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Review on Rapid Enantiomeric Analysis of Chiral Carboxylic Acids Using Visible CD Signatures

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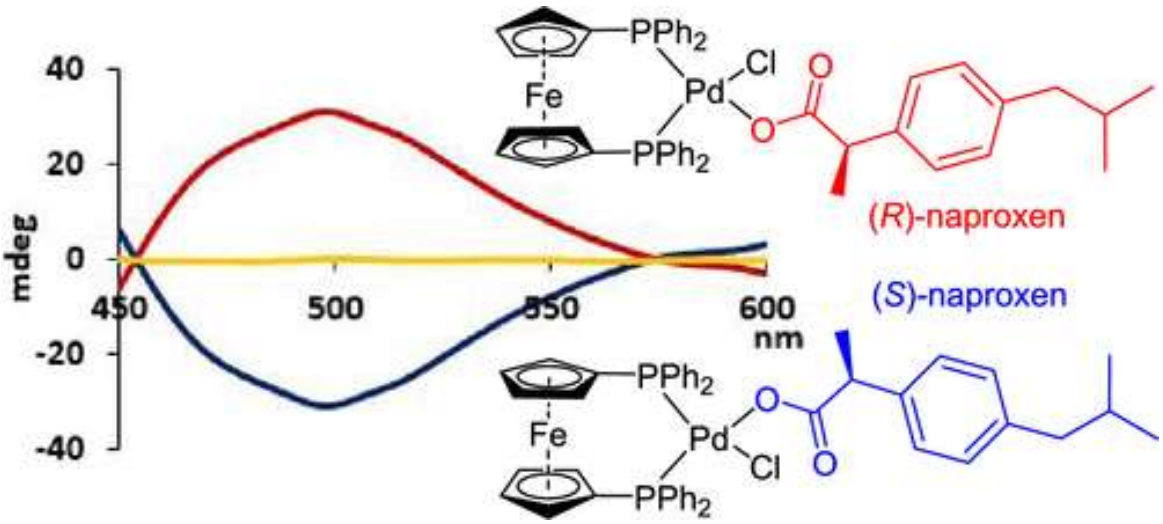
Abstract

In order to create a metal complex that is chiroptically active and exhibits strong circular dichroism (CD) responses in the visible spectrum—a crucial objective in optical sensing research—a chiral carboxylic acid reacts with 1,1′-bis(diphenylphosphino) ferrocene palladium dichloride when exposed to a mild base. Within minutes, this technique allows for the quick chiroptical measurement of carboxylic acid sample concentration and enantiomeric purity. The method is simple and adaptable, and it can be used with a wide variety of chemical substances, such as natural chemicals, medicines, hydroxy acids, and amino acids. Furthermore, because all necessary reagents are widely available, the assay is very accessible and may be easily included into any lab that focuses on chirality or high-throughput screening.



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1. Introduction

Chiral carboxylic acids are abundant in nature and are essential for many intricate biological processes as well as synthetic changes. As useful precursors and adaptable intermediates for the creation of multipurpose natural products, they are highly sought for. Pharmacologically significant compounds like dihydroartemisinic acid, tiagabine, naproxen, ibuprofen, abietic acid, and isosteviol frequently contain these chiral acids as essential structural components. These substances continue to be a primary focus of

chemical and biomedical study because of their wide range of applications, varied structures, and broad functional importance.

Accurately identifying the enantiomeric composition of carboxylic acids is a common and crucial task in many laboratories, as their chirality often correlates with their biological function or medicinal success. This should ideally be achieved using high-throughput techniques that can effectively analyze large numbers of samples. However, the time-consuming and sequential nature of traditional methods like chiral chromatography or NMR spectroscopy employing chiral derivatizing or solvating agents limits their applicability in hectic screening operations.

