Supporting Information

Tuning Enantioselective Drug Adsorption in Isoreticular Homochiral Metal-Peptide Frameworks through Proximity Pore Interactions

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Materials

4,4'-bipyridine (bipy)(>98%) 1,2-bis(4-pyridyl)ethane (bpe) (>98.0%), 4,4'-(1,4-phenylene)bipyridine (phenylbipy),benzyl alcohol (>99.0%) and L-Penicillamine (L-Pen)(98%) were purchased from TCI. Cobalt(II) acetate tetrahydrate, glycyl-L-glutamic acid (Gly-Glu)(L-GG), anhydrous methanol (MeOH)(>99.8%), N,N-dimethylformamide anhydrous (DMF)(>99.8%), 4,4′-vinylenebipyridine (vinylbipy) (97%), *meso*-1,2 bi(4-pyridyl)-1,2-ethanediol (mbped) and D-Penicillamine (D-Pen)(>98%) were purchased from Sigma Aldrich. 4,4'-bis(4-pyridyl)biphenyl (bpb)(95.0%) was purchased from Fisher Scientific. These materials were used without further purification.

Instrumentation and Characterization Protocol

Powder X-Ray Diffraction (PXRD): PXRD data were collected using a Rigaku Miniflex 600 diffractometer (monochromated Cu Kα radiation, λ = 1.54178 Å) with a tube voltage and current of 40 kV and 15 mA, respectively. Data was collected over the 2θ range of 3° – 30° at a scan rate of 0.075 degrees/min with a 0.02° step at ambient conditions.

Fourier Transform Infrared Spectroscopy (FT-IR): All FT-IR measurements were performed on a PerkinElmer Spectrum Two Spectrometer equipped with a Universal Attenuated Total Reflectance (UATR) accessory tool (single reflection diamond). Spectra were obtained with a resolution of 4 cm-1 from 400 – 4000 cm^{-1} and averaged over eight scans.

Thermal Gravimetric Analysis (TGA): TGA was performed using a standard TG-DTA analyzer from Hiden Analytical. Approximately 7-10 mg of the sample was placed in a TGA crucible. The samples were heated from 25°C – 650 °C at 10 °C/min under argon (100 ml/min).

CO_2 and N_2 Adsorption Isotherms (BET): CO_2 and N_2 sorption isotherms were collected using a 3FLEX Adsorption Analyzer from Micromeritics. Prior to CO_2 adsorption, the samples were activated using supercritical CO_2 activation. The Brunauer-Emmett-Teller (BET) surface areas were estimated from the amount of N_2 adsorbed at 77 K and 1 bar using the BET equilibrium equation.

Single-Crystal X-Ray Diffraction (SC-XRD): SC-XRD data was collected using a Rigaku Synergy equipped with dual X-ray source and HyPix 2D detector. The X–rays were generated using graphite monochromated Cu Kα radiation from a copper X–ray tube operating at 40 kV and 30 mA. The crystals were mounted on a fiber loop attached to the goniometer and data were

collected at 223K. Structure refinement was performed on OLEX and simulated crystal powder pattern generated on Mercury Software.

Solvent Stability: To test the stability of Co-L-GG(R) MOFs in varying solvents, approximately 10.0 mg of each isoreticular MOF was submerged in 1.0 mL of solvent for 24 hours. The samples were then filtered and air-dried overnight before stability was checked with PXRD. This procedure was repeated for every solvent test.

Circular Dichroism (CD): Characterization data was collected using a JASCO J-1500 spectrometer in a CD cuvette sample holder (pathlength = 0.1cm) and xenon lamp at nitrogen purge of 20L/min. Measurements were taken along the ranges of 180 – 300nm at a scanning rate of 4nm/s and the data was averaged between two scans. A methanol baseline scan was performed and subtracted from all tested solutions that contained methanol solvent.

Proton Nuclear Magnetic Resonance (^{1}H-NMR): A 50μL aliquot was extracted from the racemate solution and mixed with 600μL of deuterated solvent ($CDCl_3$ for Ibu, D_2O for Pen) in a 5mm NMR tube. ^{1}H-NMR was performed on samples using a 700MHz Ultrashield Plus spectrometer equipped with a cryocurrent probe.

Liquid-Chromatography Mass-Spectrometry (LC-MS): The D/L-penicillamine enantiomer concentrations were resolved using a liquid chromatography coupled to time-of-flight mass spectrometry (LC/TOF-MS). An Agilent 1290 Infinity HPLC and Agilent 6230 LC/TOF-MS were employed using a Dual AJS ESI source in positive-ion mode. Chromatography on an Agilent InfinityLab Poroshell 120 Chiral-T column (4.6 x 100 mm, 2.7 mm) using an isocratic mobile phase of 80% LC-MS grade MeOH and 20% LC-MS grade H2O + 0.1% formic acid at a flow rate of 0.800 mL min–1 and a column temperature maintained at 25 ˚C. Standard dilutions of D- and L- penicillamine were prepared in LC-MS grade methanol in the range of 25 to 500 ppm and collected in triplicate (0.1 mL injection volume). Respective retention times of ~2.47 min and ~1.94 min for the D- and L-isomers. Data analysis was performed using MassHunter Quantitative Analysis (for TOF) using the m/z = 150.046 peak corresponding to the [M+H] as the quantifier and qualified using the fragment peak at m/z = 115.004 (Langlois, D. K.; Lehner, A. F.; Buchweitz, J. P.; Ross, D. E.; Johnson, M. B.; Kruger, J. M.; Bailie, M. B.; Hauptman, J. G.; Schall, W. D., J. Vet. Intern. Med., 2013, 27, 1071 - 1076).

Synthesis of Isoreticular Frameworks

Co-L-GG: Cobalt(II) acetate tetrahydrate ($Co(OAc)_2 \cdot 4H_2O$) solution (0.3 mL of 0.56 M) was added to a mixture of L-GG (20 mg, 0.98 mmol) and DI H_2O (1 mL) and heated to 85 ^oC at 2.0 $^oC\ min^{-1}$ for 8 h in a sealed 3mL dram vial and allowed to cool down to room temperature at a rate of 2.0$^oC\ min^{-1}$. The resulting crystals were vacuum filtered in MeOH and air-dried overnight.

Co-L-GGbipy: In addition to the precursors for the Co-L-GG framework, a mixture of the bipy analogue (15.6 mg, 0.1 mmol) ligand in MeOH (1 mL) was added and heated under the same conditions.

Co-L-GGbpe: The same conditions above were used except that the bpe ligand was used.

Co-L-GGvinylbipy: The same conditions above were used except that the vinylbipy ligand was used.

Co-L-GGmbped: The same conditions above were used except that the mpbed ligand used. Instead of MeOH, 3.5 mL of DMF was used after sonication for 1 h within a 10 mL dram vial.

Co-L-GGphenylbipy: The same conditions above were used except that the phenylbipy ligand was used. Instead of MeOH, 3 mL of DMF was sonicated for 0.5 h in a 10 mL dram vial.

Co-L-GGbpb: The bpb ligand were sonicated in 3mL of benzyl alcohol for 0.5 h. To a 10 ml dram flask, 0.1 mL of a 3.4 M $Co(OAc)_2 \cdot 4H_2O$ solution was added with a mixture of 20 mg *L*-GG in 0.1 ml DI water. The resultant solution was replaced with MeOH and centrifuged repeatedly at 2000 rpm for 5 minutes. The solids were rinsed with MeOH and centrifuged repeatedly until the brown precipitate had disappeared and the supernatant remained clear. The solution was then vacuum filtered, and the pink MOF powder were air-dried overnight.

