JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Stereodynamic Chemosensor with Selective Circular Dichroism and Fluorescence Readout for in Situ Determination of Absolute Configuration, Enantiomeric Excess, and Concentration of Chiral Compounds

Keith W. Bentley and Christian Wolf*

Department of Chemistry, Georgetown University, Washington, D.C. 20057, United States

Supporting Information

ABSTRACT: A stereodynamic chemosensor having a parallel arrangement of a substrate-binding salicylaldehyde unit and an adjacent pyridyl *N*-oxide fluorophore undergoes rapid condensation with chiral amino alcohols and subsequent asymmetric transformation of the first kind toward a single rotamer. Crystallographic analysis shows that the concomitant central-to-axial chirality imprinting is controlled by minimization of steric repulsion and by intramolecular hydrogen bonding between the bound amino alcohol and the proximate *N*-oxide group. The substrate binding event results in strong CD effects and characteristic fluorescence changes which can be used for instantaneous in situ determination of the absolute configuration, enantiomeric composition and total concentration of a variety of chiral amino alcohols. This chemosensing approach avoids time-consuming workup and purification steps, and it is applicable to minute sample amounts which reduces the use of solvents and limits waste production.

The ever-increasing demand for new biologically active chiral compounds, in particular pharmaceuticals and agrochemicals, continues to nurture tremendous interest in asymmetric synthesis.[1] The development of enantioselective reactions for the production of chiral compounds is vigorously pursued in numerous academic and industrial research laboratories, and the pace and prospect of these efforts have increased significantly during the past 20 years with the introduction of combinatorial methods. In contrast, the analysis of the amount and enantiomeric composition of chiral compounds has become a major bottleneck in the discovery process. High-throughput screening (HTS) methods capable of analyzing large numbers of samples that can be generated overnight are required to match the productivity of parallel synthesis and other combinatorial techniques. It has been proposed that optical methods based on fluorescence, UV, and circular dichroism (CD) spectroscopy hold considerable promise toward the goal of enantioselective HTS.[2] Several optical sensing assays developed to date have been found to outperform chromatographic and NMR spectroscopic methods with regard to time-efficiency, sensitivity, and waste production.[3−5] Optical assays typically provide information on the enantiomeric excess (ee) but require independent analysis of the concentration of the substrate tested unless two chemosensors are used simultaneously or in tandem.[6] The determination of both values with a generally applicable chemosensor remains a major challenge and may require multilayer perceptron artificial neural network analysis.[7] Because a complete stereochemical analysis must reveal the absolute configuration, the enantiomeric composition, and the total concentration of a chiral compound, we have developed a widely useful, practical probe that can accomplish all three tasks with high accuracy and sensitivity.

We envisioned that this would be possible with racemic 1-(3′-formyl-4′-hydroxyphenyl)-8-(4′-pyridyl)naphthalene *N*-oxide, **1** (Figure 1). Due to the lack of bulky substituents close to the aryl−aryl bond, the enantiomers of **1** undergo fast interconversion via facile rotation about the stereogenic naphthyl-salicylaldehyde axis at room temperature. This stereodynamic probe has a salicylaldehyde ring capable of fast binding of an amino alcohol and a proximate pyridyl *N*-oxide fluorophore incorporated to report the binding event. We assumed that a condensation reaction between a chiral amino alcohol and the reactive formyl group in **1** would lead to a stereochemical bias at the stereogenic axis with characteristic chiroptical output, while hydrogen bond interactions between the alcohol moiety of the bound substrate and the *N*-oxide unit would generate a strong fluorescence response.

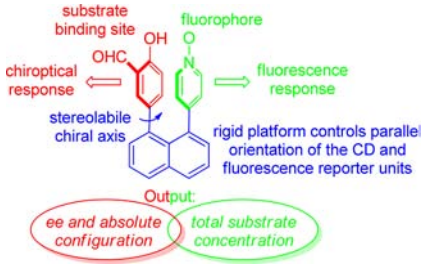


Figure 1. Design of a sensor with circular dichroism and fluorescence reporter units.

