

A Racemic Naphthyl-Coumarin-Based Probe for Quantitative Enantiomeric Excess Analysis of Amino Acids and Enantioselective Sensing of Amines and Amino Alcohols

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A new racemic naphthyl-coumarin-based probe was found to bind covalently with amino acids in MeOH-KOH system and thereby generates distinct CD responses. The induced strong CD signals allowed quantitative enantiomeric excess analysis of

amino acids and enantioselective sensing of amines and amino alcohols. The mechanism for the reaction of the coumarin-aldehyde probe with an amino acid was investigated by CD, UV-Vis, NMR, ESI-MS analyses and ECD calculation.

Introduction

Chiral amines and their derivatives are irreplaceable constituents of pharmaceutical drugs and agrochemicals. About 40% of the pharmaceuticals currently in use contain chiral amine functional groups in their structures. Amino acids are the derivatives of amines, which constitute the basic unit of life. L-amino acids, which play a vital role in many biological processes, are essential building blocks of proteins.^[1] Recently, D-amino acids such as serine, alanine and methionine have also been observed to have crucial physiological and pathological functions in the

brain and in the endocrine system of mammals.^[2,3] The amino acid enantiomers are also frequently utilized as extremely useful starting materials in chemical and pharmaceutical industries.^[4] Due to the importance and widespread application of chiral amines, several methods have been developed to identify enantiomers and quantitatively analyze the enantiomeric excess (ee).^[5] An enantioselective recognition method such as the use of fluorescent spectrometry-based sensors generally requires an optically active probe for analysis.^[6] For example, we have developed probe **1** by combining a chiral 1,1'-bi-2-naphthol (BINOL) with an achiral coumarin for enantioselective fluorescent recognition, which could determine the concentration and enantiomeric composition of Valine (Val) at two excitation wavelengths.^[7] Only a few racemic fluorescence probes have been used to determine the ee of chiral analytes.^[8] This task can also be achieved by using circular dichroism (CD) spectroscopy with achiral or racemic probes.^[9] The probes possess chromophores and can interact covalently or noncovalently with otherwise difficult to detect, "invisible" chiral analytes to induce amplified Cotton effects.^[10] You et al. reported a racemic probe with biphenyl axial chirality and dynamic covalent bonding to analyze enantiomeric composition by CD.^[10c] Pu et al. reported a terpyridine-zinc(II)-complex-based probe which showed strong CD signals when treated with chiral amines.^[10d] Wolf et al. have demonstrated that some achiral or racemic probes can determine enantiomeric composition of chiral free amino acids, amino alcohols and amines by detecting the induced CD signal.^[11] Zonta et al. reported that a modified tris(2-pyridylmethyl)amine ligand could induce the CD effect by forming a multicomplex with zinc salt and the amino acid.^[12] Wei et al. developed an achiral coumarin aldehyde probe that could determine the ee of amino acids by using CD signals.^[13] Although substantial progress has been made in optical assays and arrays, it is still very challenging to find a practical and widely useful sensing system.

Recently, we reported a BINOL-coumarin-based probe **1** (Figure 1) for enantioselective fluorescent recognition of amino acids.^[7a] The mechanistic investigation showed that the 1,2-

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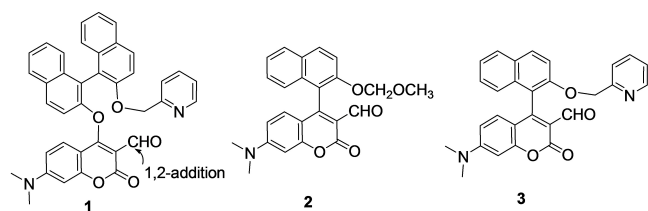
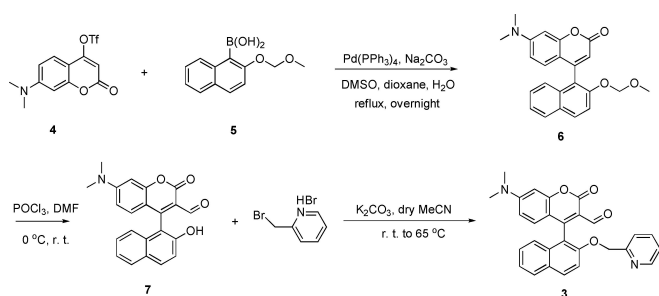


