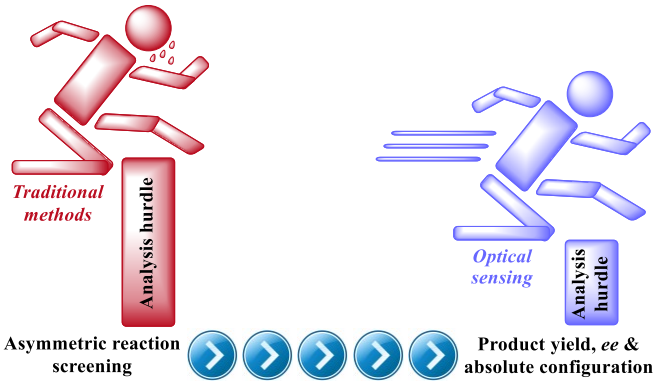
Accelerated Asymmetric Reaction Screening with Optical Assays

Diandra Safira Hassan[a]
F. Safia Kariapper[a]
Ciarán C. Lynch[a]
Christian Wolf[a]*

[a]Department of Chemistry, Georgetown University, 37th and O Streets, Washington, DC 20057, USA.

In memory of Rachel Aterrado who is sorely missed in our department.



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Abstract Asymmetric reaction development often involves optimization of several mutually dependent parameters that affect the product yield and enantiomeric excess. Widely available high-throughput experimentation equipment and optical sensing assays can drastically streamline comprehensive optimization efforts and speed up the discovery process at reduced cost, workload and waste production. A variety of chiroptical assays that utilize fluorescence, UV and circular dichroism measurements to determine reaction yields and *ee* values are now available, enabling the screening of numerous small-scale reaction samples in parallel with multi-well plate technology. Many of these optical methods considerably shorten work-up protocols typically required for traditional asymmetric reaction analysis and some can be directly applied to crude mixtures thus eliminating cumbersome separation and purification steps altogether.

Key words asymmetric synthesis, reaction development, optical methods, high-throughput screening, chirality sensing

1. Introduction

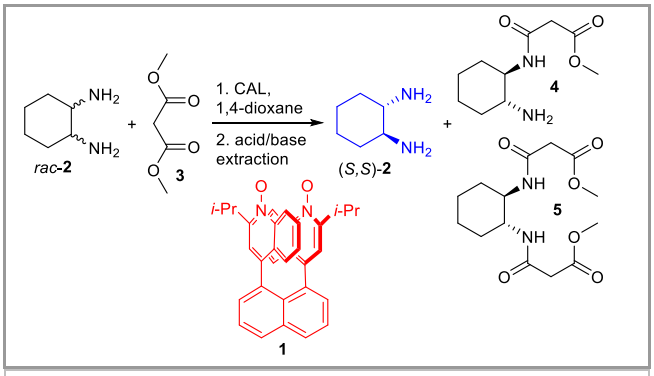
The development of new asymmetric reactions often requires comprehensive evaluation of potentially synergistic effects of different catalyst structures, additives, solvent, concentration, temperature and other parameters to identify optimal conditions that produce a chiral target compound in the highest possible yield and enantiomeric excess (*ee*). The general availability of high-throughput experimentation equipment and automated multi-well plate technology allows today's chemists to set up hundreds or even thousands of miniaturized reactions in parallel to exhaustively vary every reaction parameter. While it is no problem to conduct many asymmetric reactions simultaneously, the overall progress with the optimization task is largely diminished by the time-consuming analytical steps that follow. In fact, the yield and the enantiomeric composition of the product are generally determined separately using one sample at a time because inherently serial techniques are used. It is common practice that yields are obtained gravimetrically after product isolation or with NMR and GC methods after addition of internal standards. Chromatographic enantioseparation of purified materials on a chiral stationary phase[1] and NMR spectroscopy[2] using either a chiral solvating or derivatization agent remain the most popular means to uncover the enantiomeric excess.[3] The success of these well-established techniques is exemplified in literally countless reports. Traditional analysis, however, is too slow to process hundreds of samples which generates a major bottleneck and operational limitation in asymmetric reaction development projects. Not surprisingly, the search for more time-efficient analytical tools that are amenable to high-throughput screening of small-scale reactions is underway. Noteworthy progress has been made with the introduction of mass spectrometric,[4] IR thermographic,[5] capillary electrophoretic,[6] and biochemical assays.[7]

During the last decade, optical methods based on fluorescence, UV and circular dichroism (CD) spectroscopy have surfaced and been used to determine both yield and *ee* from crude reaction mixtures, sometimes by fully eliminating laborious, time-consuming and costly product purification.[8] The obvious speed advantages of these assays that are generally compatible with automated equipment, multi-well plate readers and parallel data collection bear tremendous potential to streamline asymmetric reaction development efforts, and many of the methods discussed herein are ready for use by the synthetic community. In this Short Review, early advances and the current state-of-the-art of this rapidly evolving field are presented with case studies that highlight the principles, experimental procedures and applications of chiroptical sensing systems that collectively aim to transform traditionally tedious asymmetric reaction analysis into a practical screening workflow. These assays generally require simple fluorescence, UV or CD measurements and calibration curves that can be obtained in parallel for quantification of reaction yield and %*ee*. The impressive diversity of optical methods that have been introduced and tested to date mirrors the variety of target compounds and asymmetric reactions that need to be analyzed. To help the interested reader who might not be familiar with the chiroptical sensing field to easily grasp the underlying sensing concept and utility, we decided to refrain from presenting

spectral details and focused on describing the asymmetric reactions and sensing protocols instead. The chiral products are always shown in blue color and the sensing components used are displayed in red to facilitate a quick overview. Key information regarding the time and preparation steps needed for the chiroptical sensing analysis are provided when they were available.

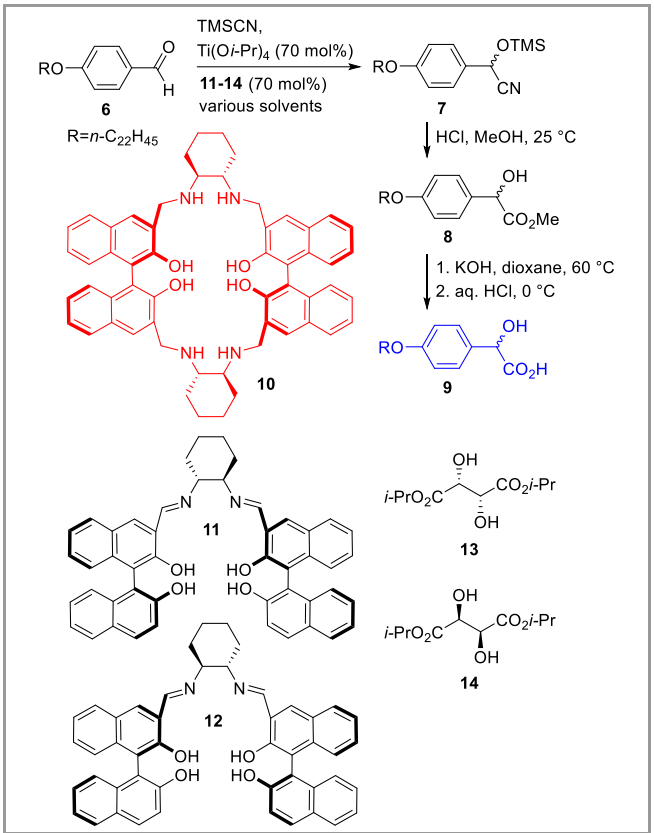
2. Fluorescence assays

Our group has had a long-standing interest in chirality sensing methodology developments and initially we introduced axially chiral 1,8-diquinolyl- and 1,8-diacridylnaphthalenes that can be used in various ways to achieve optical concentration and *ee* analysis with a variety of analytes. In 2005, we used the 1,8-diquinolylnaphthalene *N,N'*-dioxide **1** to monitor the enzymatic kinetic resolution (KR) of *trans*-1,2-diaminocyclohexane, **2**, with dimethyl malonate, **3**, in the presence of *Candida antartica* lipase, CAL (Scheme 1).[9] Small aliquots of the KR mixture were subjected to acid/base extraction but then directly subjected to **1** which undergoes hydrogen bonding interactions with **2** resulting in enantioselective fluorescence enhancement. This sensing assay gave accurate results and proved to be more time-efficient than traditional HPLC which necessitated cumbersome product derivatization toward a UV-active diamide. Interestingly, compounds **4** and **5** which are both produced in this reaction do not interfere with the fluorescence sensing analysis while the former, present in small but not negligible amounts, possibly compromised the chiral HPLC data due to co-elution issues.



