ORGANIC LETTERS

2010
Vol. 12, No. 11
2540−2543

Pattern-Based Recognition for Determination of Enantiomeric Excess, Using Chiral Auxiliary Induced Chemical Shift Perturbation NMR

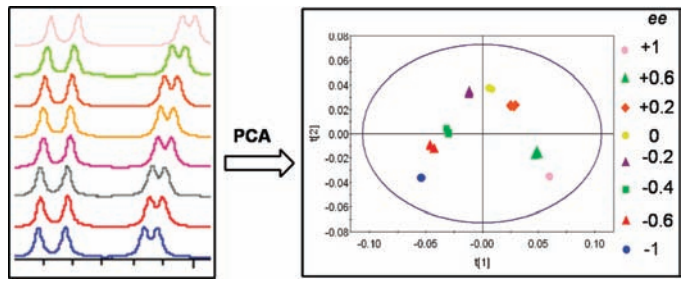
Xinxiang Lei,[*,†,‡] Lu Liu,[†] Xiaojing Chen,[§] Xiaochun Yu,[†] Lisheng Ding,[‡] and Anjiang Zhang[*,||]

College of Chemistry & Materials Engineering, Wenzhou University, Wenzhou 325035, People's Republic of China, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China, College of Physical and Electronic Informatiom, Wenzhou University, Wenzhou 325035, People's Republic of China, and Ningbo Institute of Material Technology and Engineering, Chinese Academy of Sciences, Ningbo 315201, People's Republic of China

xinxianglei@gmail.com; anjiangzhang@gmail.com

Received April 2, 2010

ABSTRACT



A protocol for the determination of enantiomeric excess of chiral carboxylic acid, using the subtle frequency shifts (chemical shift perturbation) in NMR spectra induced by chiral auxiliary, is described. The spectra were analyzed with two pattern recognition protocols. Principal component analysis demonstrated good enantioselective separation of the analytes, and partial least-squares was used to analyze ee values of unknown samples.

The dramatic growth in the importance of asymmetric synthesis has led to the demand for facile and efficient techniques for assaying the enantiomeric excess (ee) of reaction products.[1] Enantioselective analysis often remains the bottleneck in these processes because it usually entails laborious and time-consuming chromatographic techniques. The pressing need for efficient methods has shifted the focus to MS,[2] IR thermography,[3] UV and colorimetric assays,[4] fluorescence spectroscopy,[5] and circular dichroism.[6]

A very different approach is based on NMR spectroscopy that discriminates enantiomers depending on chiral auxiliaries such as chiral solvating agents (CSAs).[7] A drawback is that they need a large amount of reagent to give rise to signal splitting,[8] in many cases, while chiral reagent is commonly

[†] College of Chemistry & Materials Engineering, Wenzhou University.
[‡] Chengdu Institute of Biology.
[§] College of Physical and Electronic Information, Wenzhou University.
[||] Ningbo Institute of Material Technology and Engineering.

(1) (a) Tsukamoto, M.; Kagan, H. B. *Adv. Synth. Catal.* **2002**, *344*, 453. (b) Reetz, M. T. *Angew. Chem., Int. Ed.* **2001**, *40*, 284. (c) Reetz, M. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1335.

(2) (a) Guo, J.; Wu, J.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755. (b) Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stockigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758. (c) Markert, C.; Pfaltz, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2498. (d) Muller, C. A.; Markert, C.; Teichert, A. M.; Pfaltz, A. *Chem. Commum.* **2009**, 1607.

10.1021/ol100773s
Published on Web 05/04/2010

expensive. This has been accomplished only in some rare cases developing highly specific, very selective CSAs or synthetic receptors.[9]

Recently, Anslyn and co-workers pioneered the use of pattern recognition for enantioselective colorimetric and fluorescent indicators of chiral compounds.[4e,f,h−j,6b] Encouraged by Anslyn's success with pattern-based discrimination of enantiomeric excess, we decided to employ the combination of pattern recognition techniques and chiral auxiliaries to dissimilarly perturb the NMR spectra of the enantiomers for enantioselective analysis. Herein, we report that common chiral reagents can be used as chiral auxiliaries for accurate and highly reproducible NMR quantification of enantiomeric composition of various carboxylic acids.

