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COMMUNICATIONS

Published on Web 09/14/2006

Determination of Enantiomeric Excess and Concentration of Unprotected Amino Acids, Amines, Amino Alcohols, and Carboxylic Acids by Competitive Binding Assays with a Chiral Scandium Complex

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Received May 24, 2006; E-mail: cw27@georgetown.edu

The ever-increasing demand for enantiopure chemicals has been accompanied by significant progress in asymmetric synthesis and combinatorial methods that can produce large numbers of chiral compounds in a short time. Enantioselective analysis often remains the bottleneck in these processes because it usually entails laborious and time-consuming chromatographic techniques. The need for faster methods has shifted the focus to MS,[1] UV and colorimetric assays,[2] fluorescence spectroscopy,[3] IR thermography,[4] circular dichroism,[5] NMR spectroscopy,[6] capillary electrophoresis,[7] and biochemical assays.[8]

We have recently introduced 1,8-diheteroarylnaphthalene-derived fluorosensors for chiral recognition of carboxylic acids and N-protected amino acids.[9] A remaining drawback of this approach is the limited sensitivity due to relatively low association constants between these sensors and chiral hydrogen bond donating substrates. Anslyn's and Corradini's groups have demonstrated the usefulness of diaminocyclohexane- and β-cyclodextrin-derived copper(II) complexes for enantioselective colorimetric and fluorescent sensing of amino acids.[2b−d,10] Encouraged by Anslyn's success with indicator-displacement assays,[2b−d] we decided to employ *N*,*N*′-dioxide **1** in competitive binding assays. Herein, we report that Sc[*N*,*N*′-dioxide **1**]$_2$ can be used for accurate and highly reproducible UV−vis quantification of both enantiomeric composition and total amount of unprotected amino acids, amines, amino alcohols, and carboxylic acids in aqueous solutions.

Similar to thoroughly studied coordination complexes of pyridine and acridine *N*-oxide,[11] we rationalized that **1** should form chiral metal complexes exhibiting characteristic UV charge transfer bands. Stepwise replacement of *N*,*N*′-dioxide **1** from the metal center by chiral substrates would then provide an entry to competitive binding assays suitable for simple UV quantification of concentration and enantiomeric excess of a range of compounds. We observed that the UV absorption of **1** undergoes characteristic changes during titration with Cu(II), Zn(II), In(III), Al(III), Sn(II), Sc(III), Gd(III), and Yb(III) triflates. While absorbance decreases at 260−330 nm, it increases at 240−270 nm and a new charge transfer absorption band appears at 360−480 nm, the latter region being most strongly enhanced in the presence of scandium(III). Further UV titration experiments and Job plot analysis revealed formation of a 2:1 complex with Sc(III) having an association constant of 1.6×10^6 M^{-2} (see Supporting Information).

We expected that titration of enantiopure Sc[*N*,*N*′-dioxide **1**]$_2$ with chiral substrates would result in fast and reversible replacement of the two diacridylnaphthalene *N*,*N*′-dioxide ligands.[12] Substitution of the first *N*,*N*′-dioxide ligand by either enantiomer of a chiral substrate would generate diastereomeric complexes, while the second exchange process would produce a scandium complex devoid of ligand **1**. Since Sc(III) complexes of amino alcohols, amines, amino acids, and carboxylic acids did not show any UV absorption above 350 nm, we anticipated that the ligand exchange can easily be measured by the disappearance of the characteristic charge transfer band at 410 nm. Titration of a water/acetonitrile (1:1) solution containing 0.98×10^{-5} M of Sc[(+)-**1**]$_2$ with (*R*)- and (*S*)-valine revealed that the ligand displacements proceed with remarkable stereoselectivity. We observed that (*S*)-valine is more effective in replacing *N*,*N*′-dioxide ligands from the metal center than the (*R*)-enantiomer (Figure 1). However, when Sc[(−)-**1**]$_2$ was employed in the same UV titration experiment, ligand substitution occurred more readily with (*R*)-valine, thus proving enantioselective UV−vis sensing based on competitive ligand exchange as previously described by Anslyn.[2b−d]

