Supplementary Information

Nanocapsules of Unprecedented Internal Volume Seamed by Calcium Ions

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Materials and Method

Materials

Pyrogallol (Chem-Impex, 22779), propionaldehyde (Sigma-Aldrich, W292303), concentrated hydrochloric acid (Fisher chemicals, A144S-212), calcium chloride anhydrous (40-20 mesh, Fisher Chemicals, C614-500), calcium bromide hydrate (Acros organics, AC450632500), pyridine 99+% (Acros organics, AC13178-0025), DMF (Alfa aesar, A43465), ethyl acetate (Fisher chemicals, E195SK-4) and methanol (Fisher chemicals, A452SK-4).

Synthesis of *C*-ethylpyrogallol[4]arene (PgC_2) **1**. In a 100 mL round bottom flask containing stir bar, pyrogallol (10 g, 0.08 mol), propionaldehyde (5.74 mL, 0.08 mol), and 30 mL of ethyl acetate were added. Upon reflux, 3.5 mL of concentrated HCl was added and the reflux was allowed to continue for 24 hours, resulting in the formation of a solid suspension. The reaction was allowed to cool at room temperature and the product was filtered and washed with cold ethyl acetate followed by vacuum drying leading to the formation of solid white product (5.3 g) with a yield of 39.9%.[1]

Preparation of Calcium-seamed Nanocapsule **2** $[(Ca_4Cl)_6][(PgC_2)_{12}]$. PgC_2 (0.1 mmol, 66.4 mg) and Calcium Chloride (anhydrous, 0.4 mmol, 44.4 mg) were dissolved in 2 mL of N, N-dimethylformamide (DMF) and 2 mL of methanol (MeOH) in 8 mL glass vial. The mixture was sonicated for one hour to get a clear solution followed by addition of pyridine (1.2 mmol, 98µL). The resulting mixture was heated to 125°C for 24 h and golden colored plate-like crystals were formed and collected for single crystal X-ray analysis. The yield was found to be 0.036 g or 17.73% based on calcium chloride.

Preparation of Calcium-seamed Nanocapsule **3** $[Ca_{32}(CaNa_4(HCO_2)_5)_6(OH)_8(DMF)_{24}(OH_2)_{54}(PgC_2)_{12}]$.

PgC2 (0.1 mmol, 66.4 mg) and Calcium bromide hydrate (0.4 mmol, 80.0 mg) were dissolved in 6 mL of N, N-dimethylformamide (DMF) in 8 mL glass vial. The mixture was sonicated for one hour to get a clear solution followed by addition of pyridine (1.2 mmol, 98µL). The resulting mixture was heated to 125 ˚C for 96 h and golden colored plate-like crystals were formed and collected for single crystal X-ray analysis.

Single crystal X-ray Diffraction

The crystal structure of **2** was solved using single crystal X-ray diffraction (SCXRD) data was collected at Beamline 11.3.1 at the Advanced Light Source using 16 keV synchrotron radiation on a diffractometer equipped with a Bruker Photon 100 CMOS area detector (Bruker AXS, Madison, WI). The crystal structure of 3 was determined using data collected on a Bruker X8 Prospector diffractometer (Bruker AXS) using Cu-Kα radiation from a microfocus source. The crystals were cooled to 100 K during data collection. Data collection strategy, unit cell determination, data reduction, and absorption correction were done using the Bruker Apex3 software suite.[2]

The crystal structure of **2** was solved on a data set truncated to 1.20 Å; diffraction peaks became effectively indistinguishable from background at resolutions beyond this. The structure

nevertheless yielded an immediately recognizable solution when solved by a dual space recycling method as implemented in SHELXT.[3] At this resolution only the Ca atoms are clearly resolved, although an electron density surface map calculated from |Fobs| is clearly consistent with the determined structure. The model was refined by full matrix least squares refinement with SHELXL[4] using the following restraints due to the low data-to-parameters ratio. The pyrogallol rings were restrained to have idealized symmetry and be chemically equivalent to all others in the structure; this was done by restraining all C–C bond distances related by symmetry to have identical values (resulting in a total of three unique restraint values for all aromatic C–C bonds in the structure). All C–C single bonds associated with the ethyl groups were restrained to have an idealized distance of 1.54 Å. All Ca–O bonds were restrained to distances observed in crystal structures of 7- and 8-coordinate crown ether analogs (2.36 Å for equatorial bonds and 2.4 Å for axial bonds). For 8-coordinate Ca centers, an additional distance restraint was added to keep neighboring axial ligands at the appropriate distance from each other. The Ca and Cl atoms were refined anisotropically to convergence with the above restraints. All O and C atoms on or bridging pyrogallol rings were refined anisotropically with the thermal ellipsoids restrained to have similar values.[5] The axial O ligands and the ethyl group carbon atoms were constrained to have isotropic thermal motion. Hydrogen atoms were constrained to idealized positions and allowed to ride on the carrier atoms. Hydrogen atoms bonded to oxygen could not be located or modeled. Finally, an additional correction was made by removing the contribution of all contents of the crystallographic void space from the scattering using PLATON SQUEEZE.[6]