According to the traditional enantioselective NMR analysis method, few chiral shift reagents can be used to determine enantiomeric purities of chiral carboxylic acids accurately either because of their structural complexity or because their ^{1}H chemical shift nonequivalences are too small to realize baseline resolution.[9a] We selected commercially available and inexpensive quinidine and cinchonine as chiral auxiliaries (Figure 1A) to induce

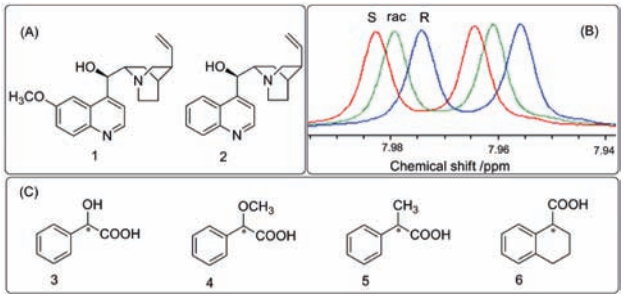


Figure 1. (A) Chiral auxiliaries of quinidine (**1**) and cinchonine (**2**). (B) Partial ^{1}H NMR spectra of 1:1 mixtures of quinidine and (*S*)-**6** (red), (*R*)-**6** (blue), and racemate (green), respectively. (C) Chiral carboxylic acids employed as analytes: (**3**) mandelic acid, (**4**) α-methoxyhpenylacetic acid, (**5**) 2-phenylpropionic acid, and (**6**) 1,2,3,4-tetrahydronaphthalene-1-carboxylic acid.

discrimination in the NMR spectra of chiral carboxylic acids. The four different carboxylic acids used for the array are shown in Figure 1C and were chosen so that each carboxylic acid was sufficiently different from one another in their structure and functionality to ensure variation among analytes. To evaluate the chiral discrimination abilities of quinidine, we measured NMR spectra for 1:1 mixtures of quinidine and chiral carboxylic acids **3**−**6** and their racemates in $CDCl_3$. Chemical shift nonequivalences were not observed. A careful comparison of the ^{1}H NMR spectra of enantiomers and racemates of various carboxylic acids showed subtle frequency shifts which were induced by the interaction between auxiliary and analytes (Figure 1B). Where we were able to obtain unique chemical shift change signatures for both analytes and chiral auxiliary, those signatures would be indicative of the chirality of an analyte. Previous studies have elucidated the chemical shift perturbations that occur during chiral auxiliary binding in situ to analytes through noncovalent, intermolecular forces, usually under conditions of exchange between the bound and the unbound forms. The associated complexes of a pair of enantiomers with a chiral auxiliary are diastereomers and are the source of perturbation in the NMR spectrum.[10]

Our first goal was to demonstrate chemo- and enantiodifferentiation of the analytes. Each carboxylic acid, as well as the enantiomers, was treated at 5 mM concentration with 5 mM chiral auxiliary **1**. The experiment was repeated four times to ensure reproducibility. A full spectrum of each sample was recorded, referenced to TMS. The data were analyzed in the aromatic region corresponding to the chemical shift range from δ 6.9 to δ 8.8 after removing the solvent resonance region (δ 7.0−7.3) prior to principal component analysis (PCA)[11] for fingerprinting. The PCA plot shows excellent discrimination for all of the carboxylic acids and their enantiomers (Figure 2). Tight clustering of identical

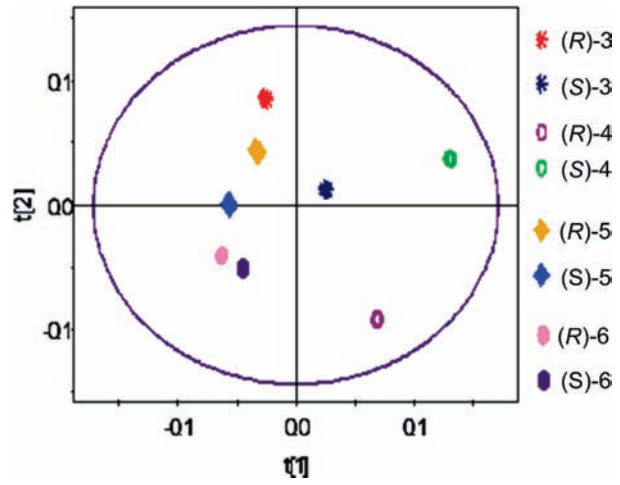


Figure 2. Response patterns for all the analytes, using chiral auxiliary quinidine obtained by PCA.

samples and good spatial resolution for all of the analytes was achieved. Using cinchonine as a chiral auxiliary led to similar chemoselectively responses (Figure S1, Supporting Information), as expected.