Experimental Procedure

Calibration of Circular Dichroism. Mixed ratios of D-Penicillamine and L-Penicillamine were weighed and dissolved in methanol to create the desired concentration. The standards were syringe-filtered prior to CD measurements. A calibration curve for Pen was generated from the max molar circular dichroism, $\Delta\varepsilon$ [mol^{-1} cm^{-1}], evaluated at 227.3 nm and corresponding enantiomer concentration [D/L] provided by CD.

Packed MOF Bed Enantioseparation Experiment. Several glass columns were packed with glass wool along with 20 mg (approximately 27 mmol) of isoreticular Co-L-GG(R) MOFs. Prior to the experiment, the MOFs were ground and soaked in anhydrous methanol. They were then air-dried and heated overnight at 80 °C to fully access their pore apertures. To each column, a racemic DL-Pen solution (46.9 mM) in methanol was slowly added dropwise and allowed to diffuse through the packed column. The resolved solution was cycled back into the packed column at least three times to ensure saturation. The saturated MOF adsorbents were washed with ethanol to remove any surface-bound molecules. The adsorbents were then soaked in methanol again and re-heated at 50 °C to provoke diffusion of any adsorbed Pen within the pore channels. This methanolic solution was syringe filtered and processed for enantioenrichment using CD. The same procedure was performed on racemic Ibuprofen. After calibrating with circular dichroism, a racemic stock solution of 33.4 mM (*RS)*-Ibuprofen (Ibu) was allowed to resolve through Co-L-GGbpe, Co-L-GGvinylbipy and Co-L-GGbpb. The resolved solutions were collected for CD to determine enantiomeric excess. The enantiomeric excess (ee) was determined with the following equation:

$$ee\% = \frac{|L - D|}{L + D}\, x\, 100$$

Where L and D (or S and R) represent the quantity of adsorbed enantiomer.

The L-enantioselectivity was determined by:

$$L - selectivity\% = \frac{(D + L\ \ adsorbed)(L - enrichment)}{D + L\ \ adsorbed}\, x\, 100$$

Where the quantity of D+L adsorbed enantiomers are determined by ^{1}H-NMR and L-enrichment of the solution was determined by the CD calibration plot

To test reuse, the packed MOF bed was washed once with methanol and adsorbent filtered. The adsorbent was then air-dried and heated in 80 ^{o}C oven overnight. The enantiomer separation experiment was then repeated.

Saturation Tests. Co-L-GGvinylbipy and Co-L-GGbpb (20 mg) were activated prior to use. Several stock concentrations of racemic Pen were made (7 mM – 87 mM) and the MOF adsorbent were added to the solution in a 3-dram vial. A magnetic stir bar was added at a stirring rate of 100 rpm for 48 h. The MOFs were then filtered and washed with ethanol to remove surface-bound molecules. The adsorbents were then soaked in anhydrous methanol and re-heated at 50^{o}C to provoke diffusion of any adsorbed Pen within the pore channels. This solution was then characterized by CD and ^{1}H-NMR

Nuclear Magnetic Resonance (^{1}H-NMR) Calculations. The total adsorbed amount of drug adsorbed within Co-L-GG(R) was determined by ^{1}H NMR. A 50 μL aliquot was extracted from the resolved solution and mixed with 600 μL of deuterated solvent ($CDCl_3$ or D_2O) in a 5mm NMR tube. The spectra were analyzed using a 700 MHz Bruker Avance spectrometer equipped with a cryocurrent probe.

The protons of methanol and designated protons of drugs were considered for the following calculations. The ratio of drug to methanol was calculated from the integration areas determined from the ^{1}H NMR spectra.

$$\frac{M_{drug}}{M_{MeOH}} = \left(\frac{I_{drug}}{I_{MeOH}}\right)\left(\frac{N_{MeOH}}{N_{drug}}\right)$$

I = Integral Area *N* = Number of Nuclei *M* = Number of Moles

The initial molar amount of methanol (16.9 mmol) was held constant through the experiments. To determine the adsorbed quantity of each drug, ^{1}H NMR was initially performed on the racemic stock solution to determine the quantity of drug introduced into each experiment. The resolved drug solution after each test was analyzed for quantity of drugs that resolved through the MOF

bed, and this amount was subtracted from the initial stock amount to determined what was retained in the adsorbent

Example: Determining adsorbed Penicillamine. The ratio of a known nuclei of Pen to that of MeOH were compared and integrals under corresponding peaks were calculated using NMR

$$M_{Pen,\ resolved} = \left(\frac{M_{Pen}}{M_{MeOH}}\right)\left(M_{MeOH,total}\right)$$

$$M_{Pen,resolved} = \left(\frac{I_{Pen}}{I_{MeOH}}\right)\left(\frac{N_{MeOH}}{N_{Pen}}\right)\left(\frac{\rho_{MeOH}}{MW_{MeOH}}\right)\left(M_{MeOH,sample}\right)$$

$$M_{Pen,resolved} = \left(\frac{1}{9395}\right)\left(\frac{3\ protons}{1\ protons}\right)\left(\frac{0.792\frac{g}{mL}}{32.04\frac{g}{mol}}\right)(1mL) = \ 7.9\mu mol$$