The X-ray diffraction data set for **3** was truncated to a resolution of 1.10 Å; data in the 1.12–1.10 Å resolution shell have a R_{merge} value of 33%, indicating the data is beginning to be significantly contaminated by noise. Higher resolution data could not be used; due to the limited size of the Apex II camera and the high 2-theta angles required for Cu-Kα data, the high angle frames consistent almost entirely of background noise and fail to integrate properly. The structure was solved using SHELXT[3] and refined by full matrix least squares refinement against F2 with SHELXL.[4] One of the atoms initially assigned as oxygen by the solution consistently refined to have a negative U_{eq} value but failed to refine well as a Ca atom, indicating that it was a third-row atom. Based on its position near anionic oxygen atoms, this peak was considered most likely to be a metal cation, and refining it as Na+ gave the greatest improvement to the R-factor among chemically reasonable choices as well as resulting in a chemically reasonable distorted square-antiprismatic coordination geometry and bond distances within the expected range for Na–O (2.20(2)-2.634(4) Å). Na^+ ions were not included in the synthesis but could be introduced into the solvent through a number of common laboratory reagents such as NaOMe. Bond distance restraints were applied to the PgC_2 C–C and C–O bond distances analogous to **2**, and a global restraint for rigid bond behavior was applied to all thermal ellipsoids.[5] All non-hydrogen atoms could be refined anisotropically except for two oxygen atoms which correspond to the coordinating atoms of disordered ligands and are considered water molecules for the determination of the formula. Hydrogen atoms bonded to carbon atoms where placed in calculated positions and constrained to ride on the carrier atoms. Hydrogen atoms bonded to oxygen atoms could not be located and were omitted from the model. Contributions from disordered solvent in the capsules and interstitial voids were analyzed and removed using PLATON SQUEEZE.[6]

For both structures Olex2 was used to construct the models for crystallographic refinement, input restraints, and to generate ellipsoid plots.[7] Packing plots were generated with CCDC Mercury,[8] and the additional graphical elements used to augment the plots were drawn using POV-Ray for Windows.[9]

Powder X-ray diffraction (PXRD)

PXRD was measured on a Bruker X8 Prospector single crystal diffractometer (Bruker AXS). The sample was first verified to contain crystals of **2** by inspection of the crystals under an optical polarizing microscope and by mounting and determining the unit cell of one of the crystals by SCXRD. The crystals were then pulverized by hand with a steel spatula while under the mother liquor. For PXRD of fresh **2**, the resulting suspension was loaded into a 0.5 mm polyimide tube. The solid material was allowed to settle into a slug which was centered into the X-ray beam for data collection. For dried **2**, a portion of the suspension was removed with a Pasteur pipette, deposited on a piece of filter paper, and allowed to dry under ambient conditions for 1 h. The dried material spontaneously clumped into pellets, one of which was mounted on a SCXRD sample loop and centered on the diffractometer.

PXRD was measured using Cu-Kα from a microfocus source (λ = 1.54178 Å; beam power = 45 kV, 0.65 mA). Scans were conducted at a fixed 2θ of 20.0º and a sample-to-detector distance of 8.0 cm. Each sample irradiated for 8 minutes by taking 8 consecutive 1-minute long scans in which the samples were rotated 360º about the phi axis. The diffraction photographs were reduced by integrating a 64º-wide sector of the photograph across the 2θ of 3 to 40º in 0.02º slices. Integration was performed using the Apex3 software suite.

Thermogravimetric analysis (TGA)

TGA was conducted with a TA Instruments TGA Q50 under a nitrogen atmosphere (40 mL min–1) with platinum pan, scanning from room temperature to 500 ºC at a heating rate of 20 ºC min^{-1}.

Transmission Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was performed using a Thermo Scientific Nicolet Summit Pro FTIR spectrometer with potassium bromide (KBr) pellets, averaging 24 scans with a resolution of 4 cm^{-1}.

Fluorescence Spectroscopy

Fluorescence Probe Studies

Fluorescent Probe Encapsulation. 4 mL of a 1 mM fluorescent dye (Nile red or pyranine, in 1:1, v/v DMF:MeOH) was added to ~36 mg of **2**. After 72 h, the supernatant was discarded and the crystals rinsed twice with 10 mL of 1:1 (v/v) DMF:MeOH using a filter funnel with vacuum filtration to remove free or surface-bound dye. To demonstrate successful solute encapsulation, ~2.5 mg of **2** containing entrapped fluorophore was completely dissolved in 3 mL of DMSO and the fluorescence spectrum acquired using 550 nm (Nile red) or 460 nm (pyranine) excitation. Fluorescence samples were prepared in 1-cm path length quartz cuvettes and measurements were

made with a Shimadzu RF 6000 fluorescence spectrophotometer using 5.0 nm excitation and emission slit widths at a scan rate of 600 nm/min and an accumulation time of 1 s.