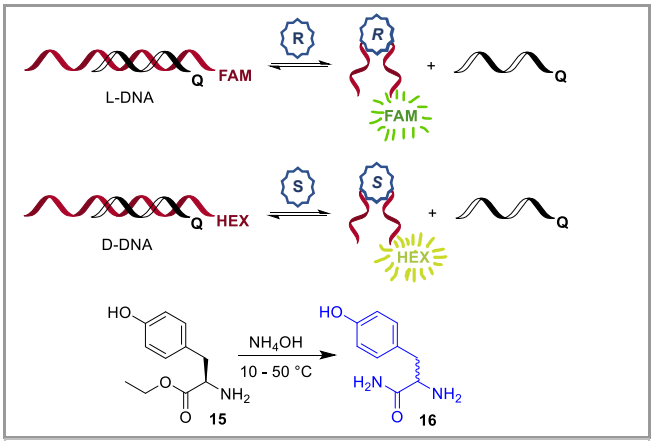
Scheme 1. Fluorescence sensing of a kinetic resolution.

Pu and colleagues have made very important contributions to the optical chirality sensing field by introducing many fluorescent BINOL derived probe designs. They were among the first to recognize the potential of enantioselective fluorescence sensing for reaction analysis. They first applied a fluorescent bisbinaphthyl macrocycle in the enantioselective analysis of the asymmetric cyanosilylation of the benzaldehyde derivative **6** with trimethylsilyl cyanide.[10] The chemosensor used in this study cannot be directly applied to compounds like **7** which was therefore hydrolyzed via ester **8** to the free α-hydroxy acid **9** (Scheme 2). The mandelic acid derivative **9** was finally isolated by selective precipitation and subjected to enantioselective fluorescence sensing with enantiopure **10** using a carefully optimized ternary solvent mixture. The corresponding diastereomeric adducts have different fluorescence intensities which provides a reliable entry to *ee* determination. With this protocol in hand, the enantioselectivity of the cyanosilylation reaction in the presence of 70 mol% of titanium complexes carrying tartrate or BINOL-derived ligands **11-14** in various solvents was investigated. The sensing results obtained with **10** were in good agreement with *ee* values determined by chiral HPLC, proving the reliability of the fluorescence measurements.



Scheme 2. Enantioselective synthesis of **7** and structure of the fluorescent chemosensor **10** that can be used for *ee* analysis of the mandelic acid derivative **9**.

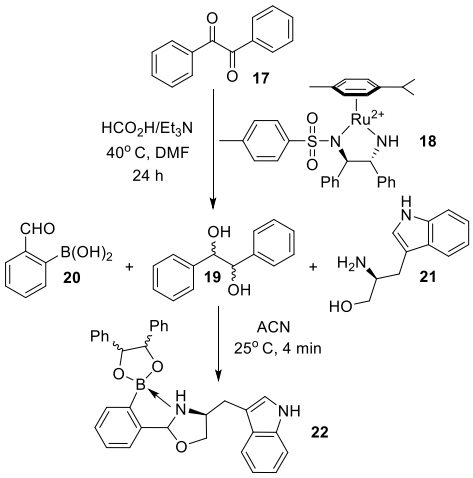
Heemstra and coworkers used enantiomeric L- and D-DNA aptamers hybridized with a short complementary strand **Q** and equipped with fluorescein (FAM) and hexachlorofluorescein (HEX), respectively, for the enantioselective recognition and quantification of tyrosinamide (Scheme 3).[11] This fluorescence assay was successfully used to monitor the aminolysis and racemization of the ethyl ester of D-tyrosine **15** to the primary amide **16** at different temperatures in the presence of ammonium hydroxide. This elegant concept allows direct concentration analysis of each enantiomer of **16** simply by measuring the emission of the orthogonal fluorophores and it is noteworthy that the underlying chemical biology could be modified to screen other asymmetric reactions in water or aqueous solution.



Scheme 3. Principle of the fluorescence analysis of the aminolysis and racemization of **1** with DNA aptamers.

Anzenbacher and colleagues analyzed the ruthenium catalyzed asymmetric Noyori hydrogenation of benzil, **17**, using a dynamic covalent assembly that was obtained by mixing the reaction product hydrobenzoin, **19**, formylphenylboronic acid, **20**, and tryptophanol, **21** (Scheme 4).[12] Multivariate data analysis of the fluorescence emission changes of the boron complex **22** allowed calculation of the enantiomeric excess and concentration of **19**. The practicality of this fluorimetric assay is quite remarkable and it impresses with several attractive features: it is very fast, generates accurate data with less than 1 mg of crude reaction mixtures, and it requires only commercially available reagents.[13]

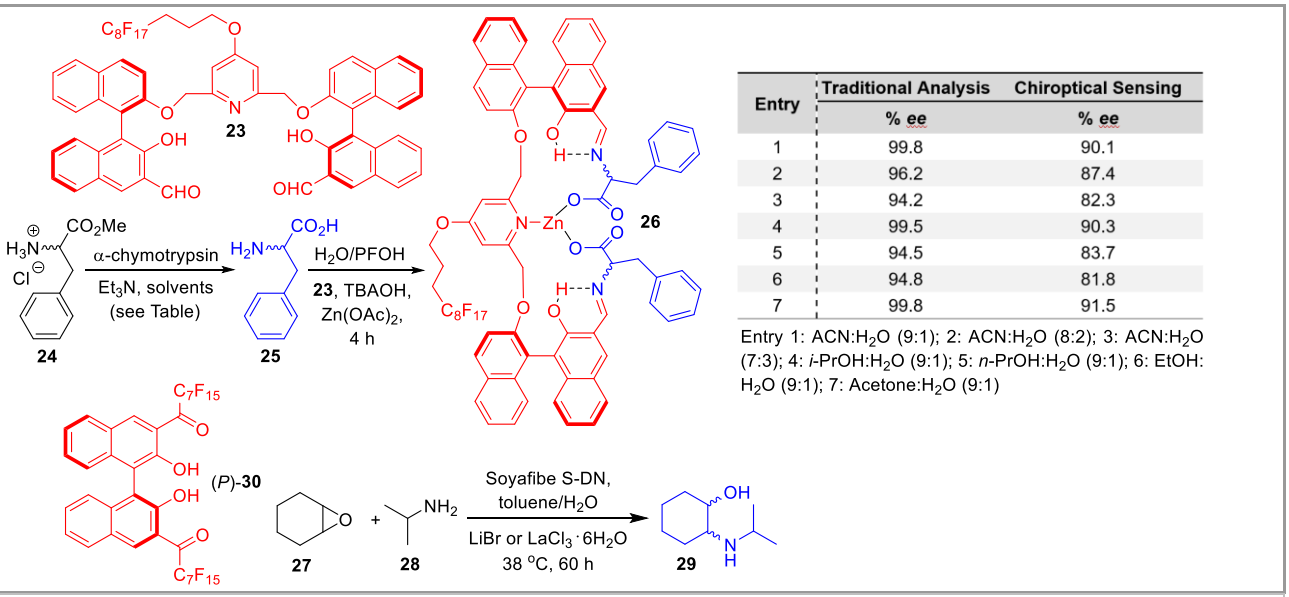
Recently, Pu and colleagues used the bis(binaphthyl)-based fluorescent probe **23** to evaluate the effect of solvent variation on the enantioselectivity of the enzymatic hydrolysis of phenylalanine methyl ester, **24** (Scheme 5).[14] The probe has a highly fluorinated alkyl chain and can be used in a biphasic aqueous/1H,1H,2H,2H-perfluoro-1-octanol (PFOH) system. Upon completion of the reaction, the free amino acid **25** is extracted into the fluorous phase where enantioselective fluorescent detection takes place, probably through the formation of zinc(II) complex **26**. The crude residues of seven reactions were first weighed out to estimate yields. This material was then applied in the biphasic assay. After stirring for 4 hours, the layers were separated and the mixture was allowed to stand for 10 minutes prior to the fluorescence analysis. Comparison to HPLC showed that the sensing result has an absolute %*ee* error margin of 8.3 to 13.0%. The same concept was successfully applied to the Soyafibe S-DN catalyzed ring opening of 1,2-epoxycyclohexane, **27**, with isopropyl amine, **28**, using the fluorinated BINOL derivative **30**.[15] Interestingly, this chemosensor can also be used in racemic form for concentration-independent %*ee* determination which very likely increases the ruggedness of this assay.



