Optical Terpene and Terpenoid Sensing: Chiral Recognition, Determination of Enantiomeric Composition and Total Concentration Analysis with Late Transition Metal Complexes

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Supporting Information Placeholder

ABSTRACT: Quantitative chirality sensing of terpenes and terpenoids exhibiting a single double bond as the only functional group, such as α-pinene, β-pinene and camphene, or two alkene moieties like limonene, valencene and β-caryophyllene is among the most difficult molecular recognition tasks. In this work, a fast chiroptical sensing method that accomplishes determination of the enantiomeric excess and overall amount of a large variety of terpenes and terpenoids using readily available phosphine derived late transition metal complexes is presented. The terpene coordination is complete within 10 minutes and coincides with spontaneous induction of strong CD signals at long wavelengths and distinct UV changes which together allow accurate *ee* and concentration quantification.

The staggering chemodiversity of terpenes in nature coincides with an immense variety of largely unexplored biological functions and effects in plants, animals, microorganisms and the surrounding ecosystem.[1] Many terpenes and terpenoids have found important applications in the pharmaceutical, food, fragrance and agricultural industries. Chiral terpenes often exist naturally as nonracemic mixtures with a characteristic enantiomeric excess (*ee*) that is related to the plant and geographic origins of these phytochemicals. Similarly, the chirality and enantiomeric composition play a fundamental role with regard to the biological activities of terpenes and their metabolites.[2] Chirality sensing of hydrocarbons exhibiting a single double bond as the only functional group, such as α-pinene, β-pinene and camphene, or two alkene moieties like limonene, valencene and β-caryophyllene is among the most challenging molecular recognition tasks.

Initial progress in this area has been achieved with supramolecular assemblies that are formed in the presence of a large excess of chiral alkenes. The desired host-guest interactions and subsequent chirality imprinting processes are typically enforced through a solvophobic environment such as acetonitrile or water, by using the chiral guest as solvent or under solvent-free conditions. Several cases of effective chirality amplification with supramolecular scaffolds and polymeric materials using limonene as solvent[3] or via solvent-free incorporation of the chiral alkene into crystalline polymer films are known.[4]

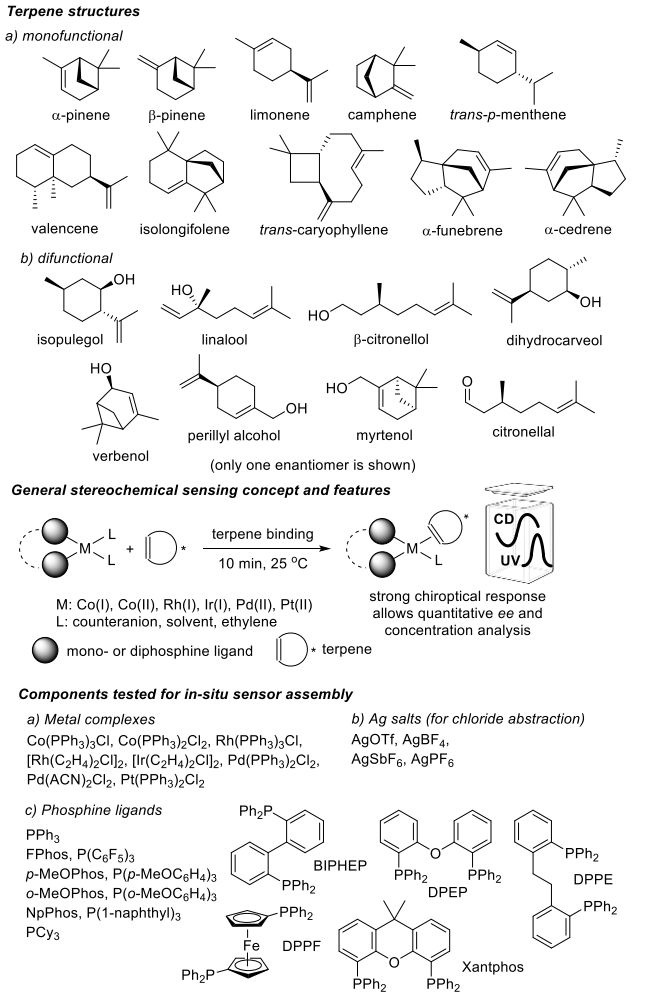


Figure 1. Structures of terpenes, general sensing strategy and sensor components.

