A Rapid and Sensitive Method for Chiroptical Sensing of α-Amino Acids via Click-like Labeling with o-Phthalaldehyde and p-Toluenethiol

Bo Li, Jie Zhang, Li Li, Gong Chen

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A Rapid and Sensitive Method for Chiroptical Sensing of α-Amino Acids via Click-like Labeling with o-Phthalaldehyde and p-Toluenethiol

Bo Li,1,2* Jie Zhang,1* Li Li,1* and Gong Chen2*

1Beijing Key Laboratory of Active Substances Discovery and Druggability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100050, China
2State Key Laboratory and Institute of Elemento-Organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

ABSTRACT. A highly practical method for comprehensive chiroptical sensing of free α amino acids with streamlined operation and high sensitivity via dual CD/UV measurements is developed. The assay takes advantage of an efficient and selective three-component labeling reaction of primary amines with o-phthalaldehyde and p-toluenethiol reagents, to derivatize the NH2 group of analytes into an isoindole chromophore. The covalent labeling generates sensitive UV and CD readouts, both of which show excellent linear relationship with the concentration of analyte. The high reactivity and the novel chromogenic reporting mechanism allow fast and accurate measurement without background interference. The sensing assay works well for a remarkably broad range of analyte concentrations, with an unprecedented lower limit at 10 micromolar. We expect this method can be readily adapted for high throughput experimentation analysis using CD instrument equipped with a multwell plate reader.

The widespread use of α-amino acids (αAAs) and other chiral amine compounds in academic and industrial laboratories have generated substantial interest in developing sensitive and convenient chiral analytical methods adaptable for high-throughput experimentation (HTE).1 Due to the operational limitations of chromatographic techniques, attention has been increasingly shifted to optical methods.2-3 Over the past decade, methods for chiroptical sensing based on circular dichroism (CD) spectroscopy with small molecule sensors have been greatly advanced by the groups of Chin, Anslyn, Wolf, Pu, Joyce, Zonta and others.4-7 By exploiting the mechanisms of dynamic covalent chemistry, supramolecular assembly, and metal complexation, these methods both amplify the CD signal and report on the concentration to allow quantitative measurement of the enantiomeric composition of analytes in high accuracy. However, despite the significant progress, truly practical methods amenable to HTE remain challenging.

Among these chiroptical methods, the combination of CD and UV is probably most desirable as the measurements can be conveniently performed in a single instrument, which can be readily modified with multiwell plate reading for parallel analysis (Scheme 1A). Notably, the Wolf group reported several powerful mix-and-measure protocols using organohalide derivatizing agents (a coumarin chloride and an aryl fluoride) that selectively reacts with the amine analytes to generate CD and UV readout (Scheme 1B).7-8 However, while labeling reactions via N-substitution can proceed cleanly, they require relatively long reaction time (1-4 hours) at high reaction concentration (typically > 1 mM). Herein, we report a new method for chiroptical sensing of various α-amino acids via a click-like three-component labeling of primary amine with o-phthalaldehyde and p-toluenethiol reagents. The resulting N-fused aromatic isoindole moiety provides strong UV and CD responses, enabling a fast, sensitive, and accurate measurement across a very broad range of analyte concentration.

The key of designing dual sensors for CD/UV-based assay is to generate strong and clear readouts for both CD and UV. While a variety of sensing mechanisms have been successfully exploited to amplify the CD signal of αAA, methods for simultaneous UV amplification with high sensitivity is surprisingly limited. The three-component condensation reaction between the amino group of αAA, o-phthalaldehyde (oPA), and alkyl thiol reagents such as 2-mercaptopropane 3a or 3-mercapto propanoic

Scheme 1. CD/UV-based quantitative chiroptical sensing of αAA with organic probes.

A) Mix-and-measure protocol using dual CD/UV measurements

B) Click-like covalent labeling of chiral amines with reporter probes

UV: clean chromogenic readout at 335 nm
CD: strong chiroptical response

Isodole via fusion of N into aromatic ring

* Fast & simple operation (negligible waiting time)
* High sensitivity & accuracy
* Broad range of working concentration (10 μM - 8 mM)
* Simple data processing (linear relationship for UV & CD)
* Inexpensive commercially available reagents

Scheme 1. CD/UV-based quantitative chiroptical sensing of αAA with organic probes.
three a cent with sorption a isoindole product were measured at 0.285 mM

Figure 1

B) Reaction characterization\(^a\)

LC-MS analysis of reaction with 3e (1 min of reaction time)

C) Stereochemical analysis and representative computed conformers of 5e\(^b\)

