Quantitative Chiroptical Sensing of Free Amino acids, Biothiols, Amines and Amino Alcohols with an Aryl Fluoride Probe

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Supporting Information Placeholder

ABSTRACT: The comprehensive determination of the absolute configuration, enantiomeric ratio and total amount of standard amino acids by optical methods adaptable to high-throughput screening with modern plate readers has remained a major challenge to date. We now present a small molecular probe that smoothly reacts with amino acids and biothiols in aqueous solution and thereby generates distinct chiroptical responses to accomplish this task. The achiral sensor is readily available, inexpensive and suitable for chiroptical analysis of each of the 19 standard amino acids, biothiols, aliphatic and aromatic amines and amino alcohols. The sensing method is operationally simple and data collection and processing are straightforward. The utility and practicality of the assay are demonstrated with the accurate analysis of ten aspartic acid samples covering a wide concentration range and largely varying enantiomeric compositions. Accurate *er* sensing of 85 scalemic samples of Pro, Met, Cys, Ala, methylpyrrolidine, 1-(2-naphthyl)amine and mixtures thereof is also presented.

Chiral amino acids play a critical role in many biological processes and are essential building blocks of peptides, proteins and other natural compounds. The structural diversity of common natural amino acids has been exploited in numerous ways across the chemical and pharmaceutical sciences.[1] Both enantiomeric forms have become invaluable starting materials for the asymmetric synthesis of biologically active compounds and new materials.[2] During recent years, the traditional focus on proteogenic *S*-amino acids has been revised as the biological significance of several *R*-antipodes has surfaced. Free *R*-amino acids perform important physiological and pathological roles in mammals, and nonracemic mixtures of standard amino acids, in particular serine, aspartic acid, alanine and cysteine, have been found in the central nervous system and in endocrine organs.[3] Although many specific biological functions remain to be fully explored, the potential of *R*-amino acids as diagnostic biomarkers and for the treatment of schizophrenia, Parkinson's, Huntington's, Alzheimer's or other neurological diseases have received increasing attention.[4]

The universal importance and widespread use of *R*- and *S*-amino acids in chemistry, biotechnology and the life sciences have generated substantial interest in the development of methods that allow quantitative enantioeselective analysis in aqueous solutions and are amenable to high-throughput experimentation equipment.[5] Despite substantial progress with optical assays and arrays,[6] the search for a practical and broadly useful sensing system that accomplishes this task has remained very challenging.[7-10]

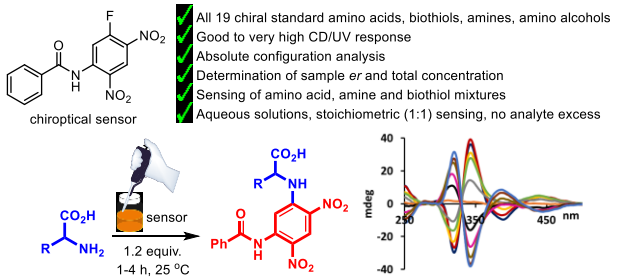


Figure 1. Enantioselective sensing of amino acids, biothiols, amines and amino alcohols with an aryl fluoride probe.

We now wish to introduce a practical sensing assay that allows determination of the absolute configuration, enantiomeric ratio and concentration of each of the 19 standard amino acids in aqueous solution (Figure 1). Our approach is based on circular dichroism and UV spectroscopic measurements which can be obtained within a single operation using generally available CD spectrophotometers or modern UV/CD multiwell plate readers. Only stoichiometric amounts of an achiral sensor that is prepared in a single step from commercially available starting materials are required. This reduces the extra work and costs typically associated with the synthesis of chiral probes that are used in enantiopure form. In addition, possible complications that may impede quantification efforts, such as undesirable kinetic resolution effects resulting from unequal formation of diastereomeric species, are avoided. To overcome these drawbacks and to streamline concentration and *er* quantification, we introduce an optical assay that relies on covalent substrate recognition with an achiral probe equipped with a chromophoric reporter moiety.[11] This strategy does not yield diastereomeric products but instead converts the amino acid enantiomers into enantiomeric sensing products with inherently the same UV signature but opposite CD activity. We show that this approach greatly simplifies the analytical task as the chromophoric probe generates distinct UV/CD outputs upon covalent substrate binding with a well-defined 1:1 stoichiometry (as opposed to sensors that rely on non-covalent interactions or reversible binding modes and require access of an amino acid); the UV response is non-enantioselective and therefore allows determination of the total amount of a nonracemic amino acid sample while the induced CD signal is used for absolute configuration assignment and enantiomeric ratio (*er*) analysis.