$$M_{Pen,\ adsorbed} = M_{Pen,sample} - M_{Pen,\ resolved}$$

$$M_{Pen,\ adsorbed} = 35.8\mu mol\ - 7.9\mu mol = 27.9\mu mol$$

Figures and Tables

Single Crystal of Co-L-GGvinylbipy

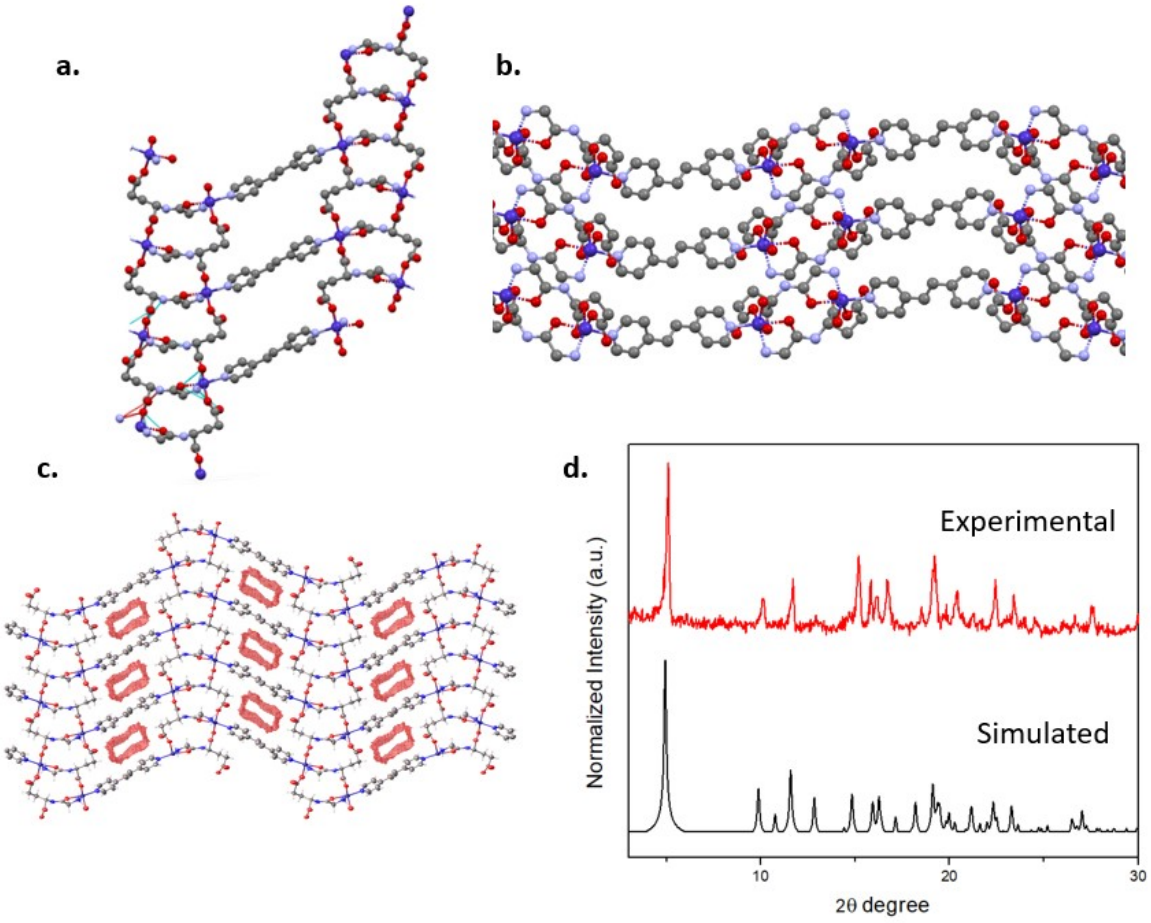


Figure S1. a. Ball-and-stick representation of Co-L-GGvinylbipy in a-axis computationally determined showing that the ethane linker spaces out peptide ladders. **b.** Co-L-GGvinylbipy structure in b-axis. **c.** The MOF is porous, with 19.7% usable pore volume corresponding to 331.6 $Å^3$. **d.** Comparison of the experimental and simulated PXRD pattern derived from single-crystal confirms that the pure phase can be obtained.

Structure of Co-L-GGbpe

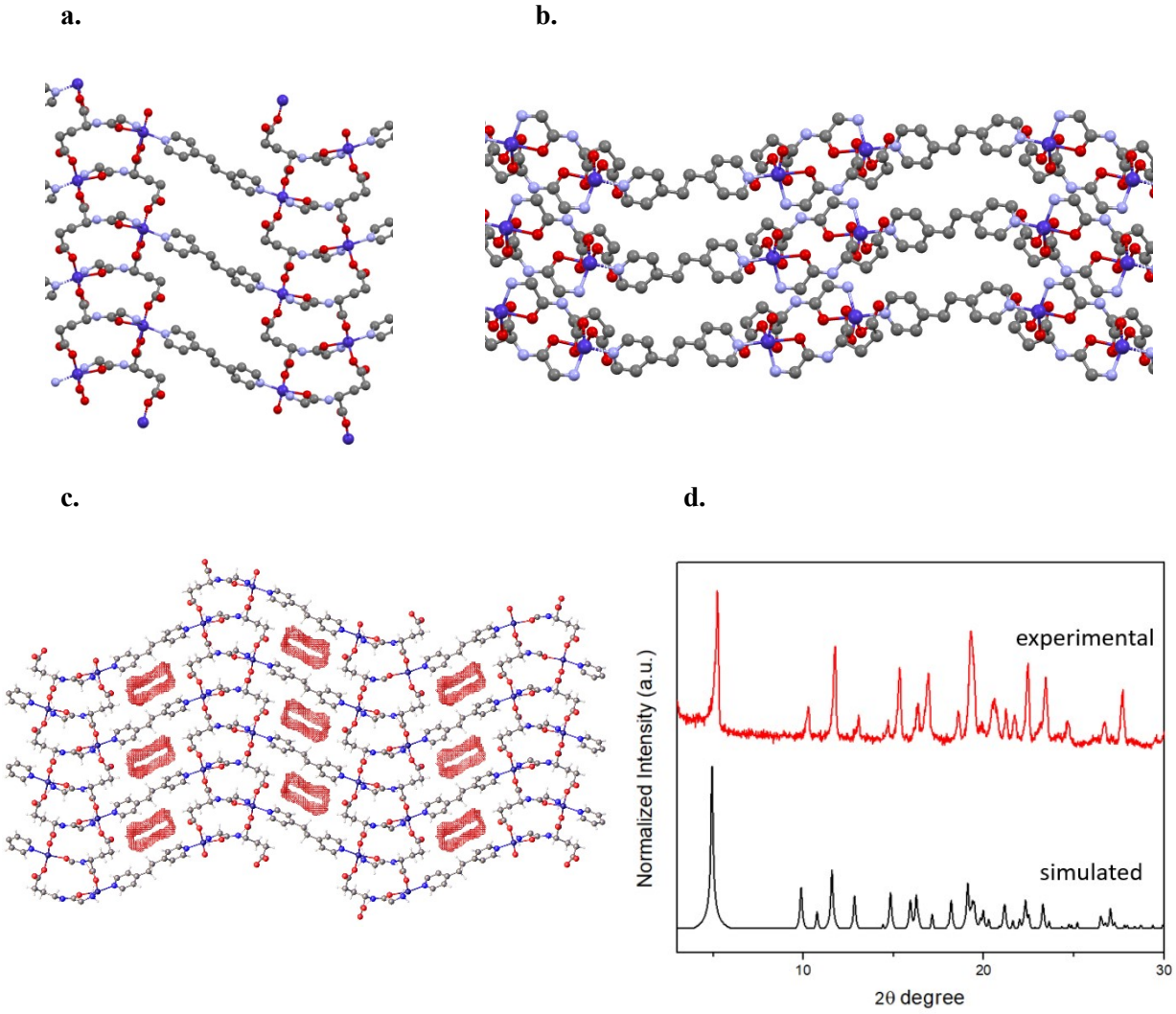


Figure S2. a. Ball-and-stick representation of Co-L-GGbpe in a-axis computationally determined showing that the ethane linker spaces out peptide ladders. **b.** Co-L-GGbpestructure in b-axis. **c.** The MOF is porous, with 17.4% usable pore volume corresponding to 294.0Å^3. **d.** Comparison of the experimental and simulated PXRD pattern derived from single-crystal confirms that the pure phase can be obtained.