Alternatively, molecular recognition of terpenoids added in 100-fold excess to oligomeric arylacetylene foldamers in a polar solvent or annealing with polyacetylenes equipped with 2,2'-biphenol units for several hours prior to dissolution and circular dichroism analysis at low temperature have been reported.[5] 2D nanosheets prepared from chiral BINOL-derived covalent organic frameworks have been used for enantioselective fluorescence sensing of α-pinene, fenthone, limonene, carvone and terpinen-4-ol and proline derived

porphyrin-ZnO hybrid materials enable quantitative CD sensing of α-pinene and limonene.[6] Recently, the possibility of qualitative differentiation between the enantiomers of α-pinene in the presence of chiral alcohols by FTIR spectroscopy was demonstrated.[7]

Few examples of NMR resolution with chiral Ag(I), Pd(II) and Pt(II) complexes have occurred in the literature but the complexity of signals observed often complicates accurate integration in particular at low sample concentrations.[8] Despite the advances mentioned above, spectroscopic *ee* analysis of chiral alkenes remains very difficult. A practical method that accomplishes this task and is also generally is amenable to high-throughput screening and multiwell plate technology has remained elusive.

A variety of small-molecule probes for optical sensing of chiral amino acids, carboxylic acids, amines, amino alcohols and a few other classes of compounds have been introduced during the last decade.[9] By contrast, chiroptical sensing of the enantiomeric ratio and overall concentration of alkenes and dienes devoid of another functionality, including α- and β-pinene, camphene, limonene, with a molecular probe has not been accomplished. At the onset of this study, we screened several Co(I), Co(II), Rh(I), Ir(I), Pd(II) and Pt(II) complexes together with a variety of mono- and bidentate phosphine ligands (PPh_3, PCy_3, FPhos, *p*-MeOPhos, *o*-MeOPhos, NpPhos, BIPHEP, DPEP, DPPE, DPPF and Xantphos) to test if alkene coordination and spontaneous induction of a strong chiroptical sensor response to the chirality of the targeted terpenes and terpenoids can be achieved (Figure 1). We reasoned that the combination of low oxidation state late-transition metals and these phosphines would result in widely soluble sensors with sufficient substrate affinity due to enhanced π-back donation with the alkene moiety.

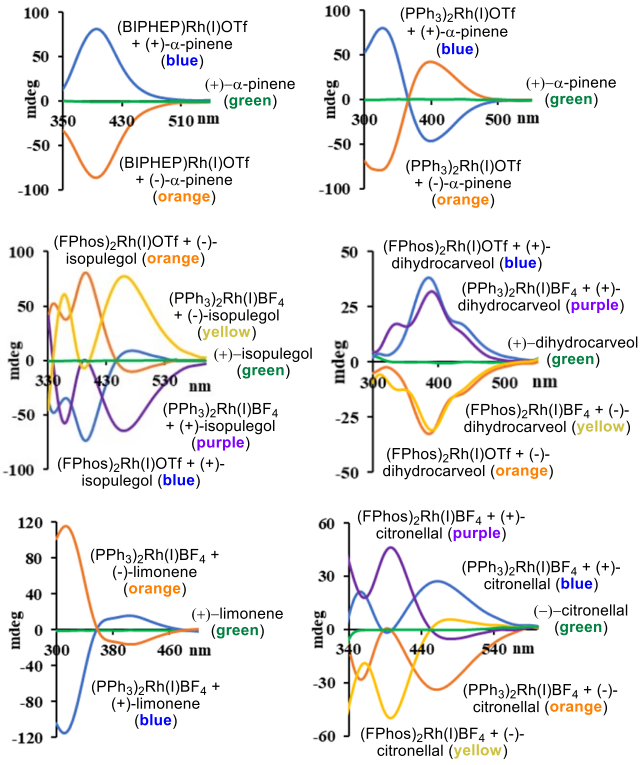
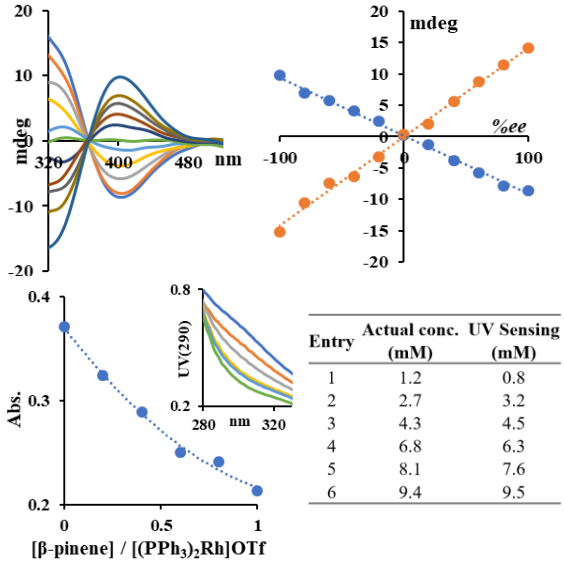


