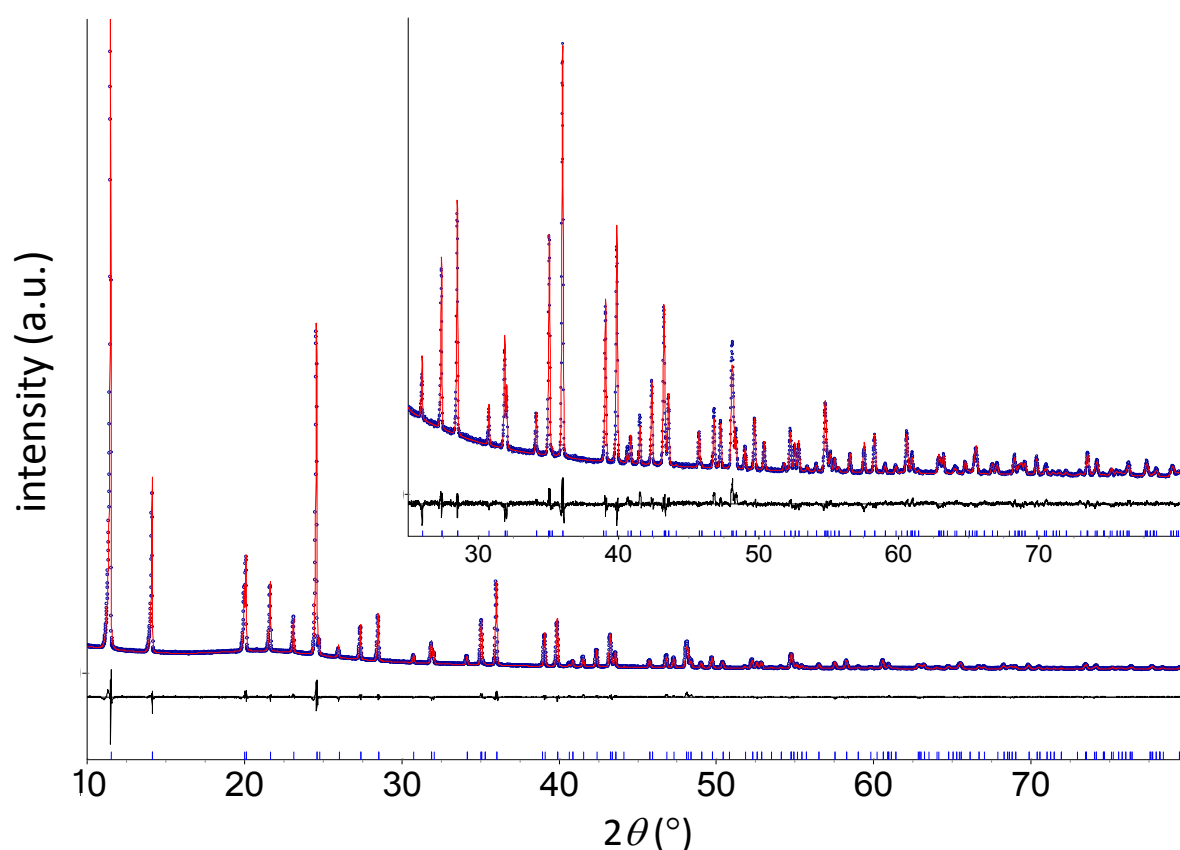


Figure S3. Final Rietveld plot of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$.

Two views of the final structure are shown in Figure S4, highlighting that the microporosity could arise both parallel (left) and perpendicular (right) to the 3-fold axis.

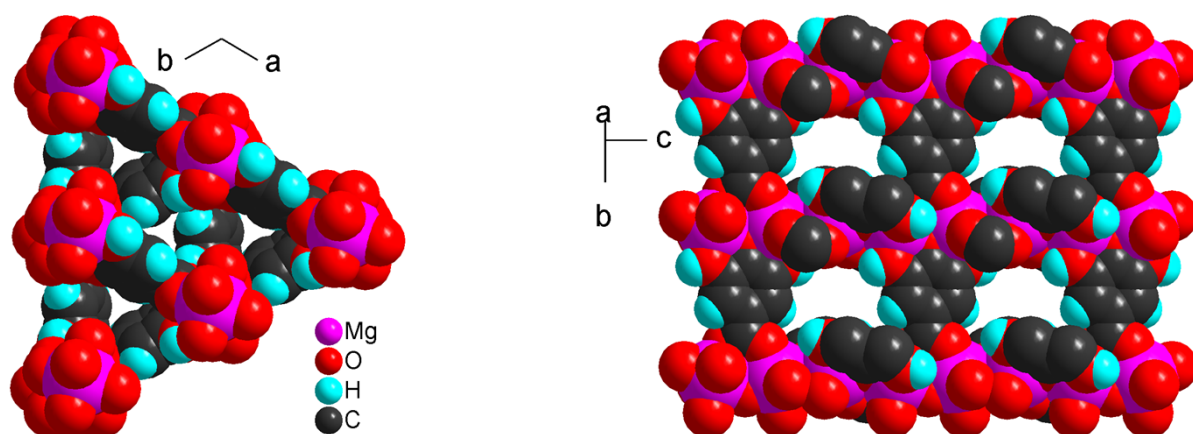


Figure S4. Van der Waals representation of the structure of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ (water molecules omitted for clarity) along (left) and perpendicular (right) to the three-fold axis.

X-ray thermodiffraction was performed using a θ - θ Bruker D8 Advance diffractometer equipped with a HTK-1200N (Anton Parr) high temperature chamber and a LynxEye XE detector (Cu radiation). Diagrams were collected every 10 °C between 20 and 200 °C. The data for $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ are shown Figure S5, and the corresponding unit-cell parameters summarized in Table S2.

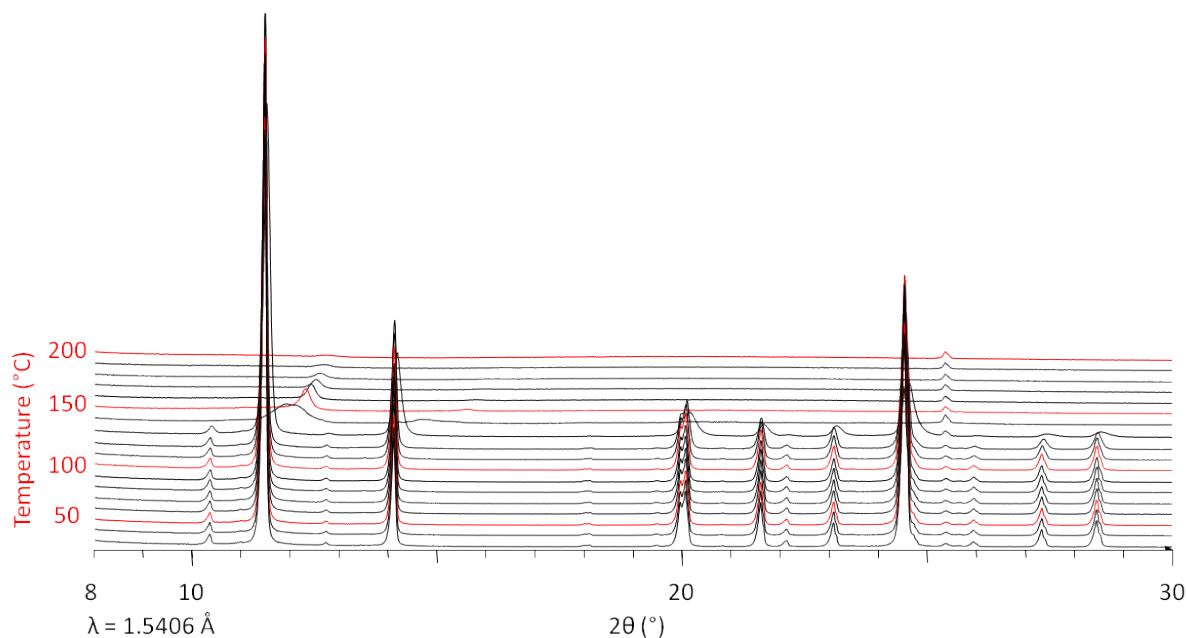


Figure S5. Thermodiffractogram of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ performed under air.

