JOC The Journal of Organic Chemistry

pubs.acs.org/joc Article

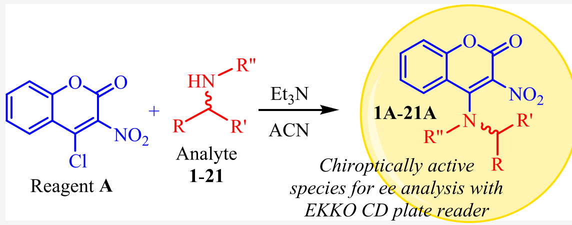
High-Throughput Determination of Enantiopurity by Microplate Circular Dichroism

Samantha L. Pilicer, Justin M. Dragna, Adam Garland, Christopher J. Welch, Eric V. Anslyn,* and Christian Wolf*

Cite This: https://dx.doi.org/10.1021/acs.joc.0c01395 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Methods for the rapid determination of enantiomeric excess (ee) in asymmetric synthetic methodology development are increasingly in demand as high-throughput experimentation protocols in academia and industry are adopted. Optical approaches have been reported, many of which rely on the use of chemical derivatization or molecular assemblies, resulting in UV/vis, fluorescence, or circular dichroism (CD) signals that report the ee values. While UV/vis and fluorescence approaches benefit from readily available 96- and 384-well plate readers, until recently, no CD plate readers existed. Herein, we report the utility of using the EKKO CD plate reader to analyze a chlorocoumarin amine derivatization methodology for the ee determination of a diverse set of chiral amines with an error margin within ±7%. Linear calibration curves of ee versus CD responses for each amine were obtained, the minimum detectable and quantifiable ee values were calculated, the technique was applied to an asymmetric hydrogenation, and various interferents expected to be present in crude samples are explored. The technique described herein is found to be suitable for high-throughput experimentation that requires a parallel and rapid ee determination step.



■ INTRODUCTION

Rapid and accurate measurement of enantiopurity is a fundamental requirement in the fields of asymmetric synthesis and catalysis and for many ancillary studies of chiral pharmaceuticals, agrochemicals, and specialty chemicals.[1] While chiral high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) have been the gold standard for these tasks for decades,[2] the use of chiroptical spectroscopy has been gaining renewed attention[3] owing to a potential advantage in sample throughput. To date, many groups have demonstrated that chiroptical analysis rivals chromatography by reducing cost, waste production, and assay development efforts.[4] A variety of methods for optical determination of enantiopurity have been reported, typically employing circular dichroism (CD), UV, or fluorescence spectroscopy.[5] The sensing approaches range from the observation of inherent chiroptical signals of the molecules of interest[6] to the use of probes that serve as reporters for chiral compounds that display little or no usable CD signal. Some chiroptical sensors are designed to undergo conformational changes upon covalent or noncovalent analyte binding that result in an induced CD signal,[7] while others are assembled in a series of chemical reactions upon exposure to the analyte.[8]

The interest in the use of CD for enantiopurity determination motivated us to explore the reagents, instrumentation, and experimental protocols that would be required for practical high-throughput enantioselective analysis. A number of researchers have reported sensor designs and assay conditions that enable reliable, reproducible, and accurate ee values for different compound types, suggesting that a manageable collection of chiroptical sensors and assay protocols might afford a general approach for enantiopurity determination.[5] While fluorescence and UV measurements have been conducted with plate readers for decades, gathering high-throughput chiroptical data in microwell plates has until recently meant interfacing a liquid handling autosampler with a CD spectrophotometer containing a flow cell, an approach that affords little or no speed advantage over conventional autosampler-limited chromatographic approaches.[9]

When developing an analytical method for chiral HPLC or SFC separation of enantiomers, multiple columns and elution conditions are often tested by trial and error before sufficient separation is achieved. Chiral amines, which are examined in this study, are a challenging class of substrates for chiral chromatography, where chemical derivatization is often used to improve the signal intensity and enantiomer resolution. By contrast, the development of chiroptical methods may be achieved with a single, generally applicable, small-molecule