We began our study by screening the electron-deficient fluoroarenes **1**-**4** with the expectation that these probes would a) undergo smooth C-N bond formation via nucleophilic aromatic substitutions with amino acids in aqueous solution at room temperature and b) generate quantifiable chiroptical signals. Bergmann's

reagent, **1**,[12] is commercially available and the derivatives **2**-**4** were prepared following literature procedures (SI).[13] Aspartic acid, one of the amino acids that appears in nonracemic form in the mammalian brain and endocrine system, was selected as test analyte for the initial sensor evaluation (Figure 2). Unfortunately, Asp sensing with **1** did not result in a CD signal but we were pleased to observe very strong CD signals above 300 nm when *N*-(5-fluoro-2,4-dinitrophenyl)benzamide, **2**, was employed for the sensing of a solution containing either (*R*)- or (*S*)-Asp in sodium borate buffer at pH 8.5. The reaction with (*R*)-Asp gave two negative Cotton effects centered at approximately 415 nm and 330 nm and exactly the opposite results were obtained by sensing of the *S*-enantiomer (SI). To avoid possible interference with CD- or UV-active impurities an optical sensor that operates above 300 nm is generally preferable and **2** clearly fulfills this requirement. We found that chiroptical sensing with the 2-naphthamide analogue, **3**, is less practical due to its low solubility in both aqueous and organic solvents and the placement of an acetamide group in **4** did not prove advantageous. We therefore continued to use **2** in all other amino acid sensing experiments.

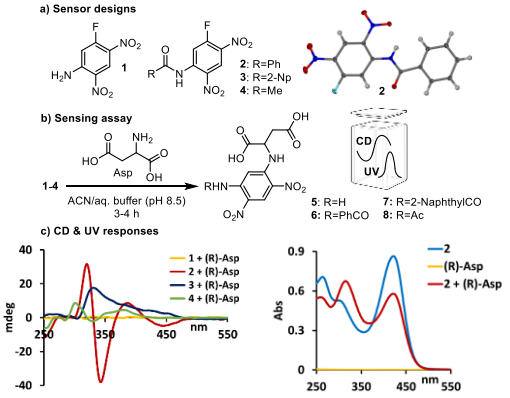


Figure 2. Structures of the aryl fluoride probes **1**-**4** and CD and UV signals obtained using (*R*)-aspartic acid as substrate. The sensing reactions were conducted at 4.0 mM with 1.2 equivalents of the sensor in ACN:sodium borate buffer (4:2.25, pH 8.5, 40 mM). UV measurements were taken at 40 µM. The CD responses with **1**, **2** and **4** were collected at 49-88 µM. For sensing with **3**, a solution of DMSO:ACN:borate buffer (8:2:2, pH 8.5, 40 mM) was used and the CD spectrum was recorded at 63 µM.

