Electronic Supplementary Information (ESI) for

Reveal of the chiral recognition for alanine and leucine in an L-phenylalanine-based metal-organic framework

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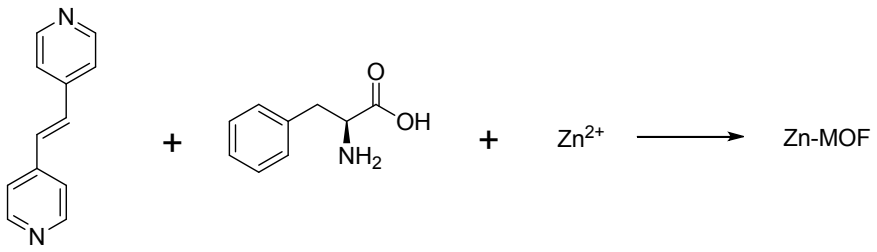
Experimental Section

General information

In this paper, zinc nitrate hexahydrate, L-phenylalanine, DL-alanine, D-alanine, L-alanine, DL-leucine, D-leucine, L-leucine, 1, 2-di(4-Pyridyl)ethylene were purchased from Energy Chemical. 4-(4,6-dimethoxytriazin-2-yl)-4-methylmorpholine hydrochloride (DMTMM), N, N-dimethyl-L-phenylalanine (Diphe), N-methylmorpholine (NMM) was purchased from Aladdin. Chromatographic grade acetonitrile from ThermoFisher Scientific. Chromatographic grade formic acid purchased from Tedia. Water was purified with Milli-Q purification equipment. Other solvents were purchased from local vendor. And all the chemical reagents were used without further purification. Single-crystal X-ray diffraction analyses of Zn-MOF were carried out on a Rigaku XtaLAB Pro diffractometer with Cu-Kα radiation (λ = 1.54184 Å) at 150(10) K. An X-ray diffractometer was applied to record the wide-angle X-ray diffraction (XRD) patterns of the prepared materials. Scanning electron microscope (SEM) was carried out on a S-4300 instrument. Thermal stability study was performed on a STA-449 F3 thermoanalyzer with a heating rate of 10 ^{o}C/min in a range of 25-800 ^{o}C under nitrogen and argon atmosphere. ^{13}C NMR spectra were recorded using a AVIII HD 400 spectrometer. X-ray photoelectron spectroscopy (XPS) results were got with an Axis Supra. Samples were analyzed on an 2D nano-LC & UPLC-QTrap-MS system. Mobile phase A was 0.1% formic acid in H_2O and mobile phase B was acetonitrile. The column is a C18 column (2.1*50 mm, 2.6 μm). The 10 min binary gradient at a flow rate of 0.3 mL min^{-1} was set as follows: 0-10 min, 5-35% solvent B. The mass spectrometric detection conditions were: ion source: ESI; ionization mode: positive ion mode; mass spectrometry mode MRM. The detection ion to leucine was 307/148 and the alanine was 265/148.



Scheme S1 Depiction of the synthesis of the Zn-MOF.

X-Ray crystallography

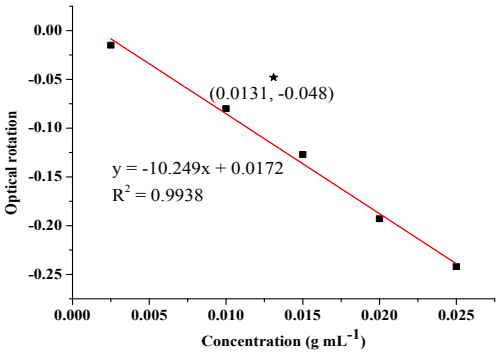
Single-crystal X-ray diffraction analyses of Zn-MOF were carried out on a Rigaku XtaLAB Pro diffractometer with Cu-Kα radiation (λ = 1.54184 Å) at 150(10) K. Data collection and reduction were performed using the program CrysAlisPro. The structures were solved by direct methods and refined by the full matrix least-squares based on F^2 using using *OLEX2,* which utilizes the *SHELXL*-2015 module. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms attached to ligands were generated geometrically, the solvent hydrogen atoms were located from difference Fourier maps and fixed isotropic displacement parameters.

Table S1 Crystallographic data and structural refinement details for the crystal Zn-MOF.