Figure 1. Previously reported fluorescent probe molecules **1** and **2** for enantioselective fluorescent recognition and the newly developed coumarin-based probe **3**.

addition of **1** with Val could generate an imine intermediate with extended conjugation between the coumarin and the naphthalene ring, which probably resulted in the highly enantioselective response at the long wavelength emission.^[7b] The first X-ray structure of a BINOL-imine-zinc(II) complex has provided significant insight into the enantioselective recognition of the BINOL-based probes toward chiral functional amines.^[14] Coumarin-based probes have been broadly applied in the fields of molecular recognition, molecular imaging, analytical chemistry, and materials chemistry due to its excellent biocompatibility, stable optical property, and good structural flexibility.^[15] More recently, we thus designed and synthesized a naphthyl-coumarin-based fluorescent probe **2** (Figure 1), which could be used as a selective Cys and glutathione (GSH) sensor by exciting at two different wavelengths, respectively, in fluorescent analysis.^[16] Herein, we report a new racemic naphthyl-coumarin-based probe **3** for quantitative enantiomeric excess analysis of amino acids and enantioselective sensing of amines and amino alcohols by using CD spectroscopic measurements.

Results and Discussion

The naphthyl-coumarin probe **3** was synthesized as shown in Scheme 1. The Suzuki cross coupling reaction of coumaryl triflate **4** with arylboronic acid **5** in the presence of Pd(PPh₃)₄ and Na₂CO₃ gave **6** in 56% yield.^[17] A mixture of POCl₃ in dry DMF was added to a solution of compound **6** in DMF to obtain compound **7** as a yellow solid in 38% isolated yield. The probe **3** was readily prepared by mixing intermediate **7** with 2-(bromomethyl)pyridine hydrobromide in the presence of K₂CO₃



Scheme 1. Synthesis of the naphthyl-coumarin probe **3**.

in dry acetonitrile, arriving at 76% isolated yield. The chemical structures of probe **3** and the intermediates were characterized by NMR spectroscopy and HRMS (see Supporting Information). Probe **3** with its axially chiral naphthyl-coumarin unit was analyzed by HPLC on a chiral OD-H column, and the result showed that probe **3** is racemic. Compound **3** can be resolved by preparative HPLC-chiral column chromatography to one of its enantiomers, which was however found to quickly racemize at room temperature.

Firstly, we measured the interaction between Val **8** and probe **3** in a KOH-MeOH solution system by conducting CD and UV spectroscopic measurements. We were delighted to observe very strong CD signals above 300 nm as shown in Figure 2. A negative Cotton effect was observed at approximately 380 nm when probe **3** reacted with L-Val (red curve in Figure 2), and the opposite result was obtained when the analytical sample was D-Val (green curve in Figure 2). The CD signal reached maximum when the L- or D-Val analyte was present in 4.0 equivalents over 5–6 h (Figure S15). No CD signal was observed above 380 nm in the absence of the probe or Val. These results indicate that the chiral CD signals depend on the configuration of Val. In addition, the two chiral CD response signals for the pair of Val's enantiomers were both preferably detected at 380 nm, which could avoid potential interferences induced by CD- and UV-Vis-active impurities.

We continued to collect the CD responses of **3** towards other common chiral amino acids **8–25** (Scheme S2 in Supporting Information). As shown in Figure S14, obvious chiral CD responses were observed above 300 nm in all examples except for proline, a cyclic amino acid, which could not undergo a condensation reaction with the aldehyde group of **3**. Almost all L-amino acids showed negative cotton effects above 300 nm, while the opposite results were obtained with D-amino acids. The trends were the same as those found for Val. Typical CD response spectra are shown in Figure 3, and very strong chiroptical signals were measured with phenylalanine (**9**), serine (**20**), alanine (**15**), and methionine (**16**).^[3] For example, the chiral CD signals depending on the configuration of Phe were amplified at 389 nm, which might indicate stronger anti-