Table S2. Unit-cell parameters of $\text{Mg}(\text{H}_2\text{gal}) \cdot x\text{H}_2\text{O}$ ($0 \leq x \leq 2$) at various temperatures (trigonal setting, space group. $P3_121$).

Temperature ($^{\circ}\text{C}$)	a ($\text{\AA}$)	c ($\text{\AA}$)
130	8.8650(4)	10.7191(7)
120	8.8780(2)	10.7658(4)
110	8.8783(2)	10.7779(3)
100	8.8779(2)	10.7822(3)
90	8.8770(2)	10.7835(3)
70	8.8763(2)	10.7812(4)
50	8.8768(2)	10.7788(3)
30	8.8773(2)	10.7791(3)

2.2 Thermogravimetric analysis

TG analyses were performed on a Mettler Toledo TGA/DSC 1, STAR®System apparatus under O_2 , at a heating rate of $2^{\circ}\text{C min}^{-1}$ up to 800°C . TGA curves for $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ and $\text{Mg}_2(\text{gal}) \cdot x\text{H}_2\text{O}$ are shown in Figure S6.

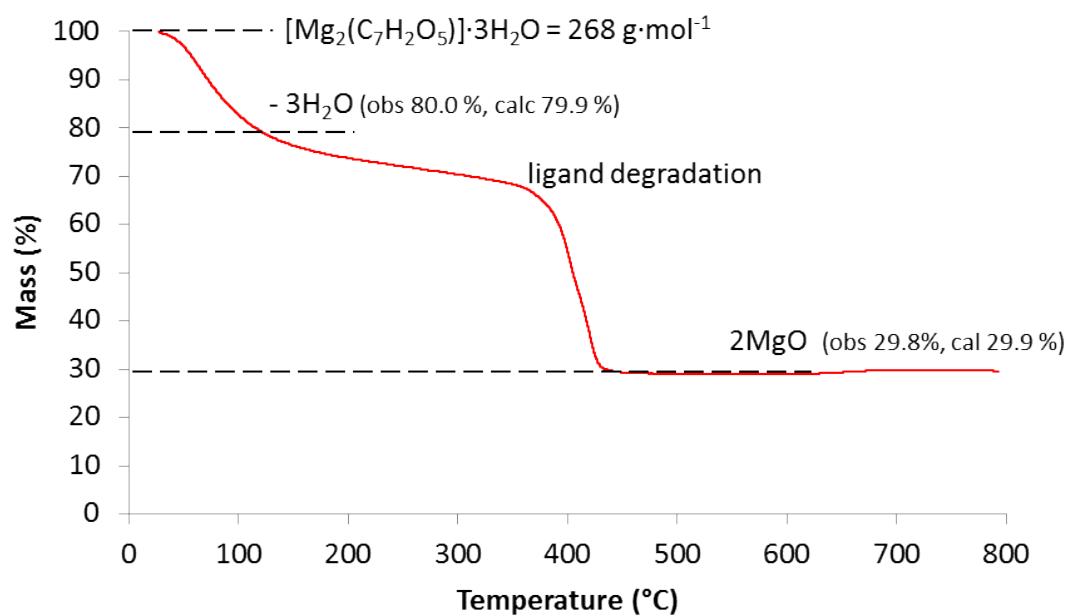
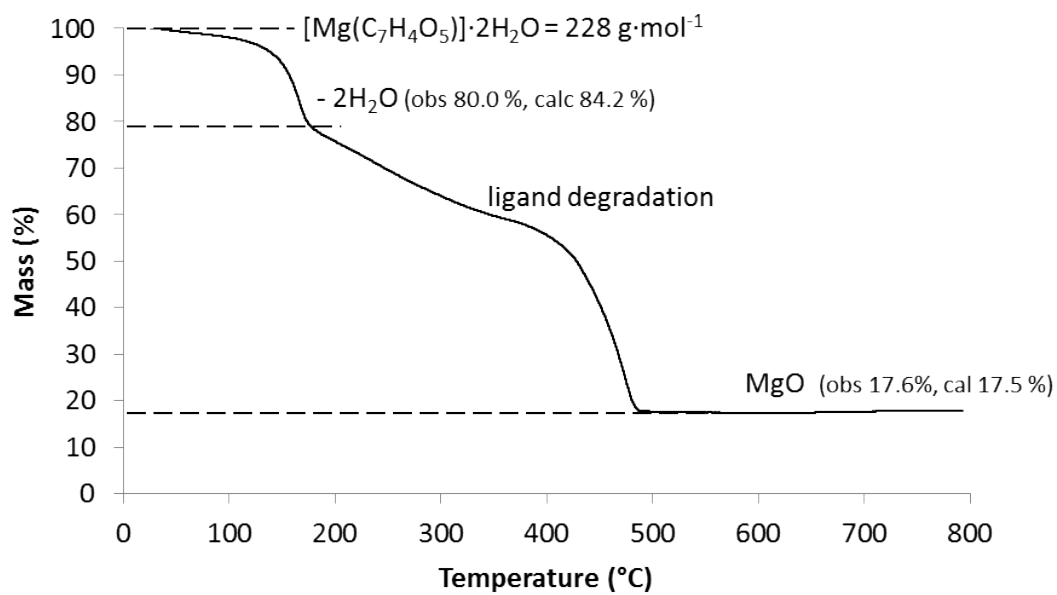


Figure S6. TGA curves for $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ (top) and $\text{Mg}_2(\text{gal}) \cdot x\text{H}_2\text{O}$ (bottom).