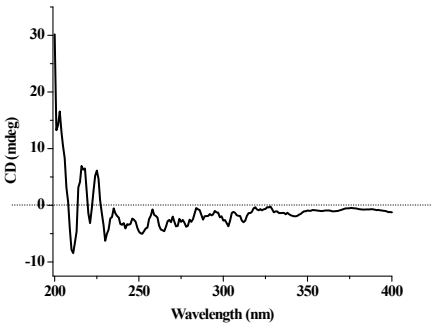
Identification code	Zn-MOF
Empirical formula	$C_{42}H_{38}N_6O_4Zn_2$
Formula weight	821.52
Temperature/K	293(2)
Crystal system	monoclinic
Space group	C2
a/Å	20.4932(17)
b/Å	25.8736(11)
c/Å	9.6520(4)
α/°	90
β/°	99.109(6)
γ/°	90
Volume/Å^3	5053.3(5)
Z	4
ρ_{calc}g/cm^3	1.080
μ/mm^{-1}	0.987
F(000)	1696
Crystal size/mm^3	0.200 × 0.200 × 0.100
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	2.013 to 29.774
Limiting indices	-28 ≤ h ≤ 28, -34 ≤ k ≤ 34, -12 ≤ l ≤ 13
Reflections collected	28483
Data/restraints/parameters	12354 / 829 / 513
Goodness-of-fit on F^2	1.086
Final R indexes [I>=2σ (I)]	R1 = 0.0539, wR2 = 0.1599
Final R indexes [all data]	R1 = 0.0869, wR2 = 0.1808
Largest diff. peak/hole / e Å^{-3}	0.847/-0.408

Characterization of the Zn-MOF

300 mg Zn-MOF were dissolved in 10 mL of 4% hydrochloric acid/H_2O solution. The molar molecular weight of Zn-MOF was 821.52 g mol^{-1}, and the concentration of L-phenylalanine amount to 0.0131 g mL^{-1}. After 72 hours, colorless crystals composed of Zn^{2+} and 1, 2-bis(4-pyridyl)ethylene formed (*Chem. Eur. J.* 2008, 14, 5329-5334). It can be confirmed by comparing infrared spectrum. The yellow supernate was gained by filtering and the value of optical rotation was -0.048°. In addition, a series of different concentrations of L-phenylalanine in 4% hydrochloric acid/H_2O solution were prepared, and the optical rotations were measured. The standard curve and regression equation were established (Figure S1a). As displayed in Figure S1a, the value of the optical rotation for the yellow liquid obtained by the dissolution of Zn-MOF by 4% hydrochloric acid/H_2O solution, was lower than that of the corresponding concentration of L-phenylalanine solution. It may be due to a certain amount of loss when L-phenylalanine in the Zn-MOF was dissolved in hydrochloric acid/methanol solution, and the color of the solution reduced the light transmittance, thereby influencing the determination of optical rotation.



(a)



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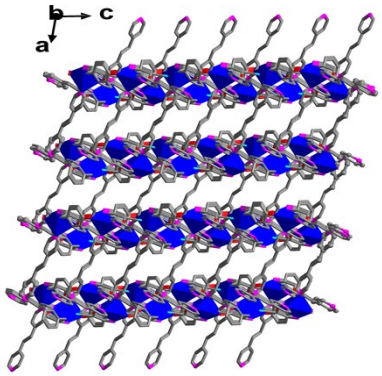

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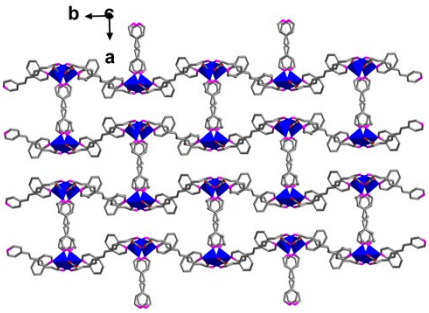

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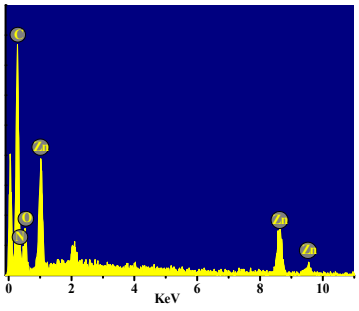
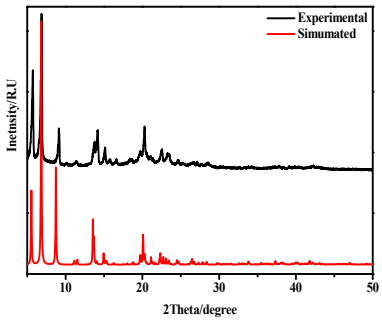