For comparison, undoped crystals of **2** (~2.5 mg) were washed as described above and promptly dissolved in 3 mL of DMSO followed by the addition of an aliquot of the desired fluorescent dye stock in DMSO.

Fluorescence-Based Time-Release Experiments

Ca-seamed crystals **2** containing encapsulated fluorescent reporter (NR or pyranine) were rinsed twice with 10-mL aliquots of 1:1 DMF:methanol to remove excess or surface-bound dye and then isolated using vacuum filtration. Rinsed crystals were added to a 1-cm path length quartz cuvette followed by the injection of 3 mL of 1:1 DMF:methanol. Fluorescence-detected time release experiments were conducted over the course of 1 h using 550 nm excitation (Nile red) or 460 nm (pyranine) with fluorescence monitored at 638 nm or 513 nm, respectively.

Scanning electron microscope (SEM) - Energy-dispersive X-ray spectroscopy (EDS)

SEM-EDS was performed using an FEI Quanta 600F Environmental Scanning Electron Microscope equipped with a Bruker Quantax 200 silicon drift detector at 10kV. Powdered samples were mounted directly onto carbon adhesive and analyzed using low-vacuum mode to mitigate charging. Backscatter and secondary electron images were collected to generate topographical and chemical phase contrast, respectively. EDS spectra were collected from representative particles to perform quantitative analysis. In addition to confirmation of the crystal structure of **2** by unit cell determination on multiple single crystals pulled from a single batch, scanning electron microscopy was used to examine particle morphology and indicates all particles are morphologically similar crystals, supporting the PXRD assignment (Figure S1-S4). Energy dispersive spectroscopy (EDS) on multiple particle surfaces indicated that they are chemically uniform (Figure S4). The discrepancy between them Cl content measured by EDS (1.40-1.45%) and that calculated from the crystal structure which excludes disordered solvent (2.2 %) was used to estimate the mass of unmodeled solvent, which was found to be 36.4 % in general agreement with TGA. The composition of this solvent likely varies from crystal to crystal and consists mostly of the crystallization solvent dimethylformamide (DMF). Assigning it exclusively to DMF results in an estimated N content of 6.99 wt. %, which overestimates that found by EDS suggesting that other solvents such as methanol and water are included in variable amounts.

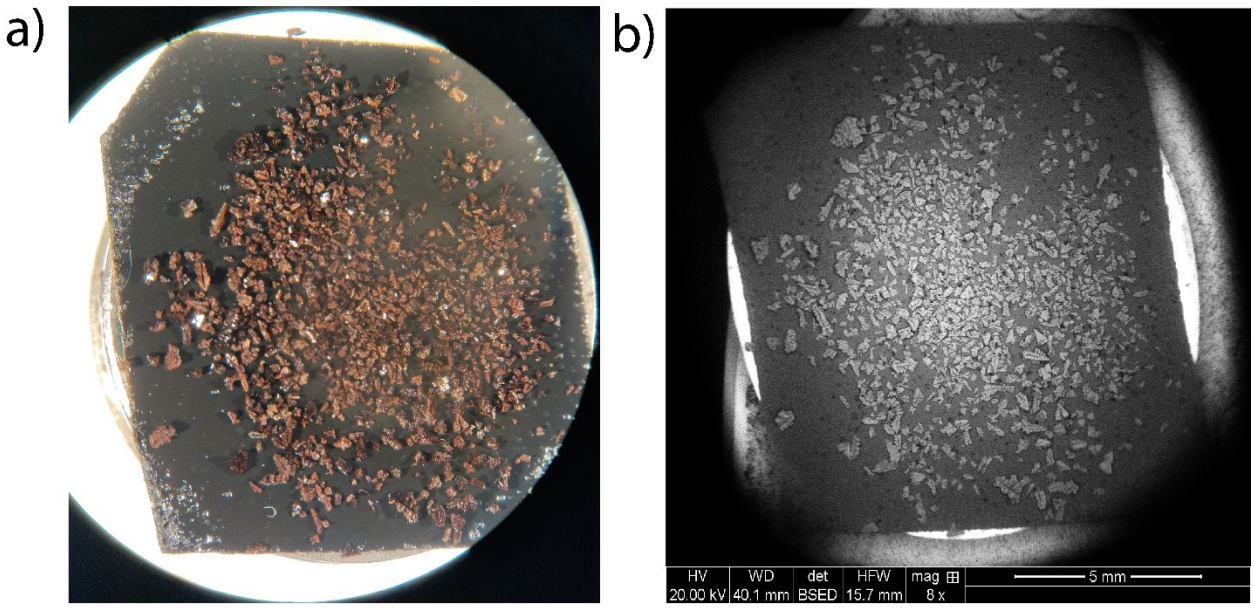


Figure S1 Optical (a) vs SEM (Back Scattered Electron Image) (b) of the calcium-seamed Nanocapsule 2 crystals . Study reveals the surface morphology and the phase contrasts. Upon further analysis, it was clear that the crystals of nanocapsule 2 were chemically uniform, and highly crystalline.