Figure 1. Labeling of L-Ala with OPA and thiois for chiroptical sens-
ging by CD/UV. a) Reaction mixture was diluted 7 times, samples were measured at 0.285 mM for CD and UV. b) DFT calculations were performed at the M062X/6-311+G(d,p) level, see SI for details.

acid 3b has long been used to derivatize \(\alpha\)AA for HPLC analysis (Figure 2A).\(^{9,11}\) The OPA labeling reactions can proceed cleanly and quickly under mild conditions to form 1-thiolate substituted isoindole product 5. The isoindole moiety has a strong UV absorption around 335 nm, offering a clean chromatographic readout with little background inference from \(\alpha\)AA and the labeling reagents. Its emission around 450 nm has also been used for fluorescent detection. Encouraged by their favorable UV properties, we questioned whether the N-fused isoindole chromophore on \(\alpha\)AA can induce useful CD signal for chiroptical sensing. The three-component condensation reaction is believed to proceed through a cyclic hemiaminal intermediate 4, which upon dehydration gives the heteroaromatic product. We commenced the investigation with the reaction of model \(\alpha\)AA L-alanine 1 and stoichiometric mixture of OPA and different thiois. To our delight, the isindolyl derivatives of Ala indeed exhibited excellent CD response around 335 nm with \(\beta\)-toluenethiol 3e giving the strongest signal among the thiol reagents tested. 3e is a commercially available solid compound and has a much weaker odor than the liquid alkyl thiols. The UV spectrum of the reaction mixture showed a distinct absorption at 335 nm (Figure 1B). As indicated by LC-MS and UV measurements, 1.2 equiv of 3e and 2 reacted cleanly with 1 equiv of 1 at 2 mM in the mixed solvents of MeOH and phosphate buffer (pH 9) (1/2) at room temperature (rt) under air atmosphere to give product 5e in >95% conversion in 1 min. Notably, the use of excess amount of 2 and 3e (e.g. 3 equiv) have negligible impact on the readout of CD and UV at 335 nM (Figure 1B). The reaction mixture was diluted for CD or UV measurement. UV and LC-MS analyses showed that product 5e is stable over a period of 12 hours.\(^{12}\) The UV and CD spectrum of 5e is not strongly affected by the solvents (see SI for assaying spectra using other organic solvents). Aqueous MeOH medium is preferred for its excellent solubilizing ability for \(\alpha\)AA and lack of interference for UV and CD measurements.

As shown in Figure 1C, our preliminary structural and spectroscopic analysis using time-dependent density functional theory (TDDFT) calculations showed the tolyl group of (S)-5e could be positioned below (type i conformers) or above (type ii conformers) the isoindole plane, forming opposite relative stereochemical arrangement of the two aryl groups along the C₁-S bond. Both types i and ii prefer near-eclipse conformations to the Cα-H bond and adopt syn and anti to the C₁-S bond respectively. Type i and ii conformers give opposite Cotton effects (CE) according to the electronic circular dichroism (ECD) simulation. Type i conformers are more stable than type ii conformer; i and ii roughly represent 70% and 30% in the conformational equilibrium mixture respectively. The overall ECD spectrum of (S)-5e matched well with its experimental data, which showed negative CE at 335 nm (See SI for details).

The new protocol was next applied to sense other optically pure proteinogenic \(\alpha\)AA and primary alkylamine samples (Figure 2A). Typically, 1 equiv of the analytes and 1.2 equiv of 2 and 3e were mixed in MeOH/buffer at 0.5-8 mM concentration for 1 min. In practice, the assay can be performed without any deliberate aging. All \(\alpha\)AAs except Cys and Pro showed a similar profile and sensitivity in both CD and UV spectra. CD and UV spectra of selected \(\alpha\)AAs are shown in Figure 2B. Pro 26 cannot form the corresponding isoindole product due to its secondary amine group. The reaction of Cys 25 mainly formed a tricyclic isoindole product 25p via the intramolecular addition of the SH side chain along with two dimeric side products (see SI). The reaction of Lys 20 with 1.2 equiv of 2 and 3e gave a mixture of mono- and bis-labeled products (e.g. 20p) at the \(\alpha\) and e amino positions (see SI for details). Side chains such as CO₂H (Glu, Asp), CONH₂ (Gln, Asn), OH (Ser, Thr, Tyr), guanidine (Arg), imidazole (His) did not interfere with the condensation reaction. Reactions of nonproteinogenic \(\alpha\)AA such as 21 gave similar results. Ala methyl ester 23 gave almost identical CD readout to Ala 1. Assaying of alaninol 24 and 1-phenylethylamine 22 gave slightly
A) αAA and other chiral amines