Received: June 12, 2020
Published: July 24, 2020

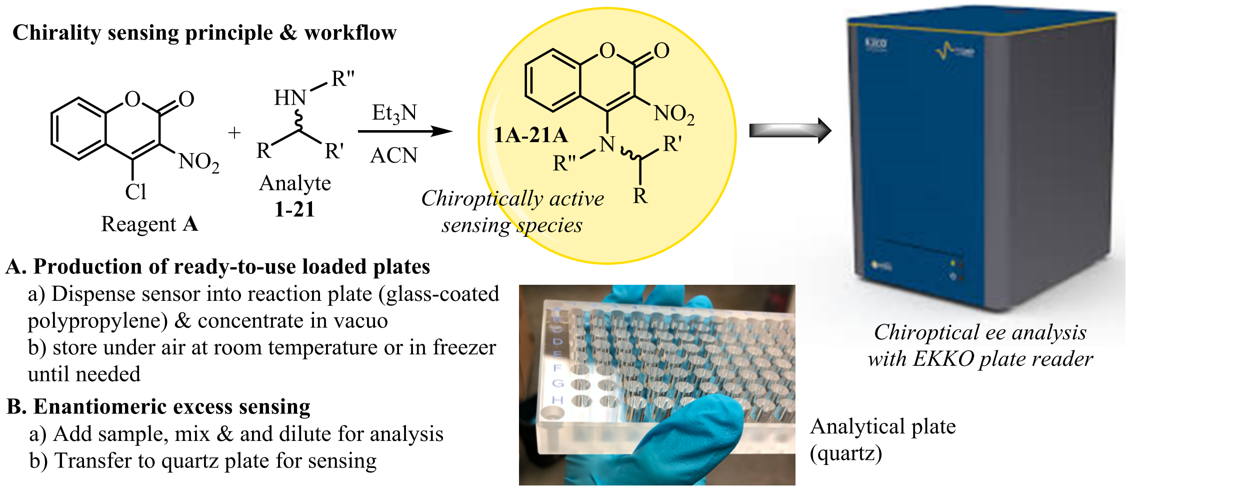


Figure 1. Overview of the workflow for high-throughput enantiopurity determination based on 4-chlorocoumarin derivatization and CD measurements using a microplate spectrophotometer.

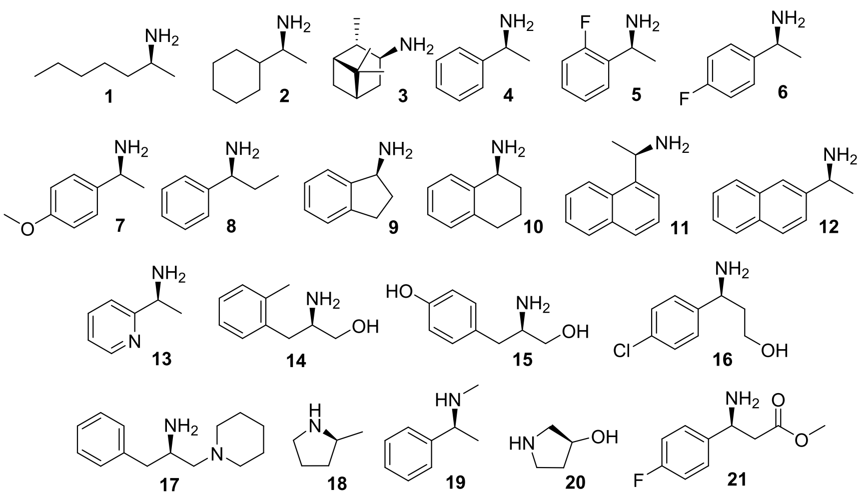


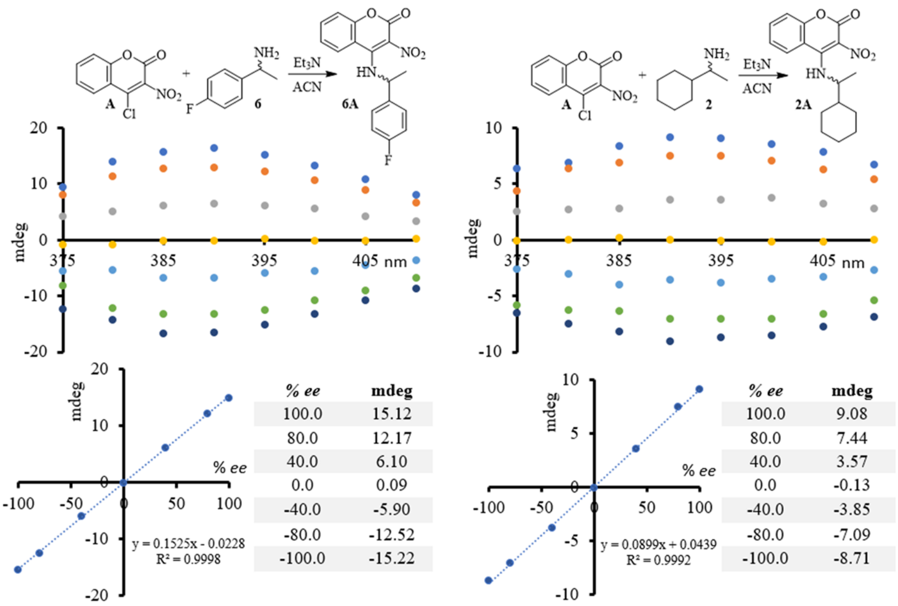
Figure 2. Structures of chiral amines successfully tested with the 4-chlorocoumarin CD assay. Only one enantiomer is shown.

sensor and relatively little optimization of solvent and other sensing parameters, which can also be carried out in parallel if desired. However, the generation of a calibration curve that correlates the measured CD signal to the sample ee values is required. We note that this can be conveniently performed together with the sample preparation when using a CD plate reader, a direction pioneered by the Anslyn and Kahr laboratories.[10] The advances of robust optical probes and user-friendly high-throughput CD sensing technology are most likely to accelerate reaction discovery and optimization efforts where large numbers of new catalysts and other parameters such as solvent, additives, or temperature need to be screened.