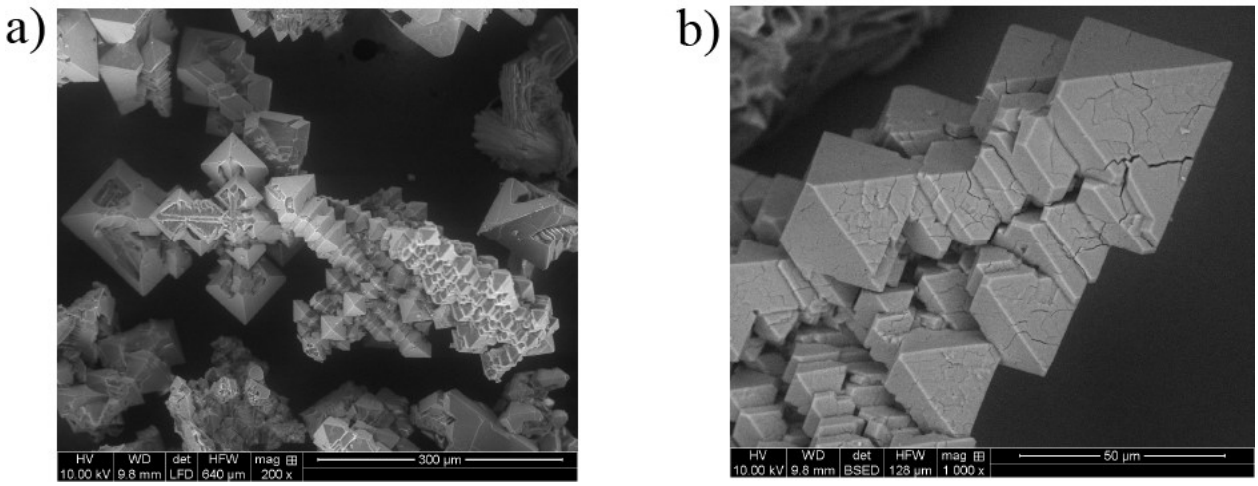


Figure S2 SEM images of the calcium-seamed Nanocapsule **2** crystals. a) Secondary Electron Image and b) Backscatter electron image.

