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Enantioselective CD analysis of amino acids based on chiral amplification with a stereodynamic probe

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ABSTRACT

The condensation between stereolabile 1,8-bis(3′-formyl-4′-hydroxyphenyl)naphthalene, **1**, and two amino acid molecules results in the formation of chiral diimines exhibiting strong CD signals. This reaction has been used to develop a chiroptical sensing method for the determination of the absolute configuration and enantiomeric composition of unprotected amino acids. This sensing approach is based on distinctive chiral amplification due to central-to-axial chirality induction within the diimine scaffold formed and does not require the use of an enantiopure ligand or metal complex.

1. Introduction

The development of molecular sensors with a potential use in high-throughput stereoselective analysis of chiral compounds has received increasing attention in recent years.[1] Due to their biological relevance and prevalence, amino acids are inarguably among the most important sensing targets. Although many examples of amino acid probes have been reported, few cases have been exploited for time-efficient determination of the absolute configuration and quantification of the enantiomeric composition. In particular, chiral receptors carrying a fluorophore that changes its signal output upon enantioselective binding to a chiral substrate have proven quite versatile. Pu et al. have introduced fluorescent BINOL-derived macrocycles that undergo enantioselective host–guest complexation with *N*-protected amino acids.[2] The success with these sensors prompted the synthesis of equally useful BINOL analogues bearing imidazolium,[3] benzoyl,[4] and chiral amino units.[5] Our group has demonstrated that 1,8-diheteroarylnaphthalenes and their *N*,*N*′-dioxide derivatives embed amino acids into a highly stereoselective cleft via hydrogen bonding interactions. The resulting fluorescence quenching allows analysis of the absolute configuration and accurate determination of the ee of a wide range of substrates.[6] Bohne realized that a pyrene–cyclodextrin inclusion complex can be used to differentiate between the enantiomers of tryptophan in the presence of alcohols or alkyl sulfates.[7] Corradini et al. have shown that incorporation of a carefully designed copper binding site and a dansyl fluorophore into β-cyclodextrin produces an intriguing class of sensors that utilizes enantioselective ligand exchange for 'switch on' detection of amino acids.[8] Noteworthy, a wide range of macrocyclic enantioselective fluorosensors,[9] including a chiral terpyridine crown ether specific for α-phenylglycine methyl ester hydrochloride,[10] a tryptophan-derived calix[4]arene for *N*-Boc protected alanine,[11] and a (+)-tubocurarine receptor suitable for the analysis of phenylalanine, have also been reported.[12] Somewhat less attractive fluorescence sensing approaches rely on covalent attachment of a dansyl or pyrene group to the amino acid substrate and enantioselective recognition using either a chiral copper(II) complex[13] or excess of α-acid glycoprotein and bovine serum albumine.[14]

Compared to the diversity of fluorescent sensors, few UV[15] and CD[16] probes for amino acids have appeared in the literature. Anslyn's group developed a practical UV–vis indicator displacement assay utilizing a chiral copper complex for the quantitative analysis of the enantiomeric composition of free amino acids in aqueous media.[17] Alternatively, this can be accomplished with several unprotected amino acids by using an axially chiral diacridylnaphthalene *N*,*N*′-dioxide-derived scandium complex.[18] Canary's group has demonstrated that induced circular dichroism measurements of a copper(II) complex carrying an amino acid derivative allows determination of both the absolute configuration and the ee based on distinct exciton coupled CD signals at 240 nm.[19] By contrast,

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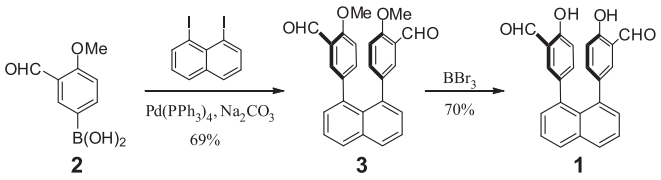
coordination of amino acids to a Cu(II) complex tethered to β-cyclodextrin or to chiral porphyrins has been reported to result in very small Cotton effects and appears less suitable for quantitative analysis.[20] In addition to fluorescence, UV and CD methods, examples of enantioselective amino acid sensing with electrochemical probes,[21] and inhibition and immunomechanical assays with tailored antibodies have been reported.[22] Chiral nanotubes fitted with a peptide have been found to be sensitive to the adsorption of the enantiomers of alanine, resulting in different resonance frequencies.[23]

Recognizing the importance and remaining shortcomings of stereochemical CD analysis of amino acids, we decided to develop a metal-free sensing method for the determination of the absolute configuration and %ee of amino acids without the need for elaborate purification. We recently introduced the stereodynamic triaryl probe **1**, which exhibits two salicylaldehyde units attached to the *peri*-positions of naphthalene.[24] Sensor **1** can easily be prepared in two steps from boronic acid **2**, Scheme 1. Since both arene rings readily rotate about the two chiral axes, the sensor exists as a mixture of enantiomeric *anti*-conformers that rapidly interconvert via a *meso syn*-isomer at room temperature.[25] This fluxional and CD silent probe, however, can favor population of a single chiral conformation upon binding to a chiral substrate. This has been shown in the case of amino alcohols.[24] Crystallographic analysis of the diimines obtained from opposite enantiomers of a chiral amino alcohol proved that the central chirality of the substrate controls the arrangement of the two pivotal aryl–aryl bonds in the probe, Scheme 2. The corresponding axially chiral structure is locked by intramolecular hydrogen bonding, which favors strong Cotton effects.



