Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 3345–3351

Investigation of a broadly applicable chiral selector used in enantioselective chromatography (Whelk-O 1) as a chiral solvating agent for NMR determination of enantiomeric composition

Michael E. Koscho* and William H. Pirkle

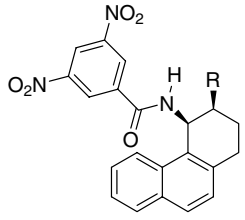
School of Chemical Sciences, University of Illinois, Urbana, IL 61801, USA

Received 5 June 2005; revised 24 August 2005; accepted 26 August 2005
Available online 20 October 2005

Abstract—A chiral solvating agent (CSA) based on the chiral selector used in the Whelk-O 1 chiral stationary phase (CSP) was prepared and its scope evaluated. This chiral selector possesses a cleft flanked with aromatic groups and produces upfield chemical shifts for analytes, which are held in this cleft. The enantiomers of each of the Whelk-O 1 resolvable analytes surveyed show non-equivalent ^{1}H NMR spectra at room temperature with the addition of only 0.5 equiv of the CSA. Similar non-equivalence is sometimes noted for enantiomers, which do not resolve on this CSP. In such cases, it is apparent that a hydrogen bond acceptor is required and higher CSA to substrate ratios and/or lower temperatures may be needed if adequate resolution of enantiomeric signals is to be obtained.

1. Introduction

Pirkle was the first to report the observation of magnetic non-equivalence for enantiomeric nuclei in a chiral solvent.[1] Since then, a great number of chiral solvating agents (CSA) have been introduced and have proven to be of great utility for the determination of enantiomeric composition by NMR as well as for the correlation of absolute configuration.[2–4] Through detailed studies of these original CSAs, was born (in many cases) an understanding of the mechanistic basis for magnetic non-equivalence of enantiomeric (and enantiotopic) nuclei. These studies, in turn, led to the development of chiral selectors, which, when covalently linked to silica gel, are capable of chromatographic enantioseparations.[5,6] We have long been interested in understanding the manner in which these low molecular weight chiral selectors differentiate between analyte enantiomers. In conjunction with numerable chromatographic studies, NMR has proven to be an invaluable tool for providing information relating to these bimolecular selector–analyte complexes. During such studies with soluble analogues of chiral stationary phase (CSP) **1** (Whelk-O 1), relatively large chemical shift differences for analyte enantiomers have been observed.[7–9] On this basis, we decided to investigate the scope of a soluble analogue of this selector, **2**, as a CSA (see Fig. 1).



(3*R*,4*S*)-CSP **1**: R = $(CH_2)_3Si(CH_3)_2O$-silica

(*S*)-CSA **2**: R = H

Figure 1. Structure of CSP **1** and CSA **2**.

In general, analytes with an aromatic group and a hydrogen bond acceptor near a stereogenic center are candidates for enantioseparation on CSP **1**. This selector has a cleft formed by the planes of the dinitrobenzoyl and naphthyl rings, a consequence of the dinitrobenzamido moiety occupying a pseudo-axial position in

* Corresponding author. Present address: Department of Chemistry, Mississippi State University, MS 39762, USA. Tel.: +1 662 325 9500; fax: +1 662 325 1618; e-mail: m.koscho@msstate.edu