- Successful analytes
  - R
  - N
  - CO₂H
  - Val 6
  - Ile 7
  - Leu 8
  - Met 9
  - Ser 10
  - Thr 11

- Problematic analytes
  - Arg 19
  - Lys 20
  - H₂N
  - Asp 12
  - Asn 13
  - Glu 14
  - Phe 15
  - Tyr 16
  - Trp 17
  - His 18

- Unusual product
  - Cys 25
  - Pro 26

B) Selected CD and UV spectra

C) Test of Ala at low concentration

Figure 2. Substrate scope at normal concentration range. Standard assaying conditions (1.2 equiv of reagents, 1 min). a) See SI for LC-MS analysis of reaction mixture. b) Samples were measured at the concentration specified.

weaker CD signal (~40%) in comparison with Ala 1.

As shown in Figure 3A, the UV absorption at 335 nm shows an excellent linear relationship with the concentration of Trp between 0.5 and 8 mM (All samples over 0.5 mM were diluted for CD and UV measurement). Moreover, the CD signals at 335 nm also showed an excellent linear relationship with the enantiomeric excess (ee) of Trp samples assayed at 2 mM using the standard protocol with 1.2 equiv of oPA and 3e. As shown in Figure 3B, plots of the g value of CD vs ee% for representative ααAs Leu 8, Glu 14, His 18, and Thr 11 measured at 0.33-1 mM showed an excellent linear relationship. The plot of UV absorption of Lys vs concentration showed a slight derivation from the linear relationship for Lys probably due to the formation of mixed labeling products (see SI). However, the plot of its CD vs ee% is still linear.

Chiroptical sensing of analytes at low concentration remains a difficult challenge for the existing methods due to low labeling reactivity and high background noise. We were pleased to find that our method worked well for αAA sample below 300 µM under slightly modified conditions with excess amount of oPA and 3e (~100 equiv) and 3 min of mixing time. As exemplified by the test of Ala, the UV signal at 335 nm showed excellent linear relationship with the concentration between 5 and 400 µM (Assaying samples below 0.5 mM were measured without dilution). Moreover, g value of CD measurement at 10 µM showed excellent linear relationship with the ee% value. Notably, excess...
amounts of reagents had negligible impact to the CD and UV signals at 335 nM, due to the unique signal amplifying mechanism of this method. Sensing of Trp sample at 10 µM gave similar sensitivity (see SI).

As shown in Table 1, our assay was next subjected to the tests of representative Ala and Trp samples of varied concentration and enantiomeric ratios. The simple linear relationships greatly simplified the calculation of the concentration and ee value from UV and CD measurements. In all cases, the absolute configuration of the major enantiomer was correctly assigned. The measured data of concentration and ee values were mostly within 5% error of actual value at both high (8 mM) and low (10 µM) assaying concentration, which are sufficiently accurate for HTE.

Table 1. Chiroptical sensing of Ala and Trp samples at varied concentration and ee.

| Sample | Conc. | ee % | Conf. | Conc. | ee % |
|--------|-------|------|-------|-------|------|
| 1 Ala-L | 2.00 mM | -100.0 | L | 1.91 mM | -98.7 |
| 2 Ala-D | 7.00 mM | 37.1 | D | 6.87 mM | 38.2 |
| 3 Trp-L | 7.00 mM | -100.0 | L | 7.10 mM | -103.4 |
| 4 Trp-D | 3.50 mM | 42.8 | D | 3.57 mM | 43.9 |
| 5 Ala-L | 4.00 µM | -60.0 | L | 3.92 µM | -61.2 |
| 6 Ala-D | 10.0 µM | 100.0 | D | 9.5 µM | 99.6 |
| 7 Trp-L | 21.0 µM | -65.0 | L | 22.0 µM | -67.2 |
| 8 Trp-D | 3.80 µM | 79.0 | D | 35.8 µM | 77.5 |

In summary, we have developed a highly practical method for comprehensive chiroptical sensing of free α amino acids which gives high sensitivity via dual CD/UV measurements using a single instrument. The click-like covalent labeling reaction rapidly fuses the NH$_2$ group of analytes into a N-heteroaromatic isoindole chromophore, which amplifies both UV and CD signals of amines with excellent linear relationship to the concentration. The high reactivity and the novel chromogenic reporting mechanism of the labeling reaction allow fast and accurate measurement with negligible aging time and background interference. The sensing assay works well for a remarkably broad range of analyte concentration with an unprecedented lower limit at 10 nM. The sensing assay is simple, robust and cheap. We expect this method can be readily adapted for high throughput experimentation analysis using CD instrument equipped with multiwell plate reader.