Having found chemo- and enantioselectivity, the next goal was to explore the ability to quantitate ee for a known analyte. For this purpose, we recorded ^{1}H NMR spectra of quinidine at given concentrations of carboxylic acids (5 mM), along with eight ee values at given concentrations (−1, −0.6, −0.4, −0.2, 0, 0.2, 0.6, and 1; expressed as % R in the data). Four replicates were done at each ee. These data were analyzed with PCA (Figure 3). Good spatial resolution was obtained in the PCA plot, which showed clustering of identical samples and spatial resolution of ee. The data sets with varying ee values were

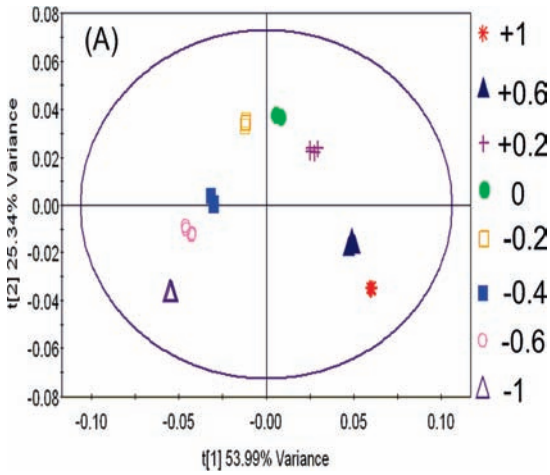


Figure 3. Two-dimensional PCA plot of chiral carboxylic acids **6** at eight different ee values (−1, −0.6, −0.4, −0.2, 0, 0.2, 0.6, 1).

clustered together in smooth curves and the ee values ranged within the region left to right from −1 to +1, respectively (Figure 3). Clearly the ^{1}H NMR technique described allows for chemo- and enantiodiscrimination. These encouraging results prompted us to immediately proceed to creating an evaluation approach.

We employed partial least squares (PLS)[11] as a protocol for prediction. To quickly test the viability of the PLS approach, the data used to generate Figure 3 were used for the PLS regression model (8 latent variables), consisting of 24 samples for which the ee values were −1, −0.6, −0.2, 0, 0.6, and 1 at four sample intervals, while the prediction set consisted of the remaining eight samples for which the ee values were −0.4 and 0.2. The linear relation between predicted and known ee values is shown in Figure 4. The

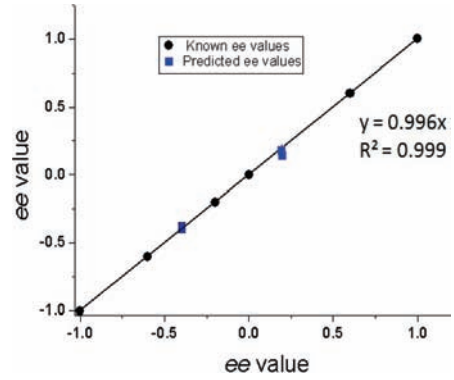


Figure 4. The linear relation between predicted and known ee values.

linear relation plot shows an excellent linear relationship between predicted and known ee values. An excellent value of 0.999 for the regression coefficient was obtained. Given this success, we expanded the approach to practical application by determining the unknown ee values of various chiral carboxylic acids.

PLS models of carboxylic acids (**3**−**5**) with quinidine were generated by using data produced by the same protocol as used for carboxylic acid **6**. Two unknown ee values of each carboxylic acid, including **6**, prepared completely separately and independent of the training set, were subjected to each PLS model respectively. The calculated values are listed in Table 1. The absolute error for ee ranges between 0.06% and 9.97%. Using cinchonine as a chiral auxiliary for prediction gave similar excellent results (Table S1, Supporting Information), as expected. The absolute error for ee ranges between 0.32% and 8.47%.

