Ninhydrin Revisited: Quantitative Chirality Recognition of Amines and Amino Alcohols Based on Nondestructive Dynamic Covalent Chemistry

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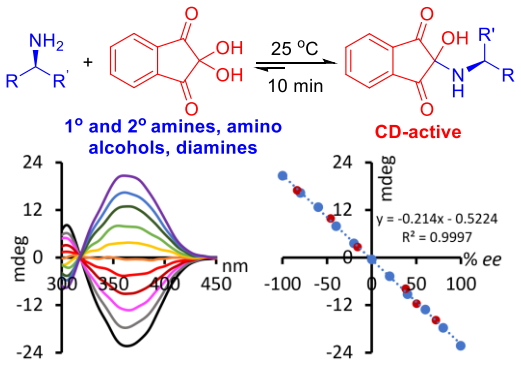


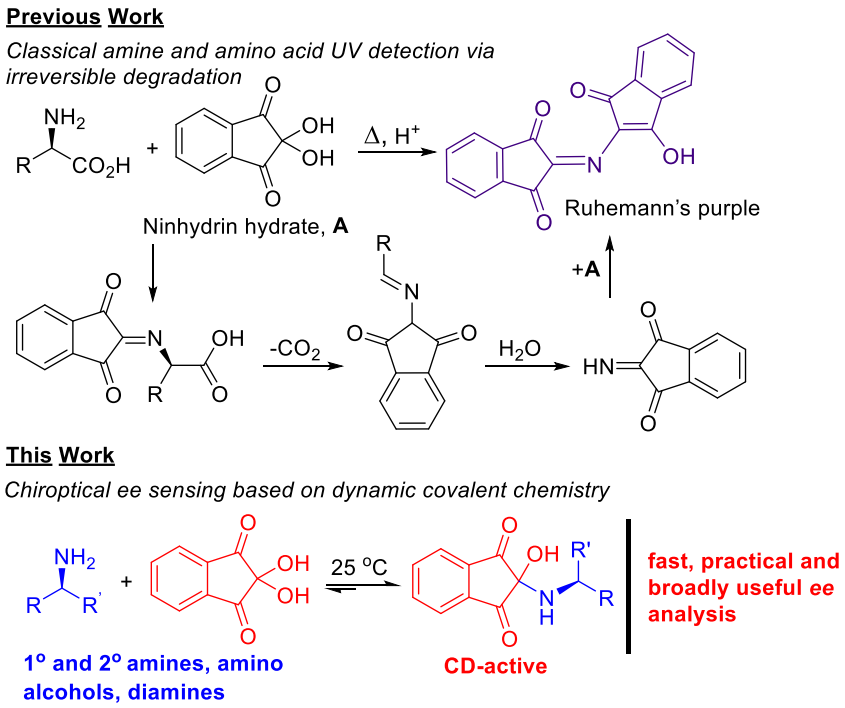
Abstract

A novel approach to chiral recognition of small molecules using the classical ninhydrin agent is introduced. Well-defined dynamic covalent chemistry with amines and amino alcohols was developed and applied to quantitative *ee* sensing with good accuracy using a straightforward mixing protocol and subsequent circular dichroism measurements. This chiroptical assay is fast, broadly useful, practical and repurposes an inexpensive reagent known for more than one hundred years in a new application.

The development of methods that allow fast detection and quantification of chiral compounds remains a major topic in the realm of analytical chemistry. Optical sensing techniques are of increasing interest due to their speed and compatibility with high throughput equipment.[1-3] Several research groups have developed circular dichroism (CD) methods with carefully designed probes that bind to a chiral target molecule and generate chiroptical signals that can be used for enantiomeric excess (*ee*) determination.[4-16] Dynamic covalent chemistry (DCC), for example reversible Schiff base formation, is one avenue that has been quite successful in this regard.[8, 17-22] The general promise and operational simplicity of chiroptical DCC sensing led us to re-examine the use of ninhydrin, an inexpensive reagent with a long history of synthetic and analytical applications.[23-27]