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Corresponding author

*Email: annaleelin@imm.ac.cn, gongchen@nankai.edu.cn.

ORCID

Li Li: 0000-0002-9496-2280
Gong Chen: 0000-0002-5067-9889

Notes

# These authors contributed equally to this work. The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures and spectroscopic data for all new compounds. Computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

References:

1. Selected review of AA sensing: (a) Zhou, Y.; Yoon, J. Recent Progress in Fluorescent and Colorimetric Chemosensors for Detection of Amino Acids. Chem. Soc. Rev. 2012, 41, 52–67. (b) Turner, A. P. F. Biosensors: Sense and Sensibility. Chem. Soc. Rev. 2013, 42, 3184–3196. (c) Tsukamoto, M.; Kagan, H. B. Recent Advances in the Measurement of Enantiomeric Excesses. Adv. Synth. Catal. 2002, 344, 453–463. (d) Pu, L. Angew. Chem. Int. Ed. Enantioselective Fluorescent Recognition of Free Amino Acids: Challenges and Opportunities. DOI: 10.1002/anie.202003969.

2. Selected reviews on optical sensing: (a) Leung, D.; Kang, S. O.; Anslyn, E. V. Rapid determination of enantiomeric excess: a focus on optical approaches. Chem. Soc. Rev. 2012, 41, 448–479. (b) Wu, J.; Kwon, B.; Liu, W.; Anslyn, E. V.; Wang, P.; Kim, J. S. Chromogenic/Fluorogenic Ensemble Chemosensing Systems. Chem. Rev. 2015, 115, 7893–7943. (c) Herrera, B. T.; Pilicer, S. L.; Anslyn, E. V.; Joyce, L. A.; Wolf, C. Optical Analysis of Reaction Yield and Enantioselective Fluorescence. A New Paradigm Ready for Prime Time. J. Am. Chem. Soc. 2018, 140, 10385–10401. (d) Li, Z.; Askim, J. R.; Suslick, K. S. The Optoelectronic Nose: Colorimetric and Fluorescent Sensor Arrays. Chem. Rev. 2019, 119, 231–292.

3. Selected examples of fluorescence sensing of AA: (a) Mei, X.; Wolf, C. Determination of Enantiomeric Excess and Concentration of Unprotected Amino Acids, Amines, Amino Alcohols, and Carboxylic Acids by Competitive Binding Assays with a Chiral Scandium Complex. J. Am. Chem. Soc. 2006, 128, 13326–13327. (b) Huang, Z.; Yu, S.; Wen, K.; Yu, X.; Pu, L. Zn(II) promoted dramatic enhancement in the enantioselective fluorescence recognition of functional chiral amines by a chiral aldehyde. Chem. Sci. 2014, 5, 3457–3462. (c) Shcherbakova, E. G.; Minami, T.; Brega, V.; James, T. D.; Anzenbacher, P., jr. Determination of Enantiomeric Excess in Amine Derivatives with Molecular Self-Assemblies. Angew. Chem., Int. Ed. 2015, 54, 7130–7133. (d) Wen, K.; Yu, S.; Huang, Z.; Chen, L.; Xiao, M.; Yu, X.; Pu, L. Rational Design of a Fluorescent Sensor to Simultaneously Determine Both the Enantiomeric Composition and the Concentration of Chiral Functional Amines. J. Am. Chem. Soc. 2015, 137, 4517–4524. (e) Shcherbakova, E. G.; Brega, V.; Minami, T.; Sheykhi, S.; James, T. D.; Anzenbacher, P., jr. Toward Fluorescence-Based High-Throughput Screening for Enantiomeric Excess in Amines and Amino Acid Derivatives. Chem. – Eur. J. 2016, 22, 10074–10080.

4. Wolf, C.; Bentley, K. W. Chirality sensing using stereodynamic probes with distinct electronic circular dichroism output. Chem. Soc. Rev. 2013, 42, 5408–5424.