0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.08.032

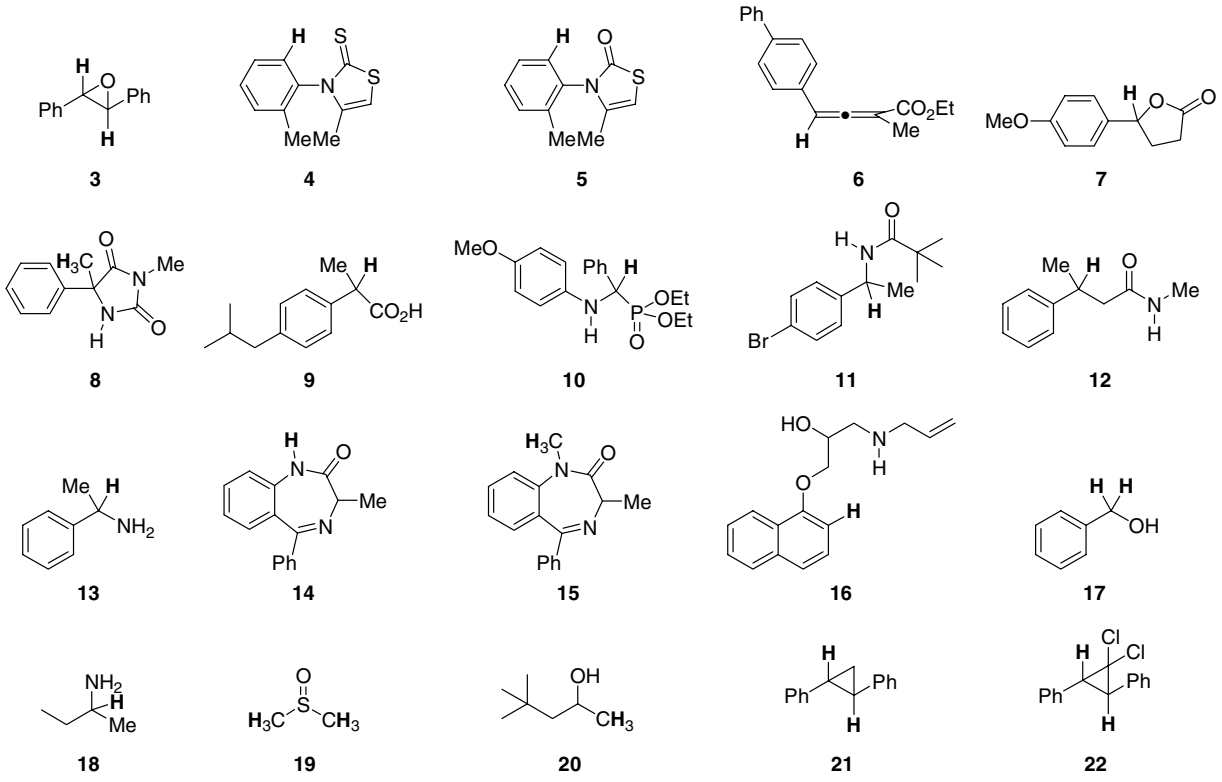


Figure 2. Structure of analytes used herein.

the saturated six-membered ring of the selector. CSP **1** selectively retains the enantiomer, which, without substantially deviating from a low energy conformation, can undergo simultaneous face-to-face π–π interaction with the dinitrobenzamide portion of the selector as well as a hydrogen bonding interaction with the amide proton of the selector. Additionally, a face-to-edge π–π interaction with an aromatic group of the analyte and the naphthyl portion of the selector increases the stability of this bimolecular complex. Nuclei that are held above the naphthyl ring, as a consequence of complexation in this manner, will be strongly shielded.

The 20 compounds chosen for this study can be divided into four groups. The first group, **3**–**12**, contains a selection of analytes, the enantiomers of which are resolvable on CSP **1**. The second group, **13**–**17**, contains compounds the enantiomers of which do not resolve on CSP **1**, although they do contain an aromatic group and a hydrogen bond acceptor. The third group, **18**–**20**, lacks the aromatic moiety, while the fourth group, **21** and **22**, lacks a hydrogen bond acceptor (see Fig. 2).

2. Results and discussion

At the outset, we expected the enantiomers of those compounds, which resolve on CSP **1** to afford non-equivalent ^{1}H NMR spectra in the presence of a single enantiomer of CSA **2**, and that the signals of the enantiomer forming the more stable complex (more retained on CSP **1**) would be shifted further upfield. For enantiomers to be separable on CSP **1**, there must be a difference in the affinity of the enantiomers for the chiral selector, thus placing each enantiomer in the cleft of the selector to different extents. The chromatographic data for analytes **3**–**12** on CSP **1** are presented in Table 1. There is little or no difference in affinity of the selector for the enantiomers of compounds which do not resolve on CSP **1**. However, a difference in affinity is not necessarily required in order to observe the spectral non-equivalence; all that is required is that the enantiomeric nuclei must be in different average magnetic environments in the diastereomeric selector–analyte complexes.

Table 1. Chromatographic data: analytes **3**–**12** on CSP **1**

Analyte	k_1	α	Mobile phase
3	0.41	3.08	A
4	1.72	1.42	B
5	1.89	1.94	B
6	0.69	3.01	A
7	3.77	1.29	B
8	0.81	2.60	B
9	0.19	1.47	C
10	5.23	1.14	A
11	1.13	10.11	B
12	12.14	1.21	A

Conditions: A—5:95 2-propanol/hexanes (v/v); B—20:80 2-propanol/hexanes (v/v); C—20:80 2-propanol/hexanes (v/v) with 1 g/L NH_4OAc. Flow rate: 2 mL/min in all cases.