In order to investigate the performance of CD microplate spectroscopy for assessment of enantiopurity, we obtained an EKKO CD microplate spectrophotometer. This instrument executes fast circular dichroism measurements using vertical optics to accommodate a series of analytes in either 96- or 384-well microplates. Using this instrument, we set out to study a variety of chiral amines using a 4-chlorocoumarin reagent recently developed by Wolf and co-workers.[11] This reagent reacts rapidly with a variety of compounds, including amines, which results in characteristic UV changes and a chiroptical signal at wavelengths greater than 300 nm, where interference from other chiral components is typically low. Herein, we report the first comprehensive assessment, method validation, and a real-time workflow protocol using this instrument. We decided to evaluate (a) the general utility of this plate reader for quantitative chiral amine analysis, (b) the tolerance for potentially interfering compounds, which is important in real-world applications, and (c) the feasibility of using a kit approach to chiroptical ee determination by performing the assay in microwell plates containing the preconcentrated 4-chlorocoumarin reagent. Altogether, this study showcases practical aspects relating to the speed, performance, robustness, and convenience of microplate measurements of enantiopurity by CD.

■ RESULTS AND DISCUSSION

As reported previously,[11] the 4-chlorocoumarin **A** reacts quickly with primary and secondary amines, yet it is stable under normal storage conditions (Figure 1). To increase the ease of use, we found that evaporation of predefined quantities of **A** in the wells of a microplate provides a loaded "kit", which could be stored at least for several weeks at room temperature, or several months in the freezer, and then used as needed (Figure 1). Preparation of the reaction plates was carried out



% ee	mdeg
100.0	15.12
80.0	12.17
40.0	6.10
0.0	0.09
-40.0	-5.90
-80.0	-12.52
-100.0	-15.22

% ee	mdeg
100.0	9.08
80.0	7.44
40.0	3.57
0.0	-0.13
-40.0	-3.85
-80.0	-7.09
-100.0	-8.71

Figure 3. Top: chiroptical sensing of **2** and **6** at 100.0, 80.0, 40.0, 0.0, −40.0, −80.0, 100.0% ee with 4-chlorocoumarin **A** at 5.0 mM in the presence of 10 equiv of triethylamine in acetonitrile. After 20 min, samples were diluted to 300.0 μM with the same solvent and transferred to the quartz plate for CD analysis. Bottom: plotting of the CD intensities measured at 395 nm vs % ee.

using a Genevac to concentrate the sensor in the 300 μL 96-well reaction plates (glass-coated polypropylene). Addition of 5.0 mM amine samples and stoichiometric amounts of triethylamine in acetonitrile to the individual wells of the reagent-loaded microplates leads to rapid dissolution of **A** and formation of a covalent derivative exhibiting a high wavelength CD signal. The preparation of the 96-well plate including sample derivatization can be completed in about 30 min. Subsequent transfer of the 96 samples to a quartz microplate of optical quality suitable for CD measurements sets the stage for the automated sample analysis. We note that the quartz plate is only needed in the CD analysis step while all other operations are performed using inexpensive glass-coated polypropylene plates. The quartz plate is easily cleaned and reused. It is advisable to run a blank plate filled with the solvent for UV and CD baseline correction prior to the analysis, but this can easily be done in parallel to the sample preparation. Variation of the scanning time per well from 1 to 25 s showed that the precision of the readings is optimal using 3 s measurements while an increase in the temperature of the plates can still be avoided. However, data that are sufficient for high-throughput screening purposes, e.g., asymmetric reaction optimization studies, where less accuracy can be tolerated, can also be obtained with 0.8 s scans. An increase in the scanning time above 3 s does not further improve the results. It takes about 1.5 s for the plate reader to move from one well to the next. Thus, for a high-speed application, CD analysis of 96 samples could be achieved in approximately 4 min using the 0.8 s scanning time.

We decided to investigate a diverse array of amine analytes (Figure 2) to explore the substrate scope of the 4-chlorocoumarin sensing assay (Figure 1) and assess its usefulness in the microplate circular dichroism technology. For this purpose, we selected the aliphatic and aromatic substrates **1−21** possessing either primary or secondary amine groups, as well as alcohol or other additional functionalities.

For the purpose of ee determination, it is not necessary to record the full CD spectra, and in fact, a single wavelength measurement is sufficient for ee analysis, as we show below. We collected single-point measurements between 375 and 410 nm. While it is possible to obtain the continuous spectra, this would unnecessarily reduce sample throughput. We therefore decided to measure the CD sensor outputs every 5 nm in ACN, which proved sufficient for the identification of the CD curve and its maximum. In a similar fashion, the concentration of the amine can be obtained by measuring UV absorbances at a few selected wavelengths for comparison with a calibration curve. Representative CD spectra obtained by derivatization of the aliphatic and aromatic amines **2** and **6** of varying enantiomeric composition at 5.0 mM and subsequent dilution to 0.3 mM for the CD analysis are shown in Figure 3. The calibration curves for the 4-chlorocoumarin-derived products **2A** and **6A** were acquired and linear correlations between the CD signals at 395 nm and the sample ee's were observed. The substrate derivatization is quantitative within 20 min and is typically performed in the presence of an equivalent of a base such as triethylamine. Comparison of the effects of Et_3N and DBU on the induced CD signals showed only minor differences and no deviation from the linear increase in the measured chiroptical response of the coumarin probe to the substrate ee. We therefore decided to use triethylamine in all other studies.