The assay workflow follows a simple mix-and-measure protocol that can be easily adapted to automated high-throughput screening equipment (Figure 3a). The substrate derivatization occurs smoothly at room temperature without formation of by-products which enables amino acid sensing using one equivalent of **2**. The clean conversion and the well-defined stoichiometry of our amino acid assay are important because this sets the stage for combined quantitative *er* and concentration analysis, *vide infra*. Monitoring of the sensing reaction by UV, CD and NMR analysis revealed that it is complete within 1-4 hours (SI). The covalent attachment of the aryl fluoride probe onto the amino acid as shown in Figure 2b was verified by ESI-MS analysis of the product obtained from **2** and aspartic acid and by superposition of the perfectly identical CD spectra of the proline sensor-tagged adduct generated *in situ* and of the isolated reference compound (Figure 3b and SI). We then continued to establish the application scope of **2** by testing all chiral standard amino acids as well as the biothiols homocysteine and glutathione in aqueous solutions. In all cases, we observed distinct chiroptical signals which underscores the general utility of this assay (SI). Representative examples of the CD effects obtained upon derivatization of alanine, proline, glutamic acid, and cysteine are shown in Figure 3. Very strong chiroptical responses were measured with aspartic acid, glutamic acid and proline. The sensing of all other amino acids with **2** occurred with good CD responses that generally suffice for quantitative *er* analysis (SI).

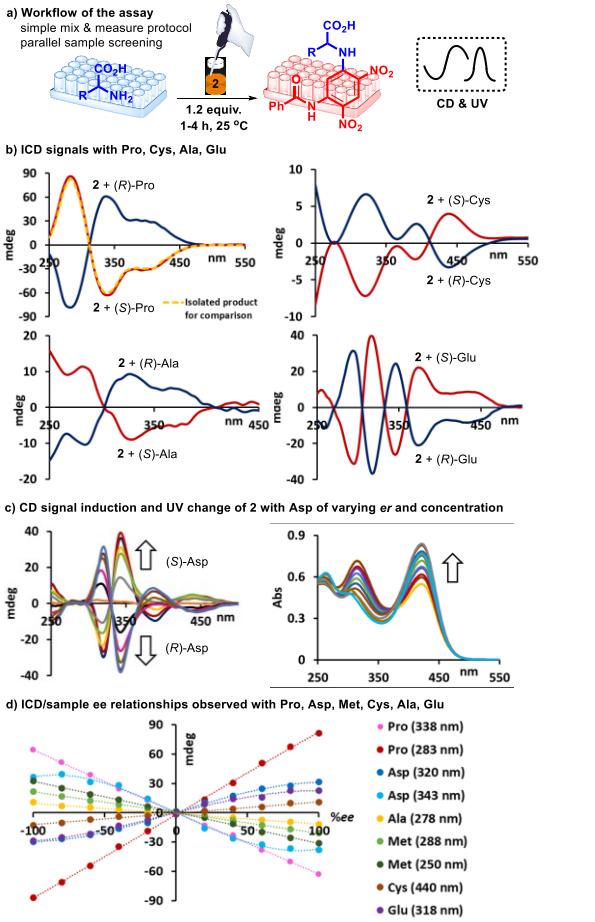


Figure 3. Selected examples of chiroptical amino acid sensing with **2**. See SI for experimental details.

As mentioned above, the induced CD signal of **2** observed upon amino acid derivatization coincides with a characteristic UV change, and we envisioned that this dual response would allow comprehensive quantitative analysis. To demonstrate the feasibility of determination of the absolute configuration, total concentration and enantiomeric composition we first collected a series of UV and CD spectra by applying probe **2** to samples of aspartic acid in varying concentrations and enantiomeric ratios in aqueous borate buffer solution at pH 8.5 (Figure 3c). The steadily increasing UV maximum observed at 315 nm is a non-enantioselective process and therefore identical for either amino acid enantiomer. The characteristic UV change therefore allows quantitative concentration analysis irrespective of the sample *er*. By contrast, the induced CD signals at 320 and 340 nm directly correspond to the enantiomeric composition of Asp. A closer look at the CD signals of **2** obtained upon sensing of nonracemic aspartic acid, glutamic acid, proline, methionine, cysteine and alanine revealed that nonlinear effects occur with Asp and Glu but not with the other amino acids (Figure 3d and SI. Scalemic mixtures of Asp and Glu may contain homochiral and heterochiral dimers in solution and this is known to result in nonlinear CD signals.[14] Importantly, nonlinear CD effects do not compromise the efficiency and accuracy of our method as demonstrated by the quantitative Asp sensing results discussed below.