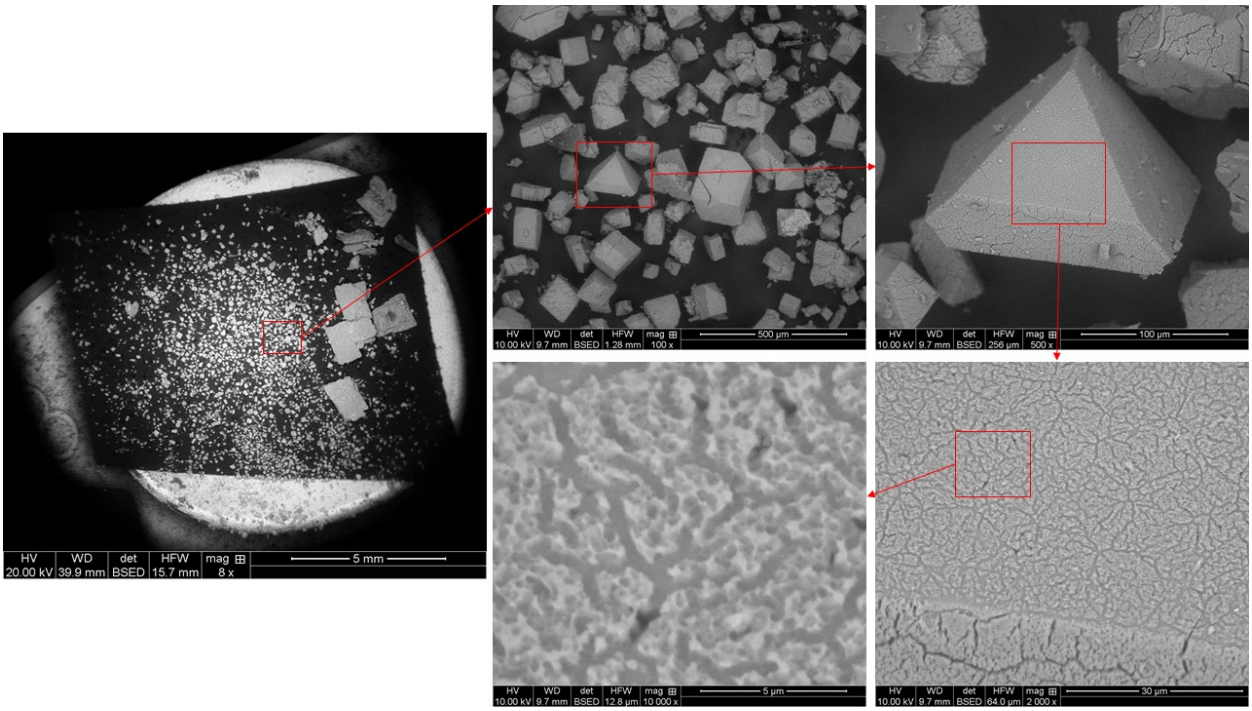
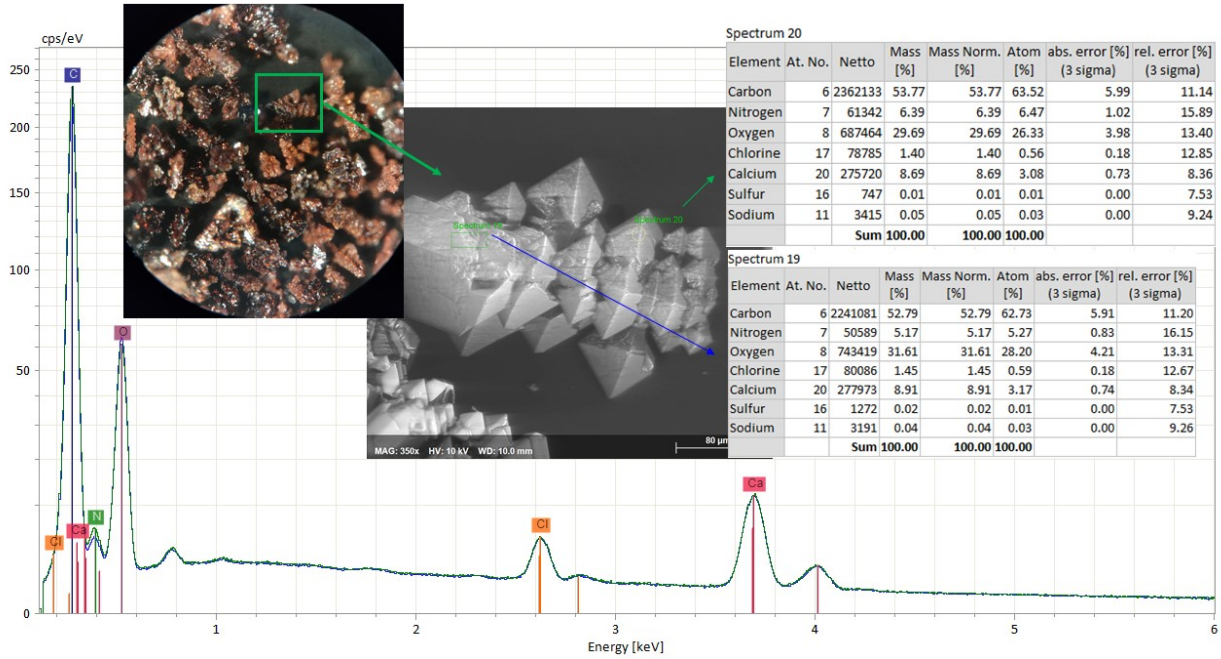


Figure S3 SEM images showing fractal calcium-seamed Nanocapsule **2** crystals.



Spectrum 20

Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (3 sigma)	rel. error [%] (3 sigma)
Carbon	6	2362133	53.77	53.77	63.52	5.99	11.14
Nitrogen	7	61342	6.39	6.39	6.47	1.02	15.89
Oxygen	8	687464	29.69	29.69	26.33	3.98	13.40
Chlorine	17	78785	1.40	1.40	0.56	0.18	12.85
Calcium	20	275720	8.69	8.69	3.08	0.73	8.36
Sulfur	16	747	0.01	0.01	0.01	0.00	7.53
Sodium	11	3415	0.05	0.05	0.03	0.00	9.24
		Sum	100.00	100.00	100.00		

Spectrum 19

Element	At. No.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (3 sigma)	rel. error [%] (3 sigma)
Carbon	6	2241081	52.79	52.79	62.73	5.91	11.20
Nitrogen	7	50589	5.17	5.17	5.27	0.83	16.15
Oxygen	8	743419	31.61	31.61	28.20	4.21	13.31
Chlorine	17	80086	1.45	1.45	0.59	0.18	12.67
Calcium	20	277973	8.91	8.91	3.17	0.74	8.34
Sulfur	16	1272	0.02	0.02	0.01	0.00	7.53
Sodium	11	3191	0.04	0.04	0.03	0.00	9.26
		Sum	100.00	100.00	100.00		

Figure S4 Energy-dispersive X-ray spectroscopy (EDS) spectra of the calcium-seamed Nanocapsule **2** crystals.