2.3 ^1H and ^{13}C Solid state NMR

The solid-state NMR spectra were recorded on an Avance Bruker 500 NMR spectrometer ($B_0 = 11.7$ T, corresponding to Larmor frequencies of 500.1 and 125.7 MHz for ^1H and ^{13}C , respectively). The sample was packed in a 3.2 mm outer diameter rotor. The $^1\text{H} \rightarrow ^{13}\text{C}$ cross-polarization (CPMAS) and two-dimensional heteronuclear (HETCOR) NMR spectra were acquired at MAS 10 kHz, using an initial ^1H 90° pulse length of 3 μs , a contact time of 3 ms, and radiofrequency (RF) fields of 60 and 50 kHz on ^1H and ^{13}C , respectively, during the polarization transfer. ^1H step small-phase incremental alternation (SPINAL-64)⁴ decoupling was applied during the ^{13}C signal acquisition (~ 70 kHz RF field). The recycle delay was 3.5 s. 192 transients were accumulated for the 1D spectrum, 16 for the 2D spectrum (60 t_1 slices). The ^1H ^1D Hahn-echo and 2D back-to-back (BABA)⁵ NMR spectra were recorded at MAS frequency of 20 kHz. The 90° pulse length was 3.0 μs , and the inter-pulse delay was synchronized with one rotor period for the Hahn-echo. For the BABA spectrum, the excitation time of the double-quantum coherence was 100 μs . The ^1H and ^{13}C chemical shifts were referenced to proton and carbon signals in TMS. The spectra were collected on the as-synthesized sample. Analysis of the spectra was done using the DMfit software.⁶ The ^{13}C CPMAS NMR spectrum of $\text{Mg}_2(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$, displayed in Figure S7 (left), shows the presence of one inequivalent gallic acid linker, in agreement with the structural model. The ^{13}C isotropic chemical shifts calculated by DFT (see the modelling section) agree well with the measured values, and allow the assignment of the ^{13}C resonances to the corresponding carbon atoms in the structure (Table S3). The ^1H MAS NMR spectrum (Figure S7, right) contains three resonances: resonance 2, characteristic of the C-H hydrogen from the gallic acid, resonance 1, which might be free and rather mobile water contained in the pores (since it does not correlate with the other protons from the structure in the 2D ^1H - ^1H double-quantum single-quantum NMR spectrum, Figure S8, left), and resonance 3, which corresponds to a particularly acidic proton (isotropic chemical shift around 11 ppm). This proton is very close in space to the C-H proton from the gallic acid as shown by the correlation peak of strong intensity on the 2D ^1H - ^1H NMR spectrum (Figure S8, left), and is also very close to one C-O carbon atom, as observed on the 2D ^1H - ^{13}C NMR correlation spectrum (Figure S8, right). These observations are consistent with the localization of this acidic hydrogen on a phenol function located in meta position of the carboxylate group. This acidity is further confirmed by the high ^1H isotropic chemical shift value calculated by DFT from the structural model, which match well the observed value (Table S4).

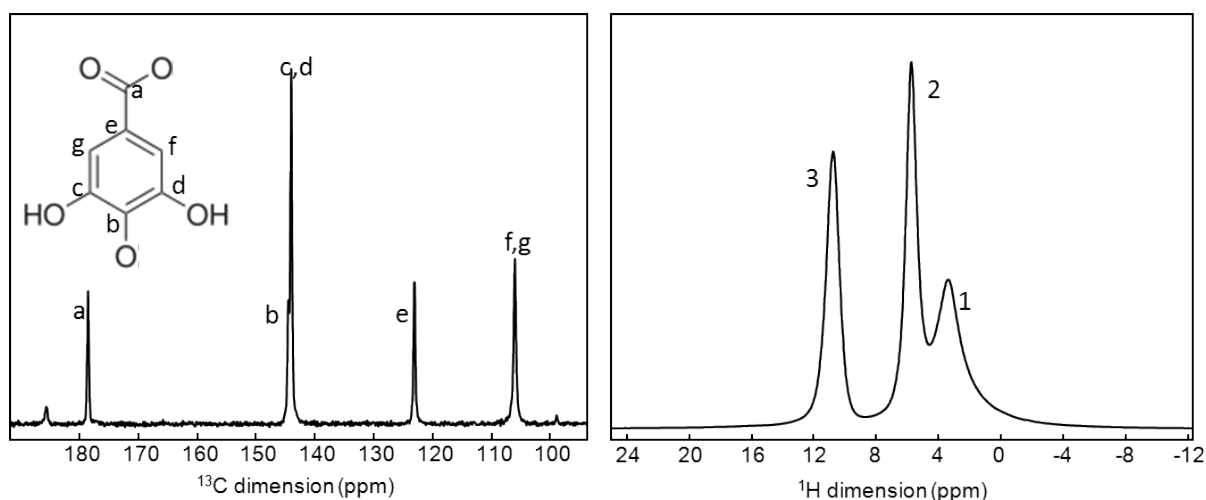


Figure S7. Left : ^{13}C CPMAS, and right : ^1H MAS NMR spectra of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$. In inset is shown the gallic acid molecule, on which the carbon atoms are labeled.

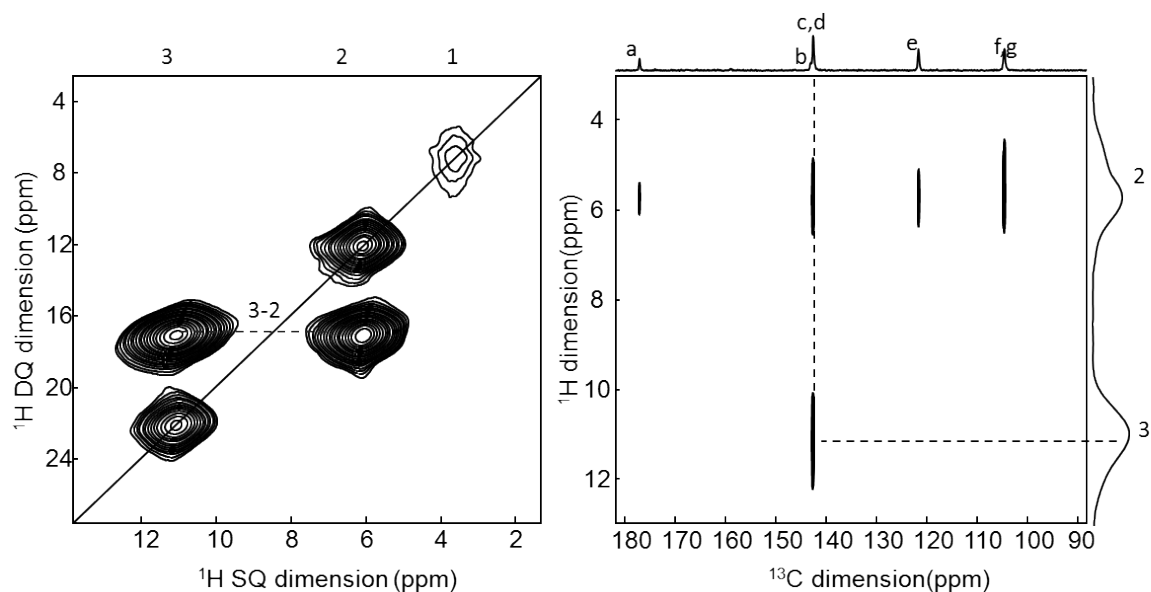


Figure S8. Left: ^1H - ^1H 2D DQ-SQ NMR correlation spectrum of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$, showing the spatial proximity (dash line) between the C-H protons (2) and the more acidic proton (3); right: ^1H - ^{13}C CP-HETCOR NMR spectrum, showing the spatial proximity (dash points) between the acidic proton and the carbon atoms in the phenol region.

Table S3. Experimental and DFT-calculated ^{13}C isotropic chemical shift (ppm) of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ (see Figure S7 for the labelling).

	a	b	c, d	e	f, g
Measured	178.4	144.3	143.9	123.0	105.9
Calculated (DFT)	183.2	148.6	146.7	120.8	107.8

Table S4. Experimental and DFT-calculated ^1H isotropic chemical shift (ppm) of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$.