Having developed a protocol for quantitative concentration and *er* determination we were able to put our assay to the ultimate test by analyzing ten samples containing aspartate in vastly different amounts and enantiomeric ratios (Table 1). In all cases, unequivo-

cal assignment of the absolute configuration of the major enantiomer present in the nonracemic samples was straightforward using the sign of the induced Cotton effects. The intensities of the CD and UV maxima at approximately 320 nm were used to quantify the *er* values and the total Asp concentration with high accuracy.

Table 1. Quantitative concentration and *er* analysis and assignment of the absolute configuration of ten Asp samples.

Entry	Sample Composition			Sensing Results		
	Abs. Config.	Conc. (mM)	Ratio *S*/*R*	Abs. Config.[a]	Conc. (mM)[b]	Ratio *S*/*R*[c]
1	*S*	2.50	82.0:18.0	*S*	2.50	80.1:19.9
2	*R*	3.00	20.0:80.0	*R*	3.09	20.5:79.5
3	*S*	3.25	78.0:22.0	*S*	3.28	79.0:21.0
4	*R*	3.50	30.0:70.0	*R*	3.50	28.2:71.8
5	*S*	3.75	65.0:35.0	*S*	3.78	67.5:32.5
6	*R*	4.00	40.0:60.0	*R*	4.27	38.3:61.7
7	*S*	4.00	75.0:25.0	*S*	4.27	75.6:24.4
8	*S*	2.00	90.0:10.0	*S*	1.73	88.3:11.7
9	*S*	4.00	95.0:5.0	*S*	3.46	92.9:7.2
10	*R*	2.50	2.0:98.0	*R*	2.31	0.7:99.3

[a]By comparison of the sign of the induced CD with a reference spectrum. [b]Based on the UV maximum at 315 nm. [c]Based on the amplitude of the CD response at 320 nm.

For example, sensing of a sample containing 2.50 mM of Asp at 82.0:18.0 *er* (*S*/*R*) gave the exact same concentration and an enantiomeric ratio of 80.1:19.9 (entry 1). Another mixture consisted of 70% of the *R*-enantiomer and a total aspartate concentration (*R*+*S*) of 3.50 mM (entry 4). Again, the absolute configuration of the major enantiomer was correctly assigned and the concentration and enantiomeric ratio were determined as 3.50 mM and 28.2:71.8 (*S*/*R*), respectively. We found that the use of probe **2** is quite universal and extends beyond Asp analysis. Chiroptical *er* sensing of nonracemic samples of Pro and even of Ala and Met, which afford moderate CD signals, was accomplished with high accuracy and precision (SI). Moreover, our chiroptical assay allows reliable determination of the absolute configuration and enantiomeric composition even at very low *er* values. Enantiomeric ratios as low as 51.0:49.0 of Pro and Met samples were accurately quantified. The sensing of a sample of Pro with a 51.0:49.0 *S*/*R* ratio gave 51.1:48.9 (*S*/*R*) and the *er* of a Met sample containing 51.0% of the *R*-enantiomer was determined as 51.5: 48.5 (*R*/*S*), see SI.