Mass spectrometry, UV spectroscopy, fluorescence, gas-phase rotational resonance, infrared spectroscopy, electronic circular dichroism (ECD), fluorescence-detected CD, and biological tests are some of the alternative analytical techniques that have been developed as a result. Chiroptical sensing techniques have become especially popular among them. Numerous chromophoric sensors that interact with chiral acids through processes including supramolecular complexation, hydrogen bonding, or ionic interactions have been presented in recent investigations; these sensors produce strong CD responses. Because achiral sensors prevent diastereomeric combinations from forming and take advantage of CD spectroscopy's built-in enantioselectivity, they are especially attractive. Additionally, it is possible to co-induce non-enantioselective UV or fluorescence signals in many systems, which allows for the simultaneous measurement of the enantiomeric ratio (er) and total analyte concentration.

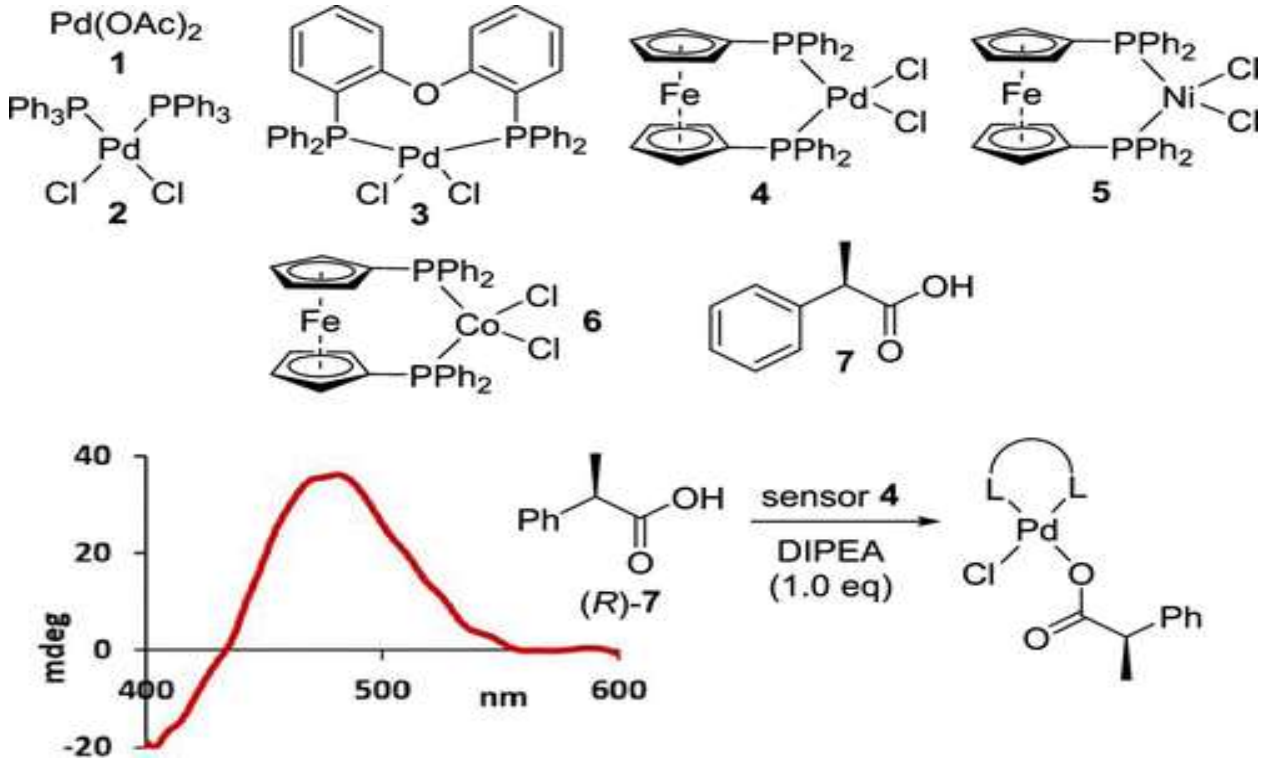

Figure 1: Using (R)-2-phenylpropanoic acid coordination with Pd complex 4, sensor structures were analyzed and CD induction was accomplished.