	C-H	O-H
Measured	5.8	10.9
Calculated (DFT)	5.2	12.5

2.4. Nitrogen sorption measurements

$\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ was heated overnight at 100 °C under primary vacuum (BEL Japan, BELSORP Prep) before recording nitrogen adsorption isotherms at 77 K using a BEL Japan Belsorp Mini apparatus. Adsorption desorption isotherms are shown in Figure S9.

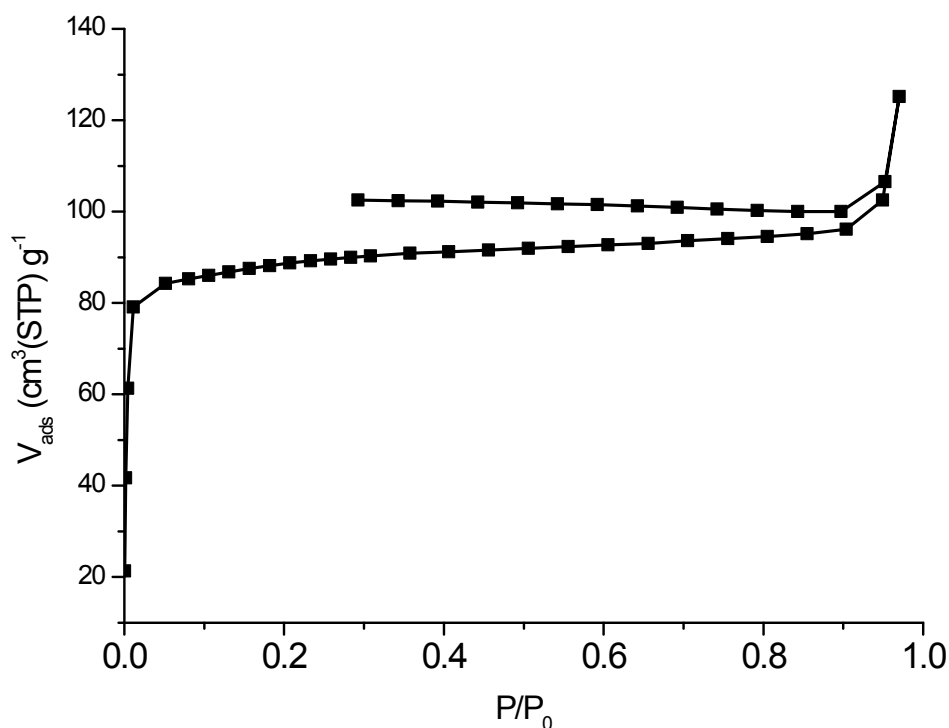


Figure S9. N₂ adsorption/desorption isotherm (at 77 K) for Mg(H₂gal). The hysteresis is likely to be associated with diffusion issues arising from the small pores (size < kinetic diameter of N₂).

2.5 Modelling

- Pore size distribution

The methodology reported by Gelb and Gubbins⁷ was used to calculate the pore size distribution (PSD) of Mg(H₂gal). In these calculations, the van der Waals parameters of the framework atoms were taken from the DREIDING force field⁸ except for Mg which was adopted from the Universal force field (UFF)⁹ as this atom is not described in the former force field. As a comparison, the calculations were also considered by using the van der Waals parameters for all atoms available in UFF. Figure S10 shows that the resulting pore diameter of this solid is comprised in the range 2.75-2.90 Å depending on the force fields employed.

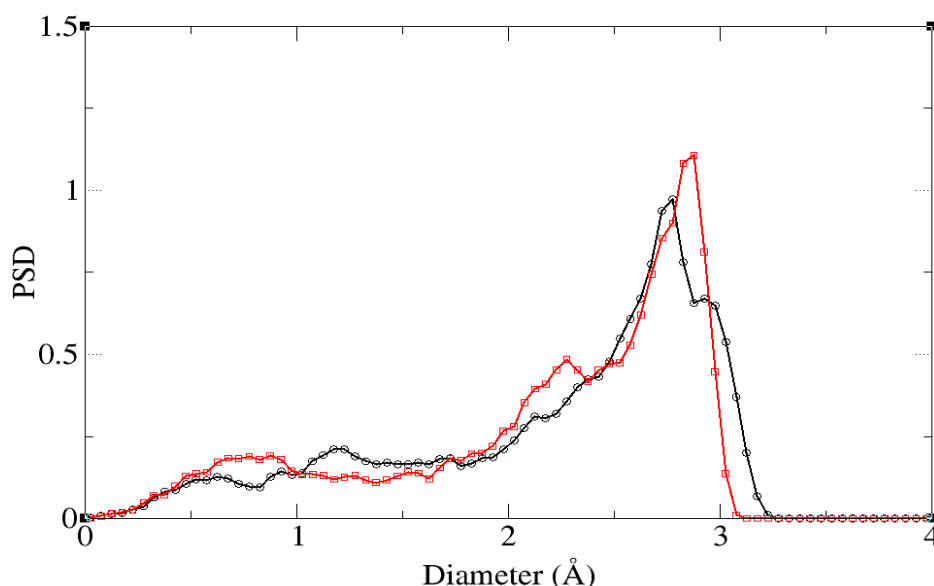


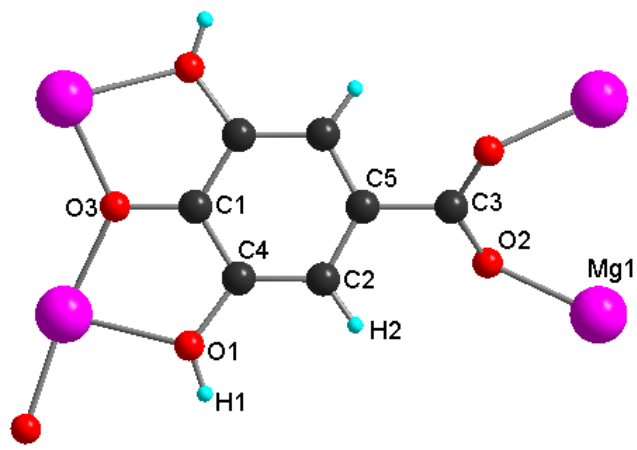
Figure S10. Pore size distribution of Mg(H₂gal) obtained using the van der Waals parameters issued from UFF for all atoms (black) and from DREIDING for all atoms except for Mg described by UFF (red).

- Theoretical surface area

We have previously pointed out that the geometric method¹⁰ using a nitrogen-sized probe molecule rolling over the framework surface does not lead to reliable calculated values for the accessible surface area when the pore size of the considered solid is comparable or smaller than the dimension of N₂ (3.64 Å).¹¹ The theoretical BET area was thus computed from the adsorption isotherm simulated for N₂. Indeed as a preliminary step, Grand Canonical Monte Carlo (GCMC) simulations using the CADSS software¹² were performed to determine the N₂ adsorption isotherms at 77 K. These simulations considered electrostatic and Lennard-Jones (LJ) interactions for N₂/N₂ and N₂/Mg(H₂gal) interactions. The N₂ molecule was described by the TraPPE force field.¹³ For the Mg-gallate, the partial charges for all framework atoms were extracted from the application of the Mulliken charge partitioning method based on periodic Density Functional Theory (DFT) calculations using PBE GGA density functional¹⁴ and the double numerical basis containing polarisation functions on hydrogen atoms (DNP)¹⁵ as implemented in the Dmol³ code. nd. The resulting set of charges is reported in Table S4 together with the labelling of the atoms. The LJ potential parameters for the framework atoms were considered in a similar way than for the calculations of the PSD. The simulation box consisted of 48 (4x4x3) unit cells and a cutoff radius of 12.0 Å was applied to the Lennard-Jones (LJ) interactions, while the long-range electrostatic interactions were handled by the Ewald summation technique. For each state point, GCMC simulations consisted of 5x10⁷ steps to ensure the equilibration, followed by 5x10⁷ steps to determine the absolute adsorbed

amounts (N_{abs}) which were assimilated to excess adsorbed amounts (N_{excess}) as the explored range of pressure is very low.