Scheme 1. Synthesis of sensor **1**.

Amino acid sensing assays are generally based on competitive formation of diastereomeric adducts with profoundly different thermodynamic stability or generation of diastereomers with individual UV, fluorescence or CD properties. However, the induction of axial and helical chirality in stereodynamic biphenyls and 2,2′-dihydroxybenzophenone, respectively, upon covalent binding of amino acids provides alternative entries to chirality imprinting and stereoselective analysis.[26] Based on these reports and our finding that **1** shows remarkable chiral amplification upon condensation reaction with amino alcohols, we envisioned that diimine formation between this stereodynamic sensor and 2 equiv of an amino acid would generate a rigid, axially chiral scaffold exhibiting a strong CD signal.

2. Results and discussion

Slow evaporation of a solution of **1** in chloroform gave single crystals suitable for X-ray analysis. Although the *anti*-conformation is generally expected to be thermodynamically favored in solution, **1** crystallizes in its *syn*-conformation, Figure 1. The splaying angle between the two cofacial salicylaldehyde rings was determined as 13.5°, which results in a centroidal arene–arene distance of 3.33 Å. The projection along the naphthalene unit shows that the two salicylaldehyde rings are almost perfectly aligned, having a torsion angle of only 4.3°.[27]

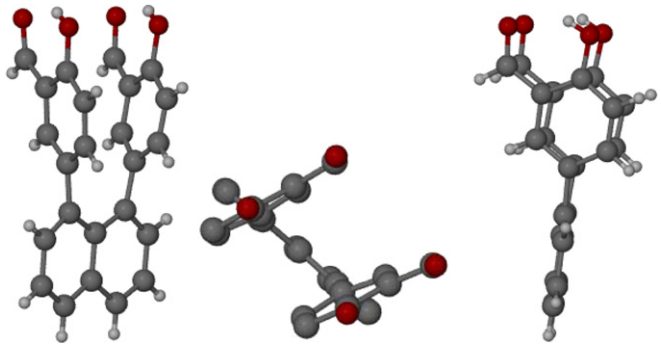


Figure 1. Single crystal structure of *syn*-**1**. The side and top views show the π-stacking of the two salicylaldehyde rings (left and middle). The view along the naphthalene ring reveals that both salicylaldehyde rings are almost perfectly aligned (right).

After screening various reaction conditions, we found that the tetrabutylammonium hydroxide (TBAOH) salts of free amino acids react with **1** in DMSO toward a diimine, which resembles results obtained with amino alcohols.[24] The condensation can easily be monitored by MS analysis and by the disappearance of the formyl peaks in the NMR spectra. Amino acids **4**–**11** were selected for this study to cover a wide range of steric bulk and to determine effects of additional functionalites such as alcohol groups. We were pleased to observe that the corresponding diimines display strong CD signals at high wavelengths, Figures 2 and 3.

Since both free **1** and the amino acids tested do not show any CD signals above 300 nm under the same conditions, the observed chiroptical activity can be attributed to the relative orientation and π–π interactions of the cofacial salicylidenimine rings formed upon

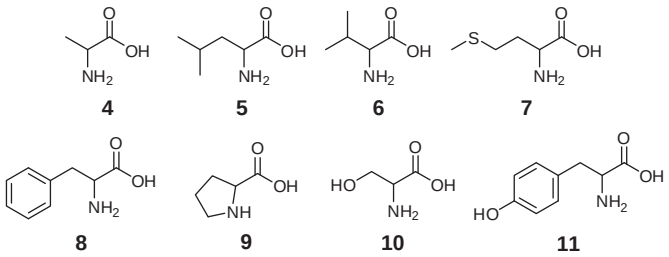
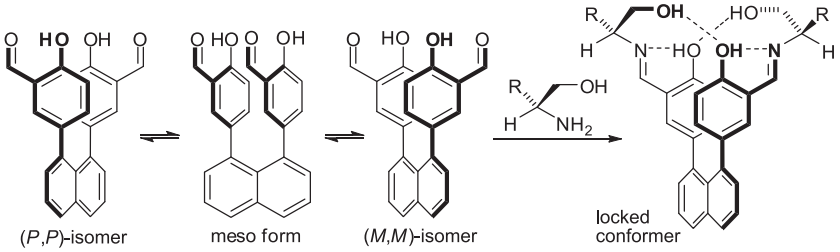


Figure 2. Structures of the amino acids used in this study.