Ninhydrin was first introduced for the detection of amino acids. [24, 25, 28, 29] Today, it can be found in numerous synthetic and biochemical laboratories where it is often used for the detection of amines,[30, 31] peptides,[32] and other natural compounds like putrescine.[33] Ninhydrin has also been integrated into important forensic applications including fluorescent ketamine detection[34] and fingerprinting analysis.[35-37] Its unique structure and reactivity has been exploited in the synthesis of a variety of biologically active compounds containing an indanone core, a spirocyclic scaffold and important heterocyclic motifs.[38-42] Typically, the reaction of ninhydrin with amines and amino acids produces ammonia, hydrindantin and the classical Ruhemann's purple which is ultimately used for qualitative and quantitative analysis.[28, 29] This process generally requires acidic conditions and elevated temperatures, and information of the original molecular structure and chirality is lost due to compound degradation (Figure 1).[25, 27, 43-45]

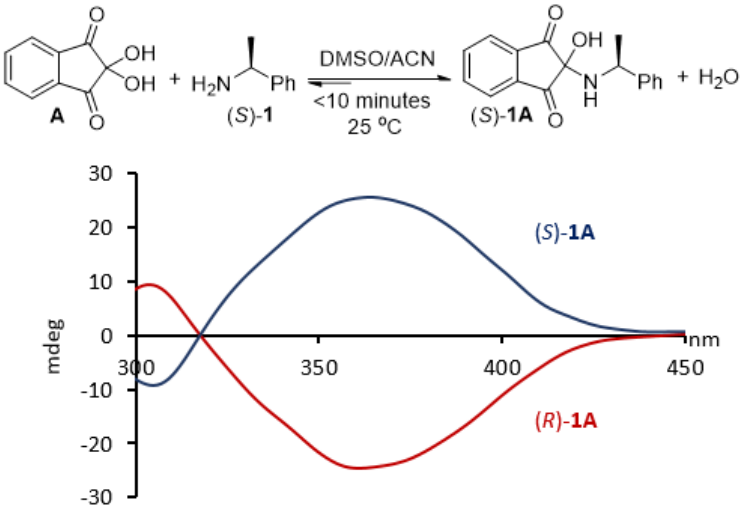
While the utility of ninhydrin lies in the vibrant colorimetric detection of compounds that form Ruhemann's purple,[43] we decided to investigate the possibility of a controlled DCC protocol with chiral amines that proceeds without degradation and loss of the molecular chirality. It was expected that *N,O*-hemiacetal formation could be achieved under mild reaction conditions, and thus generate characteristic Cotton effects arising from the covalent placement of the ninhydrin chromophore scaffold in close proximity to the chiral center (Scheme 1). We hypothesized this would allow for optical *ee* analysis based on simple CD measurements which, to the best of our knowledge, has not been demonstrated to date.



Scheme 1. Classical ninhydrin reaction with an amino acid resulting in degradation and destruction of chiral information (top). Amine capture via dynamic covalent ninhydrin *N,O*-hemiacetal formation under mild conditions (bottom).

Initially, the possibility of nondestructive DCC was examined with phenylethylamine, **1**, and ninhydrin hydrate, **A**, using anhydrous DMSO as solvent to

ensure homogenous reaction conditions (Scheme 2). Complete consumption of the free amine was observed with no detectable by-products based on ^{1}H NMR spectroscopic analysis. The reaction does not require inert atmosphere, was found to be completed within 10 minutes, which was confirmed with a variety of substrates, and titration experiments were in agreement with a 1:1 stoichiometric binding event (see SI). Further ^{1}H NMR analysis revealed a new signal consistent with the N-H proton of an *N,O*-hemiacetal at approximately 3.0 ppm. Although binding of the amine analytes to ninhydrin does release an equivalent of water, addition of molecular sieves did not affect the reaction equilibrium and time needed to completion. Importantly, the fast amine binding event generates a strong CD signal with a maximum at approximately 365 nm. The reaction of (*S*)-**1** with ninhydrin to (*S*)-**1A** produces a positive Cotton effect while the opposite is observed when the (*R*)-enantiomer is used under otherwise identical conditions. With a known reference in hand, one can thus determine the absolute configuration of **1** and many other amines and amino alcohols as is shown below by comparison of the sign of the observed induced CD signal.