The ^{1}H NMR spectrum of CSA **2** is shown in Figure 3. The spectrum contains a window for substrate signals between 3.0 and 7.2 ppm with the exception of the signal

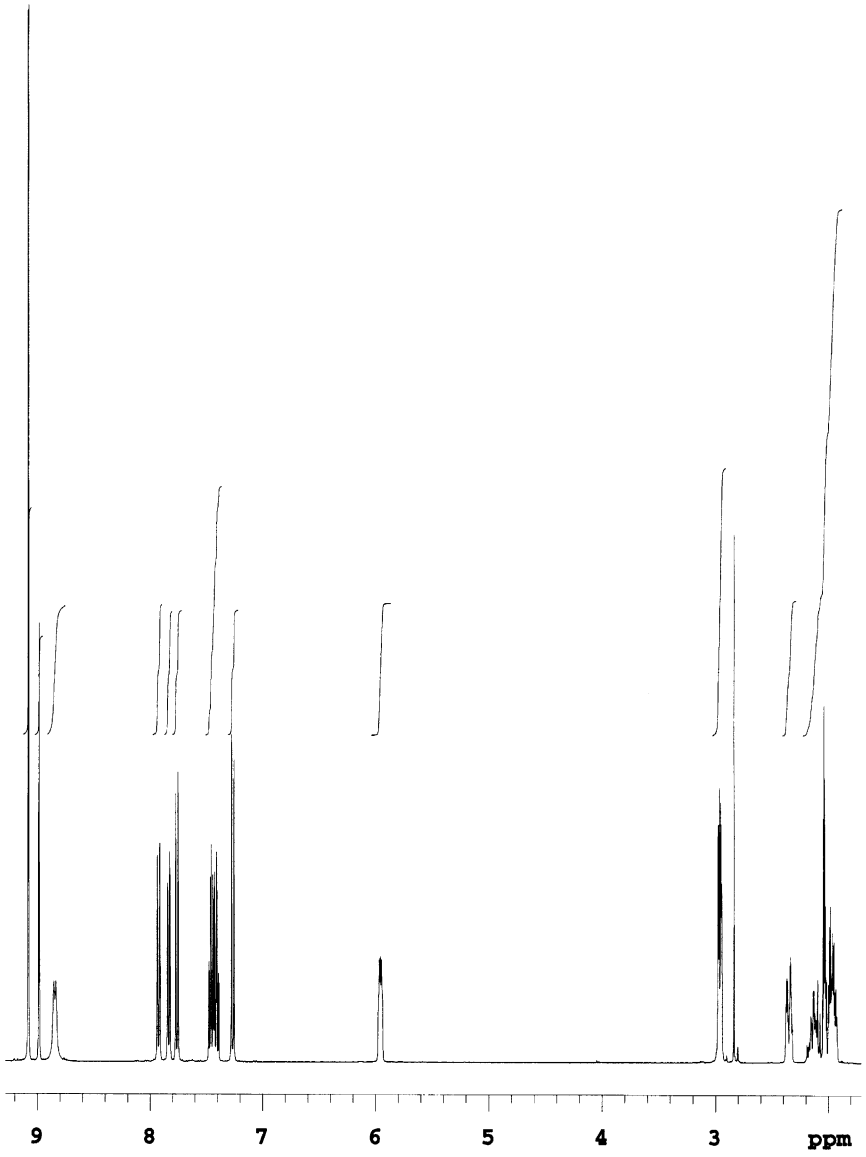


Figure 3. ^{1}H NMR spectrum of CSA **2** in d_6-acetone.

at 5.97 ppm from the proton at the stereogenic center of CSA **2**. In addition, the spectrum is devoid of signals upfield of 1.9 ppm.

Initial spectra with added CSA **2** were recorded at a racemic analyte concentration of 50 mM and CSA **2** concentration of 5 mM in deuterochloroform for analytes **3**, **4** and **5** (spectra for analyte **3** are shown in Fig. 4). This produced a chemical shift difference ($\Delta\Delta\delta$) between the two enantiomeric signals of the boldfaced protons shown in Figure 2 of 9.77, 4.65 and 21.61 Hz for analytes **3**, **4** and **5**, respectively. Though this is sufficient for the determination of the enantiomeric composition for analytes **3** and **5**, the doublet for analyte **4** ($J = 7.8$ Hz) was not completely resolved. The spectra were then recorded for these same analytes at an analyte concentration of 40 mM and a CSA **2** concentration of 20 mM; these conditions produce a complete separation of the signals of enantiotopic hydrogens.