Scheme 2. Amino alcohol-controlled central-to-axial chirality induction using sensor **1**.

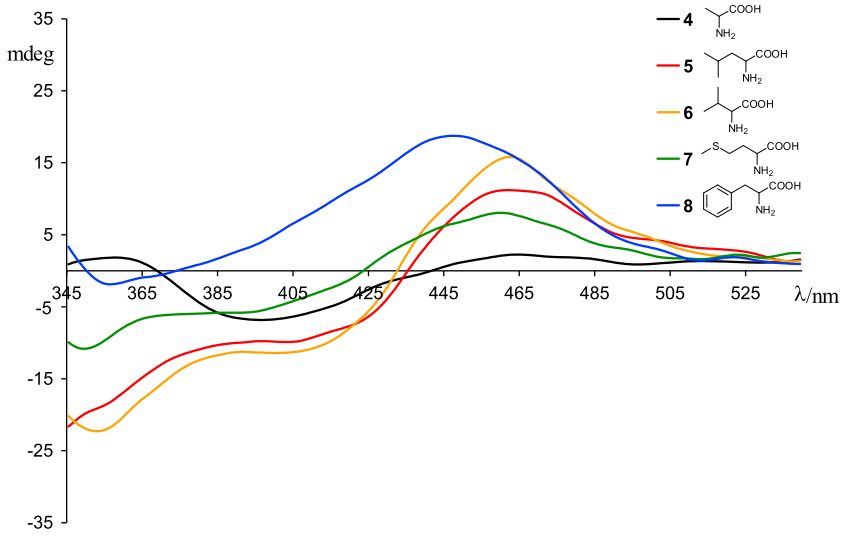


Figure 3. CD spectra of the diimines obtained from **1** and (*R*)-alanine (**4**), (*R*)-leucine (**5**), (*R*)-valine (**6**), (*R*)-methionine (**7**), and (*R*)-phenylalanine (**8**) at 2.5×10^{-4} M in $CHCl_3$.

condensation. As expected, enantiomeric substrates yielded products with opposite Cotton effects, see Figure 5 and Supplementary data. Based on the rapid enantioconversion of the sensor at room temperature and in analogy to our observations with chiral amino alcohol substrates, it is assumed that the conformational isomerism of the diimines derived from **1** is controlled by the central chirality of the amino acid. Accordingly, diimine formation perturbs the equilibrium of the stereoisomers of **1** and favors a single rotamer, which is stabilized by intramolecular hydrogen bonding, Scheme 2 and Figure 4. Upon diimine formation between the sensor and 2 equiv of an amino acid, previously treated with TBAOH to improve solubility, each carboxylate group is expected to participate in hydrogen bonding with the salicylidenimine unit to lock the stereodynamic sensor into a conformation that minimizes steric repulsion between the two substrate moieties. Importantly, employing free salicylaldehyde and various amino acids in the same experiment gave imines that proved CD-silent in the same region. The strong Cotton effects observed with the diimines obtained with **1** are therefore due to the cofacial arrangement of the two salicylidenimine units.[28] Acidification of the solutions containing the diimines derived from **4**–**8** gave blue shifted CD signals with significantly higher amplitude, Figure 5 and Supplementary data. Protonation of the imine and the carboxylate groups should facilitate hydrogen bonding between the phenol and the carboxylic acid groups in the opposite salicylideniminium ring, resulting in a rigid array with increased π-stacking, Figure 4.[29] The blue UV shift and the enhanced CD amplitudes may thus be attributed to a redirection of the hydrogen bonding motif between the proximate functionalities and an increase in the polarization of the salicylideniminium moieties. Acidification of a solution containing a diimine derived from **1** and 2 equiv of a chiral amino alcohol was found to cause a similar blue shift but gave a decreased CD amplitude while diimines obtained with amino esters remained CD silent. This implies that the presence of both the carboxylate and the free carboxylic acid functions generated under acidic conditions plays a crucial role in determining the stereochemical arrangement and the chiroptical properties of the amino acid-derived diimine scaffold. The significance of hydrogen bonding for the generation of a rigid structure and intense Cotton effects is in agreement with solvent effects. The CD amplitudes of the diimines

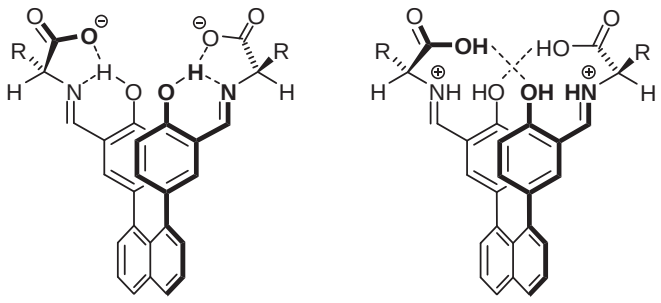


Figure 4. Hydrogen bonding motif in **1**-derived diimines obtained in the presence of TBAOH (left) and under acidic conditions (right).