Entry	^{1}H NMR Analysis		Chiroptical Sensing	
	AC	% *ee*	AC	% *ee*
1	*R*,*R*	97.5	*R*,*R*	95.4
2	*R*,*R*	≥ 99	*R*,*R*	99.5
3	*S*,*S*	99	*S*,*S*	98.3
4	*S*,*S*	≥99	*S*,*S*	99.7
5	*R*,*R*	4	*R*,*R*	3.75
6	*R*,*R*	3.7	*R*,*R*	2.87

Entries 1, 3, 5: Analysis of crude product after solvent evaporation; Entries 2, 4, 6: The diol was recrystallized from methanol; 1 and 2: (*S*,*S*)-**18** was used; Entries 3 and 4: (*R*,*R*)-**18** was used; 5 and 6: racemic **18** was used.

Scheme 4. Anzenbacher's fluorescence analysis of the Noyori hydrogenation of benzil.



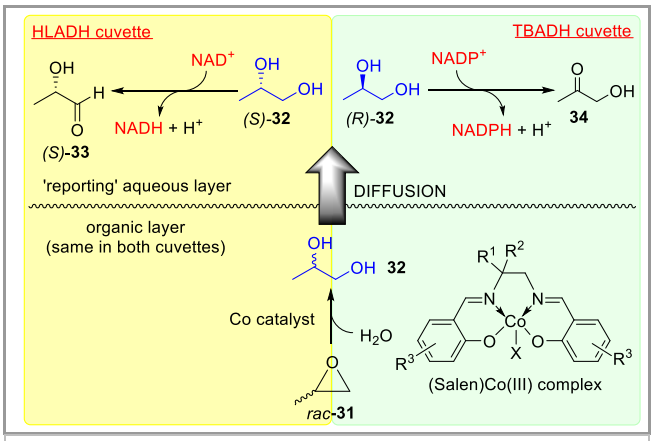
Entry	Traditional Analysis	Chiroptical Sensing
	% *ee*	% *ee*
1	99.8	90.1
2	96.2	87.4
3	94.2	82.3
4	99.5	90.3
5	94.5	83.7
6	94.8	81.8
7	99.8	91.5

Entry 1: ACN:H_2O (9:1); 2: ACN:H_2O (8:2); 3: ACN:H_2O (7:3); 4: *i*-PrOH:H_2O (9:1); 5: *n*-PrOH:H_2O (9:1); 6: EtOH:H_2O (9:1); 7: Acetone:H_2O (9:1)

Scheme 5. Enantioselective sensing of the enzymatic hydrolysis of phenylalanine methyl ester and of the Soyafibe S-DN catalyzed epoxide ring opening with isopropyl amine.

3. UV sensing methods

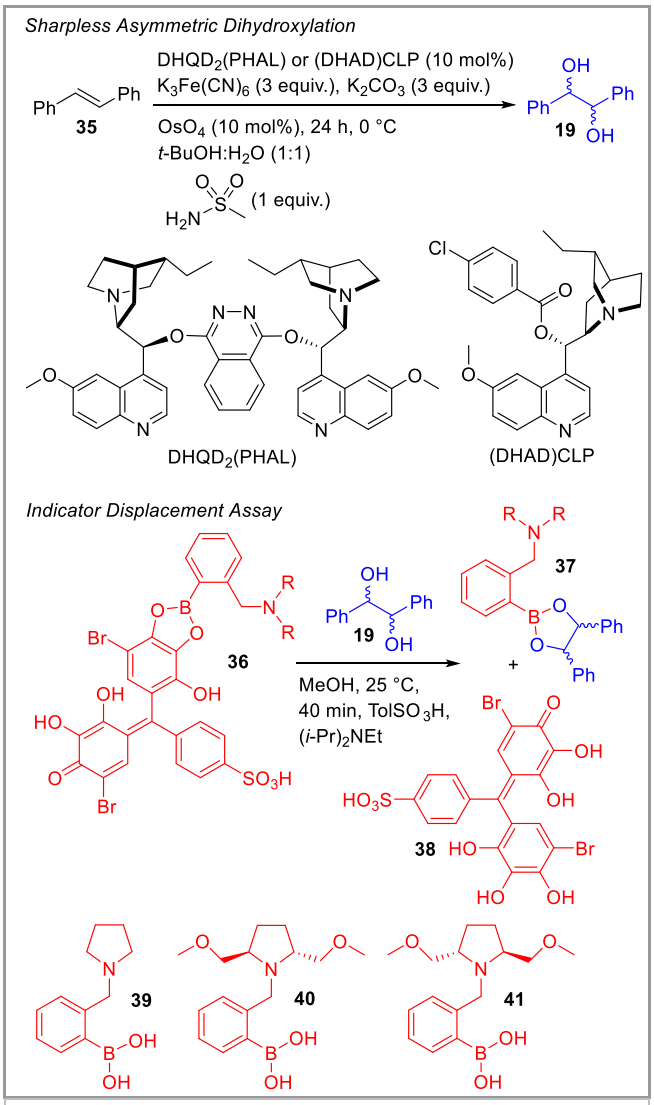
In 2015, Berkowitz and coworkers introduced an elegant enzymatic assay that allowed optical high-throughput screening of the hydrolytic kinetic resolution (HKR) of propylene oxide, **31**, toward diol **32** (Scheme 6).[16] They used two cuvettes filled with an organic layer containing the epoxide **31** and a cobalt Salen catalyst. Each cuvette also contained an aqueous layer charged with either $NADP^+$or NAD^+ together with an enantiospecific reporter enzyme. When the 1,2-diol **32** produced in the organic layer diffuses into the aqueous layer it is readily oxidized. In one cuvette, the alcohol dehydrogenase isolated from horse liver (HLADH) selectively oxidizes (*S*)-**32** to (*S*)-**33** whereas its analogue obtained from *Thermoanaerobium brockii* (TBADH) selectively oxidizes the *R*-diol to **34** in the other cuvette. These reactions either produce one equivalent of NADH or NADPH and this was monitored by an increase in the UV signal at 340 nm. Forty-nine reactions with different Salen complexes were thus screened – an impressive throughput even by today's standards. Comparison with chiral HPLC, however, revealed substantial error margins in some cases. While the double-cuvette method facilitates *in situ* reaction screening, this approach relies on the use of enzymes with complementary enantiospecificity and a biphasic setup that is only applicable to water-tolerant asymmetric reactions.



Scheme 6. Berkowitz' enzymatic high-throughput screening method.

The high impact of chiroptical assays that have been developed by the Anslyn group with a focus on real-world applications such as parallel reaction screening cannot be overstated. In 2009, they showed how one can determine the *ee* and yield of the Sharpless asymmetric dihydroxylation of *trans*-stilbene, **35**, by analyzing the diol **19** with an indicator displacement assay (IDA).[17] The reaction product was isolated and then subjected to host-indicator complexes of the general structure **36** giving rise to **37** and the free indicator **38** (Scheme 7). Chemometric analysis of the UV absorbance changes observed with hosts **39**-**41** gave *ee* values with low error margins. The total time needed for the IDA preparation, its execution and the spectral acquisitions for two asymmetric dihydroxylations performed with $(DHQD)_2(PHAL)$ and (DHAD)CLP, respectively, was only 40 minutes, and one can easily imagine that many reactions could be processed in parallel in the same time frame. The authors showed that this assay is perfectly compatible with multi-well plate technology and automation, a very important advance that proved the

possibility of streamlined asymmetric reaction screening by optical sensing more than a decade ago.

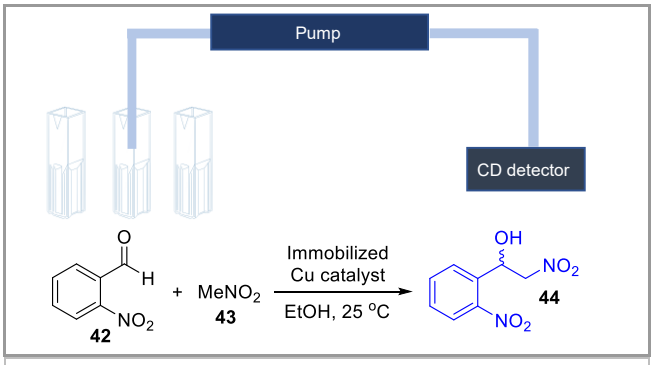


Scheme 7. Enantioselective indicator displacement assay used by Anslyn for screening of the Sharpless asymmetric dihydroxylation reaction.