Scheme 2. DCC reaction between ninhydrin hydrate, **A**, and phenylethylamine, **1**, in DMSO (5.0 mM) and CD analysis after dilution with ACN to 1.1 mM.

To maintain homogeneous conditions and a simple workflow, the acetal formation was conducted in DMSO and the CD analyses were subsequently carried out after dilution with either DMSO, acetonitrile, THF, dichloromethane or methanol to optimize the chiroptical signal induction. We found that solvents have only a minor effect on the CD signal intensity except when MeOH is used which displaces the bound substrate as expected for a DCC system based on reversible acetal formation. Dilution of the reaction mixture with acetonitrile slightly increases the CD amplitude compared to samples analyzed in neat DMSO or in one of the other solvents. The protocol using DMSO as the reaction solvent and ACN for subsequent dilution was therefore adapted in all other CD experiments (see SI). To probe the robustness and usefulness of chirality sensing with ninhydrin we monitored the stability of product samples over time and found no sign of CD signal degradation after two hours. As one would expect for a DCC assay, addition of water to a solution containing **1A** did result in partial acetal cleavage. ^{1}H NMR analysis showed regeneration of the free amine as well as substantial peak broadening of the signal at 6.49 ppm which was assigned as the exchangeable hydroxyl proton in the ninhydrin *N,O*-hemiacetal (SI).

Having established that the DCC with ninhydrin hydrate and phenylethylamine produces a stable, CD-active product under mild conditions, we applied our sensing protocol to a compound library containing a total of 26 primary and secondary amines, amino alcohols, and diamines (Figure 1). Following our simple mixing protocol, the

analyte screening was conducted in DMSO at 5.0 mM and the samples were diluted with acetonitrile to 1.1 mM for CD analysis.

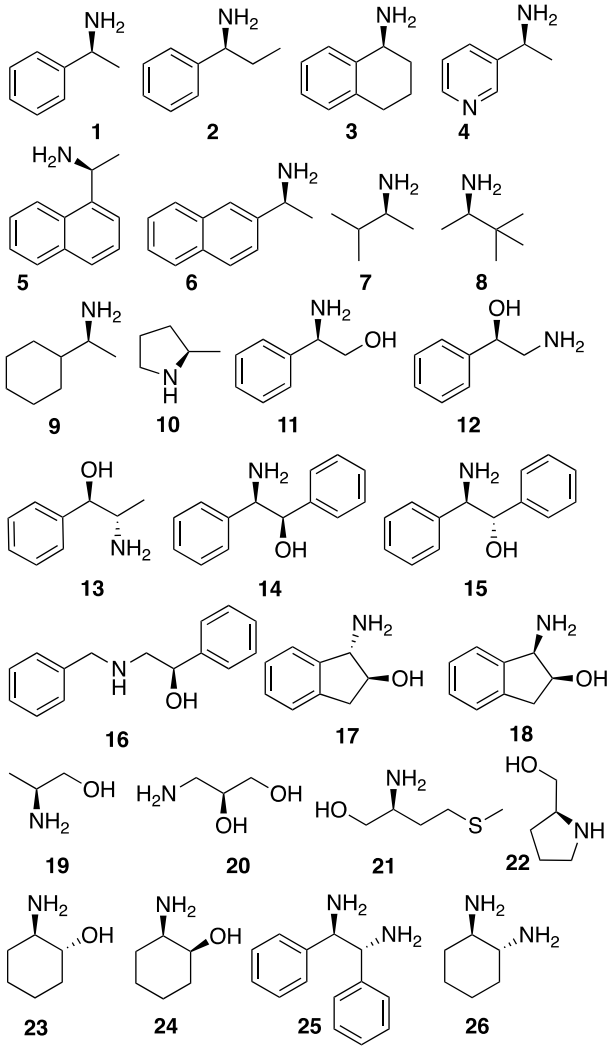


Figure 1. Structures of chiral amines, amino alcohols, and diamines examined.

