Enantioselective Hydrolysis of Amino Acid Esters Promoted by Bis(β-cyclodextrin) Copper Complexes

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It is challenging to create artificial catalysts that approach enzymes with regard to catalytic efficiency and selectivity. The enantioselective catalysis ranks the privileged characteristic of enzymatic transformations. Here, we report two pyridine-linked bis(β-cyclodextrin) (bisCD) copper(II) complexes that enantioselectively hydrolyse chiral esters. Hydrolytic kinetic resolution of three pairs of amino acid ester enantiomers (S₁–S₃) at neutral pH indicated that the “back-to-back” bisCD complex CuL₁ favoured higher catalytic efficiency and more pronounced enantioselectivity than the “face-to-face” complex CuL₂. The best enantioselectivity was observed for N-Boc-phenylalanine 4-nitrophenyl ester (S₂) enantiomers promoted by CuL₁, which exhibited an enantiomer selectivity of 15.7. We observed preferential hydrolysis of L-S₂ by CuL₁, even in racemic S₂, through chiral high-performance liquid chromatography (HPLC). We demonstrated that the enantioselective hydrolysis was related to the cooperative roles of the intramolecular flanking chiral CD cavities with the coordinated copper ion, according to the results of electrospray ionization mass spectrometry (ESI-MS), inhibition experiments, rotating-frame nuclear Overhauser effect spectroscopy (ROESY), and theoretical calculations. Although the catalytic parameters lag behind the level of enzymatic transformation, this study confirms the cooperative effect of the first and second coordination spheres of artificial catalysts in enantioselectivity and provides hints that may guide future explorations of enzyme mimics.

Because of the cooperation of the catalytic centre and the second coordination sphere, a native metalloenzyme can achieve biological transformations with remarkable efficiency and enantioselectivity under mild conditions in vivo. Over the years, significant efforts have been dedicated to the biomimetic study of metalloenzymes to understand their structures and functions and design analogues to mimic the native enzymes’ structures and functions. In the past few decades, synthetic compounds with functions resembling those of, for example, hydrolases, oxidases, and reductases have been reported. Remarkable progress has been made in mimicking the functions of the enzymes that mediate electron transfer with respect to the catalytic efficiency. However, studies of hydrolase mimetics have lagged far behind, regarding to either catalytic efficiency or selectivity. Specifically, in biomimetic chemistry, the rational design of enantioselective hydrolase mimics remains challenging.

Modified cyclodextrins (CDs) have been exploited to construct artificial enzymes since the 1970s, as introduced by Breslow. Previous studies have indicated that CDs’ host-guest interactions could result in increased substrate binding affinity and cooperative binding between intramolecular CDs. In fact, the chiral nature of the CD cavity, particularly the cooperative effects of CDs with coordinated metal ion, has been somewhat underestimated. Appropriately modified CDs can be used in enantioselective molecular binding studies, and mediate chemical and photochemical enantio-differentiating reactions. CDs play an important role in preorganizing the guest molecules through the hydrophobic interactions. A number of studies have reported the use of unmodified or simply modified CD monomers without metal ions for the enantioselective deacylation of chiral esters. However, the cooperative enantioselective catalytic effect arising from CDs’ intramolecular

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interactions with coordinated metal ions have rarely been reported, and the cooperative mechanism also remains unclear. We reported the construction of metallohydrolase mimics and superoxide dismutase mimics with CD domains5,33–39. The hydrolase activities were studied with carboxylic acid esters and phosphate esters as model, non-chiral substrates. In this study, chiral t-butyloxycarbonyl (Boc)-protected aromatic amino acid esters $S_1$-$S_3$ were chosen as substrates (Fig. 1). These substrates, which contain amino acid moieties, are biologically relevant than the previously used substrates and thus allowed us to evaluate the interactions of chiral CD cavities with naturally occurring substances. Two bisCD copper(II) complexes were utilized as enantioselective hydrolase mimics, denoted CuL$^1$ ($L^1$ = 2,6-bis(6-mono-amino-β-cyclodextrin-methyl)-pyridine, the “back-to-back” complex$^{39}$) and CuL$^2$ ($L^2$ = 2,6-bis(3-mono-amino-β-cyclodextrin-methyl)-pyridine, the “face-to-face” complex$^{40}$). The different CD orientations of the two mimics were studied in parallel to evaluate the effect of the chiral cavities on enantioselective catalysis. Electrospray ionization mass spectrometry (ESI-MS), an inhibition assay, rotating-frame nuclear Overhauser effect spectroscopy (ROESY), and theoretical calculations were performed to gain deeper insights into the underlying mechanism.