Structure of Co-L-GGmbped

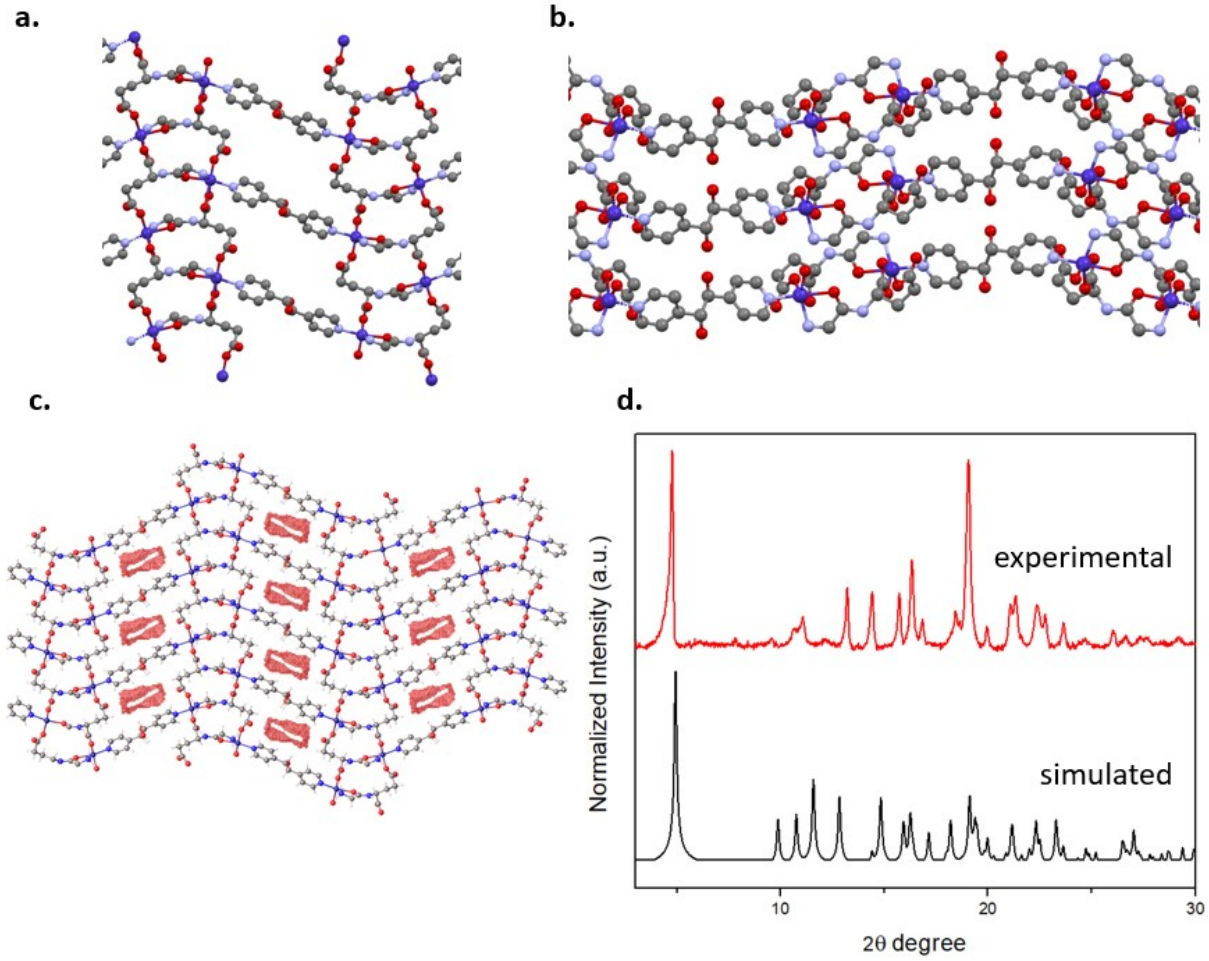


Figure S3. a. Ball-and-stick representation of Co-L-GGmbped in a-axis computationally determined showing that the ethanediol linker spaces out peptide ladders. **b.** Co-L-GGmbped structure in b-axis. **c.** The MOF is porous, with 15.3% usable pore volume corresponding to 257.6Å^3. **d.** Comparison of the experimental and simulated PXRD pattern derived from single-crystal confirms that the pure phase can be obtained.

Crystallography Data

Table S1. Lattice Parameters for Co-L-GG MOFs provided by Le Bail fitting unless noted.

*Parameters obtained from SC-XRD

**Co-L-GG-phenylbipy has two phases $P2_1/m$; P2.

Crystal data for Co-L-GG(R)	Co-L-GGvinylbipy	Co-L-GGbpe	Co-L-GGmbped	Co-L-GGphenylbipy**	Co-L-GGbpb
a [Å]	*5.5751(3) 5.555(2)	5.660(1)	*5.5599(2) 37.379(6)	*5.4924(8) 5.11(1); 5.201(2)	6.825(3)
b [Å]	*8.4418(4) 8.420(2)	8.482(2)	*36.6345(8) 5.943(1)	*9.5266(9) 8.505(2); 9.32(1)	10.759(5)
c [Å]	*35.879(2) 34.94(1)	35.13(1)	*9.5223(2) 8.176(2)	*39.268(6) 37.105(2); 37.705(5)	49.11(2)
α [°]	*90.00 90.00	90.00	*90.00 90.00	*90.00 90.00	91.06(3)
β [°]	*93.574(5) 90.96(4)	93.13(3)	*90.00 91.90(2)	*90.734(14) 90.73(3); 90.75((3)	91.00(2)
γ [°]	*90.00 90.00	90.00	*90.00 90.00	*90.00 90.00	90.090(5)
Crystal structure	*monoclinic monoclinic	monoclinic	*monoclinic monoclinic	*orthorhombic monoclinic	triclinic
Space group	P2(1)	P2(1)	P2(1)	*Pmmm P2(1)/m; P2(1)	$P1^-$
$\chi 2$	*8.56 1.32	2.09	*9.74 4.19	*11.47 2.86	1.16
Volume [$Å^3$]	*1685.32(15) 1634.1(7)	1683.6(5)	*1939.54 1815.4(5)	*2054.65 1612.5(5);1827.5(7)	3605(2)
Free volume [$Å^3$](%)	*331.6(19.7%)	294.0(17.4%)	257.6(15.3%)	-	-
Crystal color and shape	Pink, plate	Pink, spherical	Pink, plate	Pink, plate	Pink, spherical