We were delighted to observe strong induced CD signals for a variety of primary amines and amino alcohols, some exhibiting strictly aliphatic scaffolds while others also contain aromatic groups (Figure 2). For example, the ninhydrin adducts **8A** and **9A** obtained from 3,3-dimethylbutan-2-amine, **8**, and 1-cyclohexylethan-1-amine, **9**, produced strong CD signals despite the absence of a π-system in these analytes. The

CD intensities of **8A** and **9A** were actually comparable with those obtained by sensing of 2-naphthylethylamine, **6**.

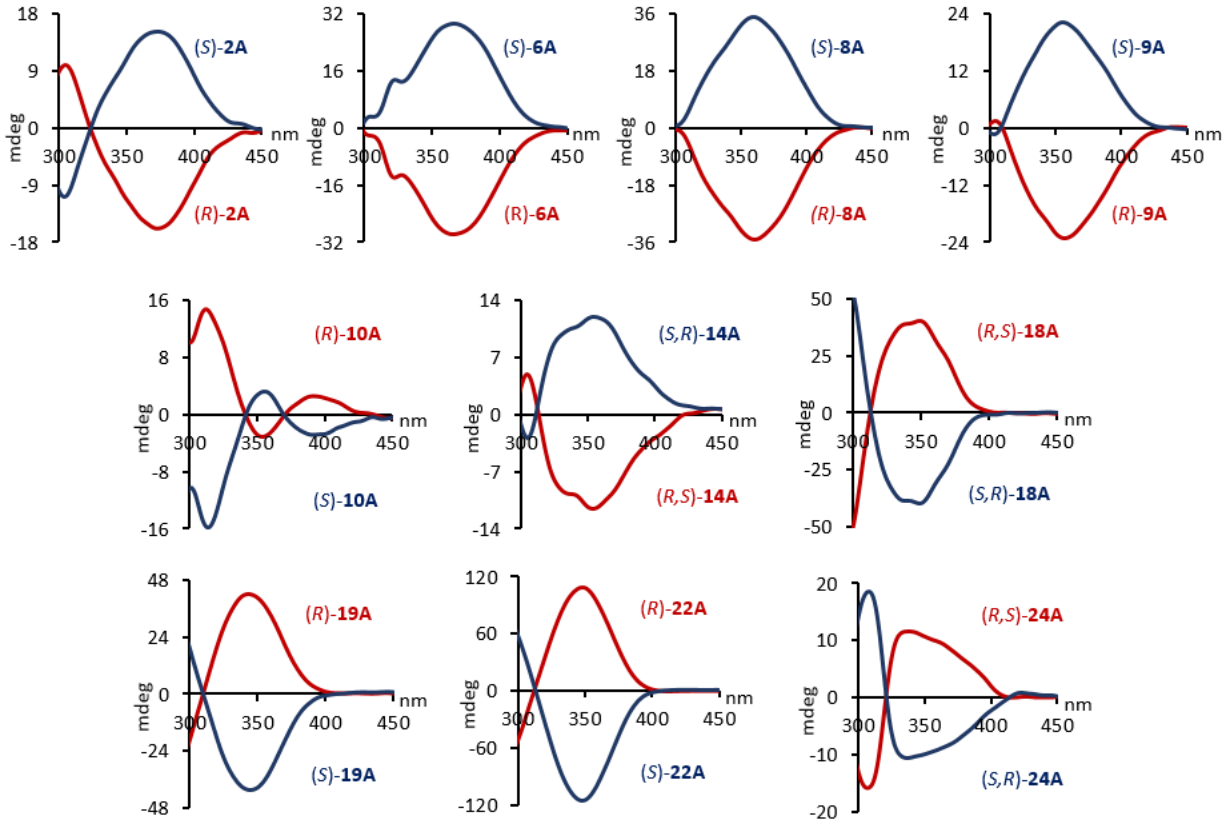


Figure 2. Exemplary CD spectra obtained using ninhydrin hydrate, **A**, and stoichiometric amounts of the chiral analytes. Reactions were carried out at 5.0 mM in DMSO and the solutions were diluted with ACN to 1.1 mM prior to chiroptical analysis**.**

Similarly, the sensing of chiral amino alcohols yielded strong CD signals regardless of the presence or absence of an aryl moiety in the analyte structure. For both **14** which has two phenyl groups and **24** devoid of an aryl ring, we observed strong CD couplets above 300 nm upon binding to **A** (Figure 2). The anisotropy g-factor of the ninhydrin adducts generally ranges from 3.0×10^{-4} to 3.0×10^{-3} (see SI). Our sensing assay with ninhydrin hydrate also produced quantifiable CD signals with secondary amines and secondary amino alcohols which cannot be analyzed with Schiff base-derived sensors.