4. Sensing with circular dichroism probes

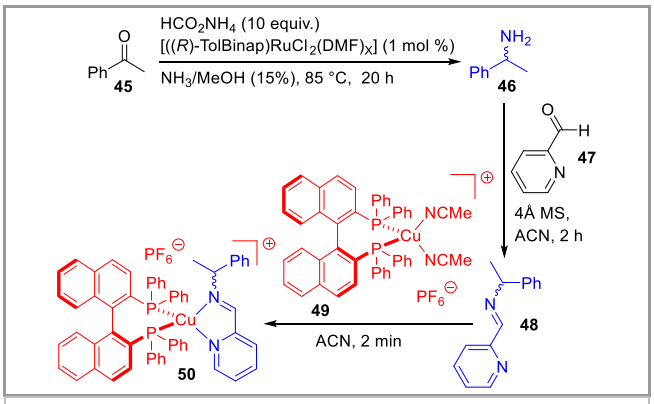
The majority of examples of optical asymmetric reaction analysis is based on circular dichroism measurements. Unlike other techniques, CD spectroscopy offers an immense advantage because it intrinsically differentiates between enantiomers. One can therefore avoid problems that may arise with UV or fluorescence methods which require the use of chiral probes to generate diastereomers for the determination of *ee* values. A variety of very practical achiral chemosensor designs that generate strong CD effects for *ee* analysis together with distinct UV or fluorescence signal changes for concomitant yield determination have emerged in the last decade. These assays have proven most useful for asymmetric reaction analysis, in particular when crude mixtures were seamlessly processed without product purification.

In 2006, Arai and colleagues investigated the copper catalyzed Henry reaction between 2-nitrobenzaldehyde, **42**, and nitromethane, **43**, using immobilized ligands to eliminate any interference of the CD-active chiral catalyst with the enantioselective analysis of **44** which was directly conducted without work-up (Scheme 8).[18,19] This method is, of course, limited to heterogeneous reactions and one cannot decipher individual yield and *ee* values because the CD signal intensities of **44** are inherently dependent on both its concentration and enantiopurity. Chiral HPLC was therefore still necessary to fully evaluate the reaction outcomes. However, the detection of a strong CD signal proved sufficiently informative to identify a case in which a high yield and high enantioselectivity were achieved, and the screening of several copper complexes led to the discovery of conditions that give quantitative amounts of **44** in 90% enantiomeric excess.

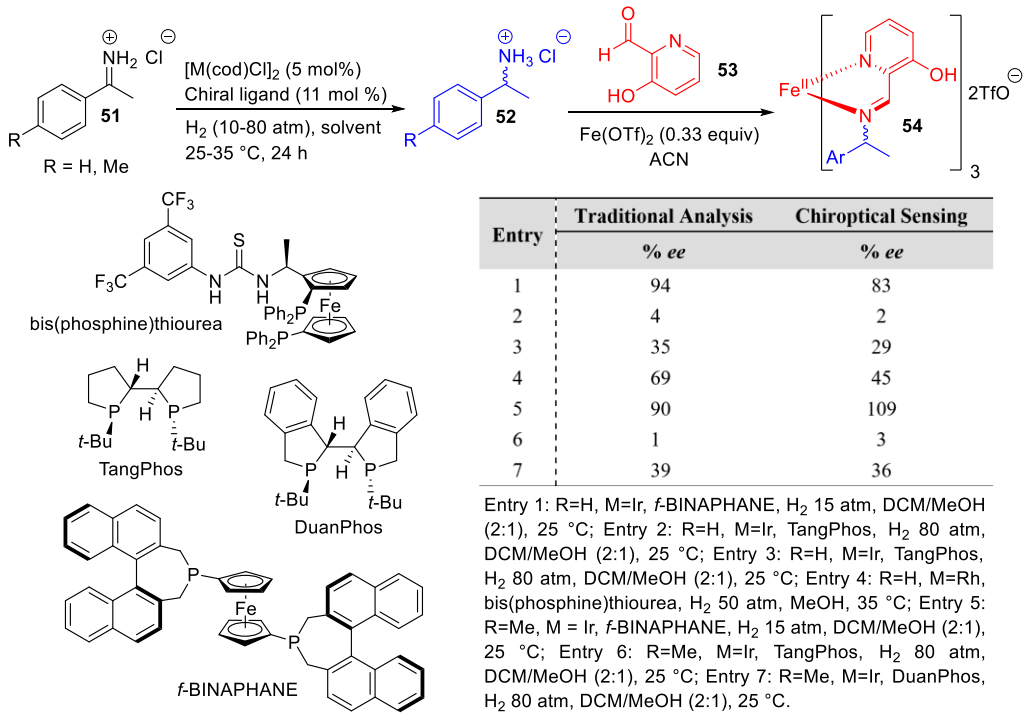


Scheme 8. Optimization of the heterogeneous Henry reaction using continuous injection CD spectrophotometry.

A few years later, Anslyn developed a chiroptical sensing protocol for the determination of the concentration and *ee* of primary amines (Scheme 9).[20] This method was tested with the ruthenium catalyzed asymmetric reductive amination of acetophenone, **45**. The crude reaction mixture containing 1-phenylethylamine, **46**, was treated with 2-pyridinecarboxaldehyde, **47**, to afford the Schiff base **48** which was allowed to coordinate to optically active [((*P*)-BINAP)Cu(MeCN)$_2$]PF$_6$, **49**. The corresponding diastereomeric complexes **50** display similar CD spectra but with different intensities. The CD spectrum of the assembly was recorded within two minutes and compared to a training data set for chemometric analysis. This sensing method relies on relatively small CD alterations that occur when **48** binds to the intrinsically CD-active (*S*)-BINAP derived copper(I) complex which might explain that the error for the concentration and %*ee* determination was 13.5% and 17%, respectively.



Scheme 9. Chiroptical sensing of the asymmetric amination of acetophenone.



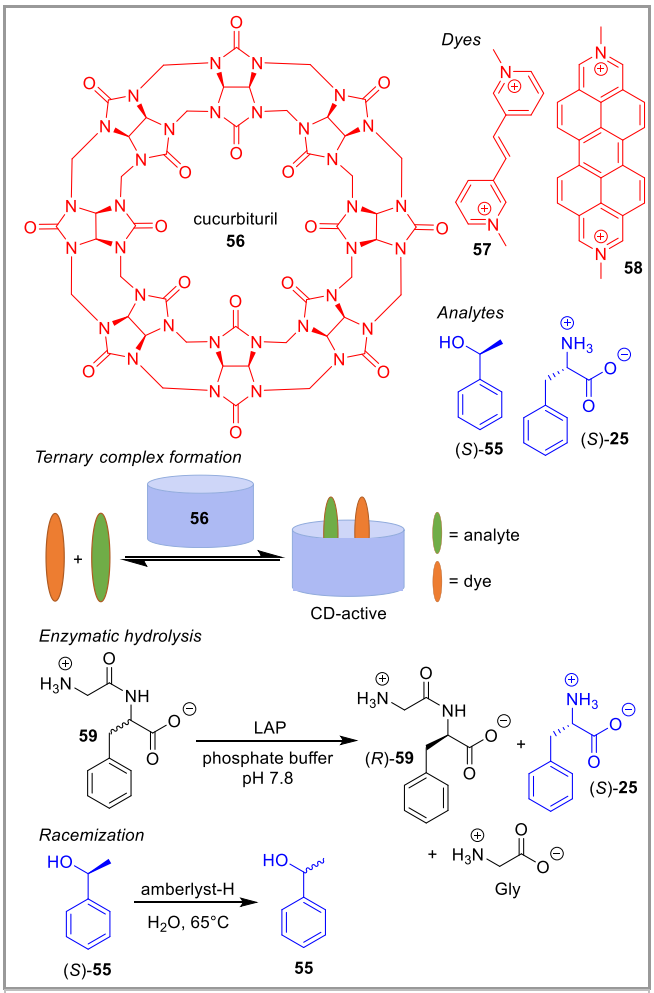
Entry	Traditional Analysis	Chiroptical Sensing
	% *ee*	% *ee*
1	94	83
2	4	2
3	35	29
4	69	45
5	90	109
6	1	3
7	39	36

Entry 1: R=H, M=Ir, *f*-BINAPHANE, H_2 15 atm, DCM/MeOH (2:1), 25 °C; Entry 2: R=H, M=Ir, TangPhos, H_2 80 atm, DCM/MeOH (2:1), 25 °C; Entry 3: R=H, M=Ir, TangPhos, H_2 80 atm, DCM/MeOH (2:1), 25 °C; Entry 4: R=H, M=Rh, bis(phosphine)thiourea, H_2 50 atm, MeOH, 35 °C; Entry 5: R=Me, M = Ir, *f*-BINAPHANE, H_2 15 atm, DCM/MeOH (2:1), 25 °C; Entry 6: R=Me, M=Ir, TangPhos, H_2 80 atm, DCM/MeOH (2:1), 25 °C; Entry 7: R=Me, M=Ir, DuanPhos, H_2 80 atm, DCM/MeOH (2:1), 25 °C.

