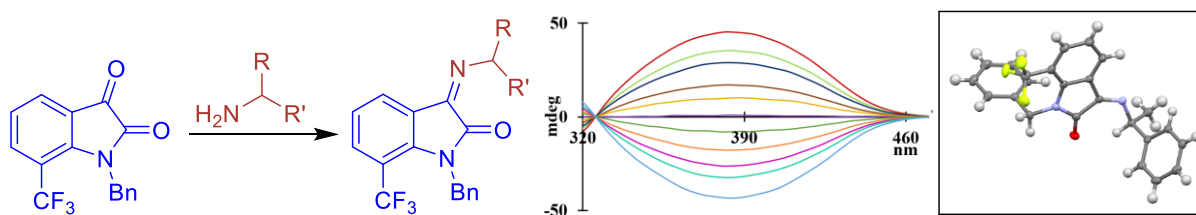


Chiroptical Sensing of Amines with Isatins

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Graphical Abstract



Abstract

Isatins are extensively researched compounds with diverse applications, particularly as synthetic precursors in pharmaceutical developments. However, their use as optical probes for enantioselective sensing of chiral amines has not been explored to date. Herein, we present a novel chiroptical assay with an optimized isatin that generates strong, red-shifted circular dichroism (CD) signals at approximately 380 nm upon ketimine formation with chiral amines. The intensity of the induced CD signal increases linearly with the enantiomeric excess of the analyte and thus allows quantitative chirality analysis. The general usefulness of this approach is demonstrated with a broad range of aliphatic and aromatic chiral amines, and by accurate determination of the enantiomeric composition of 10 samples.

Keywords: isatins, chiroptical sensing, chiral amines, Schiff bases, circular dichroism spectroscopy, enantiomeric excess determination

Introduction

Isatins have been investigated in depth over the past couple of decades due to their wide range of applications, particularly in the health sciences, and are abundant natural products frequently encountered in plants and animals.¹⁻⁷ They are commonly used synthetic precursors of anti-cancer, anti-viral, anti-bacterial and anti-inflammatory drugs among others.⁷⁻¹⁸ For example, the isatin scaffold is a key pharmacophore in the marketed anti-cancer drugs sunitinib V and toceranib.¹⁹⁻²² In addition, they play important roles as dyes in the textile and food industries.^{23,24} To the best of our knowledge, isatins have not been used as optical probes for the sensing of chiral amines. However, they have been applied to fluorescence analysis of metals.^{7,25-29}

Chiral compound analysis has become a fascinating research area and has received steadily increasing attention since the groundbreaking work by Pasteur almost 180 years ago.³⁰ Specifically, the determination of the enantiomeric ratio (*er*) of non-racemic mixtures is a crucial task in contemporary drug development.³¹⁻³⁵ While traditional methods such as NMR and chiral HPLC have been widely used, optical techniques based on circular dichroism (CD) spectroscopy that can be more efficient and are compatible with high-throughput workflows have gained increasing popularity over the past few years.^{36,37} Chiral amines undoubtedly belong to the most sought-after classes of compounds. They are routinely used in synthetic chemistry and the pharmaceutical industry and therefore remain an important analytical target.³⁸⁻⁴⁰ Among several other chiroptical sensing approaches,⁴¹⁻⁴⁷ Schiff base formation has been a privileged covalent binding motif and successfully used in various ways to date.⁴⁸⁻⁵⁰ However, the generation of red-shifted CD effects has remained a major challenge and continues to be a highly sought-after feature of chiroptical probes as it promises to avoid interferences from chiral impurities that tend to display signals below 300 nm. During recent years, we have selected isatins as invaluable starting materials in

asymmetric catalysis,⁵¹⁻⁵³ and also developed various amine sensors that operate via Schiff base formation.⁵⁴⁻⁵⁷ We now wish to report a sensing assay applicable to accurate *er* determination of a broad range of amines using isatins in a new way. The analysis is achieved by a condensation reaction with a derivative of a commercially available isatin that was found to produce strong CD signals at approximately 380 nm at millimolar concentration, which is generally considered highly advantageous in this field.