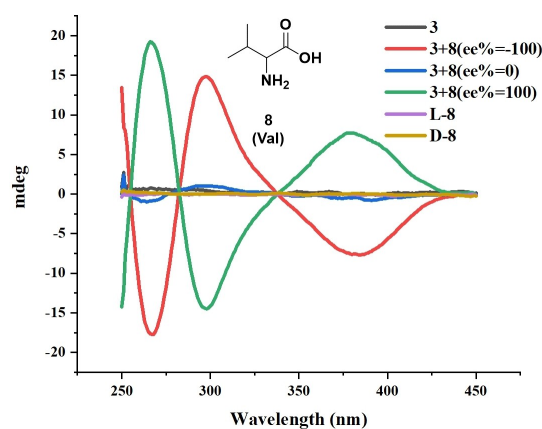


Figure 2. CD signals obtained from the reactions of the probe **3** (1.0×10^{-4} M) with *rac*- (blue), L- (red) and D-Val (green; 4.0 equiv.) at 380 nm in the KOH-MeOH system at 27 °C.

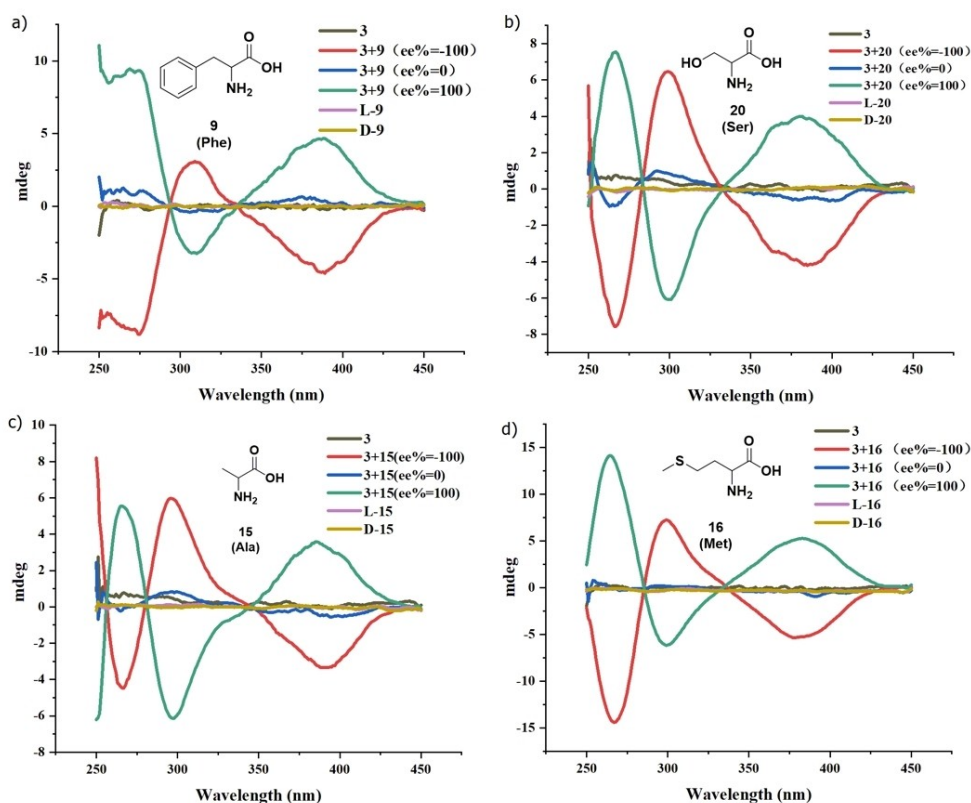


Figure 3. Selected examples of chiral amino acid sensing with the probe **3**: a) Phenylalanine, b) Serine, c) Alanine and d) Methionine (See Supporting Information for details).

interference ability for CD-active impurities compared with other CD signals at 318 nm generated by the reaction of another coumarin-based probe with Phe as previously reported in the literature.^[13]

To evaluate the feasibility of probe **3** for the determination of absolute configurations of amino acids and for ee value analysis, we acquired a series of standard CD spectra of probe **3** interacting with various enantiomeric compositions of Val. As shown in Figure 4a, the CD signals at 380 nm were directly related to the enantiomeric composition of Val (**8**). Val samples and probe **3** were used for sensing experiment analysis, and the