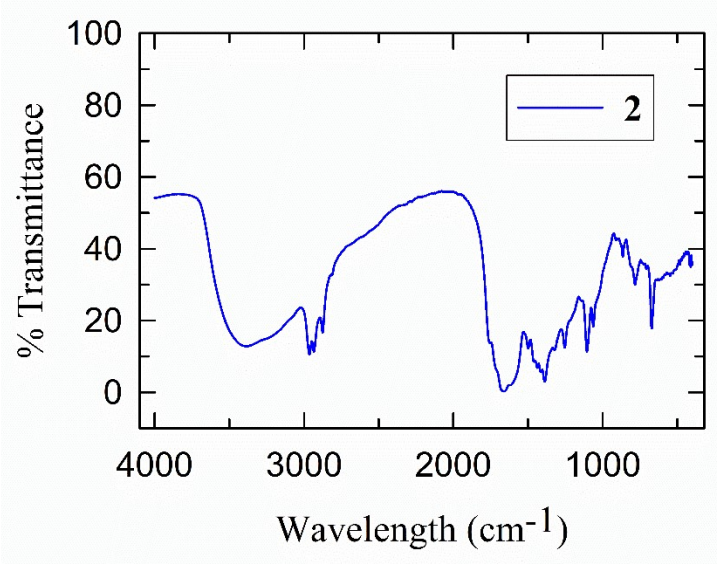


Figure S5 FTIR Spectra of the calcium-seamed Nanocapsule **2.** Signature peaks of the aromatic C–H Stretching and C–C stretching in pyrogallol[4]arene were found at ~3080 and ~1625 cm^{-1}, respectively.