		powders			powders

Le Bail Fits of Co-L-GG(R) MOFs

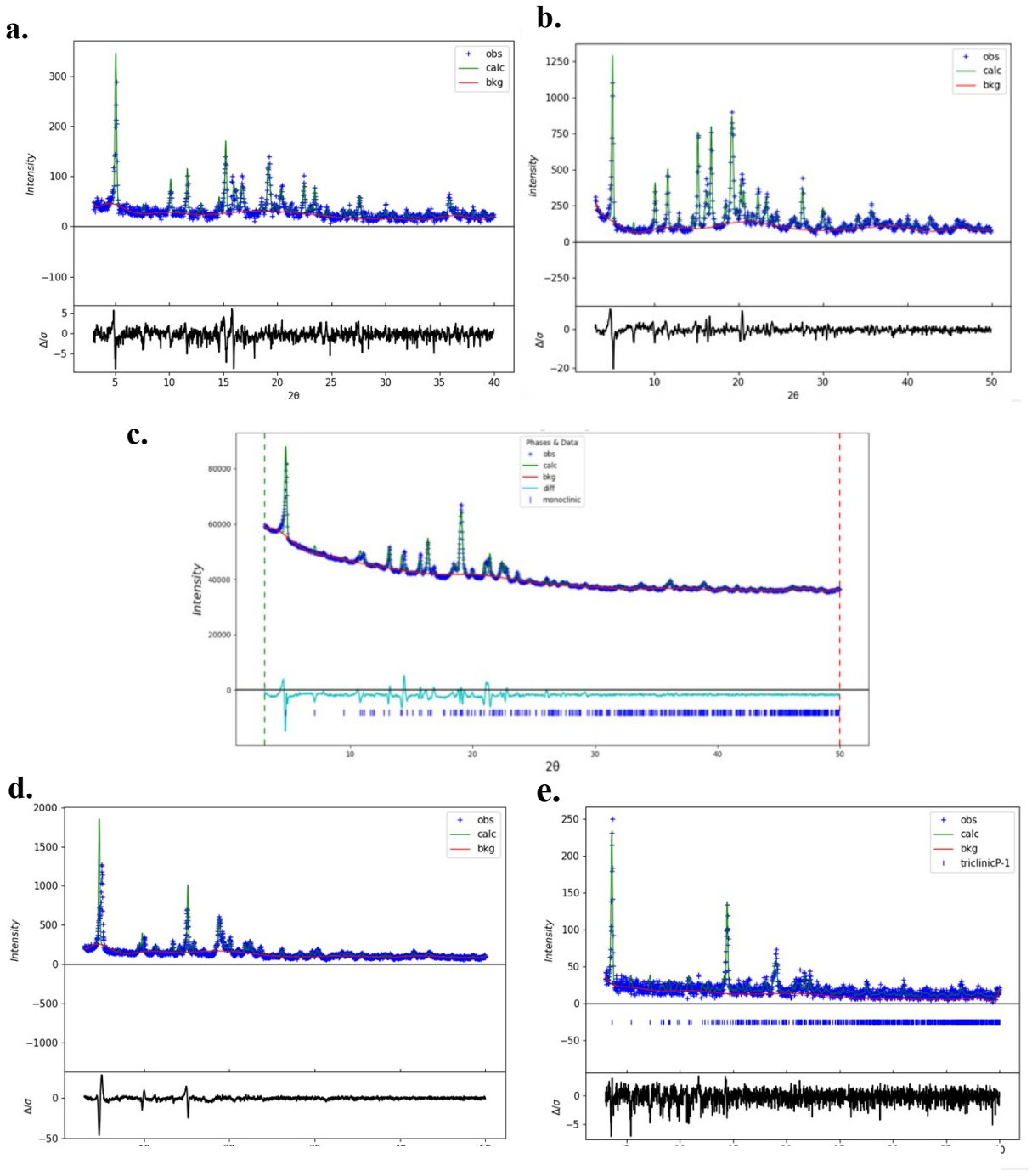
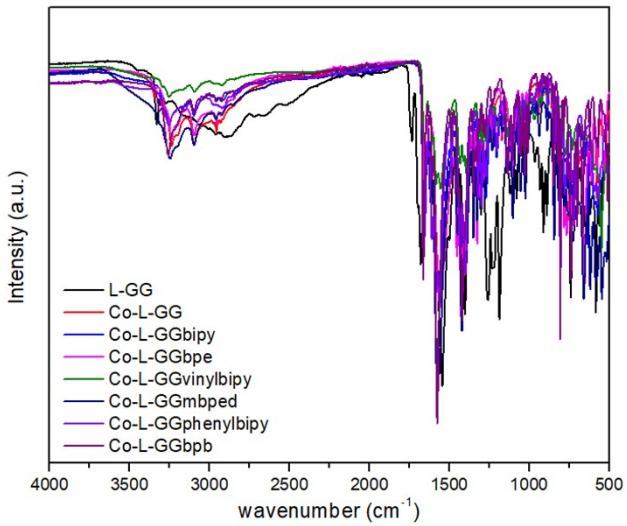


Figure S4. Le Bail Fit of **a.** Co-L-GGvinylbipy **b.** Co-L-GGbpe **c.** Co-L-GGmbped **d.** Co-L-GGphenylbipy and **e.** Co-L-GGbpb

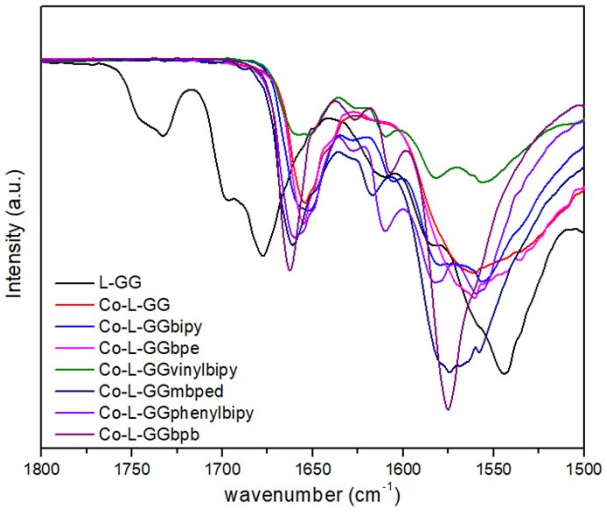
Fourier Transform Infrared Spectroscopy



a.

b.

Figure S5. a. FT-IR spectra comparison L-GG, Co-L-GG, and (R) analogues **b.** Upon coordination of L-GG and Co(II), the Co-L-GG framework is formed by evidence of the C=O band of *L*-GG around 1690 cm^{-1} shifting to 1640 cm^{-1}. The bands of the characteristic Co-L-GG framework are comparable to each isoreticular analogue.



Thermogravimetric Analysis

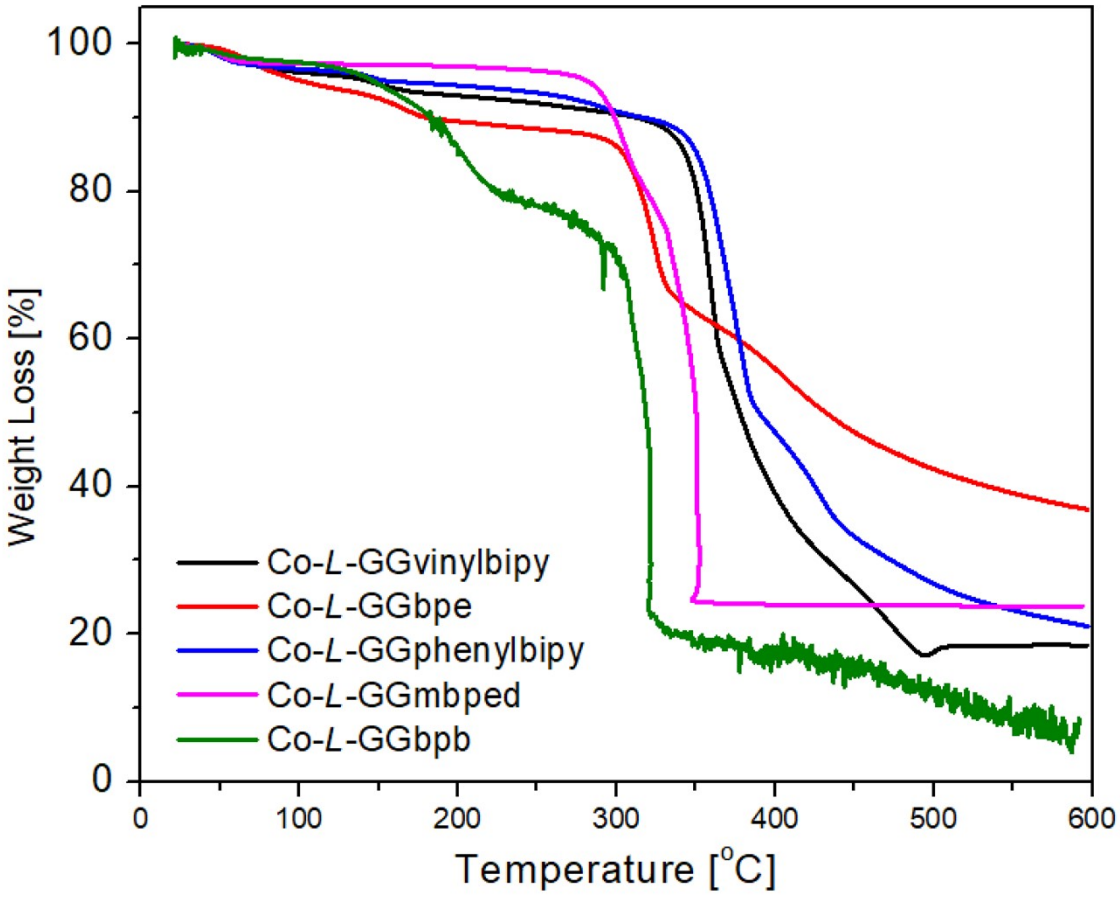


Figure S6. The TGA profile of Co-L-GG(R) MOFs shows thermal stability for at least 300 °C. Co-*L*-GGmbped shows a 19.4% weight loss at 220 °C.