Importantly, the method outlined in Figure 1 is generally applicable and the plate reader performed reliably with all analytes **1−21** including 3-hydroxypyrrolidine, **20**, which does not have the amino group directly attached to the chirality center. In general, similar CD spectra recorded after dilution of the 5.0 mM reaction mixtures to 0.3 or 0.6 mM and transfer to the quartz plate were obtained, although the exact location of the CD maximum varied slightly between analytes. The CD amplitudes of the coumarin derivatives of **1**, **7**, **8**, **10**, **15**, and **18** vary substantially, but we were pleased to find that accurate ee determination is possible even at relatively small ellipticities (see below). Again, plotting of the CD amplitudes measured at 395 nm versus the sample ee values of these coumarin

https://dx.doi.org/10.1021/acs.joc.0c01395
J. Org. Chem. XXXX, XXX, XXX−XXX

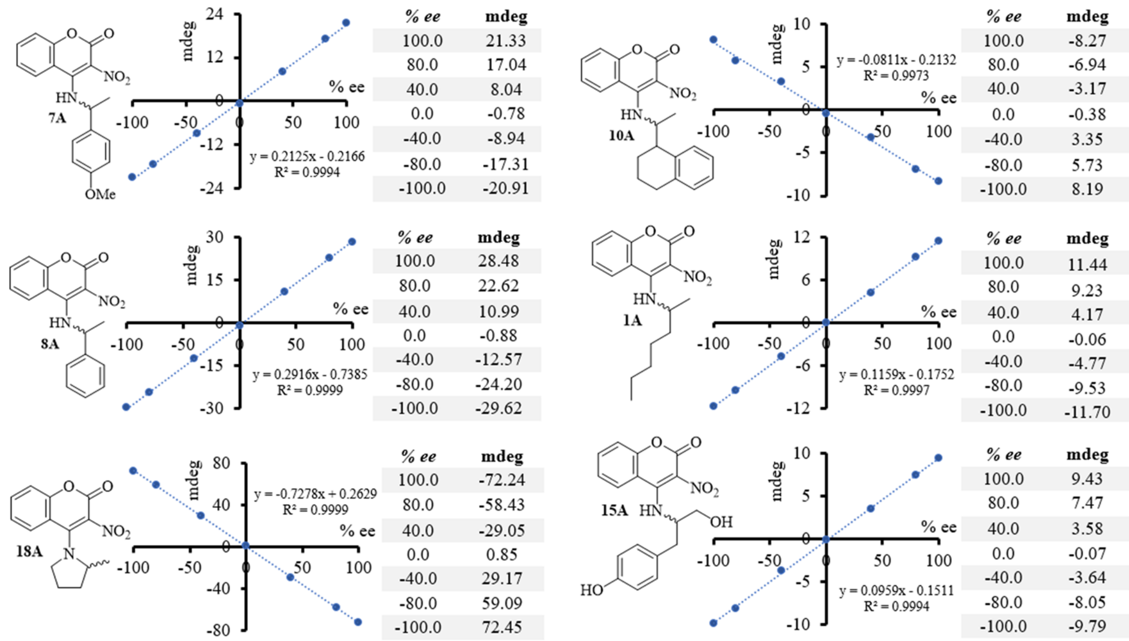


Figure 4. Linear correlation between the CD amplitudes at 395 nm and the enantiomeric excess of **1**, **7**, **8**, **10**, **15**, and **18**. The reactions between **A** and stoichiometric amounts of the amine in the presence of 1 equiv of triethylamine were performed at 5.0 mM in acetonitrile in 96-well plates. For the reaction with **15**, 10 equiv of base were added. After 20 min, the reaction mixtures were diluted to 0.6 mM (**1A**, **7A**, **8A**, and **10A**) and 0.3 mM (**15A** and **18A**) for CD analysis.

derivatives reveals linear correlations (Figure 4 and Supporting Information).

With linear regression equations in hand, 24 samples of **1**, **2**, **4**, **13**, or **19** with randomly chosen enantiomeric composition were prepared, derivatized, and analyzed as described above at a single wavelength (395 or 405 nm, Table 1). The results show that the error margin is within ±7%, which is typically acceptable for high-throughput screening applications. Standard errors were obtained from regression analysis and used to determine the minimum ee quantifiable (MEQ), as defined by eq 1. In each case, the standard error is below the MEQ, indicating that the calibration curve is reliable for the analyses. The MEQ is generally below 5%, which underscores the good sensitivity of the CD analysis.

$$\text{MEQ} = \frac{(\text{ee cal std. error}) \times 5}{2} \quad (1)$$

Having established the general analyte scope, accuracy, and speed of chirality sensing, we decided to examine the possibility of asymmetric reaction analysis with multiplate CD spectroscopy (Scheme 1). For this purpose, we chose to follow a literature protocol that produces the secondary amine **19** via hydrogenation of imine **22** using a catalyst formed from a commercially available Ir dimer and the phosphinooxazoline ligand **23**.[11] After 16 h, the crude product mixture was filtered through a cotton plug and a small 5.0 mM aliquot was applied to the 4-chlorocoumarin sensing assay, as described above. CD analysis and comparison with the calibration curve previously obtained for **19** gave 61.3% ee. In parallel, we used another portion of the reaction mixture to prepare the *t*-Boc derivative **24** for chiral HPLC analysis. The separation of the enantiomers of **24** was achieved on a Whelk-O1 column within 10 min and the enantiomeric excess was determined as 61.0%, which is in excellent agreement with the CD result.