CD amplitudes obtained at 380 nm were plotted against the ee. As anticipated, we obtained linear responses for probe **3** (dashed blue line in Figure 4c). Then, Val samples containing various enantiomeric components were analyzed by using the linear CD response relationship between probe **3** and valine for final determination. For valine, the induced Cotton effect at 380 nm was directly related to the absolute configuration of the major enantiomer in the non-racemic sample. The enantiomeric composition of valine was accurately determined by the CD signal amplitude value at 380 nm. The equation in Figure 4c was used for data calculations, and the final results are shown

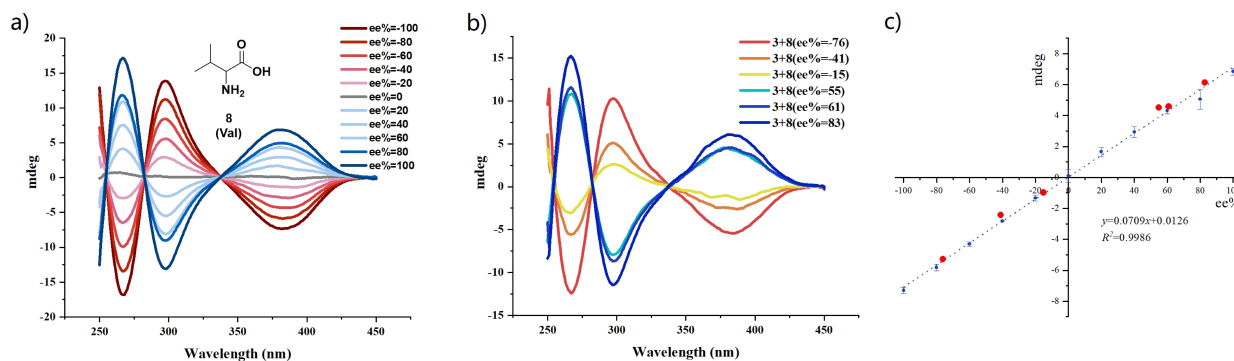


Figure 4. Chiroptical sensing of Val. a) CD response of **3** to the nonracemic sample of Val, b) CD response of **3** to samples of Val with random enantiomeric compositions, c) CD response of **3** to nonracemic samples of at 380 nm (blue) and CD signals obtained with random scalemic valine mixtures (red). Error bars represent standard deviations for three independent measurements.

in Table 1. The error margins between the calculated values and the experimental values were acceptable. For example, chiral sensing of a sample containing valine with ee value of 61% can accurately confirm that D-form was the main component, and the ee value was determined to be 64%. Probe 3 can also be used to determine the absolute configuration and ee of Phe (see Supporting Information).

We also studied the interaction of the probe 3 with aliphatic and aromatic amino alcohols and amines 26–43 (Scheme S2 in

Entry	Sample composition		Sensing results	
	Abs. config.	ee [%]	Abs. config. ^[a]	ee [%] ^[b]
1	L	76	L	75
2	D	55	D	62
3	L	41	L	35
4	D	61	D	64
5	L	15	L	14
6	D	83	D	86

[a] By comparison of the sign of the induced CD with a reference spectrum. [b] Based on the amplitude of the CD response at 380 nm.