The design of **1** has several important structural features. First, the presence of an adjacent phenol moiety is known to accelerate the condensation reaction between the formyl group

Received: June 20, 2013
Published: August 2, 2013

   dx.doi.org/10.1021/ja406259p | J. Am. Chem. Soc. 2013, 135, 12200−12203

and an amino moiety, which is important for achieving time-efficient substrate recognition. Second, the imine formation with a chiral amino alcohol was expected to induce population of a single axially chiral conformation of **1** with a distinct CD output (Figure 2). This asymmetric transformation of the first

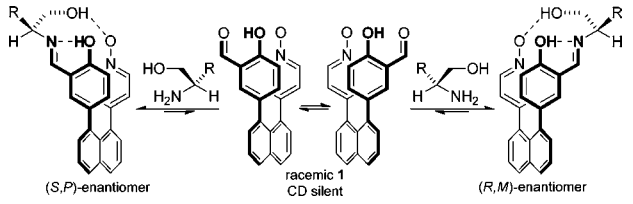
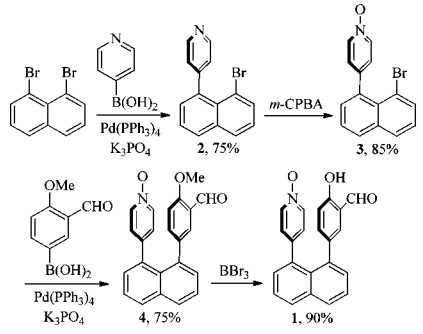


Figure 2. Anticipated asymmetric induction process upon binding of either an (*R*)- or (*S*)-configured amino alcohol locking the sensor into an axially chiral, CD-active conformation. Note that the condensation products are enantiomeric and expected to have opposite Cotton effects but identical fluorescence output.

kind would be controlled by minimization of steric repulsion and by intramolecular hydrogen bonding between the bound amino alcohol and the neighboring pyridyl *N*-oxide. Importantly, this process should occur instantaneously due to the rapid rotation about the chiral axis.[8] Third, hydrogen bonding of the alcohol moiety of the substrate to the pyridyl *N*-oxide group was expected to alter the fluorescence signal of the probe. Altogether, the initially racemic and CD-silent sensor would undergo fast amino alcohol binding, and the imine formed would exhibit (a) an axially chiral conformation with a pronounced chiroptical signal and (b) a characteristic change in the fluorescence output. The CD effect would directly correspond to the absolute configuration and ee of the amino alcohol, while the fluorescence change would not be enantioselective and therefore provide an entry to the determination of the total concentration of the substrate. Based on the short response time and the inherent sensitivity of fluorescence and CD spectroscopy, the dual readout generated by sensor **1** could then be used for complete stereochemical analysis of minute sample amounts.

We were able to synthesize **1** in four steps from 1,8-dibromonaphthalene (Scheme 1). The selective monoarylation

Scheme 1. Synthesis of Chemosensor 1



with 4-pyridylphenylboronic acid turned out to be very sensitive to temperature, solvent, catalyst loading, equivalents of the boronic acid, and reaction time. Through careful optimization of the Suzuki coupling using tetrakis-(triphenylphosphine)palladium as catalyst, we found a procedure that affords **2** in 75% yield, leaving the second aryl bromide function intact. Treatment of **2** with *m*-CPBA gave *N*-oxide **3**, and a second cross-coupling step provided precursor **4**, which was finally deprotected with boron tribromide.

The sensor was then tested with a wide range of aliphatic and aromatic amino alcohols. An immediate change of the reaction mixtures from colorless to dark yellow indicated that the condensation reactions were complete, and the samples were subjected to CD analysis without further workup.[9] The imine formation was also confirmed by NMR and ESI/MS analysis (see SI). We were pleased to find that in all cases the imines formed show strong Cotton effects even at micromolar concentrations (Figure 3 and SI). Moreover, the chemosensor

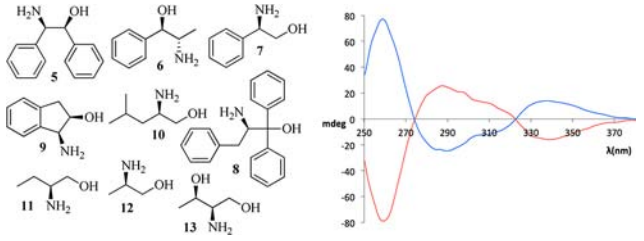


Figure 3. (Left) Structures of amino alcohols tested. (Right) CD spectra (7.50×10^{-5} M in $CHCl_3$) of the imines formed from **1** and (1*S*,2*R*)-**5** (blue) and (1*R*,2*S*)-**5** (red) at room temperature.

generates without exception a positive Cotton effect above 330 nm when an acyclic (*R*)-configured amino group is bound and a negative couplet at the same wavelength when an (*S*)-configured amine is detected. The probe can thus be used for identification of the absolute configuration of amino alcohols.