5. Selected examples of fluorescence/CD sensing assays s: (a) Bentley, K. W.; Wolf, C. A Stereodirective Chemosensor with Selective Circular Dichroism and Fluorescence Readout for In Situ Determination of Absolute Configuration, Enantiomeric Excess and Concentration of Chiral Compounds. J. Am. Chem. Soc. 2013, 135, 12200–12203. (b) Bentley, K. W.; Nam, Y. G.; Murphy, J. M.; Wolf, C. Chirality Sensing of Amines, Diamines, Amino Acids, Amino Alcohols, and α-Hydroxy Acids with a Single Probe. J. Am. Chem. Soc. 2013, 135, 18052–18055. (c) Bentley, K. W.; Wolf, C. Comprehensive Chirality Sensing: Development of Stereodirective Probes with a Dual (Chir)optical Response. J. Org. Chem. 2014, 79, 6517–6531. (d) Pilicer, S. L.; Bakhshi, P. R.; Bentley, K. W.; Wolf, C. Biomimetic Chirality Sensing with Pyridoxal-5′-phosphate. J. Am. Chem. Soc. 2017, 139, 1758–1761.

6. Selected examples of UV/CD sensing assays: (a) Huang, X.; Rickman, B. H.; Borhan, B.; Berova, N.; Nakamichi, K. Zinc porphyrin tweezer in host-guest complexation: determination of absolute configurations of diamines, amino acids, and amino alcohols by circular dichroism. J. Am. Chem. Soc. 1998, 120, 6185–6186. (b) Folmer-
CHO

MeOH/buffer (pH 9) (1:2)
25 °C, 1-3 min

N

R*

H

SH

CHO

H₂N

CO₂H

(1 : 1)

chromogenic isoindole with sensitive UV & CD readouts

Simple: mix-and-measure
Fast: little waiting time
Accurate: < 5% error
Sensitive: as low as 10 µM
Supporting Information

A Rapid and Sensitive Method for Chiroptical Sensing of α-Amino Acids via Click-like Labeling with o-Phthalaldehyde and p-Toluenethiol

Bo Li,1,2# Jie Zhang,1# Li Li,1* and Gong Chen2*

1Beijing Key Laboratory of Active Substances Discovery and Druggability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100050, China
2State Key Laboratory and Institute of Elemento-Organic Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

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1. Reagents and Instrument

Unless otherwise noted, chemicals and solvents were purchased from Sigma Aldrich, J&K Chemical, or Energy Chemical and were used without further purification. UPLC-MS analyses were performed with a Dionex UltiMate 3000 connected to a thermo scientific MSQ PLUS mass spectrometer using a Thermo Scientific C18 (1.9 μm, 2.1 × 100 mm) analytical column. Flash chromatography columns were packed with 300–400 C18 packing material with MeOH and H2O as eluents. ¹H and ¹³C NMR data were recorded using a Varian Mercury (500MHz) spectrometer with TMS as an internal standard. HRMS data were collected on a Thermo Exactive plus Orbitrap mass spectrometer. ECD spectra were recorded on a Jasco J-815 spectrometer.

2. Synthetic procedures and structural elucidation

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CHO} \\
\text{L-Ala 1} & + \quad \text{CHO} \\
& \quad \text{SH} \\
& \quad \text{MeOH/PB (pH=9)} \\
& \quad 1 \text{ min, rt} \\
& \quad \text{5e}
\end{align*}
\]

To a solution of L-Ala (1) in MeOH-phosphate buffer (PB) (1 : 2, pH = 9), p-toluenethiol (3e, 1.2 equiv) and o-phthaladehyde (2, 1 equiv) were added at room temperature without avoiding light and air. Being shaken for 1 min, the reaction mixture was directly purified by flash column chromatography packed with 300–400 C18 packing material (methanol-water 20 : 80 with 0.5% HCOOH as eluents) to afford 5e as freeze-dried yellowish powder (28 mg, 90%).

\[1^1\text{H NMR (500 MHz, DMSO-}d_6\text{)} \delta 7.79 (s, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 8.5 Hz 1H), 6.99 (d, J = 8.0 Hz, 2H), 6.96–6.93 (m, 1H), 6.91–6.88 (m, 1H), 6.80 (d, J = 8.0 Hz, 2H), 5.09-5.16 (m, 1H), 2.18 (s, 3H), 1.52 (d, J = 7.5 Hz, 3H).\]

\[1^3\text{C NMR (125 MHz, DMSO-}d_6\text{)} \delta 172.36, 136.05, 134.53, 129.67, 129.30, 125.46, 123.50, 121.94, 120.64, 120.23, 118.32, 115.78, 104.26, 57.85, 21.13, 20.48.\]
$^1$H NMR analysis of the process of three-component reaction

Supplementary Figure 1. 1 (1.0 equiv), 2 (1.0 equiv), 3e (1.2 equiv) and NaOH (1.1 equiv) were mixed in 3 mL D$_2$O/CD$_3$OD (1:1) at 10.0 mM for 1 min, after which 500 uL reaction solution was taken out for $^1$H NMR test.