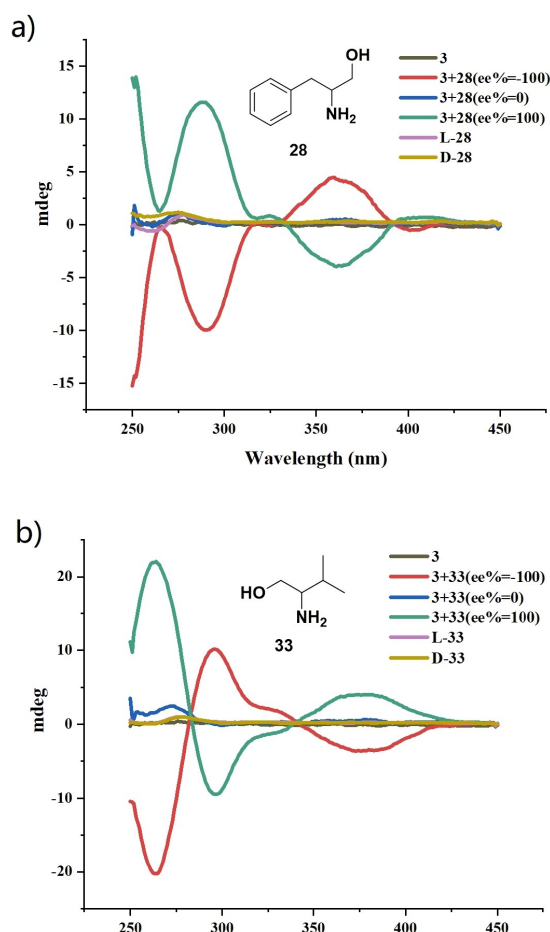


Figure 5. Representative CD spectra obtained with 3: a) Phenylalaninol and b) Valinol. The sensing reactions were conducted at 27 °C using 4.0 equiv. of amino alcohols and amines in MeOH. CD measurements were recorded after dilution with MeOH to 1.0×10^{-4} M (see Supporting Information for details).

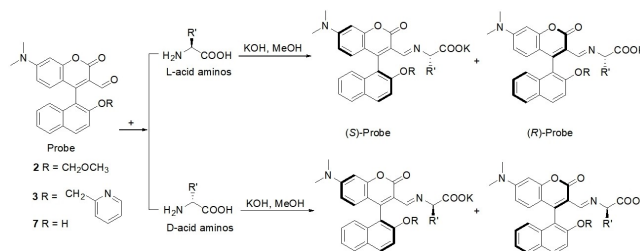
the Supporting Information). Using our simple protocol and MeOH as solvent, we observed intense CD signals in all cases (Figure S16). Representative examples of the strong CD effects obtained from the reaction of probe 3 with phenylalaninol (28) and valinol (33) are shown in Figure 5.

We hypothesized that there was a dynamic covalent reaction of the naphthyl coumarin-aldehyde probe (*S*- or *R*-configuration) with L- and D-amino acids, during which imine intermediates were produced through the aldehyde group and the amino group to induce the chiral CD signal (Scheme 2).

In order to confirm the mechanism of imine formation, we firstly compared the differences among the optical properties of these naphthyl coumarin-aldehyde probes including 2, 3 and 7 in enantioselective recognition of chiral amino acids. As shown in Figure 6, all of these probes yielded CD-silent spectra and similar UV-Vis plots. The reaction of these racemic probes with L- or D-Valine (8) induces CD signals with nearly identical symmetry dimensions (Figure 6a), and leads to achiral-related UV-Vis absorption with similar blue shift from 395 nm to 380 nm (Figure 6b). This indicates that the substitution of the hydroxyl group at the β -position of the naphthalene ring in the framework of these probes could have no obvious effect on the enantioselective recognition of chiral amino acid by using CD.

Then, we studied the reaction of 3 with D-Val-KOH by ^1H NMR spectra, HSQC spectrum and mass spectroscopic analyses. As shown in Figure 7a, when amounts of D-Val-KOH (0.5–5.0 equiv., dissolved in CD_3OD) were added to the CDCl_3 solution of probe 3, the aldehyde proton signal (10.58 ppm) of 3 gradually vanished while an imine proton signal was emerging at 8.26–8.33 ppm. Meanwhile, the H_α signal of chiral center for intermediate 3a could be found at 3.69 ppm. Correspondingly, the cross peaks with the ^{13}C NMR signal at $\delta = 160.7\text{--}160.4$ ppm for the imine carbon and $\delta = 73.7$ ppm for the chiral carbon center were observed in the HSQC spectrum after 4.0 equiv. D-Val-KOH had been added to the reaction system, which confirmed the formation of an imine product (Figure 7b). The ESI-MS spectrum of the reaction mixture is consistent with the formation of the imine product 3a as the major product with a $[\text{M}+\text{Na}]^+$ signal at $m/z = 610.1713$ (calculated $m/z = 610.1715$) as shown in Figure S23 in the Supporting Information.