We were able to grow single crystals of the imine derived from **1** and amino alcohol (1*S*,2*R*)-**5** by slow diffusion of hexanes into a concentrated chloroform solution.[10] The pyridyl and the salicylidenimine ring are both orthogonal to the naphthalene plane and undergo $\pi-\pi$ stacking. The two rings are almost perfectly parallel and show slight twisting and splaying. The splaying angle between the pyridyl and the phenyl planes in the crystal structure shown in Figure 4 was

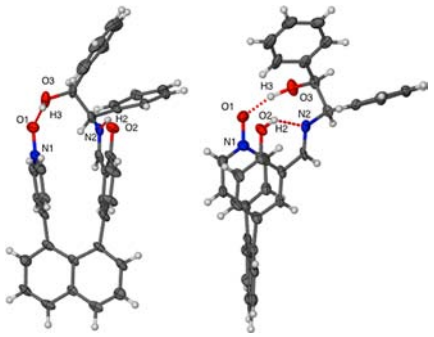


Figure 4. Views facing the naphthalene ring (left) and along the naphthalene ring (right) of the X-ray structure of the (1*S*,2*R*,*M*)-configured imine. Selected crystallographic separations [Å]: O1···H3 1.573, $\text{phenyl}_{\text{centroid}}-\text{pyridyl}_{\text{centroid}}$ 3.338.

determined as 20.6°, and the twisting angle is 4.8°. The crystallographic analysis proves that the binding of the amino alcohol moiety leads to a stereochemical bias of the chiral axis in **1** as described above. The imine formation locks the sensor into a structure exhibiting *M* torsion at the stereogenic aryl–aryl axis, which explains the distinct Cotton effects. This central-to-axial chirality induction process is guided by hydrogen bonding between the alcohol group and the

dx.doi.org/10.1021/ja406259p | J. Am. Chem. Soc. 2013, 135, 12200–12203

proximate *N*-oxide while steric repulsion is kept at a minimum (Figure 4 and SI). As a result, the orientation of the salicylidenimine plane with respect to the perpendicular naphthalene ring is controlled by the intramolecular hydrogen bonding motif, while the bulky residues of the bound amino alcohol point toward the sterically least hindered direction. The corresponding central-to-axial chirality induction in the stereodynamic sensor thus strongly favors population of a single rigid conformation and an intense CD response.

We then collected the CD spectra of non-racemic samples of the imines obtained with amino alcohols **5** and **11**, respectively, to evaluate the practical use of **1** for quantitative ee determination. In both cases, we found a perfectly linear relationship between the CD responses of **1** at 260, 290, and 340 nm, respectively, and the sample ee (Figure 5 and SI). Five

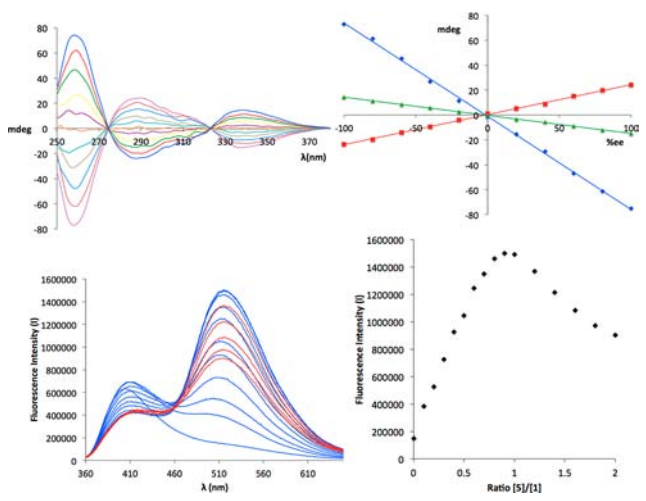


Figure 5. (Top) CD spectra of the imine obtained with non-racemic samples of **5** at 7.50×10^{-5} M in $CHCl_3$ and the corresponding plots of the CD maxima at 260 (blue), 290 (red), and 340 nm (green) vs sample ee. (Bottom left) Fluorescence change of **1** upon titration with **5**. Excitation (emission) wavelength: 340 nm (515 nm). Spectra collected in the presence of sub-stoichiometric or equimolar amounts and excess of **5** are shown in blue and red, respectively. (Bottom right) Plot of the fluorescence intensity at 515 nm vs ratio [**5**]/[**1**].

non-racemic samples of **5** covering a wide ee range were prepared and treated with sensor **1** at room temperature. Using the linear regression equation calculated from the calibration curve and the measured CD amplitudes at 260 nm, the experimentally determined ee of these samples was within 2% of the actual values. Similar results were obtained with non-racemic samples of **11** (SI).