CO_2 Adsorption Isotherms

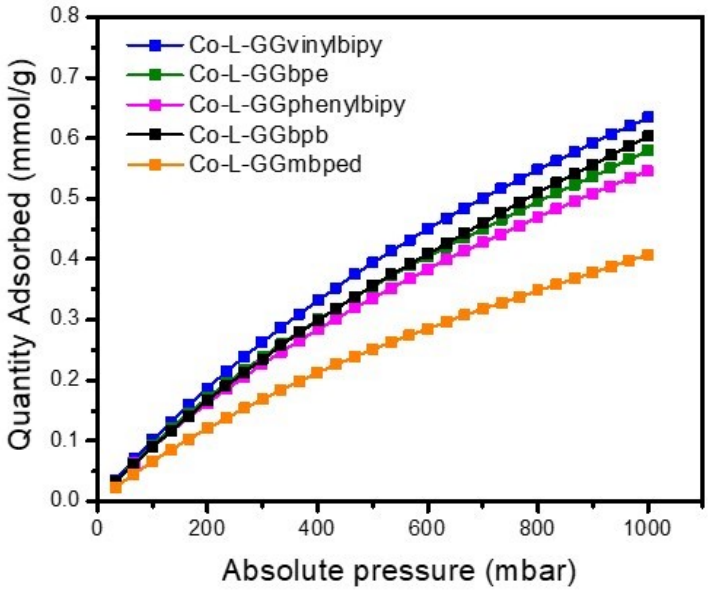
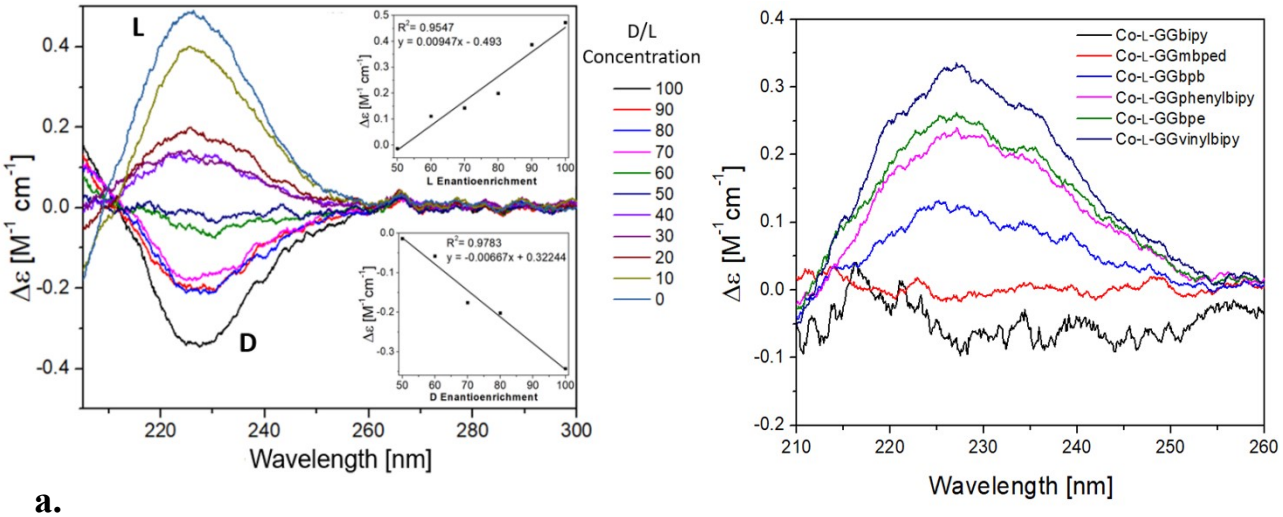


Figure S7. CO_2 adsorption isotherms collected at 298K after supercritical CO_2 activation. Nitrogen adsorption isotherms were collected at 77 K and 1 bar and revealed that all Co-L-GG(R) MOFs are nonporous to N_2.

DL-Penicillamine Experimental Circular Dichroism Spectra



a.

b.

c.

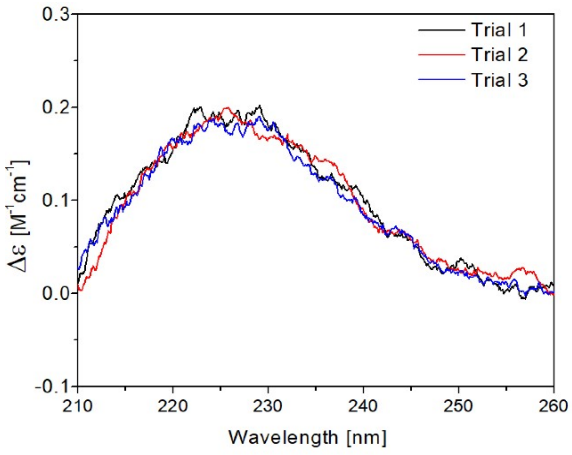


Figure S8. a. Spectra DL-Pen at various enantiomer concentrations. Calibration plots for L-enantioenrichment and D-enantioenrichment are shown. **b.** CD spectra of racemic DL-Pen (1,000 ppm) between the wavelengths of 210nm and 260nm after resolved through various Co-L-GG(R) MOFs. Co-L-GGbipy and Co-L-GGmbped resolved a racemic mixture of Pen (ee = 0%). Molar

CD for evaluation were recorded at 227.3 nm and the electronic adsorption under this wavelength ranged from 0.455-0.789, affirming reliability of CD measurements. Additionally, the HTV values for the CD experiments reported here never exceed 600 V, a value the instrument manufacturer indicates not to exceed for data quality assurance. **c.** Given similar spectra between Co-L-GGphenylbipy and Co-L-GGbpe, three CDs resulting from different trials of Co-L-GGphenylbipy were performed to demonstrate higher degree of confidence in measurement.