(e)



(f)

Fig. S1 (a) The standard curve of optical rotation for L-phenylalanine in 4% hydrochloric acid/H_2O solution; (b) Solid-state CD spectra of the Zn-MOF; (c) Two-dimensional structure of $[Zn_2(L\text{-phe})_2(bpe)_2]_n$ (side); (d) Two-dimensional structure of $[Zn_2(L\text{-phe})_2(bpe)_2]_n$ (front); (e) EDS result of the Zn-MOF; (f) PXRD of the Zn-MOF.

Thermogravimetric analysis of the Zn-MOF

In order to demonstrate the thermal stability of the synthesized Zn-MOF, the chiral material was characterized by thermogravimetric analysis (TGA). As can be seen from Figure 2, the material had a partial weight loss at 100-220 ^oC. What was lost in this temperature range was the methanol and water guest molecules filled in the pores of the material. After 220 ^oC, the material began to lose most of its mass, proving that the structure of the material began to collapse, which may have lost the organic ligand in the material. It showed that under the condition of less than 220 ^oC, the material had certain stability and can meet the requirements of use.

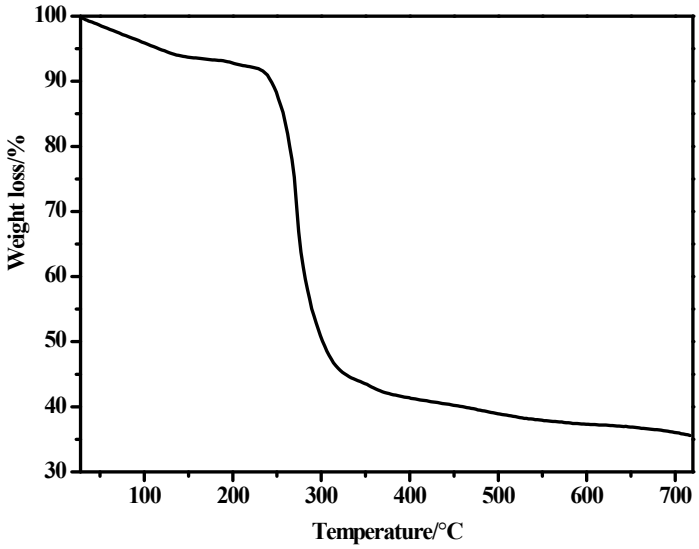


Fig. S2 Thermogravimetric curve of the Zn-MOF.

Selective adsorption of DL-leucine and DL-Alanine by the Zn-MOF

The racemic amino acid standard solution was first configured. DL-leucine and DL-alanine 6 mg were weighed into 20 mL of a 3:1 mixture of ethanol and deionized water, respectively. The obtained crystal material was carefully ground into a uniform powder using an agate mortar, and soaked in anhydrous methanol for two days. The upper liquid of methanol was discarded and fresh methanol was added to replace the high boiling solvent in the pores of the material. Then vacuum drying at 70 ^oC for 24 h was to remove the methanol molecules in the channel. The purpose of this operation was that the active sites in the Zn-MOF channel were all exposed and the best experimental results can be obtained. The prepared 10 mL racemic amino acid stock solution was added to 0.5 g of the treated material with uniform particle size, and then stirred for 30 minutes to mix the material with the racemic amino acid molecules. Next, it was allowed to stand for 24 h to achieve the adsorption equilibrium between the Zn-MOF and amino acid molecules. Finally, the supernatant was collected by centrifugation for further analysis.

Chiral derivatization of leucine and alanine solutions

In the first step, a chiral derivatization reagent was prepared. 50 Mg of DMTMM and Diphe were weighed separately and dissolved in 500 μL of DMF. Then, 19 μL of the above prepared DMTMM solution, 10 μL of Diphe solution, 2.5 μL of NMM and 21 μL of DMF were all added accurately into a 1 mL centrifuge tube, respectively. Next, the tube was placed in a dark place and shaken vigorously for 1 h. After the reaction was completed, the solution in the tube was changed from the initial colorless to pale yellow, then an amino acid derivatization reagent was prepared.