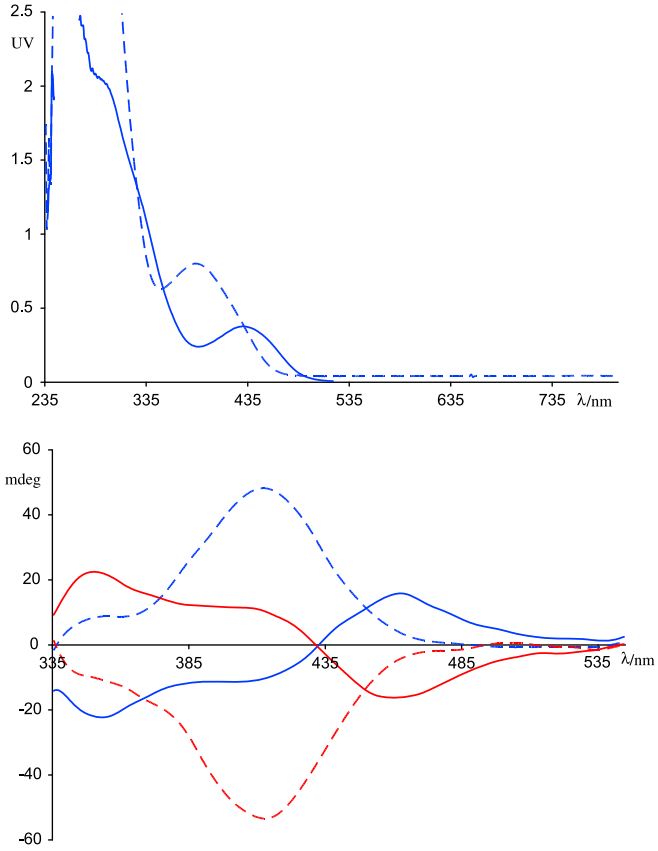


Figure 5. UV and CD spectra showing diimine products obtained with (*R*)-valine in the presence of TBAOH and upon acidification with HCl in ether (solid and dashed blue lines, respectively) and with (*S*)-valine (solid and dashed red lines). All spectra were recorded using samples having a concentration of 2.5×10^{-4} M in $CHCl_3$.

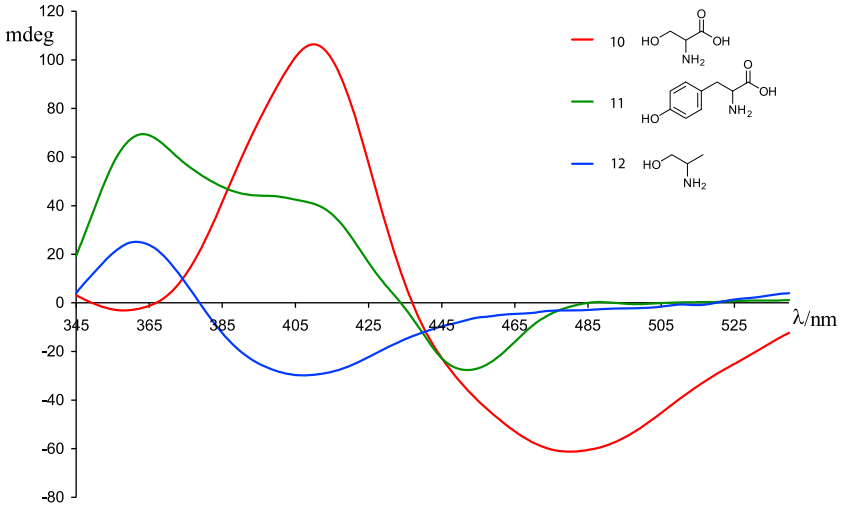


Figure 6. CD spectra of the diimines obtained with (*R*)-serine (**10**), (*R*)-tyrosine (**11**) (both 2.5×10^{-4} M), and (*R*)-2-amino-1-propanol (**12**) (5.0×10^{-4} M) in $CHCl_3$.