Having established the nature of the chiral induction process and the use of **1** for quantitative ee determination of amino alcohols, we investigated the effect of the substrate binding on the fluorescence response of **1**. We were pleased to find that the imine formation results in a strong increase in the fluorescence intensity until >1 equiv of the amino alcohol is added (Figure 5 and SI). While the fluorescence enhancement can be attributed to the formation of a rigid structure stabilized by intramolecular hydrogen bonding, the decrease in the fluorescence intensity observed in the presence of unbound substrate is probably a result of dynamic quenching. In accordance with the ee determination discussed above, the fluorescence change of **1**, i.e., a steady increase in intensity that is followed by quenching when excess of an amino alcohol is present, appears to be a general phenomenon, and we were able to use the chemosensor fluorescence response for accurate quantification of the concentration of **5** and **11** (see SI). Regression analysis of five samples containing various amounts of amino alcohol **5** demonstrated that the total amount can be determined within a 2.5% error margin.

Finally, we used the dual CD and fluorescence readout of **1** to achieve a full stereochemical analysis of non-racemic mixtures at once, i.e., determination of the absolute configuration of the major enantiomer, the ee, and the total substrate concentration. Four samples with varying concentration *and* enantiomeric composition of **5** were analyzed as described above (Table 1 and SI). The results obtained by our

Table 1. Chemosensing of Amino Alcohol 5

Sample Composition			Sensing Results		
concn (mM)	ee (%)	abs config[a]	concn (mM)	ee (%)	abs config[a]
1.20	28.0	1*S*,2*R*	1.21	24.8	1*S*,2*R*
2.14	10.0	1*S*,2*R*	1.97	13.7	1*S*,2*R*
2.70	52.0	1*R*,2*S*	2.65	52.9	1*R*,2*S*
3.04	64.0	1*R*,2*S*	3.00	62.4	1*R*,2*S*

[a]Major enantiomer.

dual sensing method deviated less than 5% from the actual values. The accuracy of the analysis performed with **1** is generally considered sufficient for HTS purposes, and it compares well with previously reported optical ee measurements achieved by using two probes simultaneously and in tandem or by multilayer perceptron artificial neural network analysis.[6,7] The optical measurements are operationally simple and fast and require only small sample and solvent amounts. In all experiments, the reaction mixtures were analyzed directly. The general simplicity and exclusion of any purification steps after imine formation underscore the practicality of chemosensing with **1**.[11]

In summary, we have designed a stereodynamic chemosensor **1** that can be used for in situ determination of the absolute configuration, ee, and concentration of chiral compounds based on substrate-controlled induction of axial chirality and a dual-mode optical readout. This probe produces a distinct CD signal upon condensation with amino alcohols which can be correlated to the substrate chirality and enantiomeric composition, while the independent fluorescence response is not enantioselective and allows quantification of the total sample amount. Screening of several amino alcohols showed that **1** has a broad substrate scope. The analysis is accurate, avoids time-consuming workup and purification steps, and is applicable to minute sample amounts which reduces the use of solvents and waste production. Finally, we note that **1** is used in racemic form which eliminates the general need for asymmetric production of enantiopure sensors previously employed in HTS analysis of chiral compounds.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic details, analytical procedures, compound characterization, MS, CD, fluorescence and NMR spectra, and crystallographic details. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

cw27@georgetown.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under CHE-1213019.

REFERENCES

(1) (a) Gawley, R. E., Aubé, J., Eds. *Principles of Asymmetric Synthesis*, Tetrahedron Organic Chemistry Series; Elsevier: New York, 1996. (b) Ojima, I., Ed. *Catalytic Asymmetric Synthesis*, 3rd ed.; Wiley: New York, 2010.