3. Optimization of sensing procedure

Base optimization

Comparison of CD signals of L-Ala using different bases at 2 mM, diluted to 1 mM for test with a pathlength of 1 mm.
Supplementary Figure 2. After screening of bases, it was found that bases had negligible influence for the sensing procedure.

Solvent optimization

Comparison of CD and UV signals of L-Ala in MeOH/PB at 2 mM, diluted to 0.285 mM for test.

Supplementary Figure 3. Mixed solvents gave the better results in CD and UV signals, so we chose PB:MeOH=(1-3) :1 (v/v) as the optimal solvent. Because the isoindole products formed by different amino acids may differ in solubility, the ratio of PB and MeOH was adjusted to guarantee the three-component reaction to proceed well.

Comparison of CD and UV signals of L-Ala in different mixed solvents at 2 mM, diluted to 0.285 mM for test.
Supplementary Figure 4. ACN= acetonitrile; IPA= isopropanol; n-PA= n-propanol
As shown in Figure 4, the UV and CD spectra of 5e were only slightly influenced by the reaction solvents. Among these assays, it was found that both H₂O/MeOH and PB/MeOH could give nearly identical results. Aqueous MeOH medium is relatively favorable because of its better solubilizing ability.

4. Stability test

CD spectra of L-Ala reacting with 2 and 3e over time

Supplementary Figure 5. The reaction was performed in MeOH/PB (1:2) at 2 mM, diluted to 0.285 mM for CD test.

UPLC traces of L-Ala reacting with 2 and 3e over time
Supplementary Figure 6. All the reactions were carried out at 5.0 mM. $t_{5e} = 9.21$ min, $\lambda = 220$ nm. LC-MS analysis showed that product 5e was stable over a period of 12 hours.

HRMS of 5e

HRMS: Calcd for $C_{18}H_{18}NO_2S$ [M+H]^+: 312.1053; found: 312.1058
5. Chiroptical sensing of various analytes

All CD spectra were measured on a JASCO J-815 automatic spectrometer (Tokyo, Japan). The mixture solvent of methanol and PB or water was used as a blank and automatically subtracted from the samples during scanning. Data were recorded from 250 to 400 nm with a scan speed of 100 nm/min and data pitch of 0.5 nm, a bandwidth of 1 nm, a response of 1 s. The reaction mixture was diluted to suitable concentration for the test. The pathlength was 1 mm and 10 mm, for high concentration (80 - 2000 μM) and low concentration (5- 400 μM), respectively. The measurements were repeated twice. OPA and p-Toluenethiol were dissolved in methanol as stock solution and were used without avoiding light and air for sensing procedure.

Due to chiroptical sensing of various AAs at relatively high concentration (> 500 μM), for assaying we used the standard condition: mixing AAs (1.0 equiv), 2 (1 equiv) and 3e (1.2 equiv) at r.t. for 1 min. For AAs at relatively low concentration (< 500 μM), the reaction time was 3 min, and 2 and 3e were excess to promote the reaction.

5.1 Successful analytes

![CD spectra of 6e](image)

All CD spectra of 6e were obtained at 0.33 mM
All CD spectra of 7e were obtained at 0.33 mM.

All CD spectra of 8e were obtained at 0.33 mM.
All CD spectra of 9e were obtained at 0.33 mM.

All CD spectra of 10e were obtained at 0.33 mM.
All CD spectra of 11e were obtained at 0.33 mM.

All CD spectra of 12e were obtained at 0.33 mM.
All CD spectra of 13e were obtained at 0.33 mM.

All CD spectra of 14e were obtained at 0.67 mM.
All CD spectra of 15e were obtained at 0.33 mM.

All CD spectra of 16e were obtained at 0.33 mM.
All CD spectra of 18e were obtained at 0.33 mM.

All CD spectra of 19e were obtained at 0.33 mM.
All CD spectra of 20e were obtained at 0.33 mM (MeOH:PB=2:1).

All CD spectra of 21e were obtained at 1.33 mM.
CD spectra of 22e was obtained at 0.33 mM (one enantiomer shown).

CD spectra of L-Cysteic acid was obtained at 0.33 mM (one enantiomer shown).
CD spectra of **L-3,3-dipheny-Ala** was obtained at 0.33 mM (one enantiomer shown).