Figure 2. Top: Chirality sensing of the enantiomers of α-pinene with BIPHEP and PPh_3 derived Rh(I) sensors generated *in situ* with $[Rh(C_2H_4)_2Cl]_2$ and AgOTf. Middle: Comparison of the sensing results obtained with isopulegol using Rh(I) complexes derived from FPhos, PPh_3, AgOTf and $AgBF_4$, and sensing of the enantiomers of dihydrocarveol with FPhos and either AgOTf or $AgBF_4$. Bottom: CD sensing of limonene and citronellal. All CD spectra were recorded at 1.1 mM in chloroform. The enantiopure terpenes were CD-silent in the spectral region of interest in the absence of the sensor.

We were pleased to find that the *in situ* assembly of a rhodium(I) complex from $[Rh(C_2H_4)_2Cl]_2$, BIPHEP, DPEP, PPh_3 or FPhos, and stoichiometric amounts of a silver salt to achieve chloride abstraction gave strong ICD effects at long wavelengths with α- and β-pinene in chloroform, Figure 2 and SI. Initially, we used 10 equivalents of the terpenes but we later observed that distinct ICD signals sufficient for quantitative *ee* analysis can be obtained with equimolar amounts, *vide infra*. The use of *p*-MeOPhos, DPPF and Xantphos as ligand under the same conditions gave relatively weak CD signals while chiroptical pinene sensing with *o*-MeOPhos, NpPhos and DPPE was unsuccessful. We also observed ICD effects, albeit of weaker intensity compared to the terpene sensors formed from $[Rh(C_2H_4)_2Cl]_2$, using the commercially available Wilkinson catalyst $Rh(PPh_3)_3Cl$ or various palladium assemblies generated from either $Pd(PPh_3)_2Cl_2$ or $Pd(ACN)_2Cl_2$. Further optimization studies revealed that the strongest CD induction can be achieved when the sensor is assembled using a 1:2 metal:phosphine ratio or one equivalent when a bidentate ligand such as BIPHEP is employed as the CD reporter. Finally, we applied AgOTf, $AgBF_4$, $AgSbF_6$ and $AgPF_6$ in CD sensing experiments with β-pinene, isopulegol and citronellal using $[Rh(C_2H_4)_2Cl]_2$ and PPh_3 to systematically investigate counteranion effects. The strongest ICD signal with β-pinene was obtained with silver triflate while $AgBF_4$ outperformed the other salts in the case of isopulegol and citronellal. Having identified the optimal silver salt for each alkene we then screened all eleven phosphine ligands. We found that PPh_3, FPhos, *p*-MeOPhos and BIPHEP are generally preferred choices and give strong CD signals in most cases (see Figure 2 and SI).



Entry	Actual conc. (mM)	UV Sensing (mM)
1	1.2	0.8
2	2.7	3.2
3	4.3	4.5
4	6.8	6.3
5	8.1	7.6
6	9.4	9.5

Figure 3. Correlation of the induced CD effects and UV changes with varying enantiomeric composition and concentration of α-pinene. Top left: CD responses of the assembly formed from $[Rh(C_2H_4)_2Cl]_2$, PPh_3 and AgOTf to α-pinene in varying %*ee*. Right: Plot of the CD maxima at 330 (orange) and 400 (blue) nm versus sample %*ee*. Bottom: Induced UV change upon binding of β-pinene to the sensor at 50.0 μM ($CHCl_3$) and quantitative analysis of six samples. All CD spectra were recorded at 400.0 μM in chloroform.