The bulk of chiroptical assays developed to date have focused on α-amino acids (23–38) and α-hydroxy acids (39–48), which are preferred bidentate structures that are especially effective at generating strong circular dichroism (CD) responses. Although several sensing platforms have been reported for

differentiating enantiomers of monofunctional compounds, there is still a lack of a general, fast-acting optical method that combines concentration measurement and enantiomeric discrimination using only far red-shifted CD signals in the visible spectrum (17–22, 49–53).

An achiral probe that can combine with chiral carboxylic acids to create a complex and produce a strong CD signal at wavelengths longer than 450 nm would be a potential solution. Avoiding the formation of diastereomeric mixtures, reducing interference from chiral impurities displaying CD activity at shorter wavelengths, and enhancing compatibility with automated CD plate readers—which frequently have trouble making accurate measurements close to the 400 nm region—are just a few benefits of such a system.

2. Result and discussion:

We examined the possibility of using metal coordination chemistry to create a molecular sensor that satisfies all the requirements using sensors with the labels 1–6 and 2-phenylpropanoic acid (compound 7) as model analytes. Notably, we discovered that a moderate CD maximum at about 320 nm is produced when (R)-7 reacts with $Pd(OAc)_2$ to generate a palladium(II) complex in the presence of a tertiary amine (see Supporting Information). Building on this first encouraging finding, we aimed to use chromophoric phosphine ligands to help carboxylate groups bind stoichiometrically. We expected this to enable accurate quantitative sensing while also producing a powerful CD signal at much longer wavelengths.

Beyond 400 nm, CD induction was demonstrated by [bis(2-(diphenylphosphino)phenyl)ether]$PdCl_2$ (compound 3) and its 1,1′-bis(diphenylphosphino)ferrocene counterpart (compound 4), while $(PPh_3)_2PdCl_2$ demonstrated very little success. Particularly noteworthy was the palladium complex 4's strong, ligand-enhanced CD induction that redshifted into the visible spectrum, which is an uncommon occurrence. At a dose of 2.65 mM, an induced CD (ICD) peak emerged at around 475 nm in tetrahydrofuran (THF) at about 40 °C (Figure 1). Complex 4's practical relevance is further demonstrated by the fact that it is soluble in common organic solvents and commercially available. Labs that specialize in chirality studies can readily modify this simple sensing method, which simply involves mixing the sensor, base, and carboxylic acid. It's interesting to note that the cobalt analogue 6 did not exhibit a similar ICD response to the corresponding nickel complex 5.

Additional research examined the experimental variables and mechanistic elements of the test. Ibuprofen (chemical 8)'s ^1H NMR spectra showed distinct up field shifts in methyl and methine proton signals, confirming quick and thorough carboxylate binding in less than 15 minutes (Figure 2). ESI-MS analysis confirmed the analyte's stoichiometric coordination (Supporting Information). Excess chiral acid had no effect on the ICD signal's strength, according to titration tests.

We used crystallographic analysis to rule out the production of bigger supramolecular aggregates or carboxylate- or chloride-bridged dimers, which would also need equimolar sensor and analyte concentrations. The inability of sensor 4 to produce single crystals from ibuprofen or other analyte coordination complexes is probably due to the considerable rotational flexibility of these acids, which prevents effective crystal lattice formation. In order to get around this, one chloride was substituted with silver benzoate by precipitating AgCl before crystallization investigations. [18]By adding a comparatively hard acid site, this substitution made it possible to successfully produce single crystals that were appropriate for X-ray diffraction. These crystallographic results are consistent with the outcomes of studies using mass spectrometry and CD titration. All things considered, the data show that the

development of a mononuclear 1:1 coordination complex, in which compound 4 replaces only one chloride ligand, is what causes the CD induction.