Encouraged by this result, we continued with investigating the effects of possible molecular interference to validate the

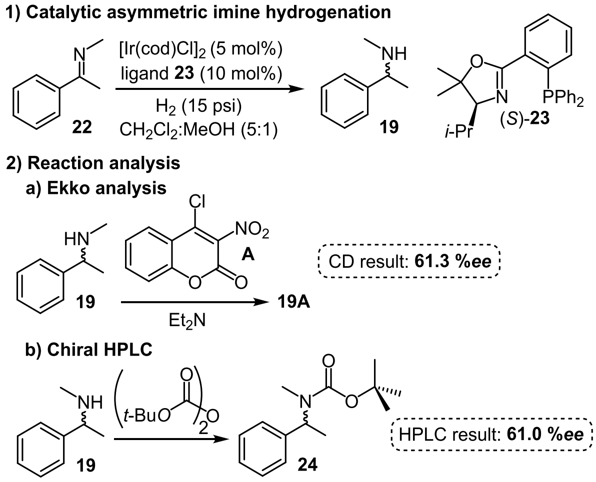
Table 1. Determination of Enantiomeric Excess of Samples of Random Enantiomeric Composition and MEQ Analysis Using CD Intensities at 395 or 405 nm Measured with the Plate Reader after Reaction with the 4-Chlorocoumarin Reagent A[a]

entry	analyte	sample (% ee)	CD (% ee)	absolute error (% ee)	standard error	MEQ
1	**1**	86.0 (*R*)	83.5[b]	2.4		
2	**1**	56.0 (*R*)	53.4[b]	2.6		
3	**1**	28.0 (*R*)	23.2[b]	4.8		
4	**1**	−46.0 (*S*)	−45.9[b]	0.1		
5	**1**	−72.0 (*S*)	−73.0[b]	1.0	2.7	3.5
6	**2**	28.0 (*R*)	24.4[c]	3.6		
7	**2**	−46.0 (*S*)	−42.1[c]	3.9		
8	**2**	−72.0 (*S*)	−70.3[c]	1.7		
9	**2**	−92.0 (*S*)	−92.7[c]	0.7	2.8	4.4
10	**4**	86.0 (*R*)	87.4[b]	1.5		
11	**4**	56.0 (*R*)	56.6[b]	0.6		
12	**4**	28.0 (*R*)	28.4[b]	0.4		
13	**4**	−46.0 (*S*)	−44.0[b]	2.0		
14	**4**	−72.0 (*S*)	−72.5[b]	0.5	1.2	1.5
15	**13**[f]	86.0 (*R*)	89.3[d]	3.3		
16	**13**[f]	56.0 (*R*)	60.9[d]	4.9		
17	**13**[f]	28.0 (*R*)	35.0[d]	6.9		
18	**13**[f]	−46.0 (*S*)	−46.5[d]	0.5		
19	**13**[f]	−72.0 (*S*)	−76.7[d]	4.7		
20	**13**[f]	−92.0 (*S*)	−91.3[d]	0.7	4.2	4.3
21	**19**	86.0 (*R*)	84.4[e]	1.6		
22	**19**	56.0 (*R*)	58.5[e]	2.5		
23	**19**	28.0 (*R*)	32.9[e]	4.9		
24	**19**	−72.0 (*S*)	−70.9[e]	1.1	2.9	3.6

[a]Solutions were prepared as described above and CD analysis was conducted at 395 nm. [b]Measurements at 0.6 mM (1 equiv of Et_3N) in ACN. [c]0.3 mM (10 equiv of Et_3N). [d]0.6 mM (10 equiv of Et_3N). [e]0.3 mM (2 equiv of Et_3N). [f]CD analysis at 405 nm.

https://dx.doi.org/10.1021/acs.joc.0c01395
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Comparison of CD and Chiral HPLC ee Analysis of an Asymmetric Imine Hydrogenation Mixture