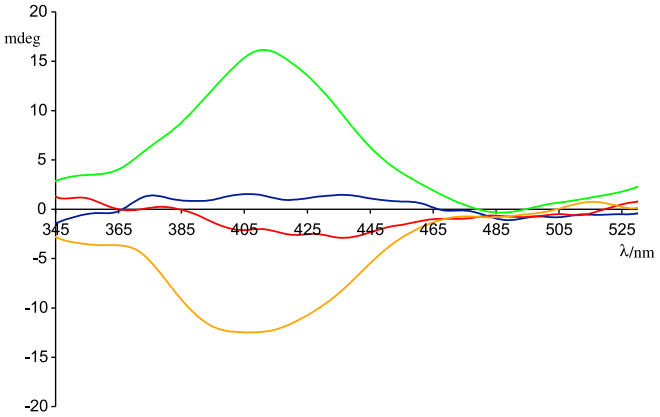


Figure 7. CD spectra of the diimines obtained from (*R*)- and (*S*)-alanine (green and orange, respectively) and (*R*)- and (*S*)-proline (blue and red, respectively) after acidification, collected at 2.5×10^{-4} M in chloroform.

formed from **1** were strongly diminished when chloroform was replaced with ethanol as solvent.

Comparison of the CD spectra obtained with the diimines derived from amino acids **4**–**8** show a consistent relationship between absolute configuration of the substrate and the sign of the Cotton effect; all (*R*,*R*)-diimines give positive Cotton effects, Figure 3. Accordingly, the induced axial chirality of the diimine product and the corresponding CD signal can be correlated to the central chirality of the amino acid used. In contrast, negative Cotton effects were obtained with the (*R*)-enantiomers of tyrosine and serine, the latter being similar to that of (*R*)-2-aminopropanol, Figure 6. The appearance of strong Cotton effects at high wavelengths is quite promising for both qualitative and quantitative CD analysis of amino acids. It is worth noting here that enantioselective sensing of proline and alanine often suffers from low selectivity.[17,21] We were pleased to find that upon acidification of the diimine obtained with alanine, our sensor provides remarkable Cotton effects with this challenging analyte, Figure 7.

To evaluate the practical use of sensor **1** for CD analysis of free amino acids, a calibration curve was obtained using leucine in varying %ee, Figure 8. To a solution of the amino acid mixture in

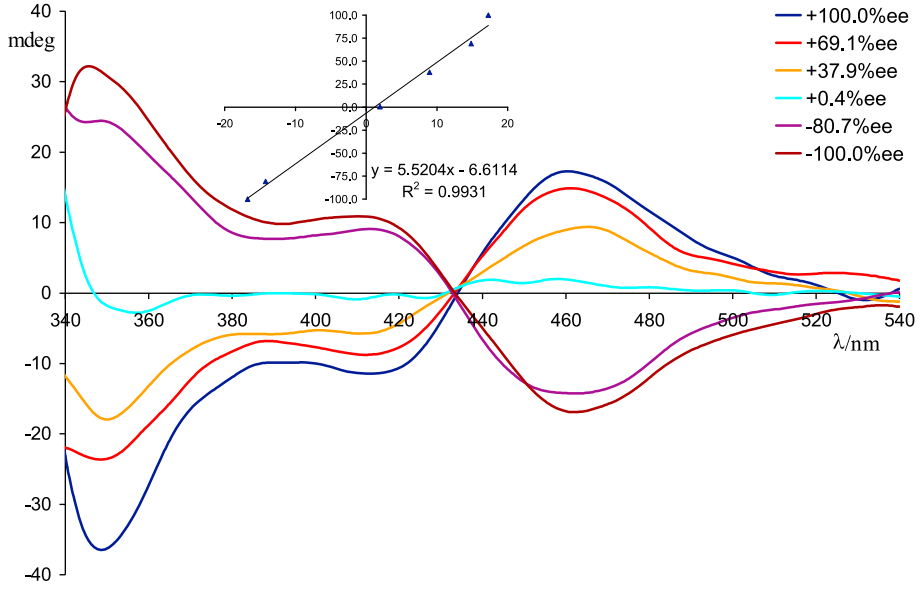


Figure 8. CD spectra obtained using leucine with varying enantiomeric composition. The amplitudes (m deg) at 460 nm are plotted vs %ee. The calibration curve shows a linear relationship between the CD amplitude of the diimine and the enantiomeric composition of leucine.

600 μL of anhydrous DMSO, a stoichiometric amount of tetrabutylammonium hydroxide (1.0 M in MeOH) was added and allowed to stir for 1 min. To **1** (4.0 mg, 0.01 mmol) in DMSO, 2 equiv of the prepared amino acid solution were added and the mixture was allowed to stir for 2 h at 70 °C. All reactions were conducted at 0.02 M and then diluted with chloroform to 2.50×10^{-4} M to collect the CD spectra in triplicate. We then tested four scalemic samples exhibiting 79.1, 53.7, −67.2, and −88.7%ee. Formation of the sensor-derived diimines and subsequent CD analysis gave 78.0, 50.1, −63.0 and −92.0%ee, respectively. These results are in good agreement with the actual values and thus show that our sensor can be used for the assignment of the absolute configuration of unprotected amino acids and for the determination of the %ee of small sample amounts.