Materials and Methods

NMR and CD spectroscopy

All chemicals used were purchased and used without purification. NMR spectra were obtained at 400 MHz (¹H NMR) in CDCl₃. Chemical shifts are reported in ppm relative to the chloroform signal. CD spectra were collected with a data pitch of 0.5 nm, a bandwidth of 1 nm, in a continuous scanning mode with a scanning speed of 500 nm/min and a response of 1 s, using a quartz cuvette (1 cm path length). The data were baseline corrected and smoothed using a binomial equation. Typically, a solution of the amine (0.1 M in chloroform, 250.0 μ L) was added to a solution of the CD probe (0.06 M in chloroform, 500.0 μ L) and the mixture was stirred for 2.5 hours at 45 °C over molecular sieves. CD measurements were taken after dilution to 0.75-2.12 mM with chloroform. For quantitative determinations of the enantiomeric composition of phenylethylamine samples, three CD measurements, each obtained with a scanning speed of 500 nm/min, were averaged.

X-ray crystallography

A single crystal was obtained by slow evaporation of a solution of **13** (50.0 mg, 0.12 mmol) in acetonitrile. Single crystal X-ray analysis was performed at 100 K using a Bruker D8 Quest equipped with a Photon 3 detector and Mo micro-focus source (λ = 0.71073 Å). Data were

integrated with the Bruker SAINT program. Structure solution and refinement was performed using the SHELXT/PC suite and ShelXle. Intensities were corrected for Lorentz and polarization effects and an empirical absorption correction was applied using Blessing's method as incorporated into the program SADABS. Non-hydrogen atoms were refined with anisotropic thermal parameters. Crystal data: C₂₄H₁₉F₃N₂O, M = 408.41 g/mol, colorless block, 0.380 x 0.392 x 0.884 mm³, space group C2, a = 23.3531(12), b = 10.3656(5), c = 8.1112(4), alpha = 90, beta = 92.565(2), gamma = 90. The CCDC number for this compound is 2382842.

Synthesis of *N*-benzyl isatin

The synthesis of *N*-benzyl isatin, **2**, was carried out according to a previously published literature protocol.⁵⁸ In an oven dried 3-neck flask, isatin (147.1 mg, 1.0 mmol) and potassium carbonate (414.6 mg, 3.0 mmol) were combined in DMF (5.0 mL). The mixture was stirred for 5 minutes followed by the addition of benzyl bromide (205.3 mg, 1.2 mmol) and overnight stirring. Upon completion, the crude mixture was extracted with dichloromethane and water at room temperature. The combined organic layers were dried over sodium sulfate and purified via column chromatography. *N*-Benzyl isatin, **2**, was obtained as an orange crystalline solid in 95% yield (224.7 mg) after purification by flash chromatography using a mixture of 5% ethyl acetate in hexanes as the mobile phase. ¹H NMR (400 MHz, CDCl₃) δ 7.62 (d, *J* = 7.5 Hz, 1H), 7.48 (ddd, *J* = 7.8, 7.8, 1.4 Hz, 1H), 7.40 – 7.28 (m, 5H), 7.10 (ddd, *J* = 8.1, 8.1, 1.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 4.94 (s, 2H).