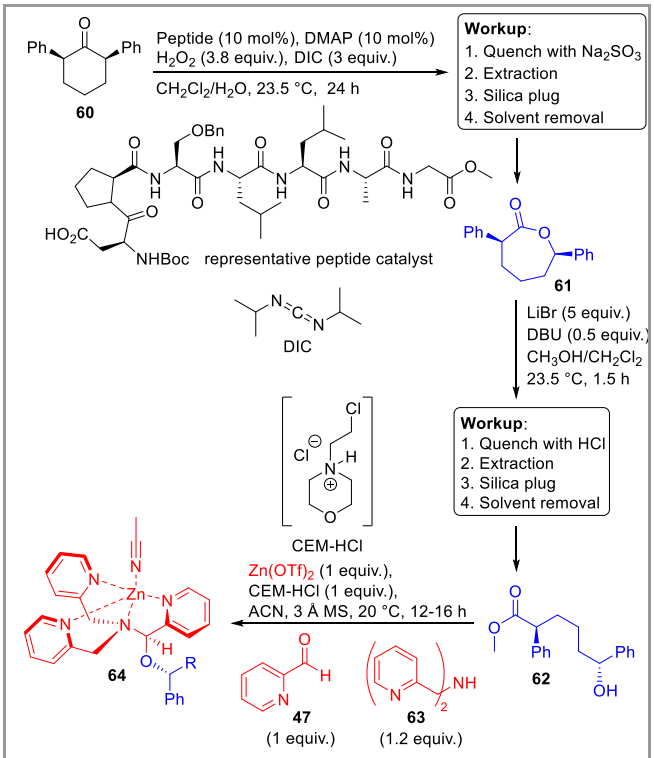
Scheme 10. High-throughput CD sensing of an asymmetric imine hydrogenation.

The same group employed CD sensing to analyze the catalytic asymmetric hydrogenation of the two unprotected imines **51** (Scheme 10).[21] The reaction solvent, metal salt, chiral ligand, hydrogen gas pressure and temperature were varied in an attempt to optimize the enantioselectivity. Samples of the amine product **52** were then subjected to Schiff base formation with 3-hydroxy picolinaldehyde, **53**, and the CD-active complex **54** was formed with iron(II) triflate to determine *ee* values. Comparison of the results obtained with seven reaction samples to traditional GC analysis showed that the use of this chiroptical assay entails significant time-savings, although the error margin was considered relatively high.

Nau and Biedermann monitored racemization and kinetic resolution reactions of (*S*)-1-phenylethanol, **55**, and the racemic dipeptide Gly-Phe, **59**, respectively, in real time (Scheme 11).[22] This was accomplished by CD analysis of ternary host-guest complexes formed from cucurbit[8]uril, **56**, a dicationic dye and the chiral analyte. The kinetics of the amberlyst catalyzed racemization of (*S*)-**55** were determined with host **56** and dye **57**, while dye **58** was used to monitor the enzymatic hydrolysis of **59** in the presence of leucine aminopeptidase (LAP). These types of transformations can either be studied synchronously in the presence of the host and dye or externally by mixing the chemosensor system with small aliquots taken from the reaction mixture. The operational simplicity and robustness of this sensing approach, which cannot only be used to determine final asymmetric reaction outcomes but also to generate real-time kinetic data, are very attractive and should find ample use among synthetic chemists.



Scheme 11. Real-time monitoring of enzymatic hydrolysis and racemization reactions with cucurbituril host guest chemistry.

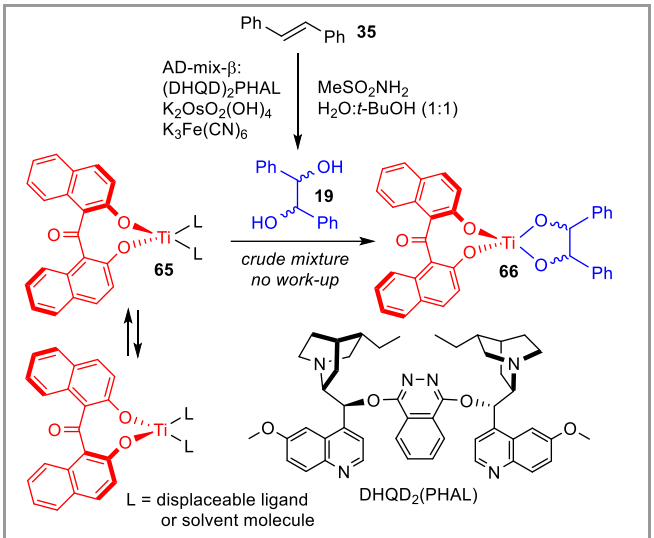


Entry[a]	Traditional Analysis	Chiroptical Sensing
	% *ee* (AC)	% *ee* (AC)
1	42 (*R*,*S*)	43 (*R*,*S*)
2	39 (*R*,*S*)	36 (*R*,*S*)
3	24 (*R*,*S*)	22 (*R*,*S*)
4	35 (*S*,*R*)	32 (*S*,*R*)
5	41 (*S*,*R*)	42 (*S*,*R*)
6	47 (*S*,*R*)	44 (*S*,*R*)

[a]Parent peptide sequence: Boc-Asp-Pro-Xaa-Xaa-Xaa-Ala-Gly-OMe. Entry 1: Leu-Phe-Thr(OBn); Entry 2: Leu-Lys(*Z*)-Thr(OBn); Entry 3: Lys(*Z*)-Lys(*Z*)-Ala; Entry 4: Ser(OBn)-Gly-Val; Entry 5: Ser(OBn)-Lys(*Z*)-Ser(OBn); Entry 6: Ser(OBn)-Leu-Ser(OBn). AC=absolute configuration.

Scheme 12. High-throughput CD sensing of a Baeyer-Villiger oxidation.

In 2015, Anslyn and Miller used a high-throughput CD sensing protocol for *ee* analysis of the peptide catalyzed Baeyer-Villiger oxidation of *cis*-2,6-diphenylcyclohexanone, **60** (Scheme 12).[23] The reaction mixtures obtained under various conditions were quenched with sodium sulfite, subjected to filtration using a silica plug and lactone **61** then underwent methanolysis to alcohol **62**. The crude reaction product **62** was extracted, filtered through another silica plug and concentrated. Upon solvent removal, the residue was applied in the reaction with $Zn(OTf)_2$, CEM-HCl, 2-pyridinecarboxaldehyde, **47**, and secondary amine **63**, giving rise to the supramolecular assembly **64** within 12-16 hours. CD analysis allowed determination of the *ee* values with an average error margin of 4% based on comparison with chiral HPLC results. This study led to the discovery of a procedure that achieves desymmetrization of **60** with 77% yield and 47% *ee*.

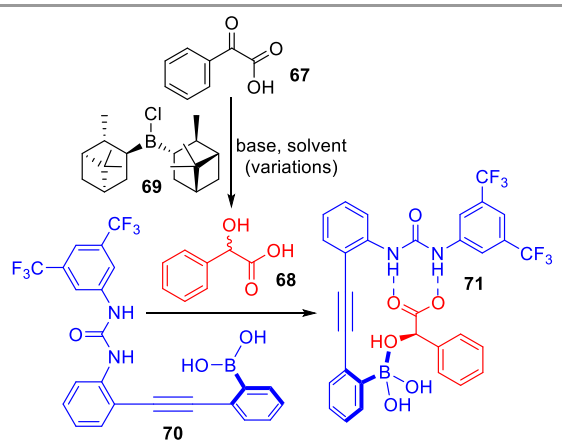


Entry	Traditional Analysis		Chiroptical Sensing	
	Yield (%)	% *ee* (AC)	Yield (%)	% *ee* (AC)
1	99.1	99.1 (*R*,*R*)	95.4	98.9 (*R*,*R*)
2	71.0	86.3 (*R*,*R*)	65.3	87.7 (*R*,*R*)
3	17.7	75.4 (*R*,*R*)	12.2	75.8 (*R*,*R*)
4	54.2	0.0 (n/a)	51.1	1.3 (n/a)

Entry 1: 0.0 °C; 2: 25.0 °C; 3: 50 °C; 4: $(DHQD)_2PHAL$ was replaced with cinchonine. AC=absolute configuration.