3. Conclusion

We have introduced a metal-free chiroptical sensing method for the determination of the absolute configuration and enantiomeric composition of unprotected aliphatic and aromatic amino acids. In contrast to previously reported enantioselective sensing of amino acids, this approach does not require the use of an enantiopure ligand or metal complex. The CD analysis with readily available **1** is based on the condensation of a stereodynamic racemic sensor exhibiting two salicylaldehyde rings with free amino acids to afford a diimine scaffold that undergoes substrate-controlled stereodivergent amplification of chirality. The cofacial arrangement of the two salicylaldehyde rings in the sensor favor intramolecular π–π interactions. The diimine formation and concomitant chiral induction is reported in the form of a sensitive CD signal. The Cotton effects of these amino acid-derived diimines occur at relatively high wavelengths, which eliminates interference with CD active analytes and impurities. After completion of the condensation reaction, the CD spectra can be obtained without further purification and the sensor can be reused after hydrolysis.

4. Experimental section

4.1. Synthetic procedures

All reagents and solvents were used without further purification. Reactions were carried out under nitrogen atmosphere and under anhydrous conditions. 1,8-Diiodonaphthalene was prepared from 1,8-diaminonaphthalene and converted to **3** as described in the literature.[24,30] Products were purified by flash chromatography on SiO_2 (particle size 0.032–0.063 mm). NMR spectra were obtained at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) using $CDCl_3$ as solvent. Chemical shifts are reported in parts per million relative to TMS.

*4.1.1. 1,8-Bis(3′-formyl-4′-hydroxyphenyl)naphthalene (**1**).* To a solution of 1,8-bis(3′-formyl-4′-methoxyphenyl)naphthalene, **3**, (0.38 g, 1.0 mmol) in 15 mL of anhydrous dichloromethane at 0 °C, BBr_3 (5.8 mL, 5.8 mmol) was added dropwise and the mixture was stirred for one hour. It was then quenched with isopropyl alcohol and then with water, and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuum. Purification by flash chromatography on silica gel (dichloromethane/hexanes 20:1) afforded **1** in 70% yield as a white solid. Anal. Calcd for $C_{24}H_{16}O_4$: C, 78.25; H, 4.38; O, 17.37. Found: C, 78.40; H, 4.31; O, 17.27.

^{1}H NMR: δ=6.64 (m, 2H), 7.00–7.25 (m, 4H), 7.42 (d, J=7.0 Hz, 2H), 7.58 (dd, J=7.0, 8.0 Hz, 2H), 8.00 (d, J=8.0 Hz, 2H), 9.62 (s, 2H), 10.75 (s, 2H). ^{13}C NMR: δ=116.6, 116.9, 119.2, 125.4, 129.3, 131.0, 134.8, 135.2, 135.5, 137.6, 137.8, 159.9, 195.9.

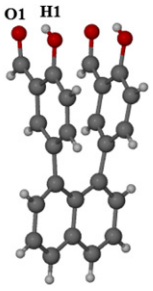
4.2. Diimine formation and MS and CD analysis

The following conditions have been optimized in terms of reaction time, solvent, concentration and equivalents. To a solution of the selected amino acid in 400 μL of anhydrous DMSO, a stoichiometric amount of tetrabutylammonium hydroxide (1.0 M in MeOH) was added and allowed to stir for 1 min. To a mixture of **1** (4.0 mg, 0.01 mmol) in DMSO, 2 equiv of the prepared amino acid solution were added and the mixture was allowed to stir for 2 h at 70 °C. Prior to each use, the CD instrument was purged with nitrogen for 20 min and the temperature was set to 25.0 °C. Spectra were collected between 335 and 545 nm with a standard sensitivity of 100 m deg, a data pitch of 0.5 nm, a band width of 1 nm, a scanning speed of 500 nm s^{-1} and a response of 0.5 s using a quartz cuvette (1 cm path length). The data were adjusted by baseline correction and binomial smoothing. Samples were diluted to 2.50×10^{-4} M with chloroform. To collect CDs under acidic conditions, a stoichiometric amount of HCl (2 M in ether, accounting for the 2 equiv of TBAOH and the two carboxylate groups in the diimine formed) was added and allowed to stir for 2 min. Samples were then diluted to 2.50×10^{-4} M with chloroform. Control experiments with salicylaldehyde were conducted to compare the CD signal of salicylidenimine with that of the sensor-derived diimine. At a concentration of 0.4 M, 2 equiv of enantiopure valine were added under the same conditions as described above for sensor **1**. The reaction was complete after 2 h, and the CD spectrum was collected at 5.0×10^{-4} M. No CD signal was detected in the region of interest for both salicylidenimine enantiomers in chloroform or ethanol, even at 10 times the concentration of the diimines obtained with **1**. Electrospray mass spectrometry (1 mg/mL in methanol, negative detection mode) of several dimines proved the formation of the desired condensation product, see Supplementary data.