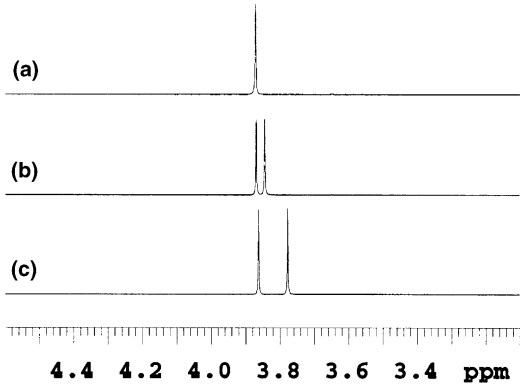


Figure 4. Partial ^{1}H NMR spectrum of **3** at (a) 40 mM; (b) 50 mM with 5 mM CSA **2** and (c) 40 mM with 20 mM CSA **2** in deuterochloroform at ambient temperature.

Subsequently, the ^{1}H NMR spectrum was recorded for each analyte at a concentration of 40 mM and CSA

Table 2. Chemical shift data[a]

Analyte	δ without CSA (m) (ppm)	δ upfield signal (ppm)	$\Delta\Delta\delta$ (Hz)
3	3.875 (s)	3.778	33.69
4	7.131 (d)	7.084	17.09
5	7.151 (d)	6.946	73.24
6	6.518 (q)	6.419	37.11
7	5.463 (m)	5.35	29.79
8	1.806 (s)	1.714	29.05
9	3.710 (q)	3.685	7.20
10	4.688 (d)	4.637	12.21
11	5.040 (quin)	4.086	368.18
12	3.296 (sextet)	3.185	21.49
13	4.120 (q)	4.034	13.43
14	9.270 (br s)	8.969	44.34
15	3.409 (s)	3.325	22.71
16	6.824 (d)	6.742	12.70
17	4.704 (s)	4.675	0
18	2.793 (sextet)	2.746	2.02
19	2.613 (s)	2.484	16.36
20	1.196 (d)	1.181	0
21	2.171 (dd)	2.168	0
22	3.241 (s)	3.237	0

[a] Data for the bold protons in Figure 2. Analyte concentration = 40 mM, CSA **2** concentration = 20 mM in deuterochloroform at ambient temperature.