Scheme 13. Determination of *ee* and yield of the asymmetric dihydroxylation of stilbene using a stereodynamic titanium complex.

Our group has introduced a variety of stereodynamic metal complexes to the chiroptical sensing field. In one application we showed how the yield and *ee* of the asymmetric dihydroxylation of *trans*-stilbene, **35**, to hydrobenzoin, **19**, can be obtained without product purification using the titanium complex **65** (Scheme 13).[24] This chemosensor is CD-silent and exists as an equimolar mixture of rapidly interconverting enantiomers. But it undergoes instantaneous asymmetric transformation of the first kind upon coordination of a chiral analyte which gives complex **66**. This coincides with a fluorescence change and appearance of induced CD (ICD) signals at long wavelengths, an important feature that eliminates the possibility of optical interferences from the catalyst, reagents and by-products. The fluorescence change can be quantitatively correlated to the product amount while the ICD intensity is correlated to the product *ee*. The accuracy of this chiroptical sensing assay which requires only one milligram of the crude reaction mixture is sufficient for high-throughput screening purposes, but it produces only 2% of the solvent waste and is almost 20 times faster than gravimetric and chiral HPLC analysis.

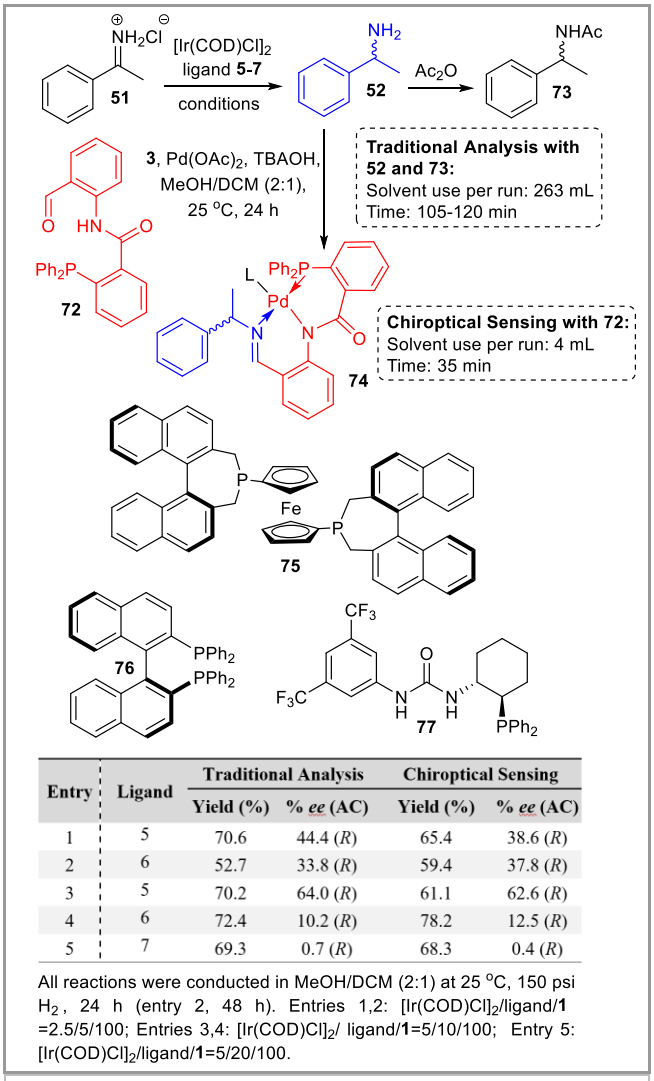


Entry	Traditional Analysis		Chiroptical Sensing	
	Yield (%)	% *ee* (AC)	Yield (%)	% *ee* (AC)
1	86.6	79.3 (*S*)	84.8	77.1 (*S*)
2	37.1	67.6 (*S*)	34.3	61.1 (*S*)
3	23.2	86.4 (*S*)	25.0	80.9 (*S*)
4	45.7	83.1 (*S*)	40.2	76.6 (*S*)
5	18.6	72.5 (*S*)	14.0	64.4 (*S*)
6	21.1	64.1 (*S*)	17.8	58.7 (*S*)

Entry 1: Et_3N, THF; 2: Et_3N, ACN; 3: Et_3N, toluene; 4: Et_3N, $CHCl_3$; 5: $(i\text{-}Pr)_2NEt$, THF; 6: $(i\text{-}Pr)_2NH$, ACN; AC=absolute configuration.

Scheme 14. Determination of *ee* and yield of the asymmetric reduction of phenylglyoxylic acid with a Brønsted/Lewis acid probe.

In 2016, we employed a stereodynamic Brønsted/Lewis acid probe to analyze the yield and *ee* of the asymmetric reduction of phenylglyoxylic acid, **67**, to mandelic acid, **68**, with (+)-DIP-Cl, **69** (Scheme 14).[25] The chemosensor **70** displays a central triple bond that connects urea and boronic acid groups for bidentate fixation of hydroxy acids. The corresponding complex **71** is locked into a chiroptically active conformation and thus provides strong UV and CD signals that are readily correlated to the reaction yield and *ee*. The reduction mixture was quenched with NaOH and H_2O_2 and the solvent was removed after acidification under vacuum. Optical analysis of 16 reactions with varying solvents and base additives was performed using just 0.5 mg of the crude residue and without any cumbersome workup steps. A comparison of the results obtained by optical and traditional analysis reveals relatively small error margins. The probe must be synthesized but there is a significant payoff in the end: the reaction analysis proved to be 70 times faster while producing only 2.5% of solvent waste in comparison to previously available protocols which also require significantly larger sample amounts.

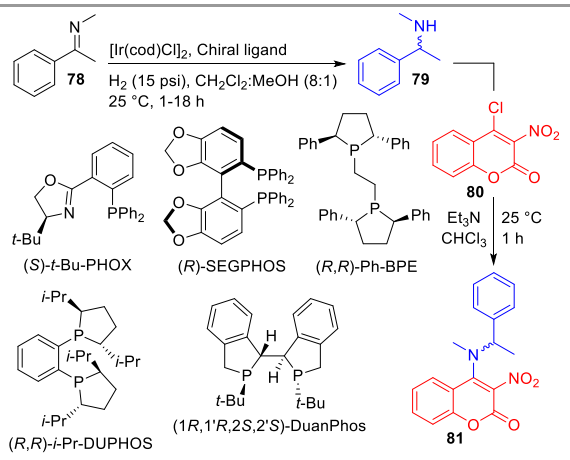


Entry	Ligand	Traditional Analysis		Chiroptical Sensing	
		Yield (%)	% *ee* (AC)	Yield (%)	% *ee* (AC)
1	5	70.6	44.4 (*R*)	65.4	38.6 (*R*)
2	6	52.7	33.8 (*R*)	59.4	37.8 (*R*)
3	5	70.2	64.0 (*R*)	61.1	62.6 (*R*)
4	6	72.4	10.2 (*R*)	78.2	12.5 (*R*)
5	7	69.3	0.7 (*R*)	68.3	0.4 (*R*)

All reactions were conducted in MeOH/DCM (2:1) at 25 °C, 150 psi H_2, 24 h (entry 2, 48 h). Entries 1,2: $[Ir(COD)Cl]_2$/ligand/**1** =2.5/5/100; Entries 3,4: $[Ir(COD)Cl]_2$/ ligand/**1**=5/10/100; Entry 5: $[Ir(COD)Cl]_2$/ligand/**1**=5/20/100.

Scheme 15. Comparison of chiroptical and traditional analysis of the asymmetric hydrogenation of iminium chloride **51**.

In the same year, our lab directly analyzed the asymmetric hydrogenation of the iminium chloride **51** to 1-phenylethylamine, **52**, with an optical assay that exploits a rapidly formed multicomponent self-assembly of the phosphine ligand **72**, palladium(II) acetate and the crude product.[26] The results are in satisfactory agreement with the gravimetric and HPLC analysis of isolated **52** and its acetyl derivative **73**, respectively, but the optical sensing approach with **74** is significantly faster and produces less than 2% solvent waste (Scheme 15). While there is no work-up needed prior to the chiroptical analysis which significantly streamlines the reaction screening, the phosphine ligand is not commercially available and needs to be synthesized.