Results

Synthesis and characterization of CuL$^1$ and CuL$^2$. The two copper(II) complexes, CuL$^1$ and CuL$^2$, were synthesized according to our previously reported procedures (see Supplementary Figs S1 and S2 for the ESI-MS results$^{35,38,39}$). Because of the relationship between coordination geometry and catalytic ability, we were particularly interested in probing the coordination geometries of the copper(II) centre by using electron paramagnetic resonance (EPR) spectroscopy, which were performed at 100 K in a frozen solution of water and dimethylsulfoxide (DMSO) (see Supplementary Fig. S3). As a result, $g = 2.25$ and $A = 160$ were obtained for CuL$^1$, and $g = 2.26$ and $A = 165$ were obtained for CuL$^2$, indicating a distorted square pyramidal coordination geometry at the copper(II) centre in both cases$^{41,42}$. This proposed geometry was also supported by the UV-Vis spectra (Supplementary Fig. S4), which showed a broad absorption band at approximately 700 nm that was assigned to the $d-d$ transitions$^{42,43}$.

Hydrolysis of Boc-protected amino acid esters. The hydrolysis of $S_1$, $S_2$, and $S_3$ at neutral pH (50-mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer containing 10% CH$_3$CN, pH 7.2) and 298 ± 0.1 K were monitored by UV-Vis spectroscopy, focusing on the absorption at 400 nm, which were proportional to the concentration of the hydrolytic product 4-nitrophenolate ($NP$). Figure 2 reveals that the concentration of $NP$ varied as a function of time during the CuL$^1$- and CuL$^2$-promoted hydrolysis of $S_1$, $S_2$, and $S_3$. The initial rate constants, $k_{in}$ (s$^{-1}$) of the substrate cleavage events are listed in Table 1. Accordingly, CuL$^1$ exhibited a higher catalytic efficiency than CuL$^2$. Moreover, the differences in the hydrolysis rates of the substrate enantiomers in the reactions with CuL$^1$ were more pronounced than those in the reactions with CuL$^2$. It should be noted that in the presence of CuL$^1$ (50 μM), $L$-$S_2$ achieved the highest hydrolysis rate ($k_{in} = 5.4 \times 10^{-5}$ s$^{-1}$) among the tested substrates (Table 1), which was 168-fold higher than the spontaneous hydrolysis rate ($k_{sp} = 3.2 \times 10^{-7}$ s$^{-1}$) (2.5 μM) (Supplementary Fig. S12). In contrast, the CuL$^1$-catalysed hydrolysis of $D$-$S_2$ was 10-fold slower than that of $L$-$S_2$ under the same conditions (Fig. 2b). The enantioselectivity was
selective catalysis. The results of these screening experiments indicated that the "back-to-back" bisCD complex substrate by both (the solid line in black). The reactions were performed in a 10% MeCN catalytic efficiency and enantioselectivity (Table 1 and Supplementary Figs S9–12).

Figure 2. Initial-rate kinetics. Hydrolysis of single enantiomers of the substrates (2.5 μM) a) S₁, b) S₂, c) S₃ by CuL¹ (50 μM) (for the L-isomer, o for the D-isomer) and CuL² (50 μM) (for the L-isomer, o for the D-isomer) and spontaneous hydrolysis (the solid line in black). The reactions were performed in a 10% MeCN solution in HEPES buffer (pH 7.2, 50 mM) at 298 ± 0.1 K.