The second step was the derivatization of amino acids. 52 μL of alanine and 65 μL of leucine supernatant were separately transferred to two small centrifuge tubes, which was placed in a freeze dryer to remove ethanol and water. Then, the amino acid-derived reagent prepared in the first step was added to the aboved two small test tubes, respectively, and the reactions were shaken for 2 h in the dark. After the reactions were completed, the colors of the yellow liquids in the centrifuge tube were deepened. Then, 10 μL of the derivatized reaction solutions were diluted with deionized water to 1/10000th of the original concentration. Lastly, the diluted liquors were passed through a 0.22 μm filter, respectively, and detected by high performance liquid chromatography-mass spectrometry. All tests were performed in triplicate.

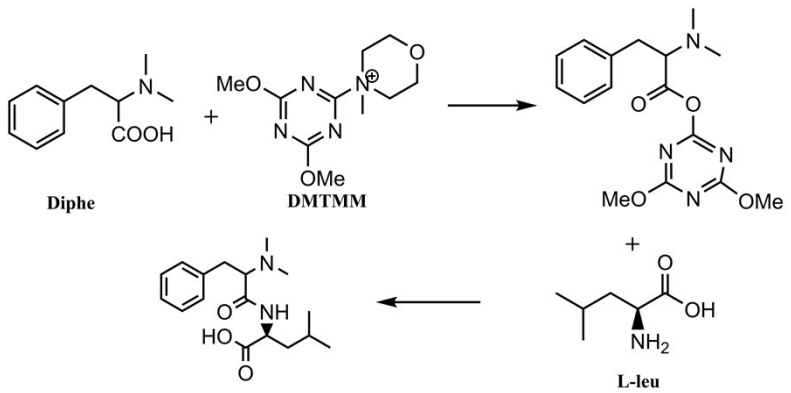
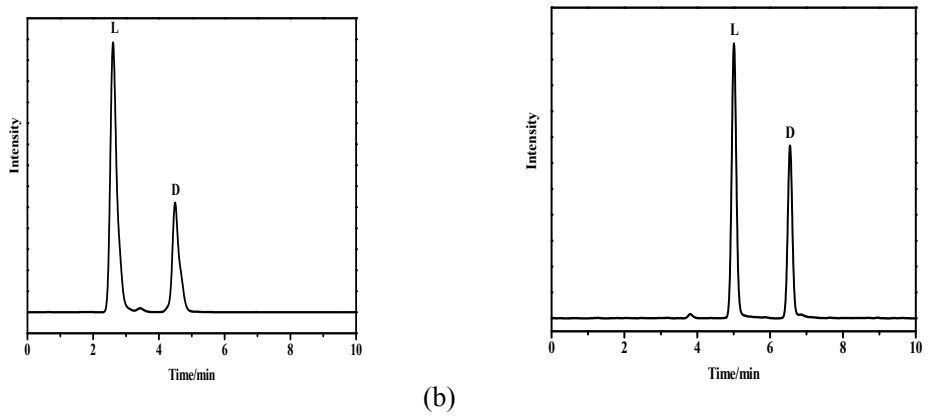


Fig. S3 Chiral derivatization of amino acids.



(a) (b)

Fig. S4 (a) HPLC-Mass separation diagram of alanine; (b) HPLC-Mass separation diagram of leucine. Chromatography condition: C18 column (2.1 × 50 mm, 2.6 μm); mobile phase A, 0.1 % HCOOH/H_2O; mobile B, acetontrile, the ratio of acetontrile increased from 5% to 35% in 0-10 minutes, flow rate, 0.3 mL min^{-1}; injection volume, 5 μL. Mass conditions: iron source, ESI;

ionization mode, positive ion model; Mass spectrometry detection mode, MRM; the detection of ion pairs for leucine is 307/148, alanine is 265/148.