All CD spectra of **23e** were obtained at 0.285 mM (MeOH : H₂O=3:1, a small amount of PB was used to dissolve Ala-OMe).
Comparison of CD signals of L-Ala and 23 (L-Ala-OMe) at 0.285 mM (MeOH : H₂O=3:1) and 23e gave the same signal compared with L-Ala at 335 nm.

All CD spectra of 24e were obtained at 0.285 mM (MeOH : H₂O=3:1, without base)
Comparison of CD signals of L-Ala and 24 (L-Alaninol) at 0.285 mM and 24e gave weaker CD signal (~40%) compared with L-Ala at 335 nm, which indicated the importance of the carboxylate group.

UPLC-HRMS analysis of the reaction of 24e

Supplementary Figure 7. UPLC trace of the reaction of L-Alaninol at 1 min using MeOH/H₂O (3:1) as solvent (without adding base)

Note: This reaction also proceeded quickly, cleanly and completely monitored by LC-MS, which indicated that the weaker CD signal of alaninol compared with Ala was due to the lack of carboxylate group.
HRMS of 24e

HRMS: Calcd for C_{18}H_{20}NO [M+H^+]: 298.1260; found: 298.1270

5. 2 Problematic analytes

All CD spectra of 25e were obtained at 0.5 mM
All CD spectra of 26e was obtained at 1.33 mM.

Chiroptical sensing of Pro cannot display any CD signals because its secondary amino group cannot form the corresponding isoindole product.

5.3 Elucidation of unusual products

Comparison of the influence of 3e for CD signals of L-Cys.

All CD spectra were obtained at 0.5 mM.

The CD signals show that 3e has small influence for the chiroptical sensing of L-Cys. Therefore, 3e may not take part in the actual reaction and we reason that the CD signals ought to be induced by the reaction of oPA and L-Cys.
UPLC-HRMS analysis of the reaction of Cys

**Supplementary Figure 8.** UPLC trace of the reaction of Cys at 1 min using the standard condition (without 3e).

**Note:** Due to the stronger nucleophilicity of SH side chain, the reaction of Cys preferred to forming tricyclic isoindole product. It is worth mentioning that the reaction system is not clean on the LC-MS spectrum and the tricyclic isoindole product 25 wasn’t stable as the time went on (decomposed within 10 min). Therefore, we couldn’t separate this product and its proposed structure was only elucidated by HRMS and the disparate UV (see Figure 2B) and CD signals.

**HRMS:** Calcd for C_{11}H_{10}NO_{2}S [M+H^+]:220.0427; found: 220.0422
**UPLC-HRMS analysis of the reaction of Lys (20)**

**Supplementary Figure 9.** UPLC trace of the reaction of Lys at 1 min using the standard condition.

**Note:** Due to the nucleophilicity of ε-NH₂ at side chain, the reaction of Lys produced a mixture of mono- and bis-labeled products which were determined by HRMS. We reasoned that the isoindole structure forming at the ε-amino terminal will not have Cotton effect when compared with α-amino position. So mono-labeled product at ε-NH₂ made negligible impact on CD signals which was buttressed by its linear relationship between g-factor and ee (see Figure 3B). But the bis-labeled product actually had a slight influence on its UV absorption of Lys vs concentration.
HRMS of mono-labeled products

**HRMS:** Calcd for C_{21}H_{25}N_{2}O_{2}S [M+H^{+}]:369.1631; found: 369.1620

HRMS of bis-labeled product

**HRMS:** Calcd for C_{36}H_{35}N_{2}O_{2}S_{2} [M+H^{+}]:591.2134; found:591.2105
6. Test of typical amino acids

6.1 Ala

UV absorption of Ala at 1-8 mM (six times dilution to 0.167-1.33 mM for test).

Linear relationship between UV absorption at 335 nm and the concentration of Ala

Chiroptical sensing of Ala at varying ee’s.

All CD spectra of were obtained at 2 mM (seven times dilution to 0.285 mM for test).
Linear relationship between g-factor and ee of Ala samples measured at 335 nm

6.2 Glu

UV absorption of Glu at 0.5-8 mM (six times dilution to 0.083-1.33 mM for test).
Linear relationship between UV absorption at 335 nm and the concentration of Glu

Chiroptical sensing of Glu at varying ee’s.

All CD spectra were obtained at 2 mM (three times dilution to 0.67 mM for test).
Linear relationship between $g$-factor and ee of Glu samples measured at 335 nm has shown in Figure 3B.

UV absorption of Leu at 0.5-8 mM (six times dilution to 0.083-1.33 mM for test).

6.3 Leu

Linear relationship between UV absorption at 335 nm and the concentration of Leu.
Chiroptical sensing of Leu at varying ee’s.