Table S4. DFT partial charges for each atom constituting the Mg(H₂gal) framework.



atom	Mg	O3	O1	O2	C1	C4	C2	C5	C3	H1	H2
charge (e ⁻)	1.157	-0.754	-0.493	-0.602	0.332	0.201	-0.144	-0.097	0.509	0.314	0.1505

We thus applied the general BET equation as defined by Bae *et al.*¹⁶

$$\frac{x}{V_{\text{excess}}(1-x)} = \frac{c-1}{V_m c} x + \frac{1}{V_m c} \quad (1)$$

In this equation, V_{excess} , expressed in cm³(STP)/g corresponds to the excess adsorbed amount of N₂ under a given equilibrium pressure P and 77 K; x is the relative pressure P/P° , where P° (=1.0 atm) is the saturation vapor pressure of N₂ at 77 K; V_m is the monolayer adsorbed amount expressed in cm³(STP)/g and c is the BET constant.

The BET area of this microporous solid was further estimated with the two consistency criteria proposed by Rouquerol *et al.*¹⁷ (i) within the pressure range chosen for a_{BET} calculation, $v_{\text{excess}}(1-x)$ should always increase with x increasing, (ii) the straight line fitted to the BET plot must have a positive intercept to yield a meaning value for the c parameter ($c>0$). The so-obtained value of V_m is then used to calculate the BET area in unit of m²/g by the following expression:

$$S_{\text{BET}} = \frac{V_m s_0 N_a}{V_{\text{STP}}} \quad (2)$$

where N_a is Avogadro's number, s_0 is the cross section area ($16.2 \text{ \AA}^2$) of one nitrogen molecule at the liquid state¹¹. V_{STP} is the molar volume of N_2 at standard temperature and pressure (273 K, 1 atm) and its value is $2.24 \times 10^4 \text{ cm}^3(\text{STP}) \text{ mol}^{-1}$. The details of the theoretical estimation of the BET area are provided in Figure S11. One can observe that the two different force fields employed for representing the MOF framework lead to a BET area of $\sim 500 \text{ m}^2 \text{ g}^{-1}$ which is slightly higher than the experimental data ($\sim 330 \text{ m}^2 \text{ g}^{-1}$). As mentioned earlier, such discrepancy may arise from an experimental underestimation of the surface area, associated with strong diffusion issue in this ultra-micropore solid.

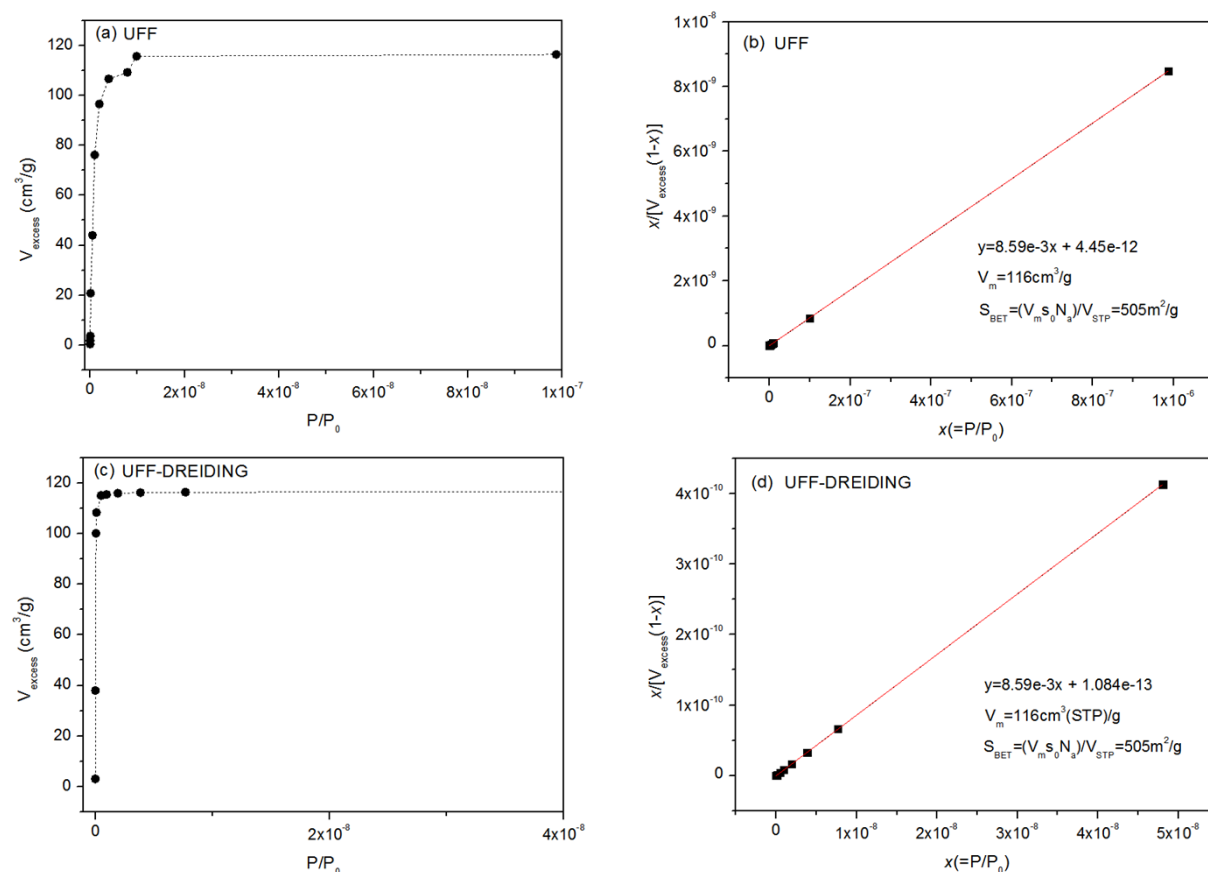


Figure S11. BET area calculation for $\text{Mg}(\text{H}_2\text{gal})$ using the simulated isotherm of N_2 at 77 K. (a,c) A plot of $V_{\text{excess}} (1 - P/P_0)$ vs P/P_0 for determining the first consistency criterion, (b,d). The selected linear plot that satisfies the second consistency criterion and the corresponding BET surface area based on the fitted red line: top: LJ parameters for all atoms of the framework taken from UFF, bottom: LJ parameters for the atoms of the framework taken from DREIDING except for Mg (UFF).