Solid-state ^{13}C NMR experiments

For NMR measurements, the activated material Zn-MOF, which were soaked in methanol and dried, were divided into five equal portions and 500 mg per aliquot. Among them, four portions of the Zn-MOF were used to load different chiral amino acids, and one was used for a control experiment. Simultaneously, the chiral amino acid solutions were then prepared. 6 Mg D-alanine, L-alanine, D-leucine and L-leucine were added to 20 mL of 3:1 mixture of ethanol and deionized water, respectively. Afterwards, four chiral amino acid solutions were added to each of the four portions of Zn-MOF above and stirred for 30 minutes. Then, they are allowed to stand for 24 h to achieve the adsorption equilibrium between MOF and amino acid molecules. Finally, the materials after adsorption of the chiral amino acids were collected by centrifugation and dried in vacuum at 70 ^{o}C for 24 hours. Then, these four samples loaded different chiral amino acid were simultaneously subjected to solid-state ^{13}C NMR experiments. Solid-state ^{13}C NMR experiments were performed on a AVIII HD 400 spectrometer.

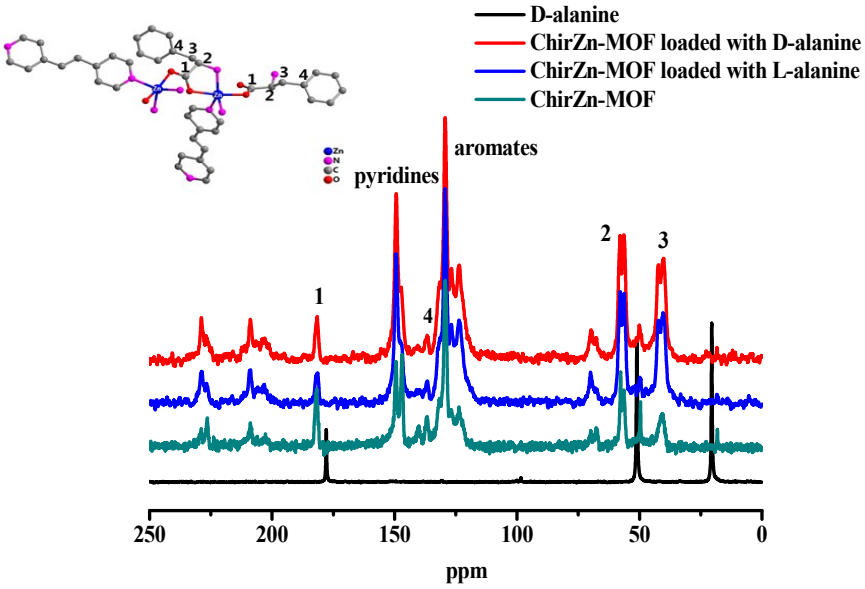


Fig. S5 ^{13}C CP MAS NMR spectra of the Zn-MOF. Top (black): sample loaded with D-alanine. Middle (red): sample loaded with L-alanine. Middle (blue): unloaded sample. Bottom (black): D-alanine. The structure of the Zn-MOF bearing the chiral side group was shown in the figure in order to define the carbon positions of the assigned signals.