Table 1. The initial rate constants for S₁–S₃ (2.5 μM) promoted by different catalysts (50.0 μM) in a 10% MeCN solution in HEPES buffer (pH 7.2, 50 mM) at 298 ± 0.1 K.

| Catalyst | kₛ (10⁻¹ s⁻¹) | S₁   | S₂   | S₃   |
|----------|---------------|------|------|------|
|          | L-  | D-  | L-  | D-  | L-  | D-  |
| CuL¹     | 3.9  | 2.1  | 5.4  | 5.2×10⁻¹ | 2.0  | 1.3  |
| CuL²     | 9.5×10⁻³  | 7.7×10⁻¹ | 1.6  | 7.2×10⁻¹ | 3.4×10⁻¹ | 1.9×10⁻¹ |
| L¹       | 6.2×10⁻¹  | 4.3×10⁻¹ | 8.5×10⁻¹ | 1.8×10⁻¹ | 2.1×10⁻¹ | 1.2×10⁻¹ |
| L²       | 4.4×10⁻¹  | 3.1×10⁻¹ | 4.8×10⁻¹ | 1.2×10⁻¹ | 4.3×10⁻¹ | 1.7×10⁻¹ |
| Cu⁺⁺     | 1.2×10⁻¹  | 6.7×10⁻¹ | 6.1×10⁻¹ |
| Buffer   | 5.0×10⁻²  | 3.2×10⁻² | 2.8×10⁻² |

reduced by changing either the substrate or the catalyst (Fig. 2a,c), while S₂ remained the most favored substrate by both CuL¹ and CuL². Additionally, changing the catalyst reduced the enantioselectivity more than that induced by changing the substrate, suggesting that the CD orientation plays a very important role in the enantioselective catalysis. The results of these screening experiments indicated that the "back-to-back" bisCD complex favoured the enantioselective hydrolysis of the amino acid-containing substrates, which was particularly evident for S₂. The control experiments using the metal or ligands separately resulted in substantially decreased catalytic efficiency and enantioselectivity (Table 1 and Supplementary Figs S9–12).

To fully assess the enantioselective hydrolysis of S₁–S₃ by CuL¹, a revised Michaelis–Menten kinetic experiment was performed in the presence of excess catalyst27,28,44–46. By increasing the initial catalyst concentration from 5.0 to 125 μM, the hydrolysis rate increased, resulting in a levelled-off curve for each substrate (Fig. 3) and implying that the catalyst-substrate complex formed prior to the catalytic reaction. The kinetic parameters were deduced by fitting the data to the Michaelis–Menten equation (Table 2)47,48. The best catalytic ability (kₛ/kₛuncat) was observed for L-S₂, which also showed the most pronounced enantioselectivity, with an enantiomer selectivity (L/D) of 15.7. Furthermore, the kₛ values for S₁–S₃ ranged from 3.3–6.2×10⁻¹ s⁻¹ for the L-isomers and 1.6–4.2×10⁻¹ s⁻¹ for the D-isomers. As a reference, the Michaelis–Menten kinetic experiment was also performed to investigate S₂ catalysis by CuL² (see Supplementary Fig. S14) and resulted in an enantiomer selectivity of 3.3, as shown in Supplementary Table S1.

To further confirm that the L-isomer was preferentially hydrolysed over the D-isomer, a chiral high-performance liquid chromatography (HPLC) analysis were performed with racemic S₂. As shown in Supplementary Fig. S16, after different ratios of racemic S₂ (10.0 μM) were consumed by CuL¹ (100 μM) at 298 K, the reaction aliquots were subjected to ethyl acetate extraction to transfer the unreacted S₂ into the organic phase49, followed by chiral HPLC analysis with CHIRALPAK® IC. When 35%, 40%, and 50% of S₂ was consumed, the remaining L:D ratios were 28:72, 23:77 and 20:80, respectively (Table 3). Based on these conversions and the remaining substrate enantiomeric ratios (e.r.), the consumption ratios of the L-S₂ and D-S₂ enantiomers were calculated to be 94.6, 92.8, and 82.18, thus indicating that the corresponding hydrolysed product (Boc-Phe-OH) were present at e.r. of 94.6, 92.8 and 82.18 (L/D) (Table 3). These results demonstrated that CuL¹ possessed high enantioselectivity for the hydrolysis of racemic S₂ and showed a preference for the L-isomers.