Table 1. PLS Analysis of Unkown Solutions[a] (ee % expressed as % R in the data) with Quinidine as a Chiral Auxiliary

analytes	given[a]	PLS[b]	error	given[a]	PLS	error
3	0.00	3.33	3.33	−60.00	−63.26	3.26
4	60.00	69.97	9.97	−40.00	−45.78	5.78
5	60.00	59.54	0.06	−60.00	−56.66	3.34
6	20.00	20.34	0.34	−60.00	−56.22	3.78

[a] Enantiomeric compositions of the solutions prepared from *R*- and *S*-carboxylic acid standards. [b] Predicted by PLS model.

In conclusion, we demonstrated a technique that fingerprints the chemical chirality of chiral carboxylic acids. Pattern recognition protocols were used to analyze the data. Excellent fingerprinting, in which the carboxylic acids were chemose-

(3) Reetz, M. T.; Becker, M. H.; Kuhling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647.
(4) (a) Eelkema, R.; van Delden, R. A.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 5013. (b) Zhu, L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 3676. (c) Zhu, L.; Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 4260. (d) Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986. (e) Folmer-Andersen, J. F.; Kitamura, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128*, 5652. (f) Zhu, L.; Shabbir, S. H.; Anslyn, E. V. *Chem.—Eur. J.* **2007**, *13*, 99. (g) Leung, D.; Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12318. (h) Leung, D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2008**, *130*, 12328. (i) Shabbir, S. H.; Joyce, L. A.; da Cruz, G. M.; Lynch, V. M.; Sorey, S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2009**, *131*, 13125. (j) Shabbir, S. H.; Regan, C. J.; Anslyn, E. V. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10487.
(5) (a) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature* **1995**, *374*, 345. (b) Zhao, J.; Fyles, T. M.; James, T. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 3461. (c) Pu, L. *Chem. Rev.* **2004**, *104*, 1687. (d) Li, Z.-B.; Lin, J.; Pu, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1690. (e) Liu, H.-L.; Hou, X.-L.; Pu, L. *Angew. Chem., Int. Ed.* **2009**, *48*, 382.
(6) (a) Ding, K.; Ishii, A.; Mikami, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 497. (b) Nieto, S.; Lynch, V. M.; Anslyn, E. V.; Kim, H.; Chin, J. *J. Am. Chem. Soc.* **2008**, *130*, 9232.
(7) (a) Parker, D. *Chem. Rev.* **1991**, *91*, 1441. (b) Wenzel, T. J.; Wilcox, J. D. *Chirality* **2003**, *15*, 256. (c) Wenzel, T. J. *Discrimination of Chiral Compounds Using NMR*; John Wiley & Sons: Hoboken, NJ, 2007.
(8) (a) Weisman, G. R. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983; Vol. 1. (b) Fraser, R. R. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983; Vol. 1.
(9) (a) Yang, D.; Li, X.; Fan, Y.-F.; Zhang, D.-W. *J. Am. Chem. Soc.* **2005**, *127*, 7996. (b) Ma, F.-N.; Lin, J.; Lin, A.; Shen, X.-M.; Zhang, C. *Org. Lett.* **2006**, *9*, 125. (c) Ema, T.; Tanida, D.; Sakai, T. *J. Am. Chem. Soc.* **2007**, *129*, 10591.
(10) Pirkle, W. H.; Hoover, D. J. *Top. Stereochem.* **1982**, *1*, 263.
(11) Jurs, P. C.; Bakken, G. A.; McClelland, H. E. *Chem. Rev.* **2000**, *100*, 2649.

lectively and enantioselectively separated, was obtained. This was achieved by using accessible chiral auxiliaries for induced chemical shift perturbation NMR. We believe that our method combines several attractive features: it depends on commercially accessible chiral auxiliaries that are nonspecific and it avoids substrate derivatization and minimizes solvent waste. Therefore, this result provides a new insight to refining and expanding the applications of existing chiral solvating agents for enantioselective analysis. We are currently working to expand the technique to a wide variety of chiral organic functional groups and to automate the pattern recognition.

Acknowledgment. The work was supported in part by grants from NSFC 30900129, ZJNSFC Y2080331, ZJST 2009C34014, and WZST H20080052.

Supporting Information Available: Experimental procedures along with copies of spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL100773S