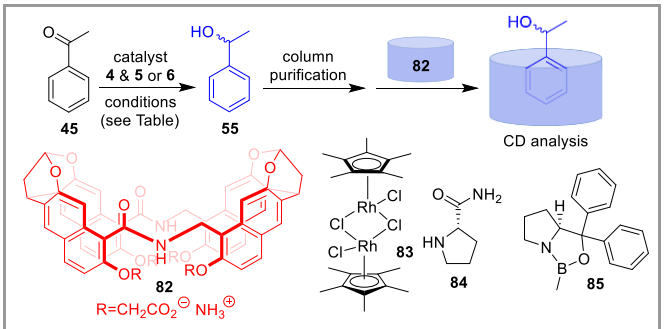


Entry	Traditional Analysis		Chiroptical Sensing	
	Yield (%)	% *ee* (AC)	Yield (%)	% *ee* (AC)
1	99.9	55.8 (*S*)	96.0	59.8 (*S*)
2	99.9	16.3 (*R*)	99.9	14.3 (*R*)
3	99.9	31.0 (*R*)	99.1	32.2 (*R*)
4	99.9	31.4 (*R*)	98.0	25.8 (*R*)
5	92.0	16.3 (*S*)	96.3	14.2 (*S*)
6	51.1	46.2 (*S*)	53.9	47.8 (*S*)
7	63.3	47.3 (*S*)	68.4	54.5 (*S*)

Entry 1: (*S*)-*t*-Bu-PHOX (5 mol%), 18 h; Entry 2: (*R*)-SEGPHOS (5 mol%), 18 h; Entry 3: (*R*,*R*)-*i*-Pr-DUPHOS (5 mol%), 18 h; Entry 4: (*R*,*R*)-Ph-BPE (5 mol%), 18 h; Entry 5: (1*R*,1'*R*,2*S*,2'*S*)-DuanPhos (5 mol%), 18 h; Entry 6: (*S*)-*t*-Bu-PHOX (2.5 mol%), 1 h; Entry 7: (*S*)-*t*-Bu-PHOX (3.25 mol%), 1 h. AC=absolute configuration.

Scheme 16. High-throughput CD sensing of an asymmetric imine hydrogenation using click chemistry.

In 2018, our group introduced a click chemistry assay and applied it to the iridium-catalyzed asymmetric hydrogenation of *N*-methyl-1-phenylethan-1-imine, **78** (Scheme 16).[27] The reduction of **78** was carried out using $[Ir(cod)Cl]_2$ and various chiral ligands, affording the secondary chiral amine, **79**. Upon completion of the reactions, 200 µL of the crude reaction mixtures were simply mixed with commercially available 4-chloro-3-nitrocoumarin, **80**, and triethylamine in chloroform for one hour. Two 40 µL aliquots of the sensing mixture were then subjected to CD and UV analysis, respectively, to determine the enantiomeric excess, absolute configuration and yield. In comparison to traditional reaction analysis, the use of **80** as chiroptical probe eliminates any work-up, reduces the amount of solvent waste by a factor of almost 100 and allows accelerated reaction screening with very small sample amounts. The accuracy, precision and robustness of this assay and its utility in asymmetric reaction analysis were later validated with automated CD microplate technology.[28] Single wavelength measurements proved sufficient and significantly increased the speed of spectral acquisition to just 3 seconds for a single sample, thus demonstrating the high-throughput nature of chiroptical sensing with **80**.



Entry	Traditional Analysis	Chiroptical Sensing
	% *ee* (AC)	% *ee* (AC)
1	50.2 (*R*)	45.9 (*R*)
2	53.8 (*R*)	51.2 (*R*)
3	84.3 (*R*)	81.8 (*R*)
4	80.5 (*R*)	82.1 (*R*)
5	72.1 (*R*)	68.2 (*R*)

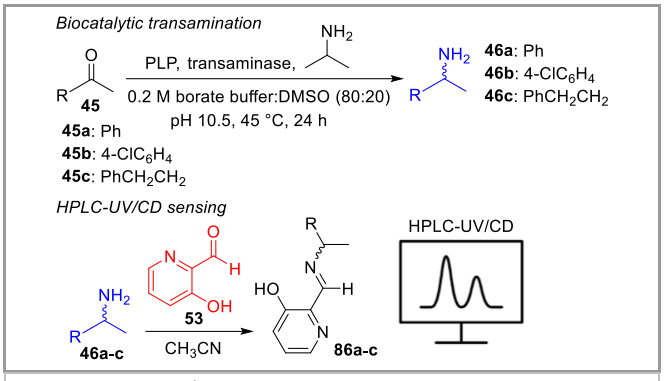
Entries 1 and 2 were performed with **83** and **84** as catalyst, KOH as base and 2-propanol as solvent. Entries 3-5 were performed with **85** as catalyst, BH_3•THF as the reducing agent and toluene as solvent. AC=absolute configuration.

Scheme 17. Analysis of the asymmetric hydrogenation of **45**.

Jiang and coworkers used the stereodynamic naphthotube host **82** to determine the enantioselectivity of the asymmetric hydrogenation of acetophenone, **45,** to 1-phenylethanol, **55**, with two different catalysts (Scheme 17).[29] Upon completion, the reaction mixture was first subjected to a thorough work-up and the product was isolated by flash chromatography prior to formation of a CD-active host-guest complex. The CD sensing protocol proved to be quite accurate and reliable. The maximum absolute %*ee* error of five test samples was found to be 4.3% in comparison to chiral GC results. This chemosensor was also used successfully for real-time monitoring of a racemization reaction.

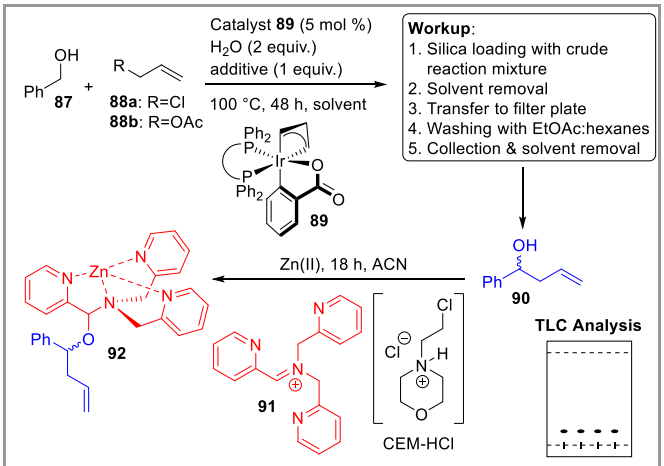
5. Hybrid approaches

The integration of chromatographic and optical methods can be an attractive venue that may combine the best of both worlds and because of the familiarity of synthetic chemists with HPLC. Welch and coworkers realized this several years ago and introduced a hybrid HPLC-UV/CD protocol for high-throughput screening of the biocatalytic asymmetric transamination of ketones **45** to the corresponding primary amines **46** in the presence of pyridoxyl-5'-phosphate, PLP, and isopropylamine (Scheme 18).[30] The transamination mixtures were transferred to a 96-well plate, extracted with dichloromethane, evaporated, and redissolved in acetonitrile prior to Schiff base formation with 3-hydroxypyridine-2-carboxaldehyde, **53**. The %*ee* of each sample was then determined with an error margin of less than 3% from the ratio of the CD/UV signals obtained upon elution of **46** from an achiral HPLC column. The chromatography step was necessary to remove a byproduct formed by condensation of excess of isopropylamine and **53** which would interfere with the optical analysis. But the method still proved quite efficient because the HPLC runs were conducted with a short C18 column and were complete in less than a minute.



Scheme 18. HPLC-UV/CD analysis of a reductive transamination of ketones.

Shortly thereafter, Anslyn and Krische integrated a tandem TLC-CD high-throughput screening method and multi-well plate technology to optimize base, solvent and other reaction parameters of the iridium-catalyzed enantioselective allylation of benzyl alcohol, **87** (Scheme 19).[31] Purification of the reaction mixtures was carried out in parallel using 96-well filter and collection plates to isolate alcohol **90**. TLCs were then obtained, photographed and compared to a calibration curve with a special software to determine the yields. The chiroptical *ee* sensing was carried out in 96-well plates by mixing the purified reaction product **90**, a zinc salt, iminium **91** and CEM-HCl to construct the multicomponent assembly **92**. After 18 hours of stirring, CD data for an entire 96-well plate were acquired within five minutes. This tandem assay allowed screening of 400 reaction samples and uncovered an improved allylation protocol that compared favorably with previously reported methods. Unfortunately, the TLC-CD analysis of samples with lower than 60% yield proved inaccurate and these reactions could not be further investigated. This limitation was not a concern because the objective was to optimize a known reaction that already produced good yields and enantioselectivities at the onset of this study. It may be a roadblock, however, if the development work needs to start at an early discovery stage where information from low-yielding transformations is often essential.