Ibuprofen Experimental Circular Dichroism Data

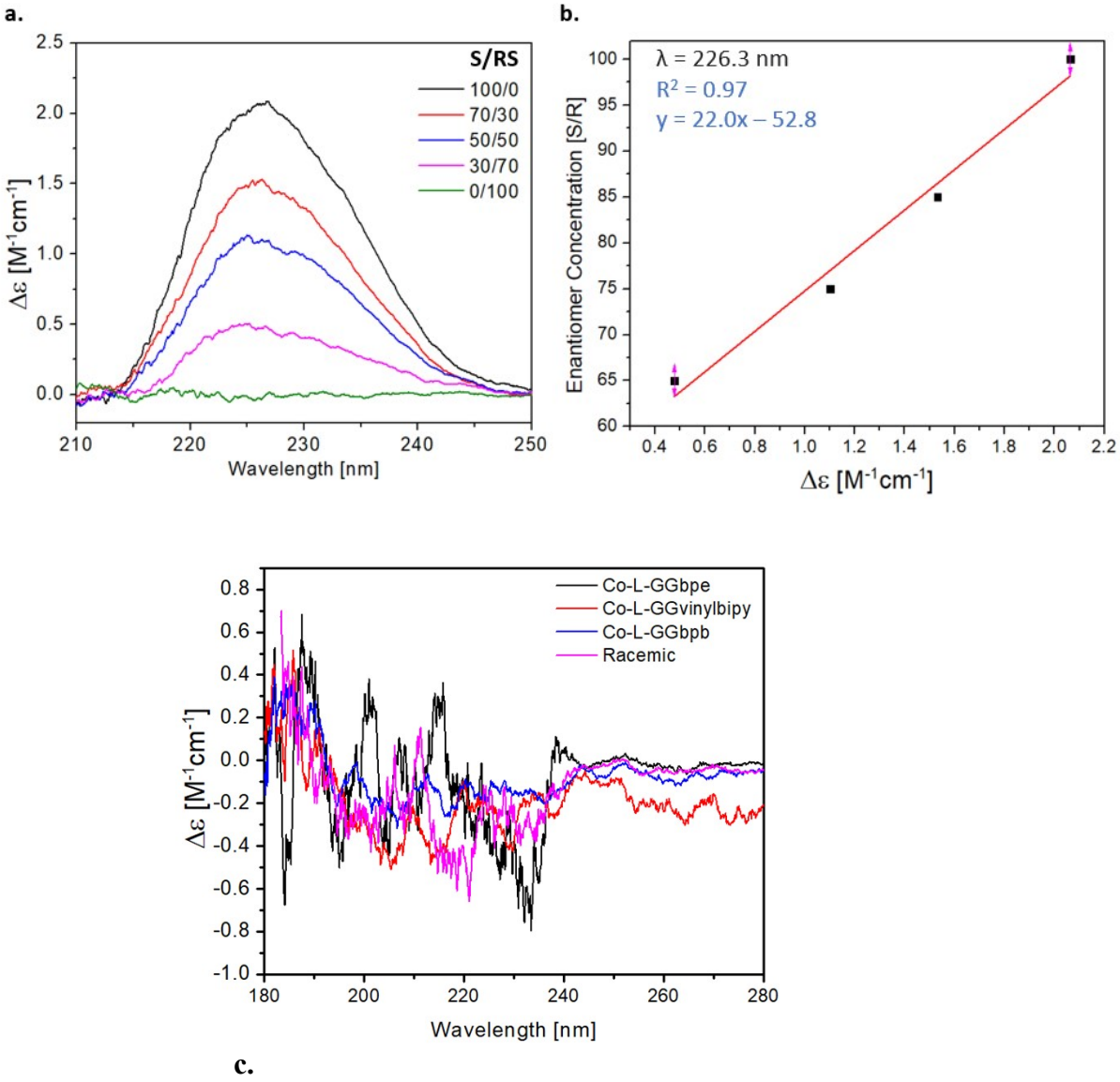
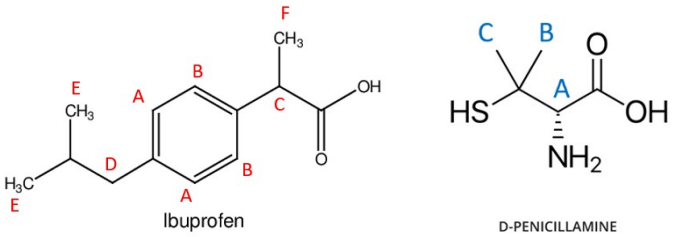


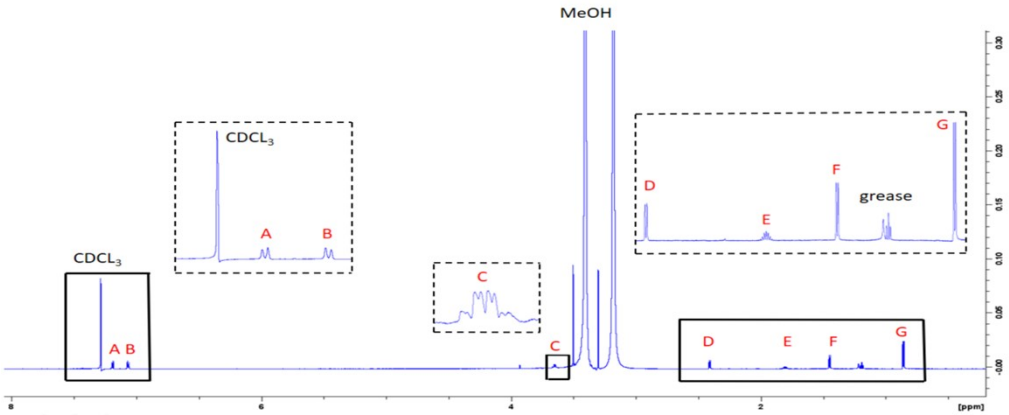
Figure 9. a. CD spectra of Ibuprofen (1,000 ppm) between the wavelengths of 210 nm and 250 nm were generated using varying ratios of *S/RS* mixtures. **b.** calibration line of *(S)*-Ibuprofen enantioenrichment generated from molar ellipticity provided at 226.3 nm **c.** CD spectra of racemic Ibuprofen resolved through Co-L-GG(R) MOFs show that the resolved solution is essentially racemic (ee = 0%).

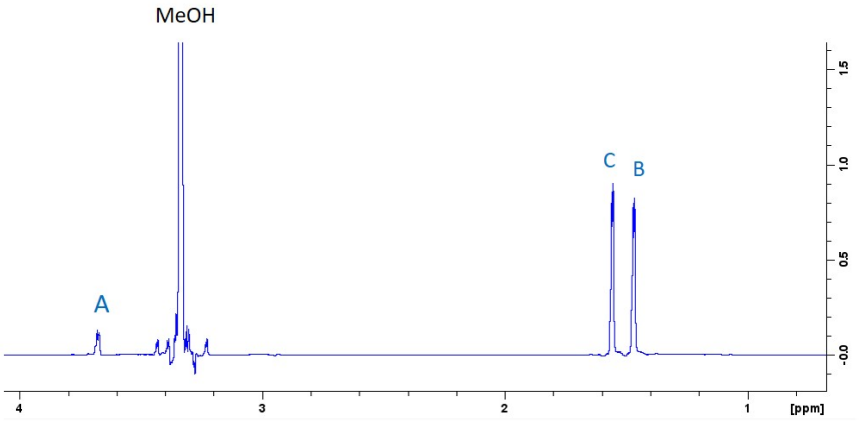
[1]H-NMR Spectra of Ibuprofen and Penicillamine

a.



b.





c.

Figure S10. a. Chemical structures of Ibuprofen and Penicillamine with labeled protons **b.** ^{1}H-NMR of *(RS)*-Ibuprofen in $CDCl_3$ and **c.** ^{1}H-NMR of DL-Penicillamine in D_2O (solvent peak surpressed) following resolution through Co-L-GGmbped.