With a practical sensing protocol in hand, we were able to significantly extend the application scope from α- and β-pinene to

other challenging terpenes and a variety of terpenoids. Strong ICD signals were recorded with camphene, *trans-p*-menthene, isolongifolene, α-funebrene and α-cedrene, all carrying a single double bond as the only functional group. Similarly, chiroptical sensing of terpenes with two alkene moieties such as limonene, valencene and *trans*-caryophyllene was successful. Clovene and aromadendrene did not give a CD signal.

We then decided to test the important group of terpenes exhibiting an alcohol group in addition to the double bond which is located remarkably remote from the chiral center in β-citronellol. Nevertheless, our sensor generated distinct CD responses to the enantiomers of this monoterpenoid as well as with isopulegol, linalool, dihydrocarveol, verbenol, perillyl alcohol, myrtenol and citronellal which carries an aldehyde function (Figure 2 and SI). CD titration experiments with our Rh(I) sensor allowed determination of the binding constants with (-)-β-pinene, (-)-limonene and (-)-isopulegol representing structures with one or two alkene units as well as those that carry a double bond together with an alcohol group as 252, 230 and 79 M^{-1}, respectively (SI). Comparison of the CD sensing of isopulegol and menthol, which has a fully saturated carbon scaffold, proved that the alkene moiety is essential for the substrate binding. While we obtained a strong ICD signal with isopulegol the solution remained CD-silent when menthol was added to the sensor. Importantly, the terpene binding and the spontaneous CD induction are very fast. We found that this process is typically complete in less than 10 minutes (SI).[10]

We noticed from ESI/MS, NMR titration studies and Job plot experiments that α- and β-pinene as well as other terpenes and terpenoids form stoichiometric 1:1 complexes with the Rh(I)phosphine probes (SI). ^{1}H NMR analysis suggests that the sensing involves the formation of a rhodium(η^2-alkene) complex.[11] With terpenoids carrying an alcohol group in addition to the double bond bidentate substrate coordination might be possible.[12] In the case of monofunctional terpenes, such as α- and β-pinene, a vacant coordination site could become available and facilitate a CH-activation pathway.[13] We expected that this might lead to rhodium chain-walking or alkene isomerization. Indeed, NMR and CD analysis revealed that coordination of β-pinene to the $(PPh_3)_2$Rh(I) sensor is followed by stereospecific transformation to the thermodynamically favored α-pinene isomer presumably via an intermediate rhodium(η^3-allyl) complex. At equilibrium, the two free terpenes coexist as a 4:1 ratio. The same result was obtained when the experiment was conducted with α-pinene as starting material. By contrast, no sign of terpene interconversion was observed when a palladium-based sensor system was used under essentially the same conditions.

Table 1. Quantitative %*ee* sensing of α-pinene (entries 1-6) and β-pinene (entries 7-12).

Entry	Sample composition		Chiroptical sensing results				Abs. error
	Abs. Conf.[a]	%*ee*	Abs. Conf.[a]	%*ee*(I)[b]	%*ee*(II)[c]	Average %*ee*	
1	(*R,R*)	83.0	(*R,R*)	85.0	83.6	84.3	1.3
2	(*R,R*)	77.0	(*R,R*)	82.3	78.8	80.6	3.6
3	(*R,R*)	43.0	(*R,R*)	43.6	48.0	45.8	2.8
4	(*S,S*)	19.0	(*S,S*)	20.9	14.3	17.6	1.4
5	(*S,S*)	39.0	(*S,S*)	36.0	36.0	35.6	3.4
6	(*S,S*)	63.0	(*S,S*)	60.7	63.2	61.9	1.1
7	(*R,R*)	83.0	(*R,R*)	81.7	79.0	80.4	2.6
8	(*R,R*)	77.0	(*R,R*)	70.8	74.1	72.4	4.6
9	(*R,R*)	43.0	(*R,R*)	46.2	40.5	43.3	0.3
10	(*S,S*)	19.0	(*S,S*)	13.8	16.6	15.2	3.8
11	(*S,S*)	39.0	(*S,S*)	38.7	47.6	43.2	4.2
12	(*S,S*)	63.0	(*S,S*)	67.5	67.7	67.6	4.6

[a]Based on the sign of the induced Cotton effects. [b]The %*ee* was calculated based on the CD maxima obtained at either 330 nm (entries 1-6) or 340 nm (entries 7-12). [c]The %*ee* was calculated based on the CD maxima obtained at 400 nm. Abs. Conf.: absolute configuration.