We conducted computational studies on the electronic circular dichroism (ECD) of the four stereoisomers formed from the combination of one chiral center (D- and L-configuration)



Scheme 2. The postulated mechanism on the dynamic covalent reaction of the racemic naphthyl-coumarin probe (*S*- or *R*-configuration) with L- and D-amino acids.

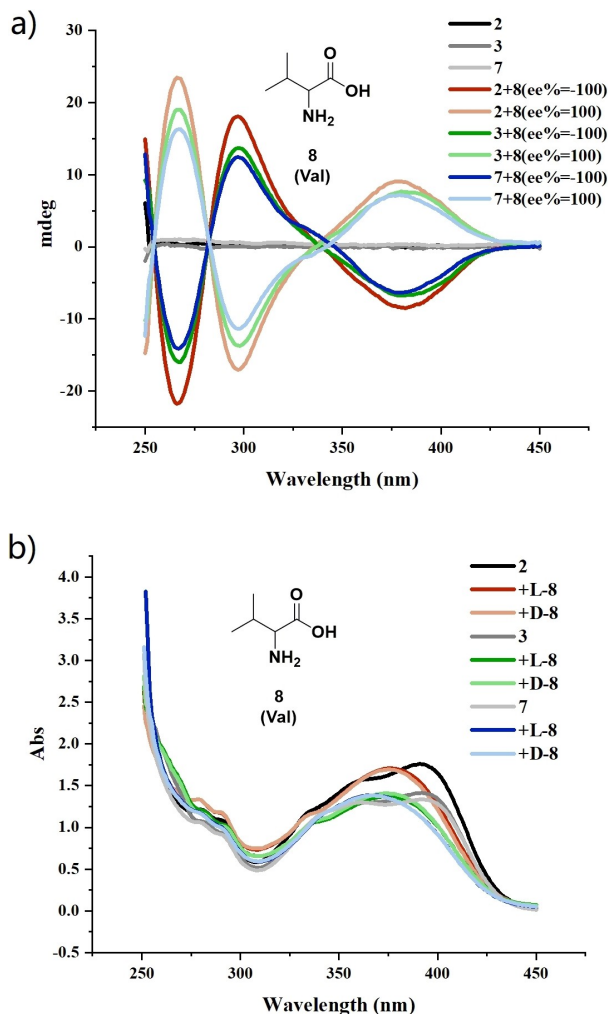


Figure 6. a) CD spectra, b) UV-Vis spectra obtained from probes 2, 3 and 7 reacting with Val (8) in the KOH-MeOH system.

along with one chiral axis (*S*- and *R*-configuration) including 3DR, 3DS, 3LR and 3LS by using Merck Molecular Force Field in Spartan's 14 software (Scheme S3 in the Supporting Information). Theoretical calculations with time-dependent DFT were conducted in methanol solution at the B3LYP/6-31 g(d) level to obtain information on the origin of the signals and the ECD spectrum of each conformation. The calculated ECD spectrum was processed according to the dominant conformation in the CD profile and labelled as Calcd, R/S-3 + L-Val(8)-KOH as shown in Figure 8. The calculated ECD results agree with the experimental data including the measured CD spectra and the assignment of configurations.

Conclusion

We designed and synthesized a novel racemic probe based on an axially chiral naphthyl-coumarin framework, which could form imines with amino acids at room temperature and induce strong CD signals around 380 nm. The induced CD responses