Synthesis of 7-trifluoromethyl *N*-benzyl isatin

In an oven dried 3-neck flask, 7-trifluoromethyl isatin (200.0 mg, 0.93 mmol) and potassium carbonate (297.0 mg, 1.86 mmol) were combined in DMF (2.0 mL). The mixture was stirred for

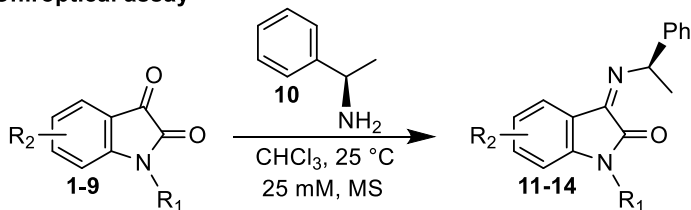
5 minutes followed by the dropwise addition of benzyl bromide (318.0 mg, 1.86 mmol) and overnight stirring. Upon completion, the crude mixture was extracted with dichloromethane and water at room temperature. The combined organic layers were washed with brine, dried over magnesium sulfate and purified via column chromatography. 7-Trifluoromethyl *N*-benzyl isatin, **9**, was obtained as an orange crystalline solid in 94% yield (264.2 mg) after purification by flash chromatography using a mixture of 5% ethyl acetate in hexanes as the mobile phase. ¹H NMR (400 MHz, CDCl₃) δ 7.90 – 7.82 (m, 2H), 7.34 – 7.20 (m, 4H), 7.16 (d, *J* = 7.2 Hz, 2H), 5.22 (s, 2H). HRMS (ESI-TOF) *m/z*: [M+Na]⁺ calcd for [C₁₆H₁₀F₃NO₂Na] 328.0556, found 328.0558.

Results and Discussion

We began our investigation by comparing CD signal inductions obtained upon condensation of the isatin derivatives **1-9** and amine **10** which was selected as a test analyte (Figure 1). Probes **2** and **9** were synthesized according to previously reported literature methods while all the other isatins were commercially available (see SI).⁵⁸ The isatins **1-4** were initially chosen to determine the effect of N-protecting groups on the CD induction. We were pleased to find that they produce CD signals above 300 nm at millimolar concentration. We then screened probes **5-8** carrying functional groups in different positions of the fused aromatic ring. This revealed that the presence of a trifluoromethyl substituent at the 7-position significantly increases the amplitude of the CD induction. We therefore modified probe **7** by benzylation in the presence of potassium carbonate to produce probe **9** which showed the strongest red-shifted CD signal upon Schiff base formation with **10**. It is noteworthy that the induced CD spectrum has a maximum at approximately 380 nm. Red-shifted chiroptical probe responses are typically favored for quantitative sensing applications because this

avoids interference from signals of chiral impurities that tend to have CD signatures in the more blue-shifted wavelength region.