Entry	Ligand	Traditional Analysis		TLC-CD Sensing	
		Yield (%)	% *ee*	Yield (%)	% *ee*
1	(*R*)-BINAP	85	92	80	90
2	(*R*)-BINAP	18	54	25	8
3	(*R*)-BINAP	52	90	68	88
4	(*R*)-BINAP	58	77	48	45
5	(*R*)-BINAP	12	47	15	11
6	(*R*)-BINAP	25	68	32	65
7	(*R*)-BINAP	43	51	22	18
8	(*R*)-BINAP	58	77	48	45
9	(*R*)-BINAP	84	98	85	94
10	(*R*)-SEGPHOS	80	94	75	90

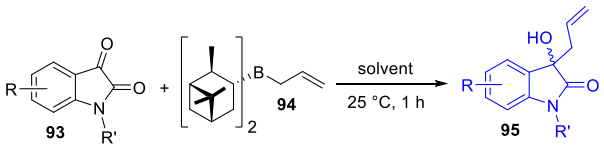
Entry 1: **88a**, 4-Cl-3-NO_2BzOH, Cs_2CO_3, THF; Entry 2: **88a**, 4-Cl-3-NO_2BzOH, Cs_2CO_3, ACN; Entry 3: **2a**, 4-Cl-3-NO_2BzOH, Cs_2CO_3, dioxane; Entry 4: **88a**, 4-Cl-3-NO_2BzOH, K_2CO_3, THF; Entry 5: **88a**, 4-Cl-3-NO_2BzOH, K_2CO_3, ACN; Entry 6: **88a**, 4-Cl-3-NO_2BzOH, K_2CO_3, dioxane; Entry 7: **88a**, 4-Cl-3-NO_2BzOH, strychnine, THF; Entry 8: **88a**, 4-Cl-3-NO_2BzOH, K_2CO_3, THF; Entry 9: **88a**, 4-CN-3-NO_2BzOH, Cs_2CO_3, THF; Entry 10: **88b**, 4-CN-3-NO_2BzOH, Cs_2CO_3, THF.

Scheme 19. Tandem TLC-CD high-throughput screening of the iridium-catalyzed enantioselective allylation of benzyl alcohol.

6. Optical analysis with intrinsically CD-active reaction products

Typically, asymmetric reaction products generate weak or blue-shifted chiroptical signals that are not suitable for yield and *ee* determination without prior isolation. In these cases, the use of a chromophoric probe that produces strong chiroptical signals at longer wavelengths where interferences from other reaction components or by-products can be excluded becomes necessary and this may be achieved in varying ways as discussed in the preceding sections. This can, however, be avoided when inherently CD-active reaction products producing sufficiently red-shifted Cotton effects are formed. To demonstrate the value of this often overlooked scenario, we used the asymmetric allylation of isatin, **93**, with the chiral allylborane **94** to show how high-throughput UV/CD screening can accelerate asymmetric reaction development without introduction of an optical chemosensor or auxiliary (Scheme 20).[32] Fifty-four combinations of nine different isatin compounds and six different solvents were conducted in parallel and the resulting mixtures containing product **95** were quenched with $NaOH/H_2O_2$ followed by HCl addition. The CD and UV spectra were then collected without further work-up and used to quantify the extent of asymmetric induction and conversion, respectively. For quantification purposes, the term normalized asymmetric induction, which takes into account the conversion calculated by UV and the maximal CD amplitude in mdeg, was used to evaluate the success

of asymmetric induction. All steps starting with the preparation of the asymmetric reaction mixtures to the final CD and UV measurements were completed within 12.5 hours. This set-up eliminates the need for time-consuming chiral HPLC analysis of each reaction and allows individual identification of optimal conditions for each substrate tested. For example, it was found that the allylation of *N*-benzyl isatin and *N*-methyl-5-fluoroisatin work best in diethyl ether and methyl *tert*-butyl ether, respectively. These reactions were then conducted at a 50 mg scale and the desired allyl alcohols were obtained in almost quantitative yields and 91-94% *ee*.



Scheme 20. Accelerated development of the asymmetric allylation of isatin.

7. Conclusion

To date, numerous small-molecule optical chemosensors that can be used for the determination of the amount, absolute configuration and enantiomeric composition of chiral compounds have been introduced. This advance has led to the introduction of practical fluorescence, UV and CD assays that achieve asymmetric reaction analysis with small sample amounts and in many cases without any cumbersome product purification steps. While the chiroptical sensing field is still under development the assays introduced so far are ready for prime time use and the instrumentation needed is generally available in a typical academic or industrial laboratory. An occasional switch from traditional and inherently serial chromatographic or NMR procedures to chiroptical methods might be quite rewarding and result in substantial time savings, reduced cost and person-hour charge per sample, less waste generation, and increased screening throughput. Although such a change undoubtedly requires an initial commitment to familiarize the lab personnel with the optical techniques and equipment, it would extend the benefits of broadly available high-throughput experimentation equipment and multi-well plate technologies from the synthesis to the equally important analytical stage. Ultimately, this can remove the current bottleneck in asymmetric reaction development projects when comprehensive screening of many parameters, e.g. catalyst structure, additives, solvents, concentrations and so on, is desirable. Despite the very attractive features mentioned above, it should be pointed out that at the current stage, the accuracy of chiroptical sensing is typically not as high as chiral HPLC analysis and error margins ranging from 5-10% are often reported. However, if hundreds to thousands of milligram-scale asymmetric reactions can be conducted and rapidly analyzed in parallel, one can easily afford to select the few cases of interest that have yields and *ee* values above the generally sought-after 90% threshold and obtain exact results for these with traditional methods. Finally, we note that in addition to the experimental advances discussed herein, increasingly powerful computational methods that allow predictive virtual screening enabling accelerated asymmetric reaction development *in silico* have surfaced in recent years.[33]

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Conflict of Interest

The authors declare no conflict of interest.

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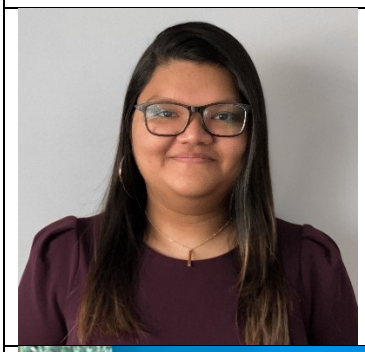
Diandra S. Hassan was born in London (United Kingdom) in 1995 and grew up in Jakarta (Indonesia). She received her B.Sc. degree in biochemistry from the State University of New York at Binghamton in 2016. After graduation, she worked in the psychiatry department at Icahn School of Medicine at Mount Sinai in New York City as Associate Researcher and then at RUCDR Infinite Biologics as Senior Laboratory Technician. In 2018, she joined Prof. Christian Wolf's research group where she integrates chiroptical sensing methodology and chemometric analysis.



F. Safia Kariapper was born in Colombo (Sri Lanka) in 1997. She graduated from NYU Shanghai with a B.Sc. in Chemistry and a minor in Business Studies in 2019. Post graduation she served as a Princeton in Asia fellow at the American University of Central Asia. Since 2020, she has been a PhD student in the Wolf research group at Georgetown University. Her current research focuses on chiroptical sensing and asymmetric catalysis.

Ciarán C. Lynch was born in Dublin (Ireland) in 1995. He received his B.S. degree in Biochemistry from the University of Scranton in Pennsylvania in 2017 and his M.S. degree in Chemistry from Georgetown University (Washington DC) in 2020. He is currently a Chemistry PhD student in the group of Prof. Christian Wolf at Georgetown University. His research interests include asymmetric catalysis, C-F bond functionalization, chiroptical sensing and small molecule drug design.

Christian Wolf was born in 1968 in Hamburg (Germany). In 1995, he obtained his Ph.D. under the supervision of Wilfried König at the University of Hamburg (Germany). After postdoctoral studies with William Pirkle at the University of Illinois, he started working in R&D at SmithKline Beecham Pharmaceuticals in 1997. Three years later, he accepted a position as faculty at Georgetown University in Washington, DC where he has developed a multidisciplinary research program with a diverse team involving several academic and industrial collaborations. His research interests comprise synthetic methodology development, asymmetric catalysis, enantioselective analysis, chiral recognition processes, optical sensing technology, stereodynamics of chiral compounds, medicinal chemistry, and drug discovery.