(2) (a) Leung, D.; Kang, S. O.; Anslyn, E. V. *Chem. Soc. Rev.* **2012**, *41*, 448−479. (b) Wolf, C.; Bentley, K. W. *Chem. Soc. Rev.* **2013**, *42*, 5408−5424. For HTS based on immunoassays, enzymatic methods, and DNA microarrays, see: (c) Reetz, M. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1335−1338. High-throughput NMR analysis: (d) Reetz, M. T.; Tielmann, P.; Eipper, A.; Ross, A.; Schlotterbeck, G. *Chem. Commun.* **2004**, 1366−1367. For electrophoretic HTS on a chip: (e) Belder, D.; Ludwig, M.; Wang, L.-W.; Reetz, M. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 2463−2466. Chromatographic methods: (f) Trapp, O. *J. Chromatogr. A* **2008**, *1184*, 160−190. (g) Troendlin, J.; Rehbein, J.; Hiersemann, M.; Trapp, O. *J. Am. Chem. Soc.* **2011**, *133*, 16444−16450.

(3) Selected examples of UV sensing of chiral compounds: (a) Zhu, L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 3676−3677. (b) Leung, D.; Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12318−12327. (c) Leung, D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12328−12333. (d) Iwaniuk, D. P.; Yearick-Spangler, K.; Wolf, C. V. *J. Org. Chem.* **2012**, *77*, 5203−5208.

(4) Selected examples of enantioselective fluorescence sensing: (a) Lee, S. J.; Lin, W. *J. Am. Chem. Soc.* **2002**, *124*, 4554−4555. (b) Lin, J.; Hu, Q.-S.; Xu, M.-H.; Pu, L. *J. Am. Chem. Soc.* **2002**, *124*, 2088−2089. (c) Mei, X.; Wolf, C. *Chem. Commun.* **2004**, 2078−2079. (d) Zhao, J.; Fyles, T. M.; James, T. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 3461−3464. (e) Mei, X.; Wolf, C. *J. Am. Chem. Soc.* **2004**, *126*, 14736−14737. (f) Li, Z.-B.; Lin, J.; Pu, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1690−1693. (g) Tumambac, G. E.; Wolf, C. *Org. Lett.* **2005**, *7*, 4045−4048. (h) Mei, X.; Martin, R. M.; Wolf, C. *J. Org. Chem.* **2006**, *71*, 2854−2861. (i) Mei, X.; Wolf, C. *Tetrahedron Lett.* **2006**, *47*, 7901−7904. (j) Wu, Y.; Guo, H.; James, T. D.; Zhao, J. *J. Org. Chem.* **2011**, *76*, 5685−5695. (k) Wu, Y.; Guo, H.; Zhang, X.; James, T. D.; Zhao, J. *Chem.—Eur. J.* **2011**, *17*, 7632−7644. (l) Yang, X.; Liu, X.; Shen, K.; Zhu, C.; Cheng, Y. *Org. Lett.* **2011**, *13*, 3510−3513. (m) He, X.; Zhang, Q.; Liu, X.; Lin, L.; Feng, X. *Chem. Commun.* **2011**, *47*, 11641−11643. (n) Wanderley, M. M.; Wang, C.; Wu, C.-D.; Lin, W. *J. Am. Chem. Soc.* **2012**, *134*, 9050−9053. For a review: (o) Pu, L. *Chem. Rev.* **2004**, *104*, 1687−1716.