- Theoretical pore volume

The pore volume of the Mg-gallate was calculated by the thermodynamic method of Myers and Monson.¹⁸ The numerical Monte Carlo integration technique was carried out using 10^7 cycles, with helium modeled as a LJ fluid ($\sigma = 2.58 \text{ \AA}$, $\varepsilon/k_B = 10.22 \text{ K}$) and the MOF framework described by the two different set of LJ potential parameters mentioned above. The corresponding pore volumes are comprised in the range $0.1612 \text{ cm}^3 \text{ g}^{-1}$ (all MOF atoms UFF) – $0.1803 \text{ cm}^3 \text{ g}^{-1}$ (all MOF atoms UFF except Mg DREIDING).

- DFT calculations of the NMR parameters

The DFT calculations of the ^{13}C isotropic chemical shifts were performed with CASTEP¹⁹ using the PBE functional and ultrasoft pseudopotentials generated “on the fly”.²⁰ The wave functions were expanded on a plane wave basis set with a kinetic energy cut-off of 610 eV. The Brillouin zone was sampled using a Monkhorst-Pack grid spacing of $0.04 \text{ \AA}^{-1}$ (6 calculated k-points). The resulting values compare very well with the experimental ones (see Table S3).

3. Bioactivity

3.1 Materials

Phosphate buffered saline (PBS) solution (0.01 M, pH=7.4), RPMI 1640 medium supplemented with GlutaMAXTM, penicillin-streptomycin (5,000 U/mL) and heat-inactivated fetal bovine serum (FBS) were provided from Gibco®-Life Technologies (see ref²¹ for composition). Similarly, dimethylsulfoxide (DMSO; $\geq 99.7 \%$) and thiazolyl blue tetrazolium bromide (MTT) were purchased by Sigma Aldrich (St Louis, MO). Whereas the phorbol 12-myristate-13-acetate (PMA) was provided by Abcam, Biochemicals), H_2O_2 (30% w/v) from Panreac (Barcelona, Spain), 2',7'-dichlorofluorescein diacetate (2.5 μM ; DCFH-DA) by InvitrogenTM and the Annexin V-FITC Apoptosis Detection Kit was supplied by Immunostep (Salamanca, Spain). All materials were used as received without further purification.

3.2 Degradation tests

The release of gallic acid from $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ was carried out in triplicate by soaking 12 mg of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ in 12 mL of aqueous solution (Milli-Q water) and in a cell culture

media (RPMI) at 37°C under continuous stirring. After different incubation times (1 / 4 / 8 / 24 h), an aliquot of supernatant was recovered by centrifugation (14500 rpm, 15 min).

The release of the gallic acid was monitored in a reversed phased high performance liquid chromatography (HPLC) system Waters Alliance E2695 separations module from Waters with a Sunfire-C18 reverse-phase column (5µm, 4.6×150 mm from Waters) and equipped with a variable-wavelength photodiode array detector Waters 2998 and controlled by Empower software. The mobile phase used for the measurements consisted of mixture of 45 % v/v methanol in PBS solution (0.04 M, pH 2.5). Injection volume was set at 10 µL, flow rate at 1 mL·min⁻¹ and temperature of the column at 25 °C. Different solutions of free gallic acid were analyzed at concentrations of: 1.00, 0.50, 0.25, 0.12, 0.06, 0.03 and 0.01 mg•mL⁻¹ as standards for the calibration curve, presented a good correlation coefficient > 0.99. The retention time of the gallic acid appeared at 2.7 min with an absorption maximum at 210 nm.²²,²³ Finally, the degradation kinetics of Mg(H₂gal)•2H₂O was represented as the wt % of the linker released (Figure 2 in the main text). The remaining solids were studied by PXRD, the corresponding XRD patterns are shown Figure S12. This study confirmed the slow degradation of the solid, with the appearance of Mg(OH)₂ concomitantly with the gallic acid release.

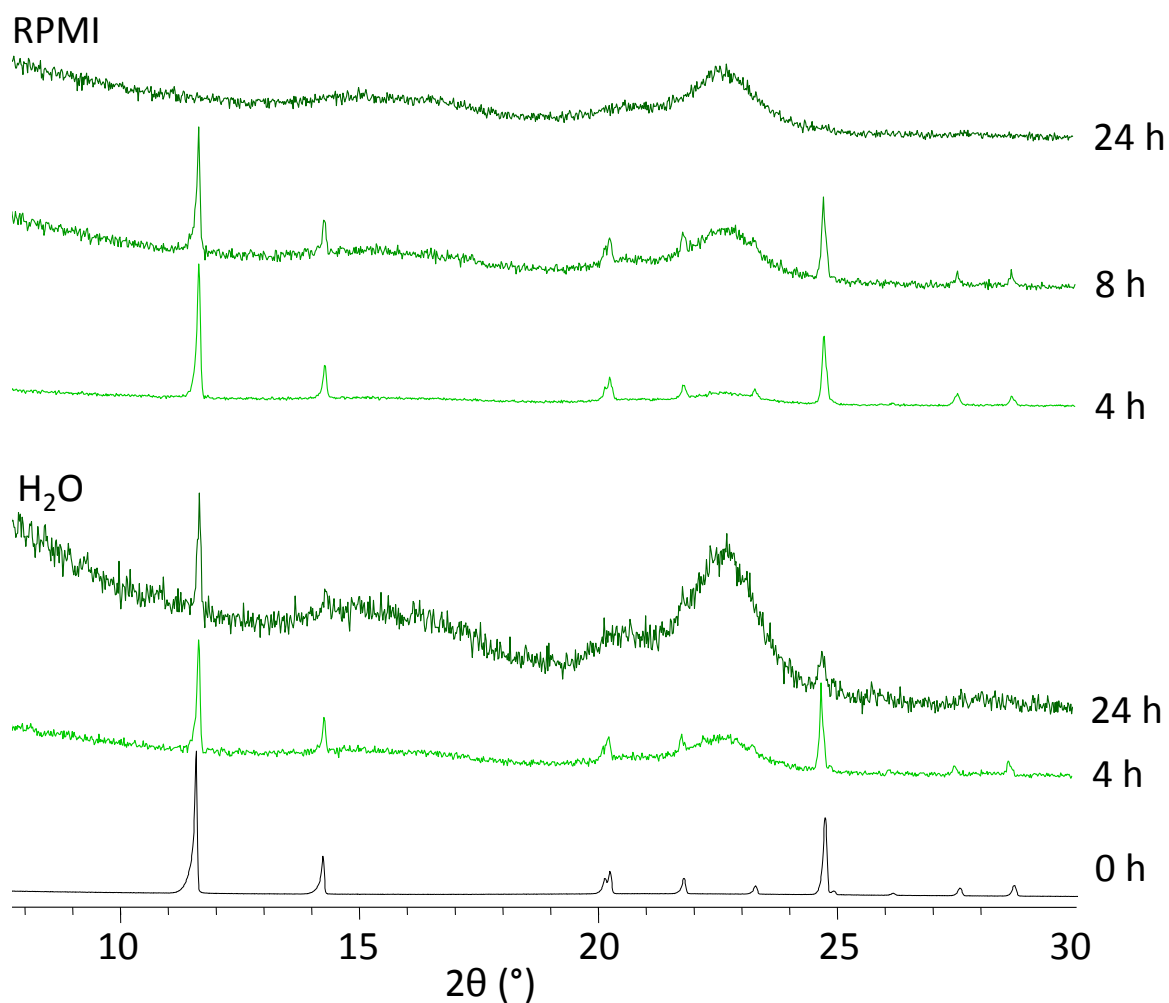


Figure S12. PXRD patterns of $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$ after incubation in water (bottom) and cell culture media (RPMI, top).