Catalytic inhibition assay. To gain more insights into the reaction mechanism, di(p-tert-butylbenzyl) amine (DBBA) was applied as a CD inhibitor to investigate the role of the CD cavity34,36. In our previous study, we have excluded the possibility that DBBA exerts its inhibitory effect through coordination with the metal ions36. The strong binding between DBBA and the CD cavity was demonstrated by ROESY. The nuclear Overhauser effect (NOE) cross-peaks could be observed as the protons are closer than 0.4 nm in space. Therefore, the
binding substituent group within the β-CD cavity could be estimated according to the relative intensity of the cross-peaks. The interactions between the aryl protons of DBBA and the protons in the CD of L1 were observed (Supplementary Fig. S17). As expected, the initial rate of the CuL1-catalysed hydrolysis of L-S2 was dramatically decreased by more than 13-fold in the presence of DBBA, suggesting that CuL1 was significantly inhibited (Fig. 4a,b). Although the hydrolysis rate for D-S2 was low, a 1.3-fold decrease was still observed in the presence of DBBA. The kinetic parameters are displayed in Supplementary Table S2. Moreover, 2D ROESY nuclear magnetic resonance (NMR) experiments of L1 with L- or D- Boc-Phe-OH, instead of the CuL1 and S2 isomers, respectively, were performed (Supplementary Fig. S18). The ROESY spectrum of L1 with Boc-Phe-OH displayed NOE cross-peak signals between H-3,5,6 of β-CD and the protons of the Phenyl and Boc groups of the Boc-Phe-OH enantiomers, indicating that the two groups could be self-included into the CD cavities of L1 from the primary side (Note: the binding constants (K_a) by β-CD for benzene and t-BuOH are 120 M⁻¹ and 48 M⁻¹, respectively, at 298 K in H2O). These observations demonstrated that the hydrophobic CD cavities were indeed involved in the catalysis of both isomers.

ESI-MS analysis of the mixture of CuL1 with each S2 enantiomer were performed (see Supplementary Figs S19 and S20). The catalyst–substrate (1:1) complexes were detected by positive-ion ESI-MS, and no catalyst–product complexes were observed, thus indicating that the product binds the catalyst more weakly than the substrate. Considering the NP group could easily be bound in the β-CD cavity, it leads to the proof that the substrate should be bound by the two cooperative hydrophobic cavities. Moreover, negative-ion ESI-MS confirmed that the hydrolysis products were N-Boc-phenylalanine (Boc-Phe-OH) and NP, and no Boc-deprotected product was observed. These observations suggested that there was enhanced affinity between the catalysts and substrates and that NP was cleaved from the substrates following the hydrolysis.

Table 2. Kinetic parameters for the hydrolysis of S1-S3 (2.5 μM) in the presence of CuL1 (5.0–125 μM) in a 10% MeCN solution in HEPES buffer (50 mM, pH = 7.2) at 298 ± 0.1 K.

Table 3. Chiral HPLC analysis of the conversion of various amounts of racemic S. [a] The enantiomeric ratio (e.r.) values of remaining substrates were determined by HPLC analysis with a chiral stationary phase. [b] e.r. values of products were calculated from the result of HPLC analysis.

**Figure 3. Saturation kinetics.** Michaelis–Menten kinetics for the hydrolysis of the substrates (2.5 μM) in the presence of CuL1 (5.0–25 μM) in a 10% MeCN solution in HEPES buffer (pH 7.2, 50 mM) at 298 ± 0.1 K.
Based on the above results, we speculate that NP ($K_a = 1503 \text{ M}^{-1}$ ($\beta$-CD, 298 K, H2O))54 and the Phenyl groups of $S_2$ were encapsulated in the cavities of CuL1 during the reaction. The enantioselectivity originated from the different geometries of the substrates that were regulated by the closely linked CD chiral cavities. The mechanism was further investigated through theoretical calculations. The structures of the $L$-$S_2$-CuL1 and $D$-$S_2$-CuL1 complexes were optimized at the ONIOM(B3LYP/lanl2dz:UFF)/IEFPCM level of theory. Color code: O, red; N, blue; Cu, magenta; C, light grey (catalysts) and cyan (substrates). Hydrogen was omitted. The intramolecular CDs were also omitted in the bottom images for clarity. The metal ions and coordinated water are shown as spheres. All labelled distances are shown in angstroms (Å).