a) Chiroptical assay



b) Probe screening

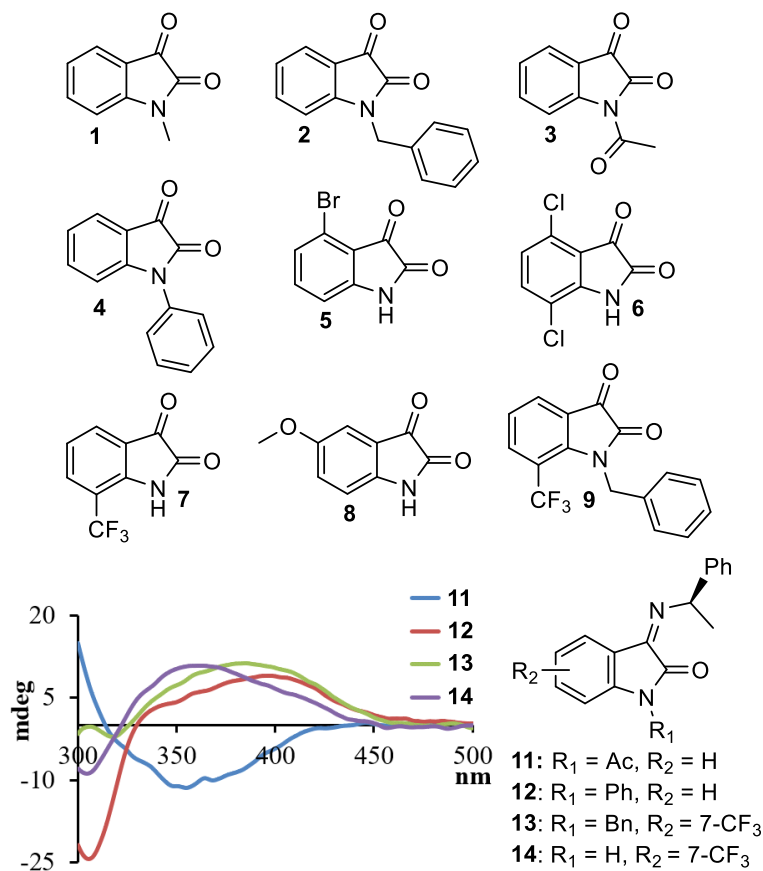


Figure 1. a) Initial probe screening conditions with *(R)*-**10**. b) Structures of probes **1-9** and examples of CD inductions obtained upon Schiff base formation. CD measurements were obtained at 0.40 mM concentration.

We then continued to optimize our chiroptical sensing assay using probe **9** and found that the strongest CD effects are obtained when the reaction is conducted for 150 minutes at 45 °C in the presence of molecular sieves (see SI). Additionally, a racemization study was conducted with isolated imine **13** to assess its stereochemical stability. The Schiff base **13** was stirred overnight in the presence of 1 equivalent of either triethylamine or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

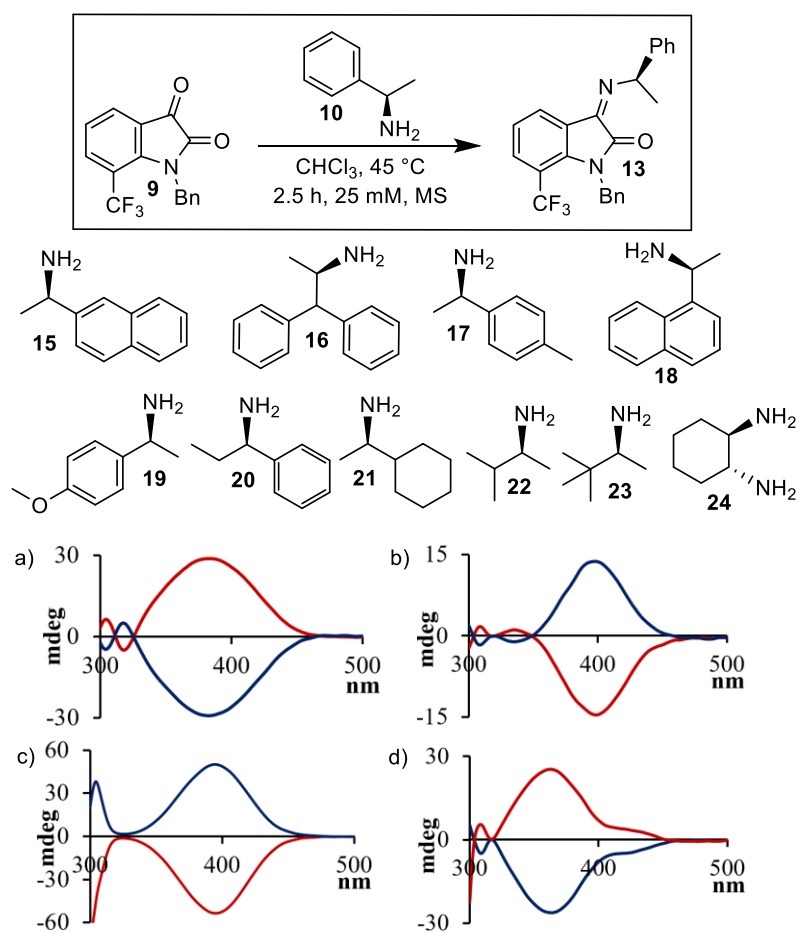


Figure 2. Optimized chiroptical sensing assay and structures of all primary amine substrates investigated (only one enantiomer is shown). CD induction measured with the Schiff base formed from probe **9** and a) amine **10**, b) amine **21**, c) amine **23**, and d) amine **24** (2 equivalents of probe

9 were used). The CD spectra obtained with the Schiff base formed from the (*R*)-enantiomer are shown in red while the (*S*)-enantiomers appear in blue. CD measurements a), b) and d) were obtained at 0.75 mM concentration while c) was obtained at 1.46 mM concentration.