3.3 *In vitro* cell studies

- Cells and culture

NCI-H460, RAW-264.7 and HL-60 cell lines (ATCC®HTB-177™, ATCC®TIB-71™ and ATCC®CCL-240™, respectively) were cultured in RPMI 1640 GlutaMAX™, supplemented with 10% FBS, and 100 units.mL⁻¹ of penicillin/streptomycin. Cell lines were grown at 37°C in a humidified 5% CO₂ atmosphere.

- Cytotoxicity assays

The cytotoxic activity of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ as well as their precursors ($\text{Mg}(\text{OH})_2$ and H_4gal) was analyzed by the MTT and Annexin assays.^{24,25} Adherent NCI-H460 and RAW-264.7 cells were seeded 24 h prior to the assay in 96-well plates at a density of 1×10^4 cells *per* well in RPMI supplemented with 10% FBS. The HL-60 cell line, with cells in suspension, was used directly at a density of 1×10^5 cells *per* well in RPMI supplemented with 10% FBS. The treatments were prepared at a 10-fold higher concentration (due to a direct 1/10 direct dilution in the well, as 30 μL of the sample in PBS were added to a final volume of 300 μL *per* well). Based in this first concentration, a dilution series of $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$ was carried out with cell culture media, obtaining different concentrations (from 250 to 1 $\mu\text{g}\cdot\text{mL}^{-1}$). Subsequently, all these *stimuli* were added into the cells for 24 h, keeping at 37°C with a 5% CO_2 atmosphere in both experiments. From one site, the cytotoxicity was determined by adding the MTT reactant (0.5 mg mL^{-1} in PBS, incubation at 37°C for 2 h) after the contact time, followed by a PBS washing with 200 μL , ending with 100 μL of DMSO added to each well, together with their measurement of the absorbance (at $\lambda = 539 \text{ nm}$) after stirring. Results are summarized in Figure S13.

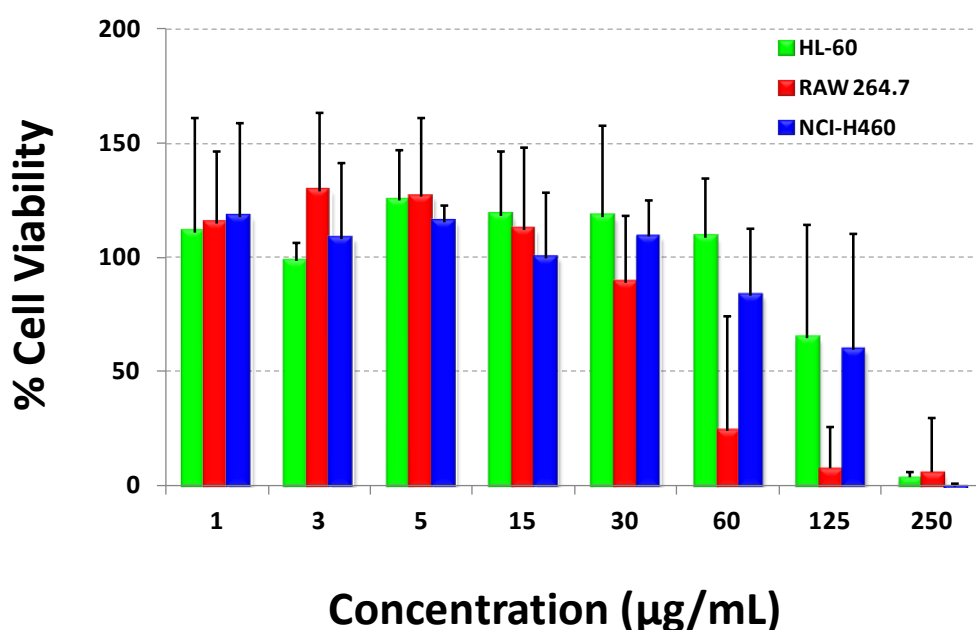


Figure S13. Cell viability of *HL-60*, *RAW 264.7* and *NCI-H460* macrophage cell lines after 24 h in contact with $\text{Mg}(\text{H}_2\text{gal})\cdot 2\text{H}_2\text{O}$.

In addition, the cytotoxicity was measured by determination of the apoptotic and necrotic index after 24 h in contact with the treatment. The cells were analyzed using Annexin-V FITC

kit (Immunostep, Salamanca, Spain), following manufacturer's instructions.^{26,27,28} Data acquisition and analysis were done by flow cytometry (BD Accuri™ C6, Biosciences) using Accuri software. For each analysis, 10,000 events were acquired. Results are summarized in Figures S14-S15.