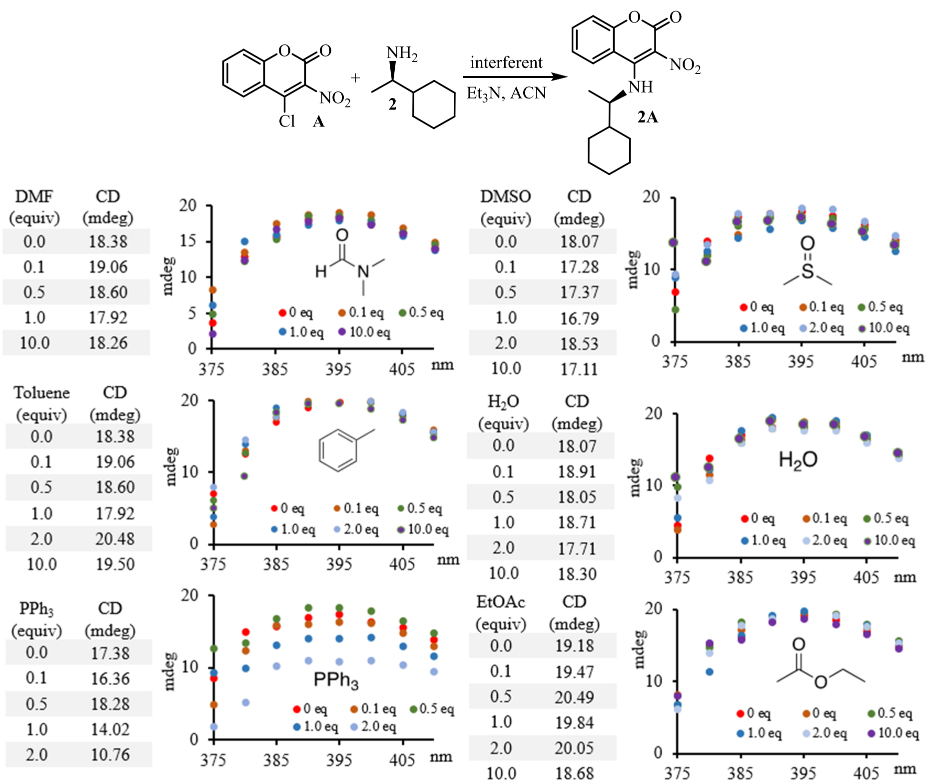
rigor of microplate CD sensing. The general robustness of chromatographic methods in particular with respect to impurities that may be separated from the targeted enantiomers during the separation process is an attractive feature and an ultimate test for optical methods. The effects of up to 10 equiv of a wide variety of possible solvents/interferents on the reaction between 4-chlorocoumarin **A** and amine **2** and the subsequent CD assay protocol were studied (Figure 5 and Supporting Information). We found that typically encountered solvents such as tetrahydrofuran, methanol, chloroform, acetone, water, ethyl acetate, toluene, dimethylformamide, and dimethylsulfoxide, and even equimolar amounts of enantiopure Binol or $[Rh(COD)Cl]_2$, are well tolerated and have only minor effects on the CD signal. The presence of excess of triphenylphosphine, a nucleophile that can be expected to compete with the amine for the reaction with **A**, resulted in a decrease in the CD signal. While this shows that interference with this particular sensing method is possible, it simply determines the application boundaries and provides valuable guidance for the user to avoid complications.

CONCLUSIONS

The studies reported herein clearly demonstrate the potential for chiroptical methods to become an important contributor in the high-throughput analysis of enantiopurity. Using an existing derivatization methodology, we found that the CD plate reader gave accurate and relatively free from interference ee values with the potential of screening 96 samples in under 4 min. While the method was applied solely to one optical assay and with only chiral amines, the workflow can be applied to the wealth of derivatization and supramolecular assembly techniques currently available to the synthetic methodology community. We can imagine parallel analysis of multiple chiral functional groups with other optical approaches to extend the utility of the method, thereby obviating the use of chiral HPLC techniques for an initial screen of ee values.

EXPERIMENTAL SECTION

General. All reagents and analytes were used as purchased. Chiral amines obtained as HCl salts were used as is and neutralized with equimolar triethylamine during the derivatization reactions. Acetonitrile was dried over 3 Å molecular sieves (heated to 170 °C for 24 h prior to use). All chiroptical analyses were performed using an EKKO CD microplate reader and a Hellma Suprasil quartz 96-well plate equipped with a quartz lid and silicon buffer mat inlay. Spectral scanning was conducted every 5 nm from 375−410 nm with 3.0 s integration time and 40 mdeg sensitivity. All stock solutions of the



DMF (equiv)	CD (mdeg)
0.0	18.38
0.1	19.06
0.5	18.60
1.0	17.92
10.0	18.26

DMSO (equiv)	CD (mdeg)
0.0	18.07
0.1	17.28
0.5	17.37
1.0	16.79
2.0	18.53
10.0	17.11

Toluene (equiv)	CD (mdeg)
0.0	18.38
0.1	19.06
0.5	18.60
1.0	17.92
2.0	20.48
10.0	19.50

H_2O (equiv)	CD (mdeg)
0.0	18.07
0.1	18.91
0.5	18.05
1.0	18.71
2.0	17.71
10.0	18.30

PPh_3 (equiv)	CD (mdeg)
0.0	17.38
0.1	16.36
0.5	18.28
1.0	14.02
2.0	10.76

EtOAc (equiv)	CD (mdeg)
0.0	19.18
0.1	19.47
0.5	20.49
1.0	19.84
2.0	20.05
10.0	18.68

Figure 5. Effects of molecular interferents on the CD sensing output.

https://dx.doi.org/10.1021/acs.joc.0c01395
J. Org. Chem. XXXX, XXX, XXX−XXX

reagent, additive/interferents, and chiral analytes were prepared using acetonitrile as the solvent.