Discussion
The initial-rate kinetic study showed that the “back-to-back” bisCD complex CuL1 exhibited higher catalytic efficiency and more pronounced enantioselectivity than the “face-to-face” analogue CuL2. These results indicated that the closely linked CD cavities on the primary face exhibit a much stronger cooperative effect with the coordinated copper ion in terms of differentiating the chiral substrates than those linked on the secondary face. However, in most cases, the enantioselectivity was significantly decreased when only $L^1$ or $L^2$ was used without the metal. $S_1$ enantiomers were an exception for $L^2$, because containing the bulk R group, $S_1$ would have an
unsuitable location on L^2 induced by the Cu^{2+} coordination with the bridge, as observed for the metal-inhibited enzymes. Besides, in the presence of either CuL\textsuperscript{1} or CuL\textsuperscript{2}, the initial hydrolysis rates of substrate L-isomers exceeded those of D-isomers in all cases, indicating that the chiral CD cavities in our models had an overall preference for the L-isomers of the amino acid esters. EIOH was tested as another cosolvent and exhibited an enantiomer selectivity similar to those of the reactions performed in MeCN. This finding indicated that a cosolvent effect of MeCN could be excluded (see Supplementary Fig. S13 and Table S4).

The $k_{\text{CuL}}/k_{\text{CuS}}$ value of 811 for L-S\textsubscript{3} was in agreement with the largest values obtained for previously reported CD-based or other simple artificial mimics with similar substrates\textsuperscript{26–28,55,56}, although some protein-based mimics produced higher values\textsuperscript{44,57–59}. The most pronounced enantioselectivity was observed for the catalysis of S\textsubscript{2} by CuL\textsuperscript{1}, with an enantiomer selectivity of 15.7. This value is comparable to that of the reported natural protein-based model\textsuperscript{44}. Although some reports have obtained better enantioselectivity with protein-modified catalysts, the complicated structure has made preparing the catalyst and performing mechanistic studies challenging\textsuperscript{29,30,57–59}. The chiral HPLC analysis of S\textsubscript{2} hydrolysis by CuL\textsuperscript{1} confirmed that the L-isomer was preferentially hydrolysed over D-S\textsubscript{2} with an e.r. of 94:6 (L:D) when 35% of the racemic S\textsubscript{2} was consumed. Finally, the S\textsubscript{2} enantiomers were determined to be the optimal substrates for CuL\textsuperscript{1} in our study, and the removal of the methylene on the Phenyl group or replacement with an Indolyl group diminished the enantioselectivity. CuL\textsuperscript{2} also exhibited the highest enantioselectivity for S\textsubscript{2} compared with the other two pairs of enantiomers, although the cooperative effect was weaker than that of CuL\textsuperscript{1}. Together, our results suggest that the substrate structure significantly affected the enantioselectivity, and S\textsubscript{2} was identified as the best substrate among those tested. The findings indicated that the catalysts recognize specific substrates.

ESI-MS proved that the catalyst formed an intermediate complex with the substrates. The 2D ROESY NMR and inhibition assay demonstrated the vital role of CDs in the catalytic pathway. Finally, the optimized structures of the catalyst-substrate complexes provided initial evidence that the enantiomers formed different geometries according to the closely linked CD chiral cavities.