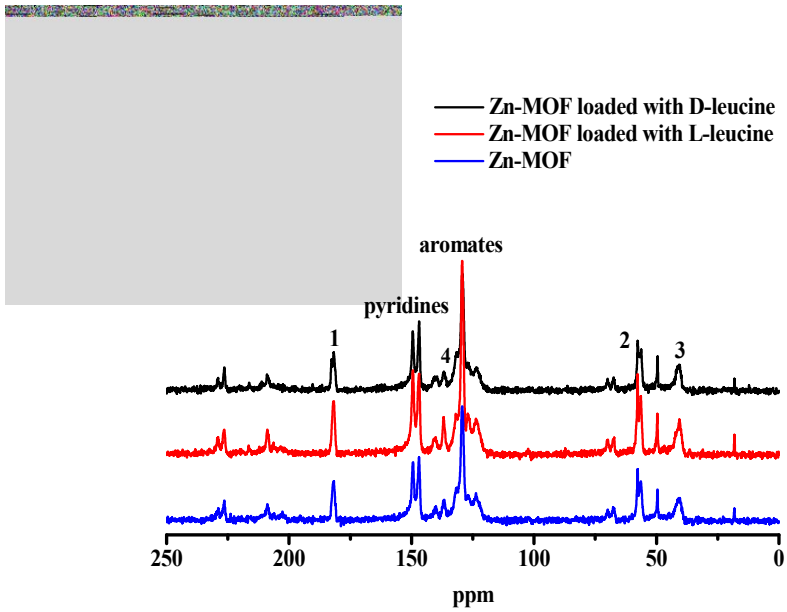
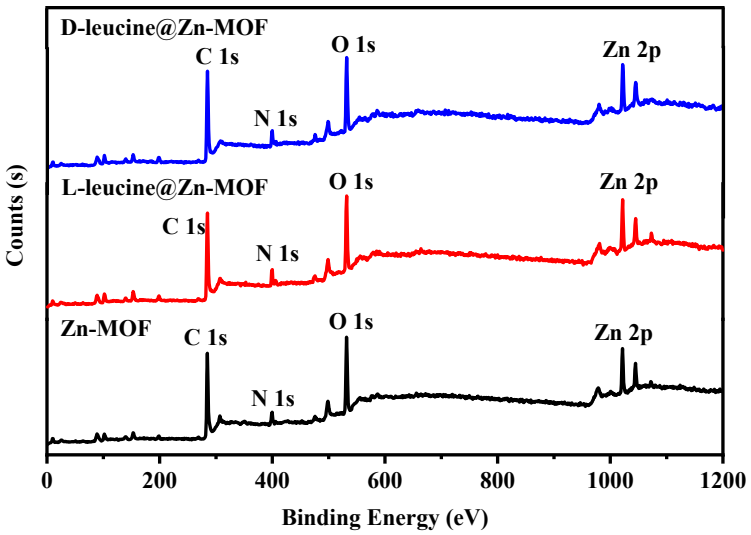
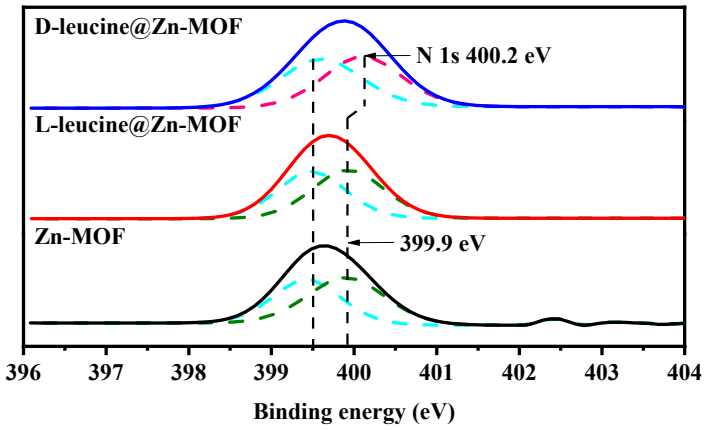


Fig. S6 (a) Solid-state ^{13}C NMR spectra of the Zn-MOF. Top (black): sample loaded with D-leucine. Middle (red): sample loaded with L-leuine. Bottom (blue): unloaded sample. The structure of the Zn-MOF bearing the chiral side group was shown in the figure in order to define the carbon positions of the assigned signals.

X-ray photoelectron spectroscopy (XPS) measurement was carried out on an Axis Supra with Al $K\alpha$ radiation as the excitation source. Binding energy for the high resolution spectra was calibrated by setting C1s to 284.6 eV. Energy step size was 0.05 eV. Dwell time was 500 ms. Number scans was 20 times and pass energy was 40 eV.

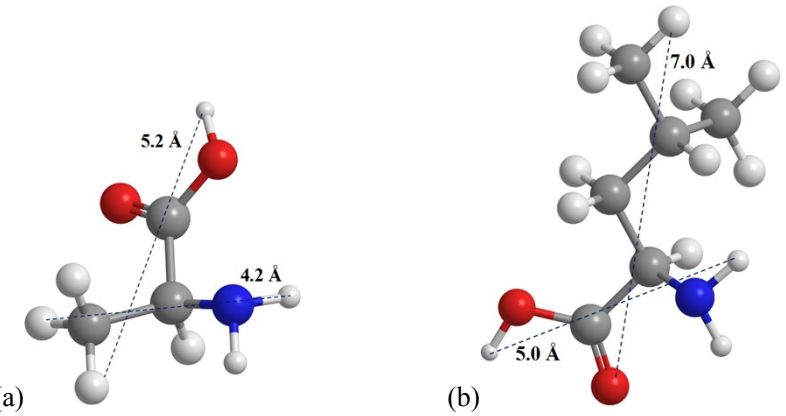


(a)



(b)

Fig. S7 (a) XPS spectra for the Zn-MOF before and after D-leucine and L-leucine adsorption; (b) comparison of N 1s XPS spectra of the Zn-MOF before and after D-leucine and L-leucine adsorption.