4.3. Crystallization and X-ray diffraction

Single crystal X-ray analysis was performed at 100 K using a Siemens platform diffractometer with graphite monochromated Mo Kα radiation (λ=0.71073 Å). Data were integrated and corrected using the Apex 2 program. The structures were solved by direct methods and refined with full-matrix least-square analysis using SHELX-97-2 software. Non-hydrogen atoms were refined with anisotropic displacement parameters. A crystal of **1** was obtained by slow evaporation of a solution of 5.0 mg of **1** in 3 mL $CHCl_3$. Crystal structure data for **1**: Formula $C_{24}H_{16}O_4$, M=368.38, crystal dimensions 0.6×0.4×0.3 mm, monoclinic, space group C2/c, a=20.4576(27) Å, b=6.8831(9) Å, c=25.3924(34) Å, α,γ=90°, β=98.623(2)°, V=3535.13 Å^3, Z=70, ρ_{calcd}=1.3841 g cm^{-3}.

O1-H1 [Å]	1.952
Phenyl-phenyl [Å] (centroid to centroid)	3.334
Splaying angle [°]	13.45
Torsion angle [°]	4.34



Acknowledgements

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Supplementary data

Synthetic procedures including NMR spectra, MS analysis of the condensation products, and CD spectra. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.04.030.