In the case of **22A**, for instance, CD intensities were particularly strong which underscores the broad utility of this chiroptical assay.

We expected that the diamines **25** and **26** would allow incorporation of a second equivalent of ninhydrin to form CD-active species denoted as **25AA** and **26AA**, respectively. As shown in Figure 3 (top), when one equivalent of **A** is introduced to **25** or **26**, the resulting Cotton effects are similar to the CD profiles observed with the monofunctional analogues. When two equivalents of **A** were added, however, the Cotton effects were remarkably red shifted (Figure 3, bottom). The appearance of CD signals above 400 nm and of additional couplets can be attributed to through-space interactions between the two bound ninhydrin chromophores which are expected to reside in close proximity in **25AA** and **26AA**. The increasingly complex CD spectrum of **25AA** can be explained with additional contributions from the two phenyl rings in this diamine.

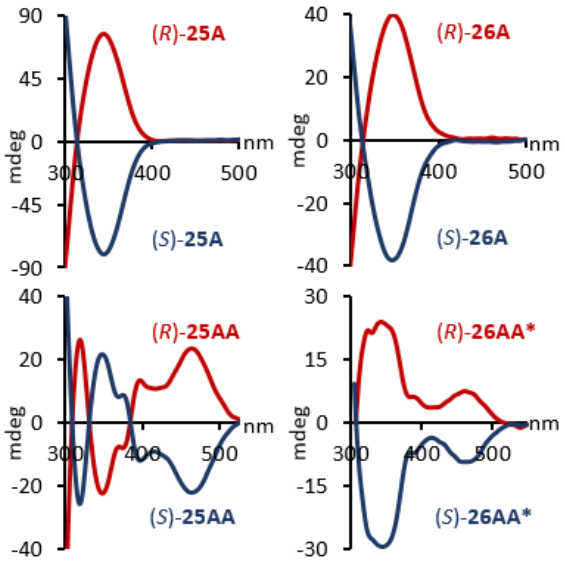


Figure 3. Chiroptical diamine sensing using one (top) and two (bottom) equivalents of ninhydrin hydrate, **A**, in ACN:DMSO (5:1) solutions at 1.10 mM. *Sensing was performed at 1.65 mM.

We then decided to further examine the reversibility of the dynamic covalent assay with a titration experiment (Figure 4). First, a 1.1 mM solution of (*R)*-**1A** was formed as described above and the CD spectrum was recorded. Then, about 0.2 molar equivalents of the free (*S)*-**1** enantiomer were added and CD measurements were taken after 30 seconds, 10, 20, and 30 minutes to monitor the equilibration process which was apparent by the decrease of the negative amplitude at approximately 365 nm (Figure 4, green CD spectra). To the same solution, approximately 1.8 equivalents of (*S)*-**1** were added and within 30 seconds, significant displacement of the initially bound (*R)*-**1** enantiomer was observed. The amine exchange was monitored for 30 minutes when the equilibrium was reached as indicated by a stable positive CD signal at the same wavelength (Figure 4, blue CD curves). These results confirm that the sensing with ninhydrin hydrate is based on DCC and that the exchange occurs within a few minutes. The reversibility of the substrate binding and rapid equilibration of interconverting enantiomeric ninhydrin adducts was confirmed with 2-methylpyrollidine **10** (see SI).