(a) (b)

Fig. S8 The structure and molecular dimension (calculated from the software of Chem3D 2004) of (a) alanine and (b) leucine.

Preparation of phenylalanine-bonded silica gel (Scheme S2)

Sililation of silica gel

Silica was immersed in hydrochloric acid/water solution (1:1, v/v) for 24 h and then was refluxed for 12 h at 120 °C under mechanical stirring. After cooling to room temperature, the product was washed with ultrapure water and ethanol to neutral and then dried overnight at 120 °C under air atmosphere. 3 g of activated silica was dispersed into 50 mL of toluene and then 10 mL of 3-aminopropyltriethoxysilane was added. The mixture was stirred and refluxed for 24 h at 110 °C under a nitrogen atmosphere before being cooled to room temperature. The mixture solution was

filtered, washed successively with toluene, methanol, dichloromethane, ultrapure water and acetone and then dried overnight at 60 °C under vacuum. Finally, 3-aminopropyltriethoxyl-bonded silica gel (NH_2-SiO_2) was obtained.

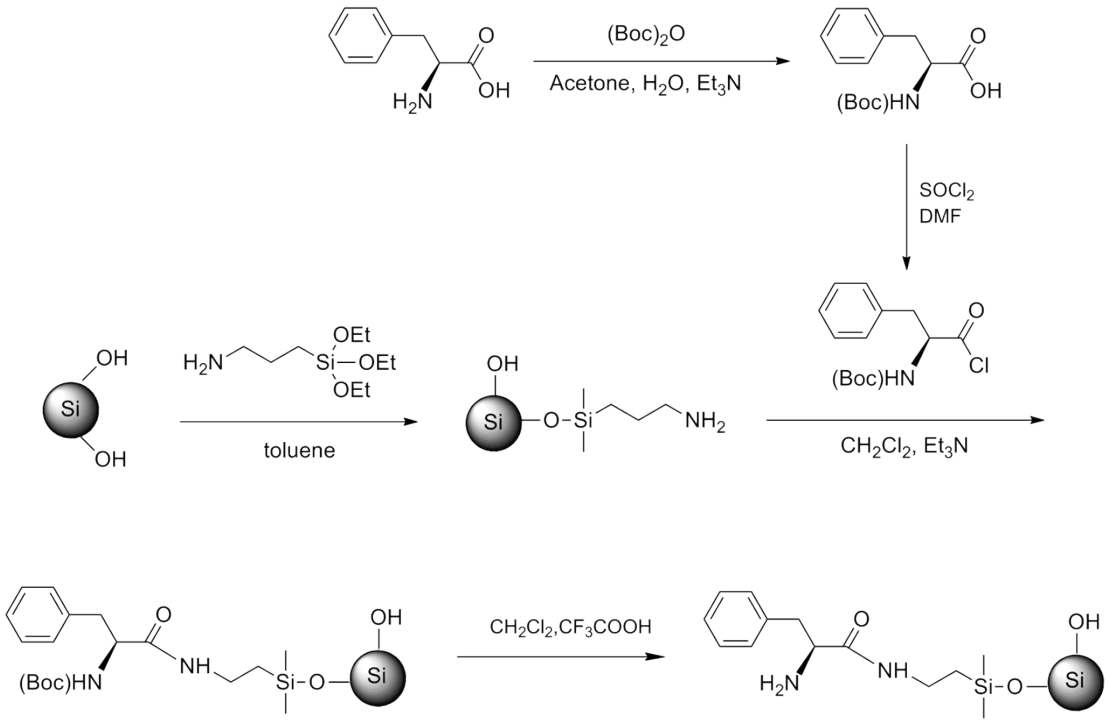
Preparation of L-Phenylalanine-bonded silica

Boc-L-Phenylalanine. 20 mmol of L-Phenylalanine, 20mL of ultrapure water and 40mL of acetone were added into a round-bottom flask followed by adding 30 mmol of Et_3N under stirring. After that, 22 mmol of $(Boc)_2O$ was added and then stirred for 6 h at room temperature. After the reaction stopped, acetone was evaporated, the water layer was extracted with ether (3×10mL), the water layer was firstly adjusted to neutral with hydrochloric acid and then extracted with ethyl acetate (4×50mL)**.** The obtained organic layer was dried by anhydrous Na_2SO_4 and then filtered and evaporated to got the product.