We suspected that our sensor is also applicable to aliphatic and aromatic amines and amino alcohols. To demonstrate the exceptional utility of **2**, we decided to screen the additional substrates **10**-**27**. Using our simple mix-and-measure protocol and chloroform as solvent we obtained strong CD spectra in all cases (Figure 4 and SI). Unlike the widely used class of optical probes that operate based on Schiff base formation with the target compound, chirality sensing with **2** is not restricted to primary amines. This is highly advantageous because it allows chiroptical sensing of amino acids, amines and amino alcohols carrying either a primary or a secondary amino function which altogether represent a large pool of important molecular targets. To this end, the very strong Cotton effects measured with the aliphatic secondary amine **17** and amino alcohol **24** are particularly noteworthy and underscore the versatility of sensor **2**. The covalent substrate binding mode was verified by mass spectrometric detection of the tagged products formed with 1-(2-naphthyl)ethylamine and 2-pyrrolidinol, and we were able to grow a single crystal of the adduct formed with (*S*)-1-(2-naphthyl)ethylamine that was suitable for crystallographic analysis (Figure 4 and SI). The smooth substrate derivatization at room temperature and the distinct chiroptical signals generated from **2** are in accordance with our standard amino acid sensing studies. We were able to prove the utility of our sensing method by accurate *er* analysis of many nonracemic samples of **11** and **17** including examples with very low enantiomeric ratios. For example, chiroptical sensing of a sample of **11** with a 51.0:49.0 *S*/*R* ratio gave 50.8:49.2 (*S*/*R*) and the *er* of a sample of **17** with a 49.0:51.0 *S*/*R* ratio was analyzed as 48.8:51.2 (*S*/*R*), see SI.

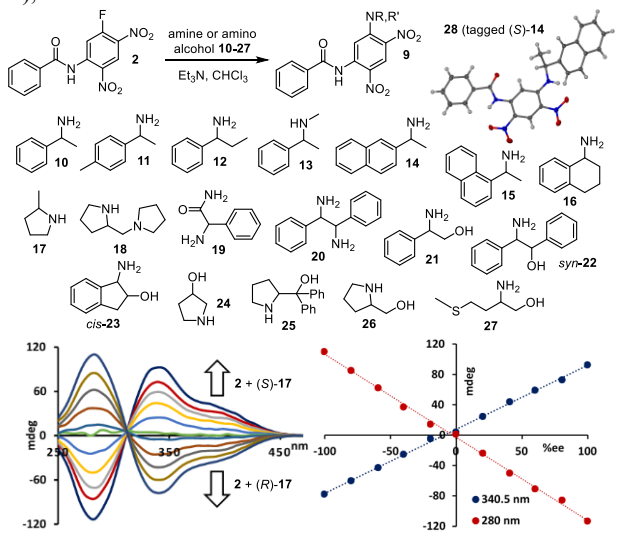
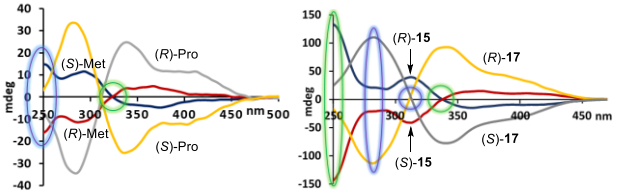


Figure 4. Structures of amines and amino alcohols analyzed, representative CD spectra obtained with **2**, and X-ray structure of the tagged 1-(2-naphthyl)ethylamine derivative **28**. The sensing reactions were conducted at 20.0 mM using 1.0 equivalent of sensor **2** and Et_3N in chloroform. CD measurements were recorded after dilution with chloroform to 60-120 μM, see SI for details.

Finally, we noticed that the chiroptical sensing with our probe affords complementary ICD signatures, for example with Pro and Met, which provides a unique entry toward selective *er* sensing of compound mixtures (Table 2). The combined analysis of the CD responses of **2** at 250 and 324 nm allowed us to quantify nonracemic samples containing both Pro and Met. Similarly, accurate *er* sensing of mixtures of the amines **15** and **17** was accomplished and we successfully determined the enantiomeric composition of Cys in the presence of the biothiols homocysteine and glutathione (SI).

Table 2. Quantitative *er* analysis of compound mixtures.