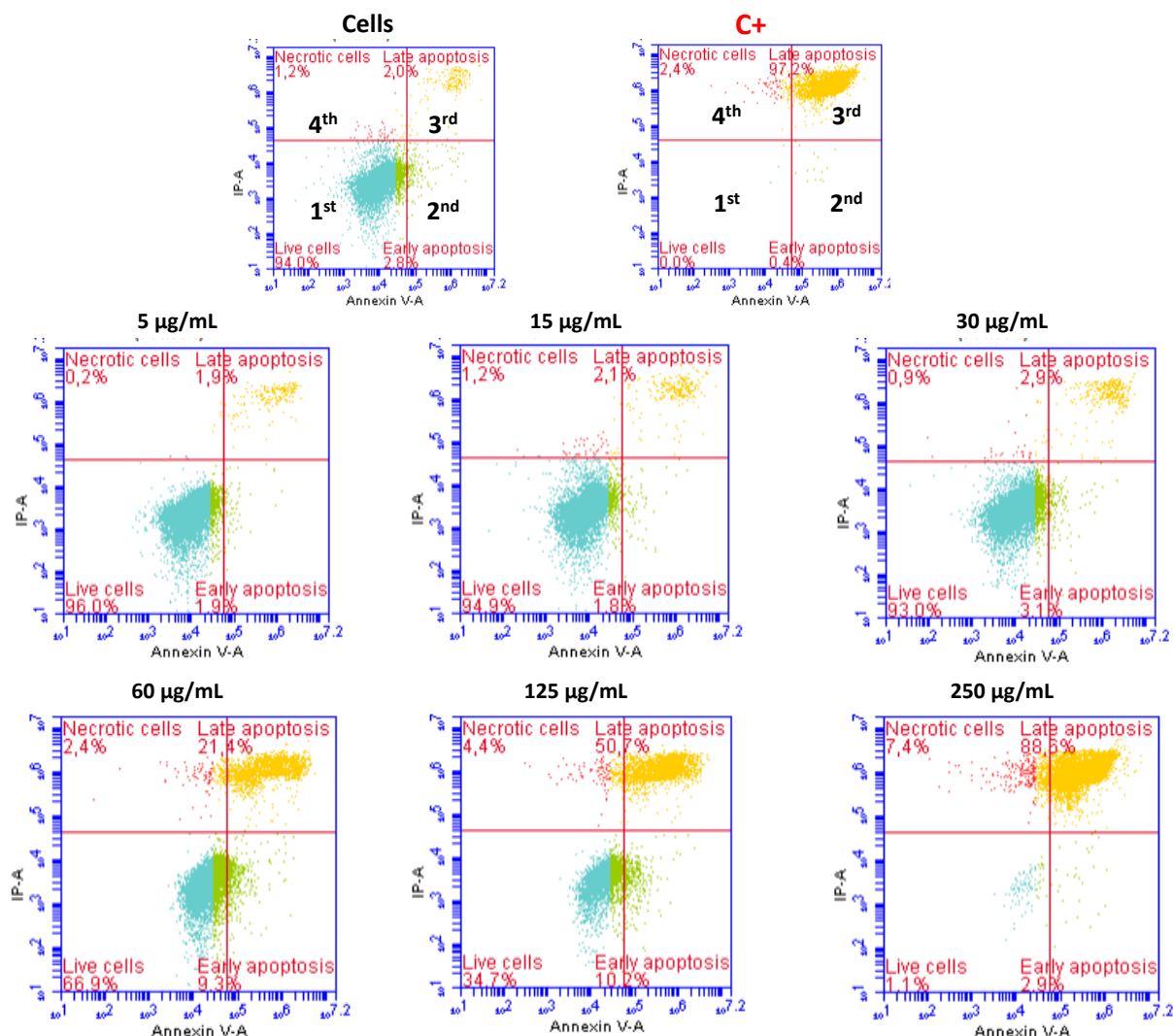


Figure S14. Annexin-V analysis of *HL-60* cell line after 24 h in contact with different concentration (5 / 15 / 30 / 60 / 125 / 250 µg.mL⁻¹) of Mg(H₂gal)•2H₂O. Negative control and positive control (C+) were considered as cells alone and cells in the presence of H₂O₂, respectively. From the 1st to 4th quadrant are represented different cell states as live, early apoptotic, late apoptotic and necrotic cells, respectively. Note that these data, corresponding to one of the triplicate experiments, are totally representative from the whole results.

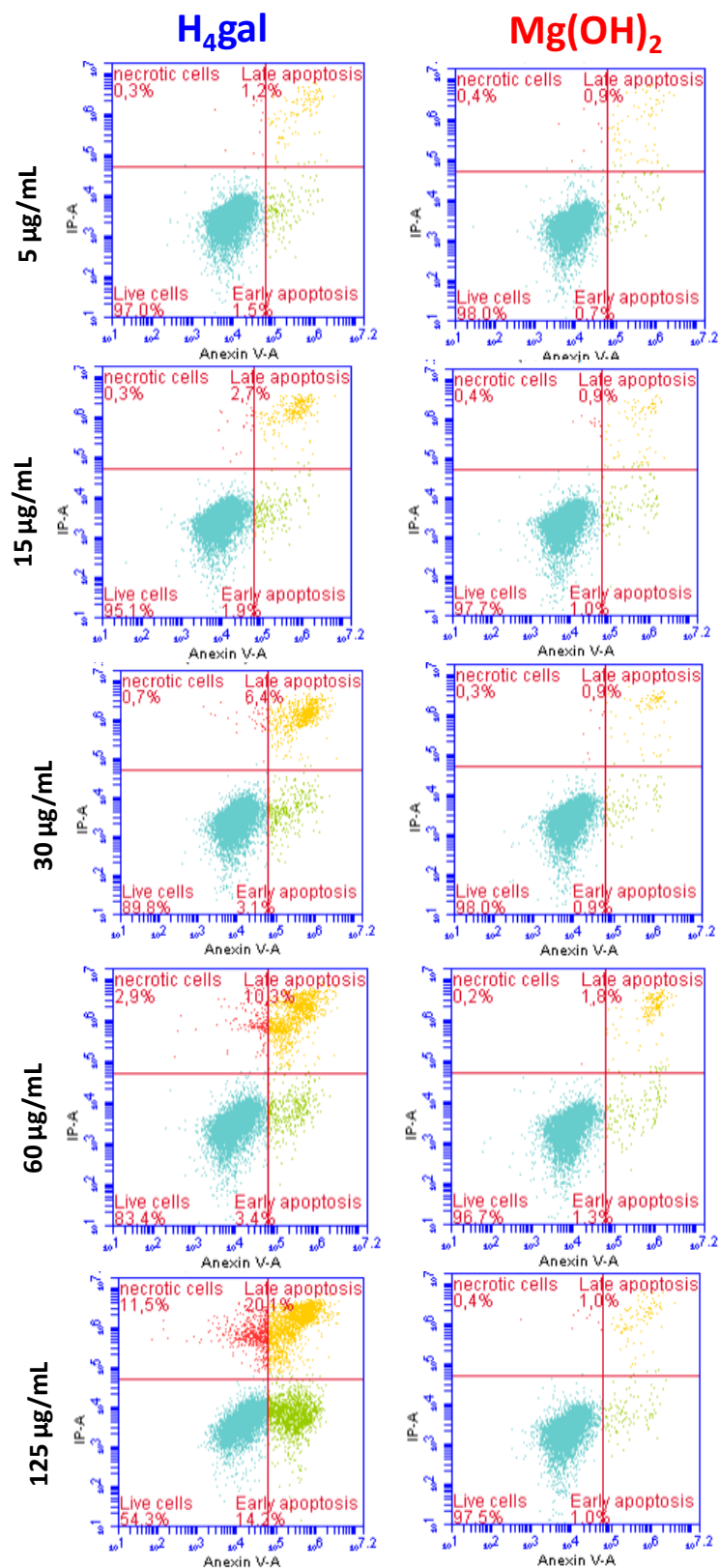


Figure S15. Annexin-V analysis of *HL-60* cell line after 24 h in contact with different concentration (5 / 15 / 30 / 60 / 125 µg.mL⁻¹) of each component of gallate (H₄gal or Mg(OH)₂). Negative control and positive control (C+) were considered as cells alone and cells in presence of H₂O₂, respectively. From the 1st to 4th quadrant are represented different cell states as live, early apoptotic, late apoptotic and necrotic cells, respectively. Note that these data, corresponding to one of the triplicate experiments, are totally representative from the whole results.

- ROS production

The cells were seeded in 96-well plates (U bottom) at a density of $1 \cdot 10^5$ cells *per* well in 100 μL of cell culture medium (RPMI supplemented with 10 % FBS). The stimulus-containing solutions in 100 μL was added to the cells to a final concentration of 5 / 15 / 30 / 60 $\mu\text{g} \cdot \text{mL}^{-1}$ of either $\text{Mg}(\text{H}_2\text{gal}) \cdot 2\text{H}_2\text{O}$, H_4gal or $\text{Mg}(\text{OH})_2$. Negative and basal controls were considered as the cells in absence of stimulus. As positive control, the cells were incubated with an oxidant component, phorbol 12-myristate-13-acetate PMA (10 μM) at 37 °C. After 8 h of incubation, cells were centrifuged (900 rpm, 5 min) and put in contact with the ROS reactant (2',7'-Dichlorofluorescein diacetate, DCFH-DA, Invitrogen™), (1 μL *per* 200 μL of cells) for 30 min at 37 °C in dark conditions. Twice PBS-washed were performed after the incubation and, finally, the 2',7'-dichlorofluorescein (DCF) fluorescence in cells was measured by flow cytometry (BD Accuri™ C6 Flow Cytometer).^{29,30}

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