(5) Selected examples of enantioselective CD sensing: (a) Superchi, S.; Casarini, D.; Laurita, A.; Bavoso, A.; Rosini, C. *Angew. Chem., Int. Ed.* **2001**, *40*, 451−454. (b) Kurtan, T.; Nesnas, N.; Koehn, F. E.; Li, Y.-Q.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **2001**, *123*, 5974−5982. (c) Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **2002**, *124*, 10320−10335. (d) 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*, 12874−12879. (e) Superchi, S.; Bisaccia, R.; Casarini, D.; Laurita, A.; Rosini, C. *J. Am. Chem. Soc.* **2006**, *128*, 6893−6902. (f) Holmes, A. E.; Das, D.; Canary, J. W. *J. Am. Chem. Soc.* **2007**, *129*, 1506−1507. (g) 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*, 5986−5992. (h) Sciebura, J.; Skowronek, P.; Gawronski, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 7069−7072. (i) Sciebura, J.; Gawronski, J. *Chem.—Eur. J.* **2011**, *17*, 13138−13141. (j) 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*, 8657−8660. (k) Waki, M.; Abe, H.; Inouye, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 3059−3061. (l) Li, X.; Borhan, B. *J. Am. Chem. Soc.* **2008**, *130*, 16126−16127. (m) Katoono, R.; Kawai, H.; Fujiwara, K.; Suzuki, T. *J. Am. Chem. Soc.* **2009**, *131*, 16896−16904. (n) Ghosn, M. W.; Wolf, C. *J. Am. Chem. Soc.* **2009**, *131*, 16360−16361. (o) Ghosn, M. W.; Wolf, C. *J. Org. Chem.* **2011**, *76*, 3888−3897. (p) Ghosn, M. W.; Wolf, C. *Tetrahedron* **2011**, *67*, 6799−6803. (q) Joyce, L. A.; Maynor, M. S.; Dragna, J. M.; da Cruz, G. M.; Lynch, V. M.; Canary, J. W.; Anslyn, E. V. *J. Am. Chem. Soc.* **2011**, *133*, 13746−13752. (r) You, L.; Pescitelli, G.; Anslyn, E. V.; Di Bari, L. *J. Am. Chem. Soc.* **2012**, *134*, 7117−7125. (s) Wezenberg, S. J.; Salassa, G.; Escudero-Adan, E. C.; Benet-Buchholz, J.; Kleij, A. W. *Angew. Chem., Int. Ed.* **2011**, *50*, 713−716. (t) Iwaniuk, D. P.; Wolf, C. *J. Am. Chem. Soc.* **2011**, *133*, 2414−2417. (u) Iwaniuk, D. P.; Wolf, C. *Org. Lett.* **2011**, *13*, 2602−2605. (v) Iwaniuk, D. P.; Bentley, K. W.; Wolf, C. *Chirality* **2012**, *24*, 584−589. (w) Li, X.; Burrell, C. E.; Staples, R. J.; Borhan, B. *J. Am. Chem. Soc.* **2012**, *134*, 9026−9029. (x) Iwaniuk, D. P.; Wolf, C. *Chem. Commun.* **2012**, *48*, 11226−11228.

(6) (a) Mei, X.; Wolf, C. *J. Am. Chem. Soc.* **2006**, *128*, 13326−13327. (b) Wolf, C.; Liu, S.; Reinhardt, B. C. *Chem. Commun.* **2006**, 4242−4244. (c) Zhu, L.; Shabbir, S. H.; Anslyn, E. V. *Chem.—Eur. J.* **2007**, *13*, 99−104. (d) Liu, S.; Pestano, J. P. C.; Wolf, C. *J. Org. Chem.* **2008**, *73*, 4267−4270. (e) Yu, S.; Pu, L. *J. Am. Chem. Soc.* **2010**, *132*, 17698−17700.

(7) Examples of optical sensing with transition metal complexes: (a) Nieto, S.; Lynch, V. M.; Anslyn, E. V.; Kim, H.; Chin, J. *J. Am. Chem. Soc.* **2008**, *130*, 9232−9233. (b) Nieto, S.; Lynch, V. M.; Anslyn, E. V.; Kim, H.; Chin, J. *Org. Lett.* **2008**, *10*, 5167−5170. (c) Nieto, S.; Dragna, J. M.; Anslyn, E. V. *Chem.—Eur. J.* **2010**, *16*, 227−232. (d) Zhang, P.; Wolf, C. *Chem. Commun.* **2013**, *49*, 7010−7012. For a sensor that can be used for analysis of a single diamine: (e) Yu, S.; Plunkett, W.; Kim, M.; Pu, L. *J. Am. Chem. Soc.* **2012**, *134*, 20282−20285.

(8) Wolf, C., Ed. *Dynamic Stereochemistry of Chiral Compounds*; RSC Publishing: Cambridge, UK, 2008; pp 84−94.

(9) The condensation reaction is complete within 10 min when molecular sieves (4 Å) and 10 mol% of *p*-toluenesulfonic acid are present in the reaction mixture.

(10) CCDC 928486 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK; fax (44) 1223-336-033; or E-maildeposit@ccdc.cam.ac.uk.

(11) This chemosensor is designed for HTS determination of the amount and ee of chiral amino alcohols, and the presence of (achiral) diastereomers or regioisomers may interfere. For stereoselective sensing of mixtures containing enantiomers and diastereomers, see ref 3d.