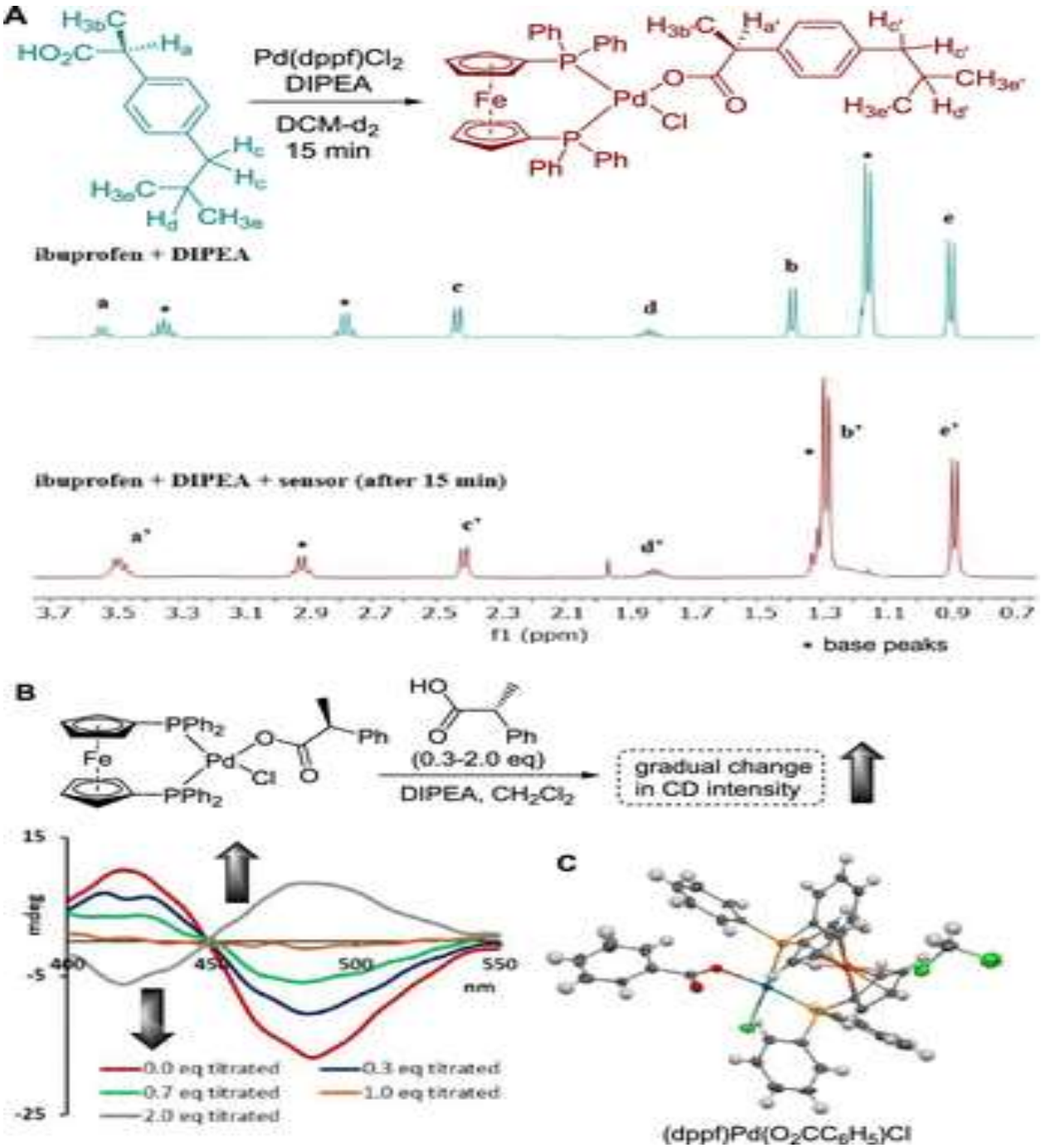

Figure 2 Research that is mechanistic. X-ray examination (ellipsoid contour 50% probability) of a benzoate complex formed from 4 (C), 1H NMR reaction monitoring with ibuprofen (A), and CD titration studies employing both enantiomers of 2-phenylpropanoic acid (B).