The CD signal of the mixture in triethylamine remained almost unchanged overnight, indicating that **13** is not susceptible to racemization in the presence of an amine base. However, the CD amplitude significantly decreased in the presence of DBU, suggesting that **13** is prone to racemization when a stronger base is added (see SI). To determine the practicality of this sensing method we extended the assay to other aliphatic and aromatic amines (Figure 2). The Schiff bases of all amines tested gave a strong CD signal around 380 nm at sub-millimolar concentrations. It is noteworthy that CD signals of similar intensity are obtained with amines carrying either an aromatic ring or just an aliphatic moiety.

We used ^1H NMR spectroscopy to monitor the formation of the Schiff base from probe **9** and amine **10** over time (Figure 3). The results indicate that the reaction is complete within 2 hours. Notably, the methine and methyl signals at 4.1 ppm and 2.4 ppm undergo a downfield shift to 6.5 ppm and 2.6 ppm, respectively, which corresponds to the formation of the Schiff base **13** from probe **9**. A single crystal of **13** suitable for X-ray analysis was grown by slow evaporation of an acetonitrile solution. The crystallography confirmed formation of the (*Z*)-imine structure as expected (Figure 4).

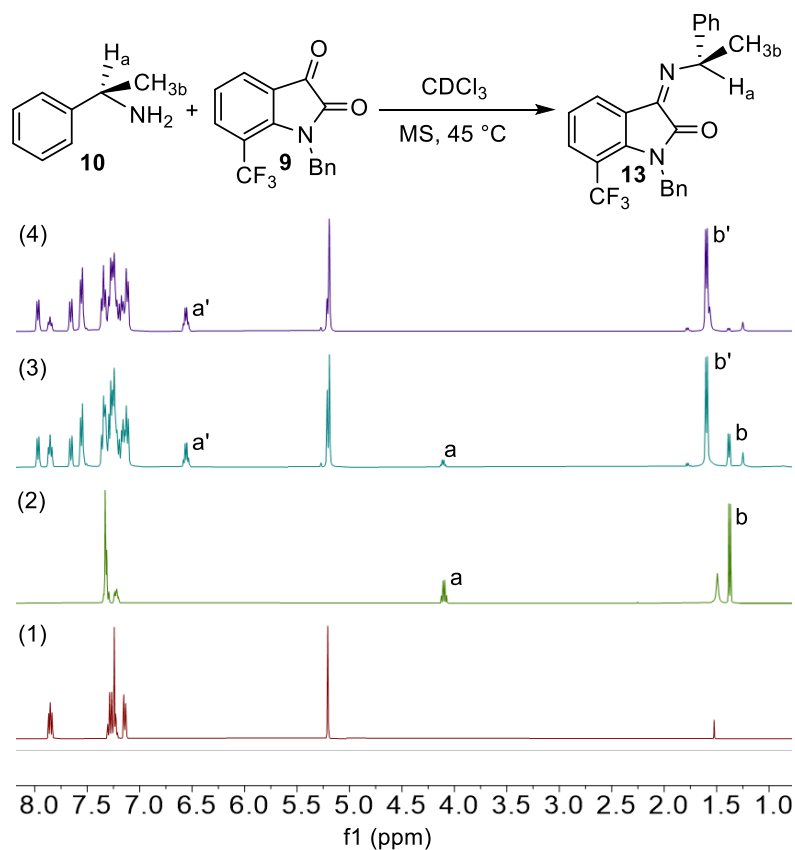


Figure 3. ^1H NMR analysis of the reaction between probe **9** and amine **10** in CDCl_3 . (1) Probe **9**; (2) amine **10**; (3) reaction mixture of probe **9** and amine **10** after 1 hour; (4) reaction mixture of probe **9** and amine **10** after 2 hours.