Chiroptical Analysis. Solutions of 4-chloro-3-nitrocoumarin, **A**, (5.00−6.00 mM) were prepared and distributed into vials. Stock solutions of each enantiomer of the chiral analyte of interest were prepared (0.025 or 0.25 M) in acetonitrile for either 10.0 or 100.0 μL additions to the reagent (1:1 **A**/analyte ratio) allowing for the click reaction to occur at 5.00 mM. The reactions were conducted in the presence of 1.0−10.0 molar equiv of triethylamine or DBU. After 20 min, aliquots of the reaction solutions were diluted to 600.0 and/or 300.0 μM. Calibrations were constructed with the quartz 96-well plate and varied ee's (−100, −80, −40, 0, 40, 80, and 100%) of the analytes. EKKO parameters were set to scan every 5 nm at 375−410 nm with 40 mdeg sensitivity, 3 s integration time using the quartz plate, and individual well blanking with acetonitrile. Determination of sample enantiopurities was achieved by comparison of the CD maxima with a calibration curve obtained under the same conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01395.

Details of the CD sensing procedures, CD spectra, and method validation (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Eric V. Anslyn − *Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, United States*; Email: anslyn@austin.utexas.edu

Christian Wolf − *Department of Chemistry, Georgetown University, Washington, DC 20057, United States*; orcid.org/0000-0002-4447-3753; Email: cw27@georgetown.edu

Authors

Samantha L. Pilicer − *Department of Chemistry, Georgetown University, Washington, DC 20057, United States*

Justin M. Dragna − *Enantiosense, LLC, Austin, Texas 78723-4648, United States*

Adam Garland − *Water Lens, LLC, Houston, Texas 77027, United States*

Christopher J. Welch − *Indiana Consortium for Analytical Science & Engineering (ICASE), Indianapolis, Indiana 46202, United States*; orcid.org/0000-0002-8899-4470

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.0c01395

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was gratefully supported by NSF SBIR (UTA18-001517), NSF (CHE-1764135), and NSF GOALI (CHE 1665040) grants, as well as the Welch Chair Regents Chair (EVA, 1-0046).

■ REFERENCES

(1) Wolf, C. *Dynamic Stereochemistry of Chiral Compounds - Principles and Applications*; RSC Publishing: Cambridge, UK, 2008, 136−179.

(2) Okamoto, Y.; Ikai, T. Chiral HPLC for Efficient Resolution of Enantiomers. *Chem. Soc. Rev.* **2008**, *37*, 2593−2608.

(3) (a)) Berova, N.; Di Bari, L.; Pescitelli, G. Application of electronic circular dichroism in configurational and conformational analysis of organic compounds. *Chem. Soc. Rev.* **2007**, *36*, 914−931. (b)) Leung, D.; Kang, S. O.; Anslyn, E. V. Rapid Determination of Enantiomeric Excess: a Focus on Optical Approaches. *Chem. Soc. Rev.* **2012**, *41*, 448. (c)) Wolf, C.; Bentley, K. W. Chirality Sensing Using Stereodynamic Probes with Distinct Electronic Circular Dichroism Output. *Chem. Soc. Rev.* **2013**, *42*, 5408.

(4) (a)) Shabbir, S. H.; Regan, C. J.; Anslyn, E. V. A general protocol for creating high-throughput screening assays for reaction yield and enantiomeric excess applied to hydrobenzoin. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 10487−10492. (b)) Nieto, S.; Dragna, J. M.; Anslyn, E. V. A Facile Circular Dichroism Protocol for Rapid Determination of Enantiomeric Excess and Concentration of Chiral Primary Amines. *Chem. − Eur. J.* **2010**, *16*, 227−232. (c)) Biedermann, F.; Nau, W. M. Noncovalent Chirality Sensing Ensembles for the Detection and Reaction Monitoring of Amino Acids, Peptides, Proteins, and Aromatic Drugs. *Angew. Chem., Int. Ed.* **2014**, *53*, 5694−5699. (d)) Joyce, L. A.; Sherer, E. C.; Welch, C. J. Imine-based chiroptical sensing for analysis of chiral amines: from method design to synthetic application. *Chem. Sci.* **2014**, *5*, 2855−2861. (e)) Giuliano, M. W.; Lin, C.-Y.; Romney, D. K.; Miller, S. J.; Anslyn, E. V. A Synergistic Combinatorial and Chiroptical Study of Peptide Catalysts for Asymmetric Baeyer−Villiger Oxidation. *Adv. Synth. Catal.* **2015**, *357*, 2301−2309. (f)) Bentley, K. W.; Proano, D.; Wolf, C. Chirality imprinting and direct asymmetric reaction screening using a stereodynamic Brønsted/Lewis acid receptor. *Nat. Commun.* **2016**, *7*, 12539. (g)) Bentley, K. W.; Zhang, P.; Wolf, C. Miniature high-throughput chemosensing of yield, ee, and absolute configuration from crude reaction mixtures. *Sci. Adv.* **2016**, *2*, No. e1501162.

(5) Herrera, B. T.; Pilicer, S. L.; Anslyn, E. V.; Joyce, L. A.; Wolf, C. Optical Analysis of Reaction Yield and Enantiomeric Excess: A New Paradigm Ready for Prime Time. *J. Am. Chem. Soc.* **2018**, *140*, 10385−10401.

(6) Thanzeel, F. Y.; Balaraman, K.; Wolf, C. Streamlined Asymmetric Reaction Development: A Case Study with Isatins. *Chem. − Eur. J.* **2019**, *25*, 11020−11025.