As expected, in the absence of a base—which is necessary to produce carboxylate ions and promote metal coordination—no circular dichroism (CD) reaction was seen. We carried out titrations by progressively adding increasing amounts of (R)-2-phenylpropanoic acid to a palladium complex that was first generated with the (S)-enantiomer and sensor 4 in order to investigate whether the binding of the analyte to the

palladium(II) center is reversible. The findings demonstrated the dynamic and reversible character of the carboxylate coordination process by showing a progressive decline in the induced CD (ICD) signal, which ultimately resulted in a reversal when the (R)-enantiomer was in excess.

The possible application of the chiral palladium complex as an enantioselective agent in NMR spectroscopy was then investigated. Under comparable circumstances, however, no discernible enantio discrimination of carboxylic acids was found when using ((R)-BINAP) $PdCl_2$.

While bases like potassium carbonate (K_2CO_3), 2,6-lutidine, and pyridine had little effect, triethylamine (Et_3N), diisopropylethylamine (DIPEA), and sodium tert-butoxide produced similar ICD signals with different intensities, according to optimization studies involving a variety of bases and solvents (see Supporting Information). DIPEA was selected for all subsequent tests because of its capacity to generate powerful ICD signals in benign circumstances. In terms of solvents, the test components were first mixed in dichloromethane (CH_2Cl_2) and then diluted with methanol (MeOH) to a concentration appropriate for CD measurements in order to get the strongest chiroptical signals. Tetrahydrofuran (THF) and acetonitrile (ACN), two other solvents that were tried, were less successful in producing strong CD reactions.

Under these ideal circumstances, we evaluated the assay's capacity to identify a wide range of chiral carboxylic acids (7–30), including both natural products like dehydroabietic acid (13), abietic acid (14), isosteviol (15), and dihydroartemisinic acid (16), as well as simple aliphatic compounds like 17 and 21 and aromatic derivatives like 10 and 11. Pharmaceutical agents like naproxen (9) and tiagabine (24). Multifunctional compounds (12, 18, 19), hydroxy acids (28–30), and different amino acids (23–27) were also successfully detected by the sensor.

When the sensor was present, we consistently saw far red-shifted CD signals between 450 and 500 nm in all of these samples. Conversely, no CD activity was shown in this spectral area by the chiral acids alone (Supporting Information). The proximity of the analyte's stereogenic center to the metal binding site significantly influenced the ICD signals' intensity and sign. This is in line with how other small-molecule sensors, such as palladium complex 4, behave. The decreasing ICD intensities from (R)-2-phenylbutanoic acid to (R)-3-phenylbutanoic acid indicate that analytes with stereocenters farther from the binding locus either produced noticeably weaker signals or failed to elicit significant enantioselective CD responses (Supporting Information).

The ICD signals found upon attaching chemicals 20–24 to complex 4 showed that the sensor had great sensitivity despite the difficulty of remote chirality centers. Interestingly, the stereocenter closest to the coordination point was invariably the source of the main chiroptical contribution. Compounds 13–15, for instance, all produced negative CD signals upon binding despite having various stereocenters and the same absolute arrangement at the carbon next to the acid group. The CD result was not considerably impacted by compound 15's distinct stereochemistry at farther centers when compared to 13 and 14.

These results demonstrate a distinct distance-dependent effect in the chiroptical sensing mechanism, where stereocenters near the binding site have a significant impact on the CD response, whereas those farther away are less significant and harder to discern. Consequently, because diastereomers with changes at distant stereocenters contribute very little to the ICD signal, the assay's capacity to distinguish between them is restricted.