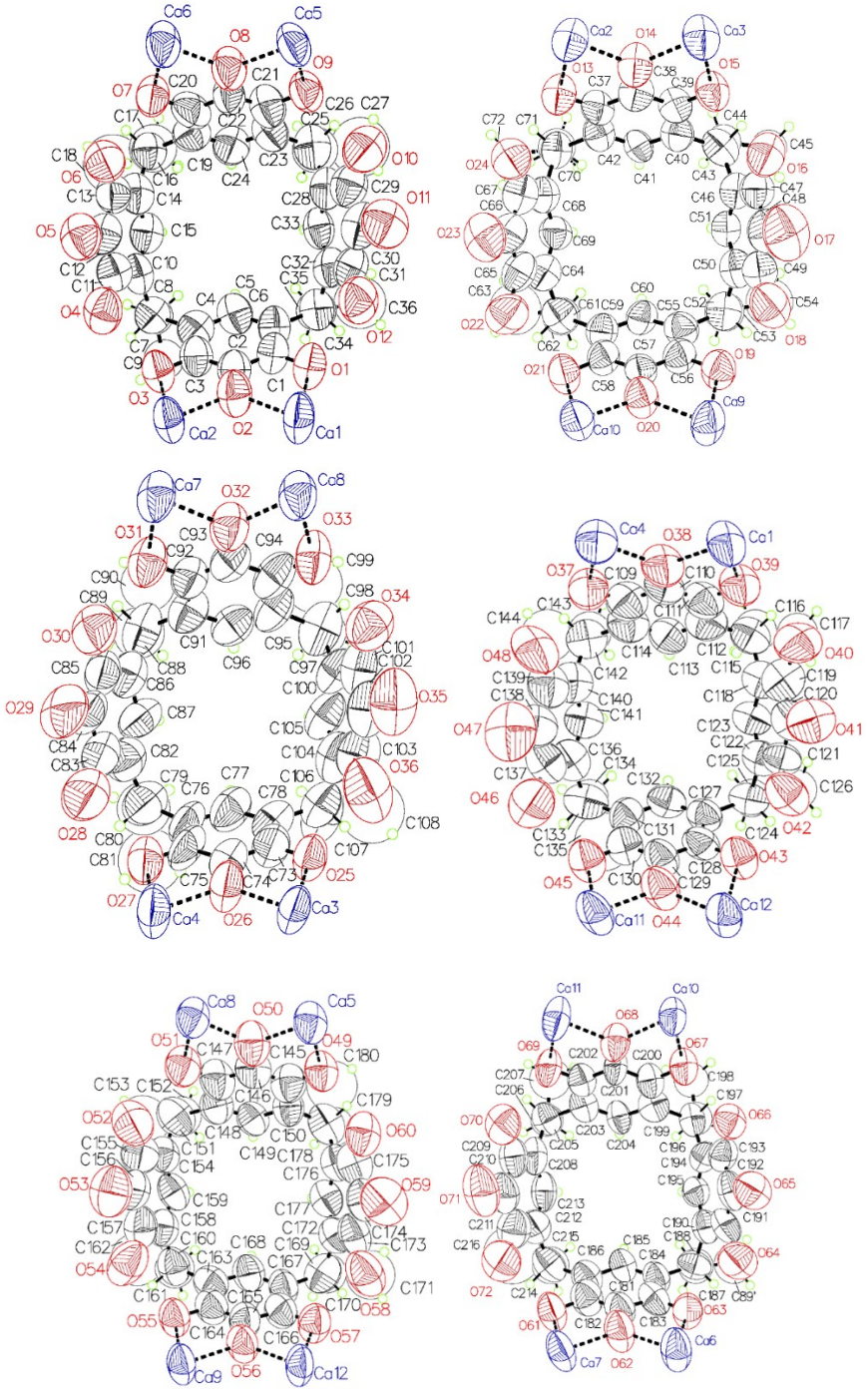


Figure S6 50% probability ellipsoid plots of all unique PgC_2 moieties in **2** and coordinating metal atoms. Elements are color coded as follows: Black = C, green circles = H, red = O, dark blue = Ca.

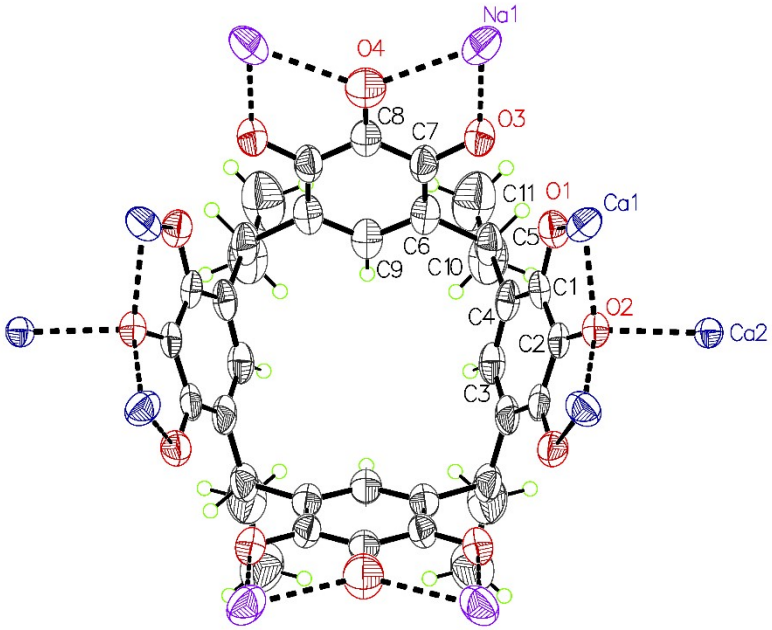


Figure S7 50% probability ellipsoid plot for PgC_2 unit in **3** and coordinating metal atoms. Non-labeled atoms are symmetry equivalents of labeled atoms. Elements are color coded as follows: : Black = C, green circles = H red = O, dark blue = Ca, violet = Na.

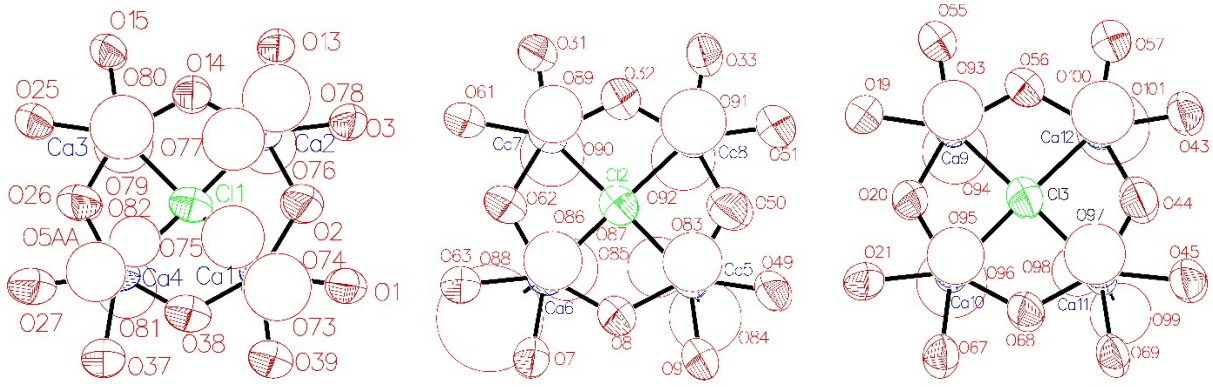


Figure S8 50% probability ellipsoid plots of all unique $\{Ca_4Cl\}$ moieties in **2**. Open circles represent atoms refined with isotropic thermal motion. Elements are color coded as follows: Ca = dark blue, Cl = green, O = red.