(7) (a)) Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi, K.; Berova, N. Absolute Configurational Assignments of Secondary Amines by CD-Sensitive Dimeric Zinc Porphyrin Host. *J. Am. Chem. Soc.* **2002**, *124*, 10320−10335. (b)) Superchi, S.; Bisaccia, R.; Casarini, D.; Laurita, A.; Rosini, C. *J. Am. Chem. Soc.* **2006**, *128*, 6893−6902. (c)) Dutot, L.; Wright, K.; Gaucher, A.; Wakselman, M.; Mazaleyrat, J.-P.; De Zotti, M.; Peggion, C.; Formaggio, F.; Toniolo, C. The Bip Method, Based on the Induced Circular Dichroism of a Flexible Biphenyl Probe in Terminally Protected -Bip-Xaa*- Dipeptides, for Assignment of the Absolute Configuration of β-Amino Acids. *J. Am. Chem. Soc.* **2008**, *130*, 5986−5992. (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. (e)) Zardi, P.; Wurst, K.; Licini, G.; Zonta, C. Concentration-Independent Stereodynamic g-Probe for Chiroptical Enantiomeric Excess Determination. *J. Am. Chem. Soc.* **2017**, *139*, 15616−15619. (f)) De los Santos, Z. A.; Joyce, L. A.; Sherer, E. C.; Welch, C. J.; Wolf, C. Optical Chirality Sensing with a Stereodynamic Aluminum Biphenolate Probe. *J. Org. Chem.* **2019**, *84*, 4639−4645. (g)) De los Santos, Z. A.; Lynch, C. C.; Wolf, C. Optical Chirality Sensing with an Auxiliary-Free Earth-Abundant Cobalt Probe. *Angew. Chem., Int. Ed.* **2019**, *58*, 1198−1202. (h)) Shirbhate, M. E.; Kwon, S.; Song, A.; Kim, S.; Kim, D.; Huang, H.; Kim, Y.; Lee, H.; Kim, S.-J.; Baik, M.-H.; Yoon, J.; Kim, K. M. Optical and Fluorescent Dual Sensing of Aminoalcohols by in Situ Generation of BODIPY-like Chromophore. *J. Am. Chem. Soc.* **2020**, *142*, 4975−4979.

(8) (a)) Joyce, L. A.; Maynor, M. S.; Dragna, J. M.; da Cruz, G. M.; Lynch, V. M.; Canary, J. W.; Anslyn, E. V. A Simple Method for the Determination of Enantiomeric Excess and Identity of Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2011**, *133*, 13746−13752. (b)) Wezenberg, S. J.; Salassa, G.; Escudero-Adán, E. C.; Benet-

Buchholz, J.; Kleij, A. W. Effective Chirogenesis in a Bis-(metallosalphen) Complex through Host-Guest Binding with Carboxylic Acids. *Angew. Chem.* **2011**, *50*, 713−716. (c)) Joyce, L. A.; Canary, J. W.; Anslyn, E. V. Enantio- and Chemoselective Differentiation of Protected α-Amino Acids and β-Homoamino Acids with a Single Copper(II) Host. *Chem. − Eur. J.* **2012**, *18*, 8064−8069. (d)) Dragna, J. M.; Pescitelli, G.; Tran, L.; Lynch, V. M.; Anslyn, E. V.; Di Bari, L. In Situ Assembly of Octahedral Fe(II) Complexes for the Enantiomeric Excess Determination of Chiral Amines Using Circular Dichroism Spectroscopy. *J. Am. Chem. Soc.* **2012**, *134*, 4398−4407. (e)) Jo, H. H.; Gao, X.; You, L.; Anslyn, E. V.; Krische, M. J. Application of a high-throughput enantiomeric excess optical assay involving a dynamic covalent assembly: parallel asymmetric allylation and ee sensing of homoallylic alcohols. *Chem. Sci.* **2015**, *6*, 6747−6753. (f)) De los Santos, Z. A.; Wolf, C. Chiroptical Asymmetric Reaction Screening via Multicomponent Self-Assembly. *J. Am. Chem. Soc.* **2016**, *138*, 13517−13520. (g)) Badetti, E.; Wurst, K.; Licini, G.; Zonta, C. Multimetallic Architectures from the Selfassembly of Amino Acids and Tris(2-pyridylmethyl)amine Zinc(II) Complexes: Circular Dichroism Enhancement by Chromophores Organization. *Chem. − Eur. J.* **2016**, *22*, 6515−6518.

(9) Welch, C. J. Are We Approaching a Speed Limit for the Chromatographic Separation of Enantiomers? *ACS Cent. Sci.* **2017**, *3*, 823−829.

(10) Metola, P.; Nichols, S. M.; Kahr, B.; Anslyn, E. V. Well plate circular dichroism reader for the rapid determination of enantiomeric excess. *Chem. Sci.* **2014**, *5*, 4278−4282.

(11) Thanzeel, F. Y.; Balaraman, K.; Wolf, C. Click chemistry enables quantitative chiroptical sensing of chiral compounds in protic media and complex mixtures. *Nat. Commun.* **2018**, *9*, 5323.