The UV−vis sensing method is based on a two-step ligand exchange process involving the formation of intermediate diastereomeric complexes. Displacement of the first *N*,*N*′-dioxide ligand from the metal center by (*R*)- or (*S*)-valine generates Sc[(+)-**1**(*R*)-**2**] and Sc[(+)-**1**(*S*)-**2**]. The first step can be monitored by the initial decrease of the charge transfer band at 410 nm and is described by individual exchange constants K_{1S} and K_{1R}, respectively. Further addition of the substrate then results in the replacement of the second *N*,*N*′-dioxide ligand and formation of equienergetic valine-derived scandium complexes and free ligand **1**. The latter step results in the disappearance of the charge transfer band and is described by individual exchange constants K_{2S} and K_{2R}. The conversion of Sc[(+)-**1**]$_2$ to the ternary Sc[(+)-**1**(*R*)-**2**] complex upon addition of (*R*)-**2** to a solution of the sensor in water/acetonitrile (1:1) was detected by ESI-MS (Supporting Information). The stepwise replacement of the *N*,*N*′-dioxide ligands from Sc[(+)-**1**]$_2$ by (*R*)-valine can be described as follows:

$$\text{Sc[(+)-1]}_2 + (R)\text{-2} \overset{K_{1R}}{\rightleftharpoons} \text{Sc[(+)-1(}R\text{)-2]} + (+)\text{-1} \quad (1)$$

$$\text{Sc[(+)-1(}R\text{)-2]} + (R)\text{-2} \overset{K_{2R}}{\rightleftharpoons} \text{Sc[(}R\text{)-2]}_2 + (+)\text{-1} \quad (2)$$

We conducted competitive binding experiments with substrates **3**−**12** (Chart 1). Using Sc[(+)-**1**]$_2$ as the sensor, we observed stereoselective replacement of dextrorotatory **1** in all cases (see Supporting Information). The titration results reveal two remarkable features of enantioselective sensing with Sc[*N*,*N*′-dioxide **1**]$_2$. First, this sensor differentiates between the enantiomers of a range of compounds and is suitable for UV analysis of unprotected amino acids, amino alcohols, amines, and carboxylic acids in aqueous solution. Second, minute amounts of the scandium complex are sufficient for enantioselective sensing of micromolar concentrations of chiral substrates.

The diastereomeric complexes Sc[(+)-**1**(*S*)-**2**] and Sc[(+)-**1**(*R*)-**2**] have different thermodynamic stabilities and molar extinction coefficients at 410 nm, whereas the final exchange products, Sc[(*S*)-**2**]$_2$ and Sc[(*R*)-**2**]$_2$, do not absorb at 410 nm and therefore do not interfere with UV measurements. The exchange processes described for (*R*)-**2** in eqs 1 and 2 have been analyzed for all substrates **2**−**12** as described by Connors based on the assumption

10.1021/ja0636486 CCC: $33.50

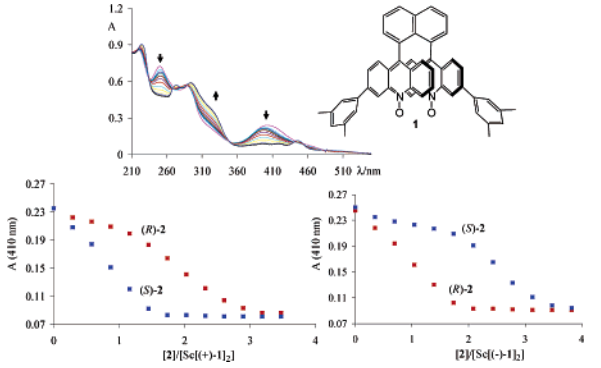
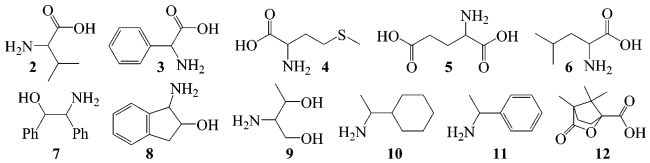


Figure 1. Enantioselective UV sensing. Left: UV−vis spectra of Sc[(+)-**1**]$_2$ obtained by titration with (*R*)-valine. Right: UV sensing of the enantiomers of valine using Sc[(+)-**1**]$_2$ and Sc[(−)-**1**]$_2$. The sensor concentration was 0.98×10^{-5} M in acetonitrile:H_2O (1:1).