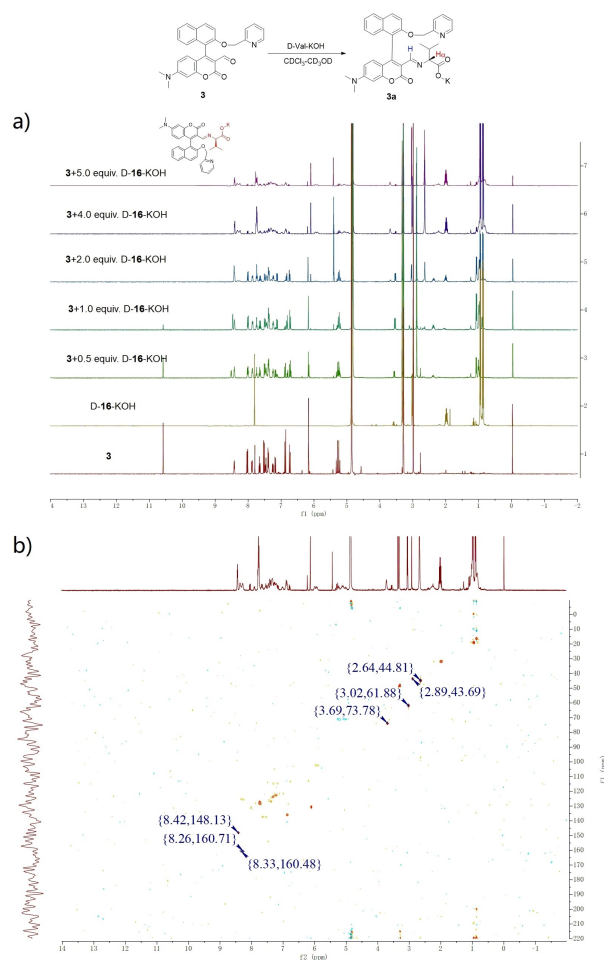


Figure 7. a) ^1H NMR spectrum of probe 3 (60 mM in CDCl_3 , 100 μL) with varying equivalents of D-Val-KOH (0.5–5.0 equiv. in CD_3OD , 500 μL). b) HSQC spectra of probe 3 (60 mM in CDCl_3 , 100 μL) with D-Val-KOH (48 mM in CD_3OD , 500 μL).

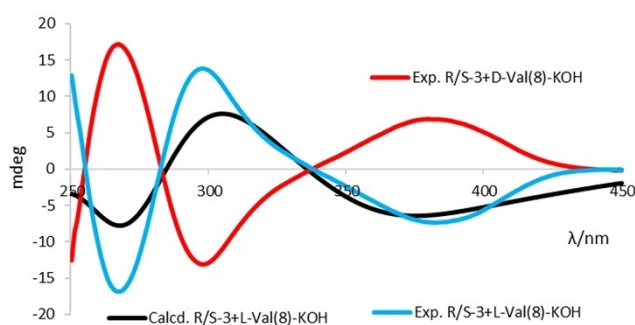


Figure 8. Comparison between the experimental CD spectra for the reaction of the probe 3 with D- or L-Val(8)-KOH and the calculated CD spectrum for the reaction of the probe 3 with L-Val(8)-KOH.

could be applied to determine the enantiomeric composition of amino acids and reflect the absolute configuration of the major enantiomer. The racemic probe is readily available and suitable for chiroptical analysis of each of the 18 standard amino acids, and 18 amino alcohols and amines. The mechanism on the imine formation from the reaction of the coumarin aldehyde

probe with the amino acid was confirmed by CD, UV-Vis, NMR, ESI-MS analyses and ECD calculation.

Experimental Section

Materials: NMR spectra were recorded on a Bruker AV II-400 spectrometer. Chemical shifts for ^1H NMR spectra were recorded in parts per million (ppm) relative to solvent signals at 7.26 ppm for CDCl_3 , 3.31 ppm for CD_3OD and 2.50 ppm for $\text{DMSO}-d_6$. Chemical shifts for ^{13}C NMR spectra were recorded relative to the centerline of a triplet at 77.23 ppm for CDCl_3 . Mass spectra were recorded on Sciex X500R Q-TOF and Agilent 6200 series TOF/6500 series Q-TOF. HPLC spectra were recorded on a Shimadzu LC-20AT spectrometer, using the chiral chromatographic column purchased from DAICEL (OD-H, 4.6 mm I.D \times 250 mL). Circular dichroism (CD) spectra were recorded on a Applied Photophysics Chirascan spectropolarimeter. The UV-Vis spectra were measured by Agilent Technologies Carry 60 UV-Vis spectrometer. CDCl_3 , CD_3OD , and $\text{DMSO}-d_6$ were purchased from Adamas. All other reagents were obtained from commercial sources and were used without further purification, unless indicated otherwise. All of the solvents were HPLC grade in the optical spectroscopic studies.