Acyl chlorination of Boc-L-Phenylalanine. 18mmol of Boc-L-Phenylalanine was dissolved in 20mL of $SOCl_2$ and then 1mL of DMF was added. The mixture was refluxed for 8 h at 85 °C with magnetical stirring. The solvent was then evaporated under vacuum to provide the desired product as an yellow oily substance.

Boc-L-Phenylalanine-bonded silica. 3 g of 3-aminopropyltriethoxyl-bonded silica gel was suspended under nitrogen in freshly distilled dichloromethane and 12mmol of trimethylamine was added. Acyl chlorination product was dissolved in 100 mL of dry dichloromethane and added dropwise into the suspension. The reactive mixture was stirred under nitrogen for 24 h at room temperature. The mixture was then filtered. The bonded silica gel was washed sequentially with dichloromethane and dried overnight under vacuum at 60 °C.

L-**Phenylalanine-bonded silica gel.** 5.7 g of trifluoroacetic acid and 3g of Boc-L-Phenylalanine-bonded silica were added into 10 mL of dichloromethane, The mixture was stirred for 4 h at room temperature. Finally, the product was washed sequentially with dichloromethane and dried overnight under vacuum at 60 °C.



Scheme S2 Preparation scheme of phenylalanine-bonded silica gel

Table S2 Elmental analysis results of the bonded silica gel.

Material	C(%)	H(%)	N(%)	Bonded amount (μmol/m^2)
NH_2-SiO_2	4.08	1.06	0.93	-
Boc-L-Phe-SiO_2	10.4	1.42	1.87	-
L-Phe-SiO_2	9.19	1.31	1.35	1.77

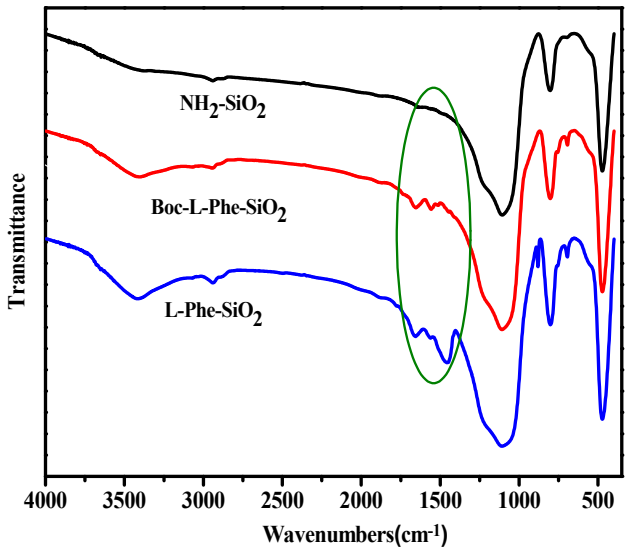


Fig. S9 FTIR spectra of NH_2-SiO_2, Boc-L-Phe-SiO_2, L-Phe-SiO_2.

The obtained materials were analyzed by FTIR and elmental analysis. Compared with amino silica, Boc-L-Phenylalanine bonded silica and L-Phenylalaninethe bonded silica, the spectrum of latter two showed the characteristic peaks of the benzene ring at 1676 cm^{-1}, 1561 cm^{-1} indicating the successful bonded of L-phenylalanine on silica gel (Fig. S8). Table S1 showed the elemental analysis results of amino silica, Boc-L-Phe-SiO_2 bonded silica and L-Phenylalanine bonded silica, which confirmed that the L-Phenylalaninethe bonded silica was immobilized on the silica gel successfully. Besides, the increase of the peak at 3300 cm^{-1} in IR spectrum and the decrease of the carbon content for L-Phenylalanine-bonded silica by comparison with Boc-L-Phenylalanine bonded silica in elmental analysis, indicating the successful removal of Boc. According to the carbon content of the bonded silica gel stationary phase, the bonded amount of L-phenylalanine was calculated is 1.77 μmol/m^2.