Sample Composition				Sensing Results[a]			
Abs. Config.	Pro *S*/*R*	Abs. Config.	Met *S*/*R*	Abs. Config.	Pro *S*/*R*	Abs. Config.	Met *S*/*R*
R	15.0:85:0	*S*	65.0:35.0	*R*	15.4:84.6	*S*	67.2:32.8
R	25.0:75.0	*S*	75.0:25.0	*R*	26.0:74.0	*S*	74.5:25.5
R	35.0:65.0	*S*	85.0:15.0	*R*	35.9:64.1	*S*	84.2:15.8
S	75.0:25.0	*R*	25.0:75.0	*S*	75.0:25.0	*R*	27.5:72.5
S	85.0:15.0	*R*	35.0:65.0	*S*	87.3:12.7	*R*	33.2:66.8
S	95.0:5.0	*R*	45.0:55.0	*S*	95.3:4.7	*R*	42.5:57.5
S	55.0:45.0	*R*	5.0:95.0	*S*	55.2:44.8	*R*	9.7:90.3
S	65.0:35.0	*R*	15.0:85.0	*S*	66.0:34.8	*R*	19.9:80.1
Abs. Config.	**15** *S*/*R*	Abs. Config.	**17** *S*/*R*	Abs. Config.	**15** *S*/*R*	Abs. Config.	**17** *S*/*R*
R	5.0:95.0	*S*	95.0: 5:0	*R*	6.2:93.8	*S*	95.6:4.4
R	15.0:85.0	*S*	85.0:15.0	*R*	11.1:88.9	*S*	84.0:16.0
R	25.0:75.0	*S*	75.0:25.0	*R*	25.0:75.0	*S*	74.0:26.0
R	35.0:65.0	*S*	65.0:35.0	*R*	34.0:66.0	*S*	64.4:35.6
R	45.0:55.0	*S*	55.0:45.0	*R*	42.4:57.6	*S*	56.5:43.5
S	85.0:15.0	*R*	15.0:85.0	*S*	85.3:14.7	*R*	16.0:84.0

[a]The wavelengths that were selected for orthogonal *er* sensing are highlighted with circles. See SI for details.

In summary, we have developed a small-molecule probe that irreversibly binds amino acids in aqueous solution with a well-defined 1:1 stoichiometry and thereby generates distinct chiroptical signals that allow assignment of the absolute configuration and quantification of the sample concentration and enantiomeric ratio. The UV response of the sensor is non-enantioselective and therefore allows determination of the total amount of an amino acid sample independent of the enantiomeric composition while the induced CD signals are in response to the chirality of the target compound and can be used for absolute configuration and *er* analysis. The achiral sensor is readily available, inexpensive and suitable for chiroptical analysis of each of the 19 standard amino acids, homocysteine and the tripeptide glutathione as well as aliphatic and aromatic amines and amino alcohols. The sensing method is operationally simple, based on a mix-and-measure protocol, adaptable to high-throughput screening with modern UV/CD plate readers, and the data analysis is straightforward. The utility and practicality of the assay were demonstrated with the successful analysis of ten samples containing aspartic acid over a wide concentration range and with largely varying enantiomeric compositions. The wide scope, selectivity and ruggedness of our sensing method was further verified with accurate *er* sensing of 85 scalemic samples of Pro, Met, Cys, Ala, methylpyrrolidine, 1-(2-naphthyl)amine and mixtures thereof.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, compound characterization, optical measurements and crystallographic data. The Supporting Information is available free of charge on the ACS Publications website.

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Notes

The authors declare no competing financial interests.

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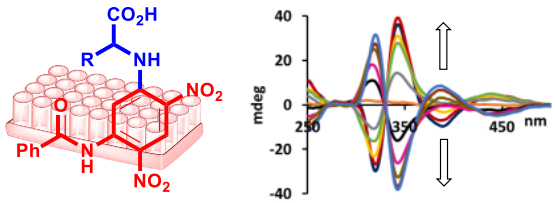

- All 19 chiral standard amino acids, biothiols, amines, amino alcohols
- Good to very high CD/UV response
- Absolute configuration analysis
- Determination of sample *er* and total concentration
- Sensing of amino acid, amine and biothiol mixtures
- Aqueous solutions, stoichiometric (1:1) sensing, no analyte excess