Preparation and Characterization of Probe 3: A mixture of compound **7** (81 mg, 0.23 mmol), 2-(bromomethyl)pyridine hydrobromide (175 mg, 0.69 mmol) and K_2CO_3 (160 mg, 1.15 mmol) was dissolved in dry acetonitrile (50 mL). The reaction was refluxed at 65 °C for 12 h. After cooling to room temperature, the reaction mixture was extracted with EtOAc. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. Flash chromatography of the residue through silica gel (petroleum ether: ethyl acetate = 7:1 \rightarrow 5:1 \rightarrow 4:1 \rightarrow 3:1 \rightarrow 2:1 \rightarrow 1:1) gave **3** as a yellow solid (79 mg, 76%). ^1H NMR (400 MHz, CDCl_3) δ 10.76 (d, $J=0.7$ Hz, 1H), 8.53 (ddd, $J=4.9, 1.8, 1.0$ Hz, 1H), 7.97 (d, $J=9.1$ Hz, 1H), 7.87 (dt, $J=7.5, 2.5$ Hz, 1H), 7.58 (td, $J=7.7, 1.8$ Hz, 1H), 7.52–7.48 (m, 1H), 7.45–7.39 (m, 3H), 7.18 (ddd, $J=7.7, 4.9, 1.1$ Hz, 1H), 7.15–7.12 (m, 1H), 6.91 (d, $J=9.2$ Hz, 1H), 6.61 (dd, $J=9.3, 0.8$ Hz, 1H), 6.27 (s, 1H), 5.36–5.24 (m, 2H), 3.01 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 186.4, 160.6, 159.4, 156.8, 154.6, 152.6, 152.4, 149.1, 136.9, 132.1, 131.6, 131.2, 128.9, 128.2, 127.6, 124.5, 124.2, 122.8, 120.9, 118.1, 114.3, 112.4, 112.3, 110.1, 109.9, 77.2, 71.7, 44.2. HRMS: m/z calcd. for $\text{C}_{28}\text{H}_{23}\text{N}_2\text{O}_4[\text{M} + \text{H}^+]$: 451.1652, found: 451.1655.

Quantitative Enantiomeric Excess Analysis: A stock solution containing **3** (2 mM) in MeOH was prepared and 150 μL aliquots were placed into small vials. To each vial, 150 μL of a solution of substrate (e.g. valine, 8 mM) of varied ee was added. The enantiomeric excess compositions of the substrate solutions were 100, 80, 60, 40, 20, 0, –20, –40, –60, –80, and –100 ee. Each mixture was stirred for 6 h at 27 °C prior to CD analysis. All CD measurements were taken after dilution to 1.0×10^{-4} M with MeOH.

Mechanistic Studies: The CD and UV-Vis spectra on the reaction of probes **2**, **3**, and **7** with chiral amino acids (Val, Phe, Ala, Met and Ser) were investigated in a MeOH-KOH system at 27 °C. The 1D NMR, HSQC, NOESY, and ESI-MS spectra on the reaction of probe **3** with D-Val-KOH were obtained from in $\text{CD}_3\text{OD}-\text{CDCl}_3$ solution at 27 °C. After the initial determination of the formation of imine product, ECD calculation was carried out for the possible four diastereomeric structures, and the simulated results were compared with the actual CD spectrum.

Computational Studies: Monte Carlo conformational searches were carried out by means of the Spartan's 14 software using Merck Molecular Force Field (MMFF). The conformers with Boltzmann-population of over 5% were chosen for ECD calculations, and then

the conformers were initially optimized at B3LYP/6-31 g level in gas. The theoretical calculation of ECD was conducted in MeOH using Time-dependent Density functional theory (TD-DFT) at the B3LYP/6-31 + g (d, p) level for all conformers of compounds (including **3DS**, **3DR**, **3LS** and **3LR**). For each of these compounds, rotatory strengths for a total of 30 excited states were calculated. ECD spectra were generated using the program SpecDis 1.6 (University of Würzburg, Würzburg, Germany) and GraphPad Prism 5 (University of California San Diego, USA) from dipole-length rotational strengths by applying Gaussian band shapes with $\sigma = 0.3$ eV.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: chiral amine · circular dichroism · enantioselective recognition · naphthyl-coumarin · racemic probe

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