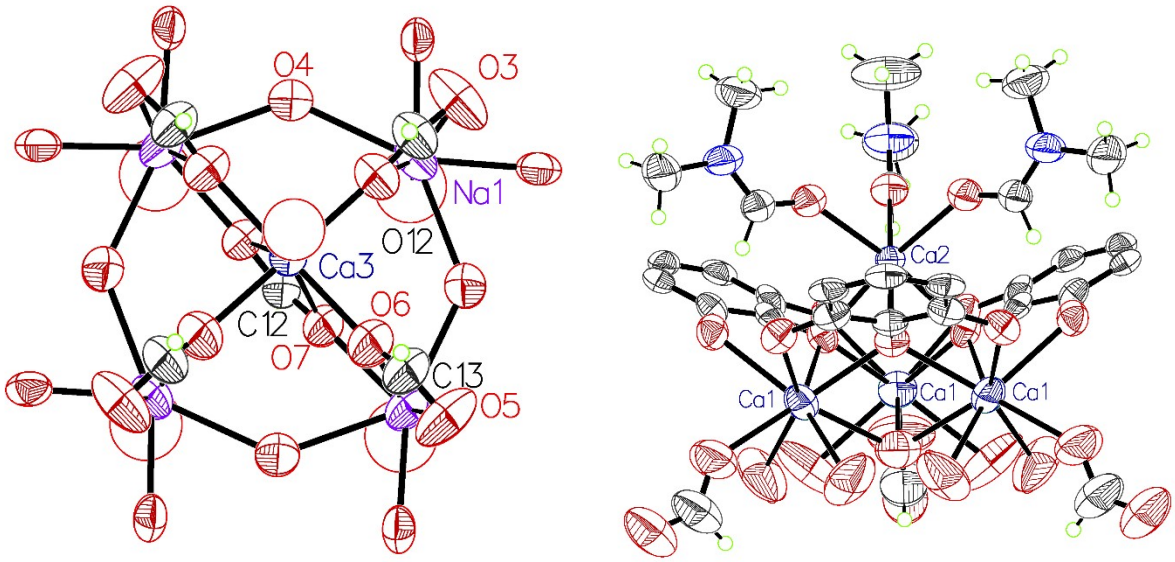


Figure S9 Polyatomic anions in **3.** *Left* – Cross shaped $\{CaNa_4(HCO_2)_5\}$ moieties; unlabeled atoms are symmetry equivalents of labeled atoms. *Right* – tetrahedral $\{Ca_4(HCO_2)_4(DMF)_3\}$ moieties; only metal atoms are labeled for clarity. Elements are color coded as follows: C = black, H = green circles, N = blue, O = red, Ca = dark blue, Na = violet.

Crystallographic Information

Table S1 Summary of crystallographic data

Crystal Structure	**2**	**3**
CCDC Number	1955663	2071418
Empirical formula	$C_{4320}H_{334}Ca_{24}Cl_6O_{186}$	$C_{534}H_{732}Ca_{38}N_{24}Na_{24}O_{290}$
Formula weight	9675.69 g/mol	14202.19 g/mol
Temperature	100.0(1) K	100.0(1) K
Wavelength	0.7749 Å	1.54178 Å
Crystal system	Monoclinic	Cubic
Space group	$P2_1/n$	*Im*-3*m*
Unit cell dimensions	a = 31.196(3) Å	36.2373(1) Å
	b = 31.607(3)	36.2373(1)
	c = 44.006(4)	36.2373(1)
	α= 90°	α= 90°
	β= 91.733(2)°	β= 90°
	γ= 90°	γ= 90°
Volume	43371(7) $Å^3$	47585(5) $Å^3$
Z	2	2
Density (calculated)	0.741 g/cm^3	0.991 g/cm^3
Absorption coefficient	0.266 mm^{-1}	2.512 mm^{-1}
F(000)	9301	14896
Crystal size	0.14 x 0.14 x 0.14 mm	0.18 x 0.18 x 0.18 mm
Theta range for data	1.230 to 18.838°	2.44 to 42.90°

collection		
Index ranges	-25<=h<=25 -26<=k<=26 -36<=l<=36	-32<=h<=32 -32<=k<=32 -32<=l<=32
Reflections collected	269848	198100
Independent reflections	26222 [R(int) = 0.0784]	1837 [R(int) = 0.0943]
Completeness	99.8 % to θ = 18.838°	99.8% to θ = 44.563°
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.7443 and 0.6749	0.7488 and 0.5738
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	26222 / 4409 / 2629	1837 / 318/ 212
Goodness-of-fit on F^2	2.606	2.184
Final R indices [I>2sigma(I)]	R1 = 0.1663, wR2 = 0.4734	R1 = 0.1136, wR2 = 0.3689
R indices (all data)	R1 = 0.1893, wR2 = 0.5175	R1 = 0.1333, wR2 = 0.4261
Extinction coefficient	n/a	n/a
Largest diff. peak and hole	1.302 and -0.516 e·Å^{-3}	1.200 and -0.357 e·Å^{-3}

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