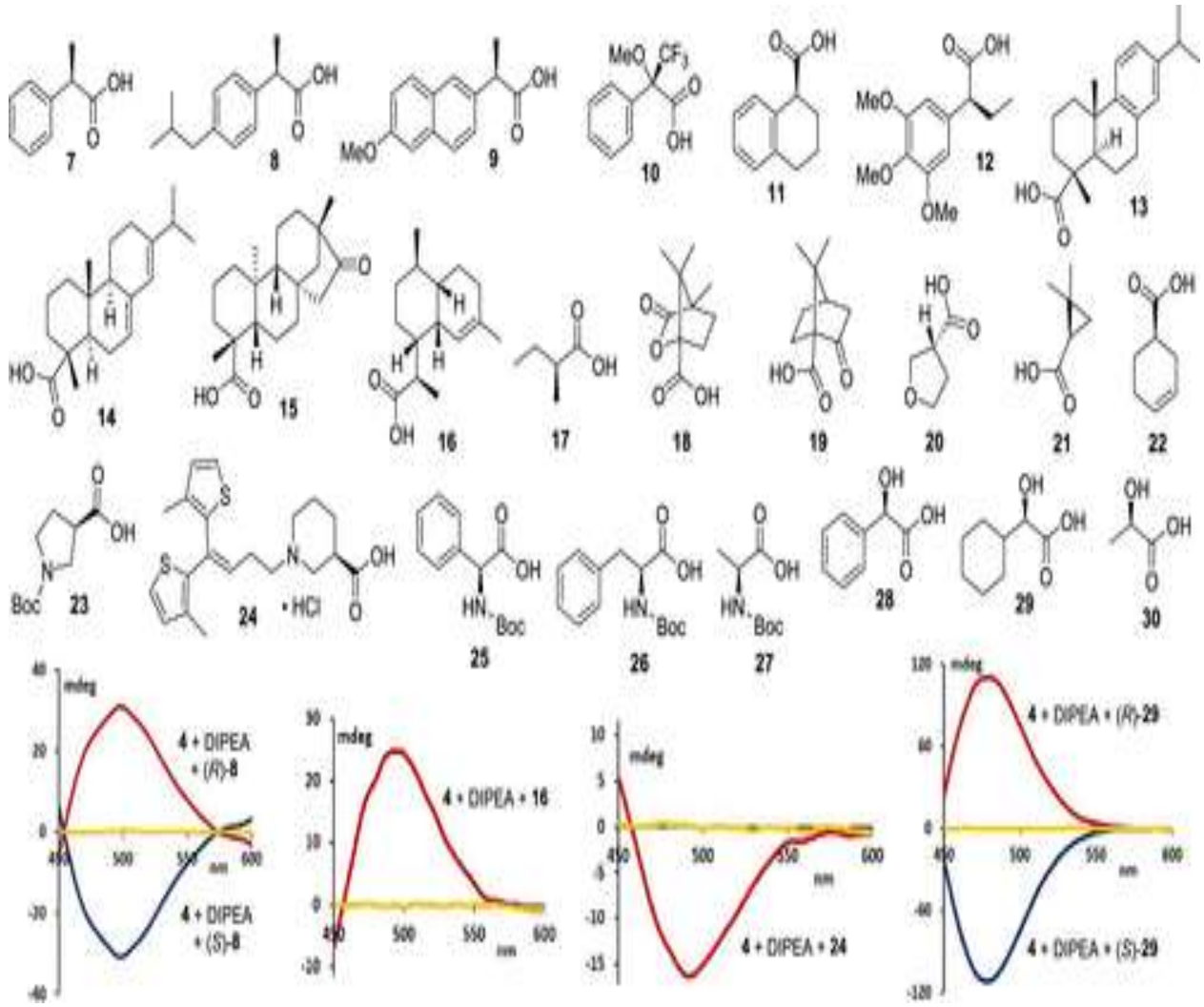

Figure3. This study looked at carboxylic acid compounds and a variety of ICD spectra with or without 4 (yellow, red, or blue). Refer to the SI for further details.