Experimental Adsorption of Penicillamine in Various MOFs

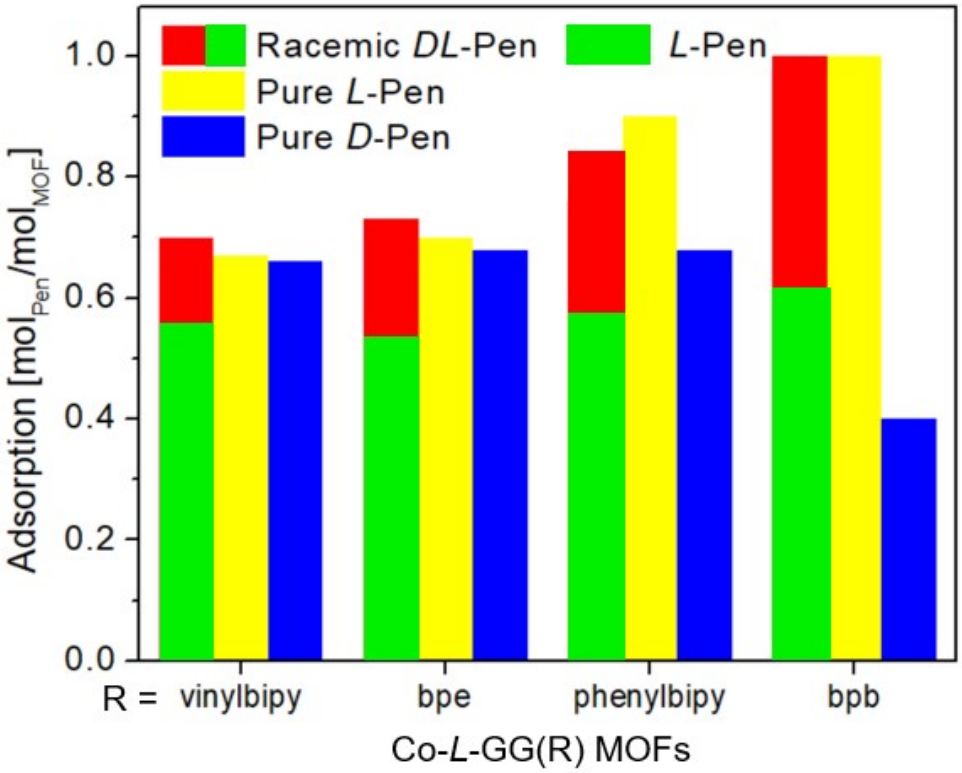
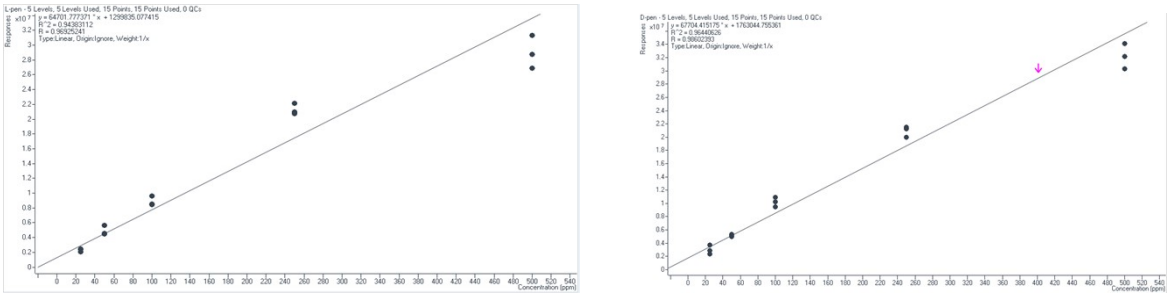


Figure S11. Adsorbed quantities of Pen in various MOFs. In a racemic mix, L-Pen is enriched but the ratio of L to D decreases with increasing pore size. Pure L-Pen is more selective than pure D-Pen in the case of Co-L-GGbpb where saturation capacity is not limiting adsorption, indicating that pore size and shape influences selectivity of diffusing stereoisomer.

Liquid-Chromatography Mass-Spectrometry (LC-MS)

a.



b.

c.

d.

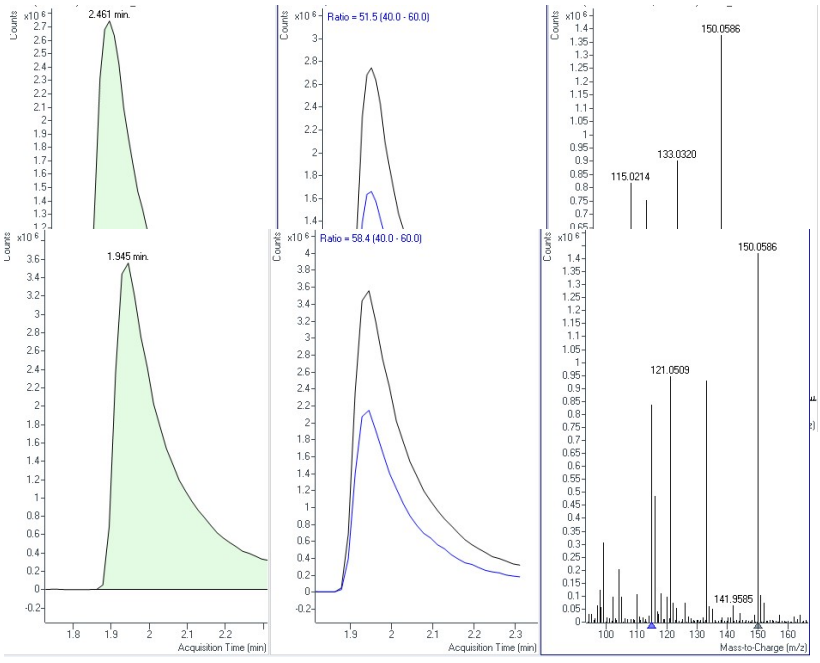


Figure S12. LC-MS Calibration of **a.** 20-500ppm L-Pen, $R^2 = 0.943$ and **b.** 20-500ppm D-Pen, $R^2 = 0.964$. Experimental LC-MS peaks with charge-to-mass ratio of adsorbed **c.** L-Pen and **d.** D-Pen

in Co-L-GGvinylbipy. Data analysis was performed using MassHunter Quantitative Analysis (for TOF) using the m/z = 150.046 peak corresponding to the [M+H] as the quantifier and qualified using the fragment peak at m/z = 115.004

Powder X-Ray Diffraction Post-Adsorption

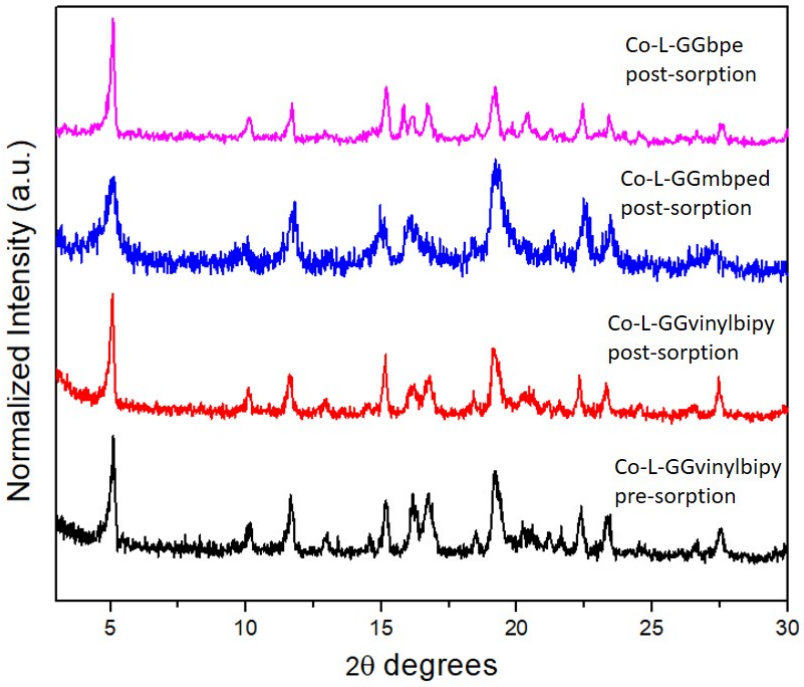


Figure S13. PXRD of Co-L-GGvinylbipy, Co-L-GGmbped and Co-L-GGbpe post-racemic Penicillamine sorption after 3 cycles. Co-L-GGvinylbipy pre-sorption (black) is provided to show the MOFs are stable following the experiment.