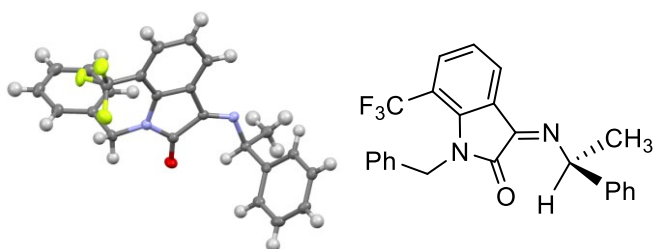


Figure 4. X-ray structure of Schiff base **13**.

Finally, we attempted to demonstrate the usefulness of chiroptical amine sensing with isatins for *er* determination (Figure 5). It is important to note that the use of an achiral probe like

9 avoids the formation of diastereomers. This simplifies quantitative analysis efforts by eliminating errors that could arise from undesirable kinetic resolution and unequal formation of diastereomeric derivatives. A calibration curve generated with probe **9** and amine **10** showed that the magnitude of the induced CD signal increased linearly with the enantiomeric composition of the analyte (Figure 5).

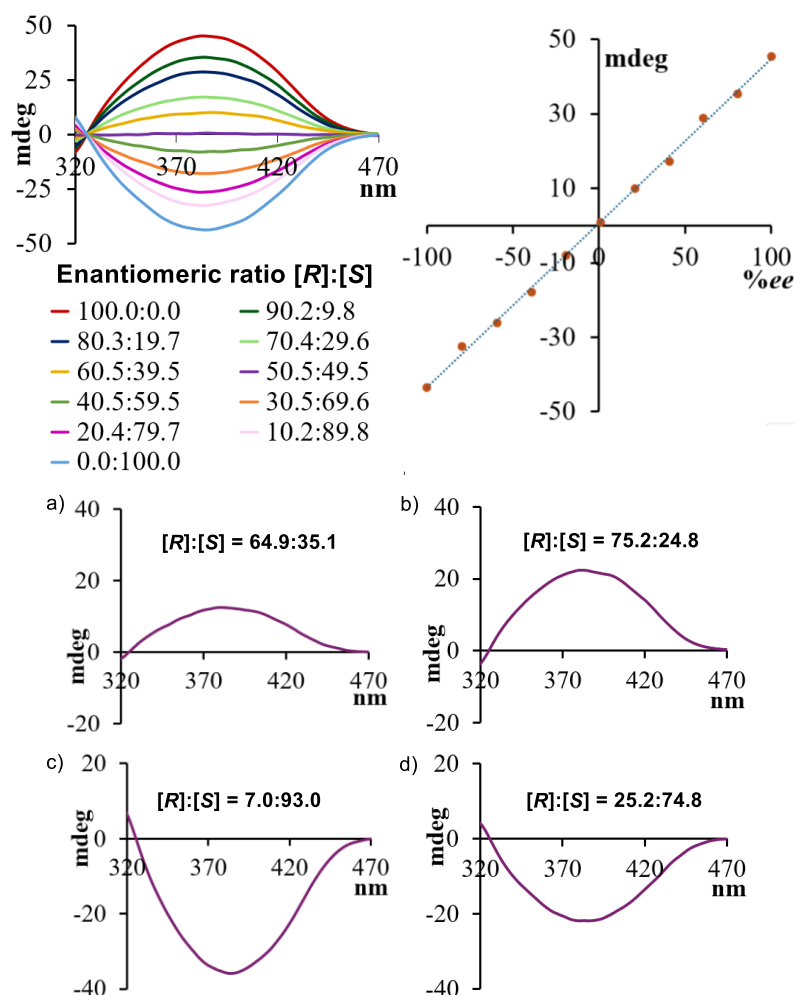


Figure 5. Linear CD response of the isatin probe to the sample enantiomeric composition. Top: Induced CD signals obtained with probe **9** and amine **10** at varying *er* (left). Plot of the CD signal at 383.5 nm and varying *ee* values (right). All CD measurements were obtained at 0.98 mM. The “–” sign indicates that (*S*)-(-)-1-phenylethylamine was in excess. Bottom: Selected examples of