In order to show how well our circular dichroism (CD) sensing approach works, we made ten distinct samples that included either 1,2,3,4-tetrahydronaphthalene-1-carboxylic acid or ibuprofen. The enantiomeric ratios ([R]:[S]) and total concentrations ([R] + [S]) of these samples differed. with a straightforward procedure that allows for the simultaneous determination of the enantiomeric ratio (er) and total acid concentration with only four CD measurements, each sample was examined using our chiroptical assay with sensor 4. Crucially, unlike previous approaches our research developed, this method does not require extra UV or fluorescence measurements or calibration curves.[4]

Consider the first sample, which had an enantiomeric makeup of 65% (S) and 35% (R) and included enantioenriched (S)-ibuprofen at a total concentration of 35.0 mM. Four 100.0 µL aliquots of this material were treated with different concentrations of sensor 4 and an amine base in order to perform the test. Then, using CH_2Cl_2, the reaction mixtures were diluted to a level of 2.5 mL. Before CD measurements were conducted, 250.0 µL samples of these solutions were further diluted with 2.0 mL of methanol (MeOH) following 15 minutes of stirring.

The matching CD signal intensities obtained at 490 nm were plotted against the predicted concentrations of sensor 4 in the original undiluted sample, taking into account both dilution phases. A definite positive

association (shown by a blue line) was obtained by linear regression of the CD signals obtained when the analyte was in excess over the sensor. On the other hand, the CD signal plateaued and formed a horizontal line parallel to the x-axis (shown in red) when sensor 4 was present in excess. As long as sensor 4 is above the carboxylic acid concentration, this plateau is the highest induced CD (ICD) response that can be obtained for that specific sample.

The initial carboxylic acid concentration in Sample #1 was found to be 32.9 mM based on the intersection of these two lines. By comparing the measured CD signal strength (in millidegrees) with the anticipated value from a reference sample of known enantiopurity at the same concentration, the enantiomeric ratio was determined after the concentration was determined. An enantiomeric ratio of 65.5% (S) to 34.5% (R) was obtained from this investigation.

The predominant ibuprofen enantiomer's absolute configuration was determined by the sign of the observed CD signals. This assignment needs to be cross-referenced with a reference chemical with known absolute stereochemistry when employing sensor 4. It is difficult to assign absolute configuration based only on induced CD effects unless there is a strong correlation—often through exciton-coupled CD mechanisms—between the sign of the Cotton effect and the three-dimensional structure of the analyte. Without this information, it is advised to validate the stereochemical designations using complementary experimental methods or computer modeling.

Overall, the thorough examination of the concentration and enantiomeric ratio shows excellent precision and accuracy, with error margins that are similar to those found in earlier research using different chiroptical sensing techniques.

Conclusion

In summary, we have created a reliable chiroptical sensing technique for carboxylic acids that produces potent visible spectrum circular dichroism signals. In order to create a well-defined 1:1 metal complex quickly—within minutes—this method depends on the coordination of carboxylate ions to 1,1′-bis(diphenylphosphino)ferrocene palladium dichloride in the presence of a moderate base. This effective ligand exchange process is reflected in the resultant CD signals, which are seen between 450 and 500 nm. Accurately identifying the enantiomeric ratios and quantities of various carboxylic acid materials, such as significant medications, natural compounds, amino acids, and hydroxy acids, showed the method's usefulness. This technique's broad application, long-wavelength CD response, and ability to offer simultaneous concentration and enantiomeric analysis using only CD measurements are some of its main advantages over earlier approaches. The assay is simple, requires easily accessible ingredients, and is simple to apply in chirality research labs. Additionally, it is scalable to high-throughput formats, including multi-well CD plate readers, and compatible with ambient conditions, enabling the effective parallel analysis of several samples.

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