Chart 1. Substrates Tested



that Beer's law is followed by all UV-active species (see Supporting Information).[13] Accordingly, the first *N*,*N*′-dioxide ligand of Sc[(+)-**1**]$_2$ is more readily replaced by the (*S*)-enantiomer of valine because Sc[(+)-**1**(*S*)-**2**] is more stable than Sc[(+)-**1**(*R*)-**2**]. As a result, ligand exchange occurs at lower concentrations of (*S*)-**2** than with (*R*)-**2**, which explains the enantioselective decrease of the intensity of the charge transfer absorption at 410 nm shown in Figure 1.

We then decided to develop a method that allows accurate measurements of both the total amount and the enantiomeric excess of chiral compounds. We found that this can be accomplished by combination of two simple assays. First, the total concentration of a chiral substrate is analyzed by UV−vis sensing with racemic Sc(*N*,*N*′-dioxide **1**)$_2$. As expected, addition of either enantiomer of valine to a solution of the racemic sensor affords superimposable UV titration curves which can be exploited for quantitative nonstereoselective analysis (Supporting Information). The enantiopure scandium complex is then used to uncover the enantiomeric composition in a succeeding assay. To evaluate the accuracy and reproducibility of this method, we prepared nine micromolar samples of valine, camphanic acid, α-cyclohexylethylamine, and threoninol having different concentrations and enantiopurities ranging from 15 to 95%. The UV signal of the racemic Sc complex in the presence of each sample was then measured and compared to a calibration curve for determination of the individual concentrations.

Having determined sample concentrations, we were then able to reveal enantiomeric compositions using enantiopure Sc[(+)-**1**]$_2$ in essentially the same UV experiment. In all cases, results obtained by our UV−vis sensing method were in excellent agreement with actual values (Figure 2 and Supporting Information). For example, the measured (actual) total concentration and amount of (*R*)-enantiomer of a valine sample were 1.58×10^{-5} M (1.60×10^{-5} M) and 97% (95%). The data demonstrate the high reproducibility and accuracy of this method which is applicable to the screening of samples exhibiting a wide range of enantiopurity.

In summary, we have developed a practical UV−vis sensing method for enantioselective microanalysis of unprotected amino acids, amines, amino alcohols, and carboxylic acids in aqueous

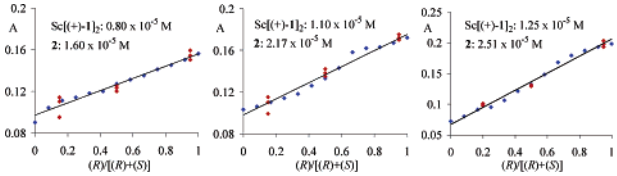


Figure 2. UV absorbance of Sc[(+)-**1**]$_2$ as a function of the enantiomeric composition of valine **2** at three different concentrations (blue, calibration; red, actual measurements).

solution. Using racemic and enantiopure Sc[*N*,*N*′-dioxide **1**]$_2$ in two competitive binding assays, we observed superior sensitivity and a wider application spectrum compared to that in previously reported fluorescence assays. The intrinsic sensitivity and selectivity of this new sensor simplifies quantitative analysis of minute sample amounts. We believe that our method combines several attractive features: it is suitable for microanalysis of a wide range of chiral compounds; it allows determination of both concentration and enantiomeric excess; it depends on two simple assays that provide accurate results; it avoids substrate derivatization, and it utilizes sensitive UV−vis spectroscopy, minimizing solvent waste.

Acknowledgment. Funding from the National Science Foundation (CAREER Award, CHE-0347368) and the Petroleum Research Fund (PRF40897-G4) is gratefully acknowledged.

Supporting Information Available: Synthesis of **1** and details of all UV and MS experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0636486