sensing samples with $[R]:[S] = 64.9:35.1$ (a); 75.2:24.8 (b); 7.0:93.0 (c); 25.2:74.8 (d). See SI for details.

The linear correlation established above was then utilized to determine the *er* of ten amine samples. The results are shown in Table 1. We were pleased to find that we could correctly determine the *er* and absolute configuration of all ten samples within a relatively small margin of error. For example, samples 3 and 9 had an $[R]/[S]$ ratio of 75.2:24.8 and 14.3:85.7, while the calculated *er* values obtained by CD sensing were 74.7:25.3 and 13.7:86.3, respectively. The results show that this assay works effectively across a wide range of enantiomeric compositions.

Table 1. Chiroptical sensing of the *er* of 10 unknown samples.

Sample number	Sample composition		Sensing results	
	Configuration	<i>er</i>	Configuration	<i>er</i>
1	<i>R</i>	92.2:7.8	<i>R</i>	94.0:6.0
2	<i>R</i>	83.5:16.5	<i>R</i>	84.2:15.8
3	<i>R</i>	75.2:24.8	<i>R</i>	74.7:25.3
4	<i>R</i>	64.9:35.1	<i>R</i>	63.5:36.5
5	<i>R</i>	53.7:46.3	<i>R</i>	51.2:48.8
6	<i>S</i>	43.7:56.3	<i>S</i>	44.3:55.7
7	<i>S</i>	34.1:65.9	<i>S</i>	36.1:63.9
8	<i>S</i>	25.2:74.8	<i>S</i>	24.4:75.6
9	<i>S</i>	14.3:85.7	<i>S</i>	13.7:86.3
10	<i>S</i>	7.0:93.0	<i>S</i>	8.6:91.4

See SI for details. The absolute configuration was determined by comparing the sense of CD induction with a reference sample.

Conclusion

In summary, we have developed an efficient chiroptical sensing assay for chiral primary amines utilizing readily available 7-trifluoromethyl *N*-benzyl isatin. This probe reacts with amines to form a Schiff base that give a strong CD signal with a maximum at approximately 380 nm. The generation of a red-shifted CD signal is considered an important improvement over other chiroptical probes that do not exhibit the advantageous isatin scaffold and thus produce CD effects at lower wavelengths that may be more prone to interferences from chiral impurities. The intensity of the induced CD signal can be correlated with the enantiomeric composition of the analyte and thus enables quantitative chirality analysis. The scope of this assay was demonstrated with various aliphatic and aromatic primary amines and accurate determination of the *er* of ten samples containing 1-phenylethylamine was shown. This assay is expected to streamline modern chiral compound analysis workflows and is amenable to high-throughput experimentation equipment, which can enable parallel analysis of hundreds of samples.

Author Contributions

C.W., F.S.K. and F.M. designed the experiments and analyzed the data. FSK, F.M. and S.L.P. performed all experiments. C.W. and F.S.K. wrote the manuscript and supporting information. C.W. and S.L.P. conceived the idea and C.W. and F.S.K. supervised the project. All authors discussed the results and commented on the manuscript.

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Conflict of Interest

All authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supporting information of this article.

Supporting Information

Synthetic protocols and compound characterization, CD spectra, mechanistic analysis, X-ray crystallography data and details of the optimization studies may be found in the online version of this article at the publisher's website.

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