In conclusion, we presented a hydrolase mimetic study focusing on chiral substrates, in which two bisCD-based copper(II) complexes were developed for the hydrolysis of Boc-protected amino acid esters under physiological conditions (pH = 7.2). Accordingly, the adjacent chiral CD cavities had an overall preference for binding L-isomers. In addition, the "back-to-back" bisCD complex CuL\textsuperscript{1} exhibited much better catalytic efficiency and enantioselectivity towards the chiral amino acid esters than the "face-to-face" analogue CuL\textsuperscript{2}. Mechanistic studies showed that the two closely linked chiral CD cavities played a vital role in mediating the enantioselective hydrolysis by regulating the different isomer geometries during the reaction, in which the hydrophobic groups of the substrate were embedded in the two intramolecular CD cavities. These findings indicate a cooperative effect of the first and second coordination spheres of artificial catalysts on the enantioselectivity and also provide hints that may guide future exploration of enzyme mimics.

### Methods

**Materials.** Reagent-grade β-CD was recrystallized twice from H\textsubscript{2}O and dried in vacuo for 12 h at 373 K. All of the amino acid esters or precursor enantiomers, N-Boc-phenylalanine 4-nitrophenyl ester (S\textsubscript{2}), N-Boc-phenylglycine (Boc-Phg-OH), and N-Boc-Tryptophan (Boc-Trp-OH) were purchased from GL Biochem (Shanghai) Ltd. Dicyclohexylcarbodiimide (DCC) was purchased from Aladdin. Di-p-tert-butylbenzyl) amine (DBBA) was synthesized with previously reported methods\textsuperscript{34}. DMF was superdry grade and stored over a molecular sieve. Common organic reagents were reagent grade and redistilled before use. Milli-Q water was used in all physical measurements. CuL\textsuperscript{1} and CuL\textsuperscript{2} were synthesized as described in previous studies\textsuperscript{35,38,39}. All compounds were confirmed by elemental analyses, ESI-MS, and 1H-NMR spectra.

**General methods.** The 1H NMR spectra were recorded on Mercury plus 300 spectrometers. The 2D NMR spectra were recorded on a Bruker Avance III 600 spectrometer. The elemental contents were analysed with a Perkin–Elmer 240 elemental analyser. The ESI-MS spectra were collected on a Thermo LCQ-DECA-XP spectrometer. The UV/Vis spectra were monitored with a Varian Cary 100 UV/Vis spectrophotometer equipped with a temperature controller (±0.1 K). The HPLC analyses were performed on an Agilent 1200 HPLC with CHIRALPAK® IC 250 × 4.6 mm column. The EPR spectra were recorded on a Bruker A300-10-12 spectrometer.

**Synthesis of the amino acid esters.** The amino acid esters were prepared according to a previously described method\textsuperscript{34}, with some modifications. Boc-L-Phg-OH (3.5 g, 13.9 mmol) and 4-nitrophenol (1.9 g, 13.9 mmol) were dissolved in dry DMF (15 mL). Aliquots of the mixture were added to a stirred and cooled (−10°C) suspension of DCC (2.9 g) in dry DMF (50 mL). The reaction mixture was magnetically stirred at −10°C for approximately 2 h, then allowed to stand at room temperature for 5 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The crystallization of the residue from 1:2 (v/v) ethyl acetate/petroleum ether produced pure Boc-L-Phg-ONp (L-S\textsubscript{1}) with a 78% yield. 1H-NMR (300 MHz, CDCl\textsubscript{3}): 8.22 (1H, d, J = 9.2, NP-H), 7.42 (2H, dd, J = 9.3, 3.6, Ph-H), 7.20 (1H, d, J = 9.0, NP-H), 5.52 (1H, d, J = 6.9, CH-N), 5.43 (1H, d, J = 7.0, CH-N), 1.47 (4H, s, CH\textsubscript{2}); elemental analysis (calcld., observed for C\textsubscript{19}H\textsubscript{20}N\textsubscript{2}O\textsubscript{6}: C (61.28, 60.94), H (5.41, 5.48), N (7.52, 7.52).

D-S\textsubscript{1} was prepared through the same procedure. 1H-NMR (300 MHz, CDCl\textsubscript{3}): 8.22 (1H, d, J = 9.2, NP-H), 7.42 (2H, dd, J = 9.3, 3.6, Ph-H), 7.20 (1H, d, J = 9.0, NP-H), 5.52 (1H, d, J = 6.9, C-NH), 5.43 (1H, d, J = 7.0, CH-N), 1.47 (4H, s, CH\textsubscript{2}); elemental analysis (calcld., observed for C\textsubscript{19}H\textsubscript{20}N\textsubscript{2}O\textsubscript{6}: C (61.28, 60.94), H (5.41, 5.48), N (7.52, 7.52).