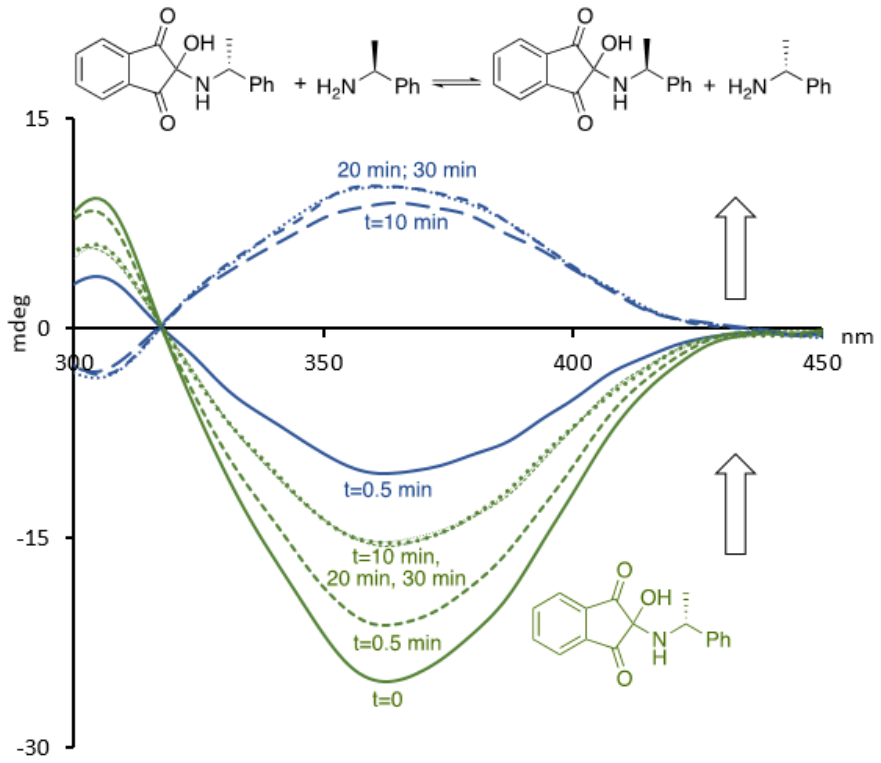
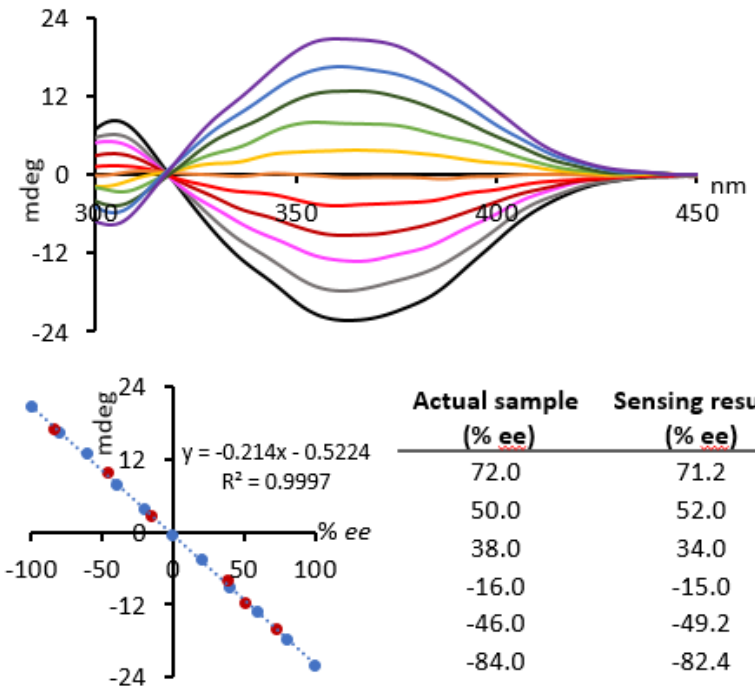


Figure 4. Titration of (*S*)-**1** (0.0-2.2 mM) into a solution of (*R*)-**1A** (1.1 mM) and CD analysis of the DCC process observed in ACN:DMSO (5:1).

Finally, we pursued quantitative *ee* sensing by exposing solutions of varied enantiomeric ratios of **1** to **A** (Figure 5). The linearity of the CD responses (CD intensity vs. %*ee*) obtained is in agreement with 1:1 stoichiometric amine binding and suggests that compound aggregation phenomena, which may result in nonlinear effects,[46] are very unlikely under the sensing conditions.