References and notes

1. Pu, L. *Chem. Rev.* **2004**, *104*, 1687.
2. (a) Lin, J.; Li, Z.-B.; Zhang, H.-C.; Pu, L. *Tetrahedron Lett.* **2004**, *45*, 103; (b) Li, Z.-B.; Lin, J.; Zhang, H.-C.; Sabat, M.; Hyacinth, M.; Pu, L. *J. Org. Chem.* **2004**, *69*, 6284; (c) Li, Z.-B.; Lin, J.; Sabat, M.; Hyacinth, M.; Pu, L. *J. Org. Chem.* **2007**, *72*, 4905.
3. Lu, Q.-S.; Dong, L.; Zhang, J.; Li, J.; Jiang, L.; Huang, Y.; Qin, S.; Hu, C.-W.; Yu, X.-Q. *Org. Lett.* **2009**, *11*, 669.
4. Xu, K.-x.; Qiu, Z.; Zhao, J.-J.; Zhao, J.; Wang, C.-j. *Tetrahedron: Asymmetry* **2009**, *20*, 1690.
5. He, X.; Cui, X.; Li, M.; Lin, L.; Liu, X.; Feng, X. *Tetrahedron Lett.* **2009**, *50*, 5853.
6. (a) Mei, X.; Wolf, C. *Chem. Commun.* **2004**, 2078; (b) Wolf, C.; Liu, S.; Reinhardt, B. C. *Chem. Commun.* **2006**, 4242; (c) Mei, X.; Wolf, C. *Tetrahedron Lett.* **2006**, *47*, 7901; (d) Mei, X.; Martin, R. M.; Wolf, C. *J. Org. Chem.* **2006**, *71*, 2854.
7. Yang, H.; Bohne, C. *J. Photochem. Photobiol. A: Chem.* **1995**, *86*, 209.
8. (a) Pagliari, S.; Corradini, R.; Galaverna, G.; Sforza, S.; Dossena, A.; Marchelli, R. *Tetrahedron Lett.* **2000**, *41*, 3691; (b) Corradini, R.; Paganuzzi, C.; Marchelli, R.; Pagliari, S.; Sforza, S.; Dossena, A.; Galaverna, G.; Duchateau, A. *Chirality* **2003**, *15*, S30; (c) Pagliari, S.; Corradini, R.; Galaverna, G.; Sforza, S.; Dossena, A.; Montalti, M.; Prodi, L.; Zaccheroni, N.; Marchelli, R. *Chem.—Eur. J.* **2004**, *10*, 2749; (d) Corradini, R.; Paganuzzi, C.; Marchelli, R.; Pagliari, S.; Sforza, S.; Dossena, A.; Galaverna, G.; Duchateau, A. *J. Mater. Chem.* **2005**, *15*, 2741.
9. Webb, T. H.; Wilcox, C. S. *Chem. Soc. Rev.* **1993**, 383.
10. Wong, W.-L.; Huang, K.-H.; Teng, P.-F.; Lee, C.-S.; Kwong, H.-L. *Chem. Commun.* **2004**, 384.
11. Qing, G.-y.; He, Y.-b.; Wang, F.; Qin, H.-j.; Hu, C.-g.; Yang, X. *Eur. J. Org. Chem.* **2007**, 1768.
12. Godoy-Alcántar, C.; Nelen, M. I.; Eliseev, A. V.; Yatsimirsky, A. K. *J. Chem. Soc., Perkin Trans. 2* **1999**, 353.
13. Corradini, R.; Sartor, G.; Marchelli, R.; Dossena, A.; Spisni, A. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1979.
14. (a) Yan, Y.; Myrick, M. L. *Anal. Chem.* **1999**, *71*, 1958; (b) Kumar, C. V.; Buranaprapuk, A.; Chou Sze, H. *Chem. Commun.* **2001**, 297.
15. Karakaplan, M.; Aral, T. *Tetrahedron: Asymmetry* **2005**, *16*, 2119.
16. Araki, K.; Inada, K.; Shinkai, S. *Angew. Chem., Int. Ed.* **1996**, *35*, 72.
17. (a) Leung, D.; Folmer-Anderson, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12318; (b) Leung, D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12328.
18. Mei, X.; Wolf, C. *J. Am. Chem. Soc.* **2006**, *128*, 13326.
19. (a) Zahn, S.; Canary, J. W. *Org. Lett.* **1999**, *1*, 86; (b) Holmes, A. E.; Zahn, S.; Canary, J. W. *Chirality* **2002**, *14*, 471.
20. (a) Corradini, R.; Dossena, A.; Impellizzeri, G.; Maccarrone, G.; Marchelli, R.; Rizzarelli, E.; Sartor, G.; Vecchio, G. *J. Am. Chem. Soc.* **1994**, *116*, 10267; (b) Ogoshi, H.; Mizutani, T. *Acc. Chem. Res.* **1998**, *31*, 81.
21. (a) Sheridan, E. M.; Breslin, C. B. *Electroanalysis* **2005**, *17*, 532; (b) Yin, X.; Ding, J.; Zhang, S.; Kong, J. *Biosens. Bioelectron.* **2006**, *21*, 2184; (c) Budnikov, G. K.; Evtyugin, G. A.; Budnikova, Y. G.; Al'fonsov, V. A. *J. Anal. Chem.* **2008**, *63*, 2.
22. (a) Hofstetter, O.; Hofstetter, H.; Schurig, V.; Wilcheck, M.; Green, B. S. *J. Am. Chem. Soc.* **1998**, *120*, 3251; (b) Dutta, P.; Tipple, C. A.; Lavrik, N. V.; Datskos, P. G.; Hofstetter, H.; Hofstetter, O.; Sepaniak, M. J. *Anal. Chem.* **2003**, *75*, 2342.
23. Vardanega, D.; Picaud, F.; Girardet, C. *J. Chem. Phys.* **2009**, *130*, 114709.
24. Ghosn, M. W.; Wolf, C. *J. Am. Chem. Soc.* **2009**, *131*, 16360.
25. 1,8-Diphenyl- and 1,8-dipyridylnaphthalenes are known to undergo fast rotation about the two aryl–aryl bonds at room temperature. In general, the enantiomeric *anti*-isomers of these triaryls rapidly interconvert via the thermodynamically less stable *meso syn*-intermediate with a rotational energy barrier of 60–75 kJ/mol. See: *Dynamic Stereochemistry of Chiral Compounds*; Wolf, C., Ed.; RSC: Cambridge, 2008; pp 84–94.
26. (a) Mazaleyrat, J.-P.; Wright, K.; Gaucher, A.; Toulemonde, N.; Wakselman, M.; Oancea, S.; Peggion, C.; Formaggio, F.; Setnicka, V.; Keiderling, T. A.; Toniolo, C. *J. Am. Chem. Soc.* **2004**, *126*, 12874; (b) Mazaleyrat, J.-P.; Wright, K.; Gaucher, A.; Toulemonde, N.; Dutot, L.; Wakselman, M.; Broxterman, Q. B.; Kaptein, B.; Oancea, S.; Peggion, C.; Crisma, M.; Formaggio, F.; Toniolo, C. *Chem.—Eur. J.* **2005**, *11*, 6921; (c) Dutot, L.; Wright, K.; Gaucher, A.; Wakselman, M.; Mazaleyrat, J.-P.; De Zotti, M.; Peggion, C.; Formaggio, F.; Toniolo, C. *J. Am. Chem. Soc.* **2008**, *130*, 5986; (d) Kim, H.; So, S. m.; Yen, C. P.-H.; Vinhato, E.; Lough, A. j.; Hong, J.-I.; Kim, H.-J.; Chin, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 8657.
27. Crystallographic data of **1** have been deposited with the Cambridge Crystallographic Data Center (CCDC 765565). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, United Kingdom (fax: 44 1223 336033 or email: deposit@ccdc.cam.ac.uk).
28. All attempts to obtain a crystal structure of the amino acid-derived diimines have been unsuccessful.
29. Based on the basicity of salicylidenimines, we expect that the imine groups are protonated prior to the formation of free carboxylic acid groups. See: Leach, B. E.; Leussing, D. L. *J. Am. Chem. Soc.* **1971**, *93*, 3377.
30. House, H. O.; Koepsella, D. G.; Campbel, W. J. *J. Org. Chem.* **1972**, *37*, 1003.