The prospect of stoichiometric 1:1 sensing and the elimination of the common requirement of using excessive substrate amounts in terpene recognition studies were very exciting to us because this suggested an unprecedented opportunity for quantitative stereoselective analysis. In fact, remarkably strong CD signals were still obtained when only one pinene equivalent was added to our sensors (Figure 3 and SI). Encouraged by these findings, we evaluated the CD response of the triphenylphosphine and BIPHEP derived rhodium complexes to varying enantiomeric compositions of equimolar amounts of α- and β-pinene. The induced chiroptical responses increased linearly with the %*ee* of the samples. A representative example is shown in Figure 3. When we altered the terpene concentration we discovered a steady decrease in the UV absorption spectrum which we expected would provide an opportunity to determine the total amount of both pinene enantiomers irrespective of the sample *ee*. This was demonstrated with 6 samples containing β-pinene in various concentrations ranging from 0.8 to 9.6 mM. For example, the UV sensing of the samples having β-pinene at 4.3 and 9.4 mM gave 4.5 and 9.5 mM, respectively (entries 3 and 6).

We then put our chiroptical sensing method to the ultimate test and attempted quantitative %*ee* sensing of 12 samples containing either α- and β-pinene in vastly different enantiomeric compositions, Table 1. Based on the sign of the induced Cotton effects we were able to correctly assign the absolute configuration of the major pinene enantiomer in all cases by comparison with a reference. Employing the measured ICD maxima in straightforward linear regression analysis allowed us to quantify the enantiomeric compositions of all samples with good accuracy and absolute %errors below 5%. The CD sensing of the α-pinene solutions having the (*R,R*)-enantiomer in 83.0 and 43.0 %*ee*, respectively, gave 84.3 and 45.8 %*ee* (entries 1 and 3). The stereochemical analysis of β-pinene samples containing the (*R,R*)-enantiomer and the (*S,S*)-enantiomer in 83.0 and 19.0 %*ee* afforded 80.4 and 15.2% *ee*, respectively (entries 7 and 10).

In summary, we have introduced a practical sensing method that allows fast stereochemical analysis of terpenes and terpenoids. This was achieved by screening the chiroptical responses of cobalt, rhodium, iridium, palladium and platinum complexes assembled *in situ* with readily available phosphine ligands. The optical alkene sensing with late transition metal complexes is broadly useful and applicable but not limited to 18 important terpenes and terpenoids including of α-pinene, β-pinene, limonene, isopulegol and β-citronellol. The alkene coordination is complete within 10 minutes and coincides with spontaneous induction of strong CD signals at long wavelengths and distinct UV changes which provide the means for accurate quantification of the overall concentration and enantiomeric sample composition. This approach thus provides an attractive solution to a long-standing problem, and the simple mix-and-measure sensing protocol is adaptable to high-throughput experimentation equipment, multiwell plate technology and automation if desired.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

Experimental details including quantitative UV/CD analysis, and NMR data. The Supporting Information is available free of charge on the ACS Publications website.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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11) The chloride abstraction with a silver salt is essential for the sensing and no ICD signals were observed otherwise. The ethylene molecule is generally considered to be labile and easily replaced. It was not detected by ESI-MS analysis of the phosphine-rhodium complexes in the absence or presence of a terpene. However, our NMR analysis indicates that ethylene is not replaced even when a large excess of the terpene was added. For comparison, see Vaughan, B. A.; Webster-Gardiner, M. S.; Cundari, T. R.; Gunnoe, T. B. A Rhodium Catalyst for Single-Step Styrene Production from Benzene and Ethylene. *Science* **2015**, *348*, 421.

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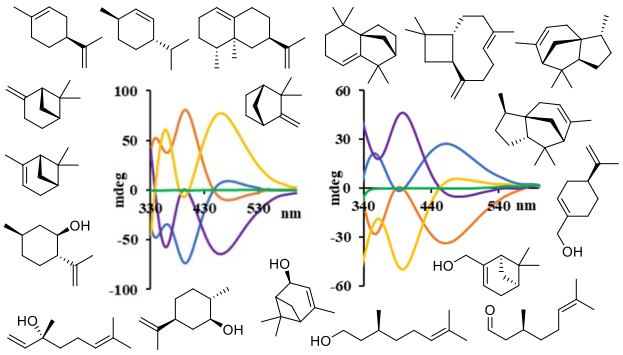
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