Actual sample (% ee)	Sensing results (% ee)
72.0	71.2
50.0	52.0
38.0	34.0
-16.0	-15.0
-46.0	-49.2
-84.0	-82.4

Figure 5. Quantitative *ee* sensing of phenylethylamine, **1**, with ninhydrin hydrate, **A**. Top: CD spectra obtained by sensing of **1** in varied enantiomeric compositions of (100.0, 80.0, 60.0, 40.0, 20.0, 0, -20.0, -40.0, -60.0, -80.0, -100.0 *%ee*). Bottom: Linear correlation between the chiroptical responses of **A** at 360 nm and the analyte *ee*'s (blue) and sensing of samples with random *ee* compositions of 1-phenylethan-1-amine (red). Positive *ee* values in the table refer to samples enriched in (*S*)-**1** and negative values to the samples with excess of the (*R*)-enantiomer.

A second series of samples prepared in parallel were subjected to our assay under identical conditions and their *ee*'s were calculated from the calibration experiments. The analysis of samples with high *ee* values showed sensing results as close as 0.2% *ee* to the actual values. At low enantiomeric excess where signal-to-noise ratios can affect accuracy more substantially, the error margin was still within 4% *ee* and, with the -16.0% *ee* sample, the calculated value deviated by only 1%. We note that the accuracy of our assay surpasses typical expectations for most high-throughput applications.

Conclusions

We have described a practical assay that enables the chiral analysis of primary and secondary amines, amino alcohols and diamines by repurposing ninhydrin hydrate as an effective CD sensing agent in nondestructive chemistry. While previously known UV and fluorescence detection methods with ninhydrin are based on irreversible analyte degradation and formation of Ruhemann's purple, our approach exploits mild, reversible acetal formation and chiroptical analysis of a variety of chiral small molecules that are CD-silent in the absence of the sensing agent under the same conditions. Using a simple mixing protocol, we have demonstrated a broadly useful DCC chirality sensing assay with 26 substrates and showed that fast absolute configuration analysis and quantitative *ee* determination is possible with good accuracy.

Experimental Section

General. All reagents, analytes and solvents were used as purchased and experiments were conducted under air atmosphere. Ninhydrin and the substrates were mixed in anhydrous DMSO at 5.0 mM and the solutions were then diluted with acetonitrile to 1.1 mM unless indicated otherwise. The general scope of the ninhydrin chiroptical sensing was evaluated using stoichiometric amounts of primary amines and amino alcohols and the reactions were examined by circular dichroism, UV and NMR spectroscopy.

Chiroptical analysis. CD spectra were collected with a standard sensitivity of 100 mdeg, a data pitch of 1.0 nm, a band width of 1.0 nm, a scanning speed of 500 nm min^{-1} and a response of 1 s using a quartz cuvette (10 mm path length). Scans were corrected using a binomial smoothing function. The chiral substrates surveyed do not produce a CD profile above 250 nm at the experimental conditions in the absence of the ninhydrin sensor. For quantitative *ee* sensing, a solution of **A** (10.0 mM) was prepared in DMSO

and 500.0 μL amounts were distributed into vials containing 500.0 μL of DMSO to generate reaction concentrations of 5.00 mM. Equimolar amounts of 1-phenylethan-1-amine dissolved in DMSO with varied *ee* values (100.0, 80.0, 60.0, 40.0, 20.0, 0, -20.0, -40.0, -60.0, -80.0, -100.0 *% ee*) were subsequently added (0.005 mmol, 10 μL). CD measurements were then taken by dispensing 550 μL aliquots of the reaction mixtures into a quartz cuvette and dilution to 2.5 mL with acetonitrile to afford a calibration curve. A second set of ninhydrin sensing reactions using random *ee* mixtures of the same amine (72.0, 50.0, 38.0, -16.0, -46.0, -84.0 %*ee*) were conducted following the same procedure. The enantiomeric compositions of these mixtures were then determined by comparison of the individual CD maxima obtained at 360 nm with the calibration curve.

Supporting Information. Details of the CD sensing procedures, optimization studies, spectroscopic reaction analysis, NMR, CD and UV spectra.

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