All CD spectra of were obtained at 2 mM (four times dilution to 0.50 mM for test).

Linear relationship between g-factor and ee of Leu samples measured at 335 nm has shown in Figure 3B

6.4 His

UV absorption of His at 0.5-8 mM (six times dilution to 0.083-1.33 mM for test).
Linear relationship between UV absorption at 335 nm and the concentration of **His**

Chiroptical sensing of **His** at varying ee’s.

All CD spectra were obtained at 2 mM (six times dilution to 0.33 mM for test).
Linear relationship between g-factor and ee of His samples measured at 335 nm has shown in Figure 3B

6.5 Thr

UV absorption of Thr at 0.5-8 mM (six times dilution to 0.083-1.33 mM for test).

Linear relationship between UV absorption at 335 nm and the concentration of Thr
Chiroptical sensing of Thr at varying ee’s.

All CD spectra were obtained at 2 mM (two times dilution to 1 mM for test).

Linear relationship between g-factor and ee of His samples measured at 335 nm has shown in Figure 3B.

6.6 Lys

UV absorption of Lys at 0.5-8 mM (six times dilution to 0.083-1.33 mM for test).

MeOH/PB=2:1 as solvent
Linear relationship between UV absorption at 335 nm and the concentration of Lys.

Chiroptical sensing of Lys at varying ee’s.

All CD spectra were obtained at 2 mM (six times dilution to 0.33 mM for test).

MeOH/PB=2:1 as solvent.
Linear relationship between g-factor and ee of Lys samples measured at 335 nm has shown in Figure 3B

6.7 Trp

UV absorption of Trp at 5-320 μM without dilution for test

Linear relationship between UV absorption at 335 nm and the concentration of Trp
Chiroptical sensing of Trp at varying ee’s.

All CD spectra of were obtained at 10 μM without dilution for test

Linear relationship between g-factor and ee of Trp samples measured at 335 nm
7. DFT calculations for stereochemical analysis

Preliminary conformational search of (S)-5e was performed in the MMFF94 molecular mechanics force field via the MOE software package. Twelve conformers were identified within an energy window of 6 kcal/mol, and were further optimized using density functional theory (DFT) and M06-2X hybrid functional at the 6-311+G(d,p) basis set level. These conformers degenerated to seven real minima of potential energy surface and no vibrational imaginary frequencies were found. Boltzmann populations of all conformers were calculated according to their relative free energies (ΔG) from the M06-2X/6-311+G(d,p) approach. Solvent effects were considered by adopting polarizable continuum model (PCM) and the solvation model based on density (SMD) for water. One hundred lowest electronic transitions were obtained for all the conformers using B3LYP/6-311+G(d,p) approach. The overall ECD spectra were then generated at the bandwidth of 0.3 eV according to the Boltzmann weighting of each conformer using SpecDis1.71 software. All quantum computations were carried out by using Gaussian 16 program package.

Table S1. Main conformers of (S)-5e in water and their Boltzmann distribution

| Conformers | ΔG (kcal/mol)a | P (%)a | ΔG (kcal/mol)b | P (%)b |
|------------|----------------|--------|----------------|--------|

S36
| Image | Value 1 | Value 2 | Value 3 | Value 4 |
|-------|---------|---------|---------|---------|
| ![Image](image1) | 0.00    | 49.19   | 0.00    | 30.29   |
| ![Image](image2) | 0.49    | 21.69   | 0.72    | 8.94    |
| ![Image](image3) | 0.71    | 14.82   | 0.23    | 20.42   |
| ![Image](image4) | 1.06    | 8.19    | 0.60    | 11.08   |
| ![Image](image5) | 1.49    | 3.97    | 0.96    | 6.01    |
| ![Image](image6) | 2.07    | 1.50    | 0.16    | 23.07   |
| ![Image](image7) | 2.56    | 0.65    | 3.03    | 0.18    |

a) using the SMD/water/B3LYP/6-311+G(d,p)/M06-2X/6-311+G(d,p) approach; b) using the PCM/water/B3LYP/6-311+G(d,p)/M06-2X/6-311+G(d,p) approach.
**Supplementary Figure 10.** Comparison of experimental and calculated ECD spectra of 5e.

[1] MOE2009.10, Chemical Computing Group Inc.
[2] T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Pecitelli, SpecDis version 1.71, Berlin, Germany, 2017, https://specdis-software.jimdo.com.
[3] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian 16, Revision B.01, Gaussian, Inc., Wallingford CT, 2016.