Boc-L-Trp-ONp (L-S\textsubscript{2}) and Boc-D-Trp-ONp (D-S\textsubscript{2}) were synthesized as described above. L-S\textsubscript{2}: 1H-NMR (300 MHz, CDCl\textsubscript{3}): 8.17 (3H, d, J = 9.1, NP-H, Indole-H), 7.58 (1H, d, J = 7.8, Indole-H), 7.40 (1H, d, J = 8.1, Indole-H), 7.26–7.20 (3H, m, Indole-H, CHCl\textsubscript{3}), 7.17–7.08 (2H, m, NP-H), 6.98 (2H, d, J = 9.1, Indole-H), 5.14 (1H, d, J = 7.5, C-NH), 4.85 (1H, dd, J = 13.5, 5.9, CH-N), 3.42 (2H, qd, J = 14.3, 6.4, CH\textsubscript{2}), 1.45 (9H, s, CH\textsubscript{3}); elemental analysis (calcld., observed for C\textsubscript{23}H\textsubscript{23}N\textsubscript{3}O\textsubscript{6}: C (62.11, 62.34), H (5.45, 5.50), N (9.88, 9.77).
Kinetics of amino acid ester hydrolysis. The hydrolysis rates of the amino acid esters in the presence of CuL¹ and CuL² were measured with an initial slope method by monitoring the increase in the 400-nm absorption of the released NP. At this wavelength, the absorbance of the ester substrate was negligible. The reaction solution was maintained at 298 ± 0.1 K. HEPES buffer (pH = 7.2, 50 mM) was used, and the ionic strength was adjusted to 0.10 M with NaClO₄. The amino acid esters were prepared as solutions in CH₃CN, and the buffer and catalyst solutions were freshly prepared in water.

In a typical experiment, a small amount of aqueous catalyst solution was spread evenly in a 600-μL cuvette containing 10% (v/v) CH₃CN buffer solution, and the reactions were always initiated by injecting a small amount of substrate CH₃CN solution, followed by complete mixing. All of the solutions were equilibrated to 298 ± 0.1 K. The initial first-order rate constants (kₑ (s⁻¹)) for the substrate cleavage were obtained directly from a plot of the NP concentration versus time by using the initial rate method. The errors in the kₑ values were less than 5%. The ε value for NP at pH 7.2 was 10,398. The inhibition study was performed by following a similar procedure, except for the pre-equilibrium incubation after mixing DBBA with CuL¹.

Kinetic resolution of racemic S₂. The hydrolysis of racemic S₂ was performed using a method similar to that described in the previous section. Racemic S₂ (10.0 μM) was mixed with CuL¹ (100 μM) under the same conditions as those used in the kinetic studies. The reaction process was monitored with a UV/Vis spectrometer. Then, upon mixing of the reaction mixture with ethyl acetate, the products (Boc-Phe-OH and 4-nitrophenol) and remaining S₂ were completely extracted into the organic phase. The organic phase was evaporated to dryness and the residue was dissolved in ucinanol for HPLC analysis. The chiral HPLC analyses were performed at room temperature with a CHIRALPAK® IC 250 × 4.6 mm column and monitored by a UV-Vis detector at 254 nm; the elution solvent was 40/30/30 H₂O (0.1% formic acid)/CH₃CN/ETHOH, and the flow rate was 1.0 mL/min.

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Author Contributions
S.-S.X., M.Z., J.W., Z.-W.M. and L.-N.J. conceived and designed the experiments. S.-S.X., Z.-F.K., B.-C.C., H.S. and Z.-K.C. performed the experiments. S.-S.X., J.W. and Z.-W.M. analyzed the data. S.-S.X., M.Z., J.W. and Z.-W.M. wrote the paper. All authors discussed the results and commented on the manuscript.

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