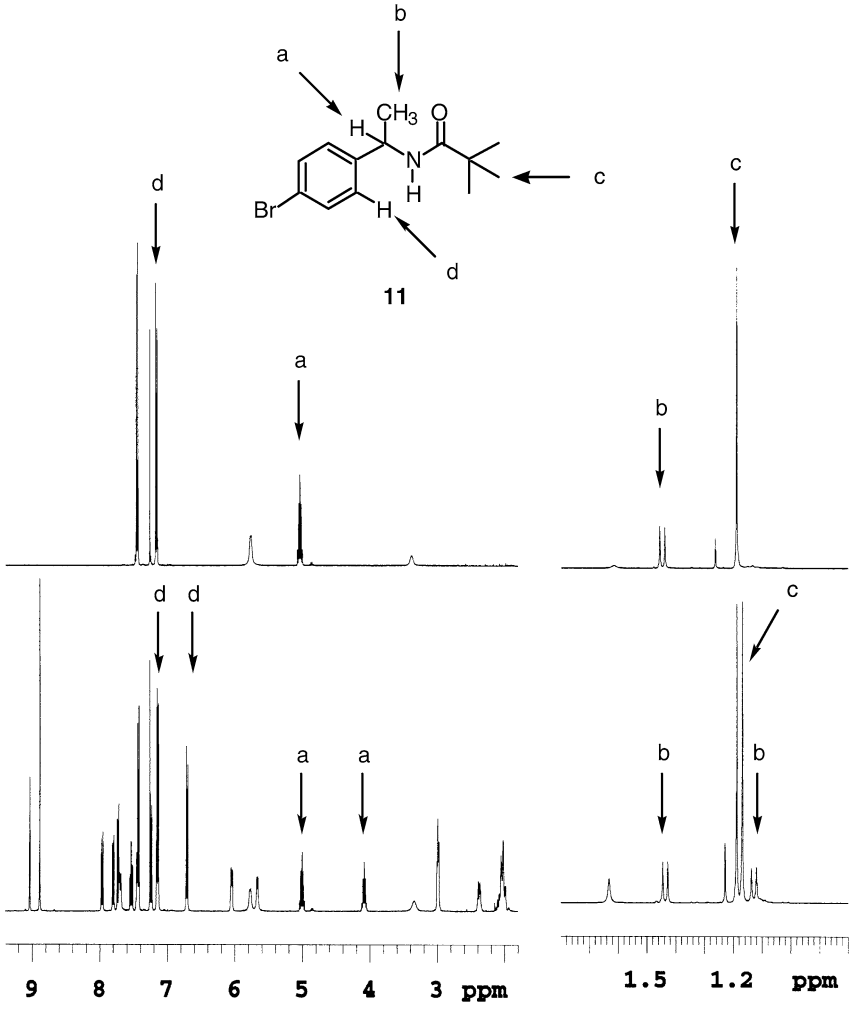


Figure 5. ^{1}H NMR spectrum of **11** without (top) and with (bottom) CSA **2** at a concentration of 40 mM for **11** and 20 mM for (*S*)-CSA **2** in deuterochloroform at ambient temperature. The regions between 1.8–9.3 ppm and 0.8–1.8 ppm are shown to different scales for clarity. One of the signals from proton d and the other ring proton are obscured by signals from the CSA.

concentration of 20 mM in deuterochloroform. The chemical shift data thus afforded are presented in Table 2. Under these conditions, 16 of the 20 chosen analytes displayed non-equivalence of the enantiomeric (or enantiotopic) nuclei in the ^{1}H NMR spectra. Of the 16 analytes that display non-equivalence, the chemical shift difference between the resonances of the indicated enantiomeric nuclei was sufficient for the determination of the enantiomeric composition in 14 cases.

Additionally, many of these spectra showed non-equivalence for more than just the indicated signal. Figure 5 shows the spectrum of **11** with and without CSA **2** to illustrate this point.

The spectra of analytes **9** and **13**, the two analytes which did not show complete spectral resolution, were then recorded at a probe temperature of −50 °C, conditions which afforded complete separation of the multiplets (Figs. 6 and 7).

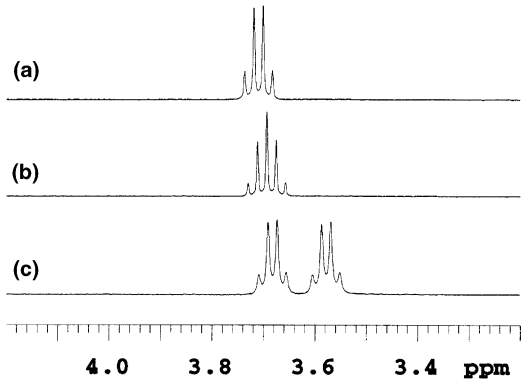


Figure 6. Partial ^{1}H NMR spectrum of **9** at 40 mM (a) at ambient temperature; (b) with 20 mM CSA **2** at ambient temperature and (c) with 20 mM CSA **2** at −50 °C in deuterochloroform.

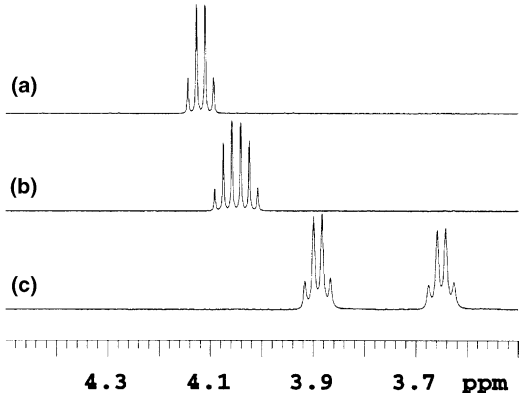


Figure 7. Partial ^{1}H NMR spectrum of **13** at 40 mM (a) at ambient temperature; (b) with 20 mM CSA **2** at ambient temperature and (c) with 20 mM CSA **2** at −50 °C in deuterochloroform.

The spectrum for analytes **17** and **20**, the two analytes that did not show non-equivalence under the initial conditions, were then recorded at a concentration of 3.3 mM and CSA concentration of 20 mM at −50 °C. Under these conditions, spectral non-equivalence was observed to the extent of 3.0 and 3.4 Hz for the indicated enantiomeric protons of analytes **17** and **20**, respectively. Analytes **21** and **22** did not display non-equivalence under any of the conditions used. In fact, the chemical shift differences between spectra recorded with and without CSA **2** are very small, indicating a very low affinity of the selector for these compounds.

In order to determine the sense of non-equivalence for the analytes, which are separable on CSP **1**, a small

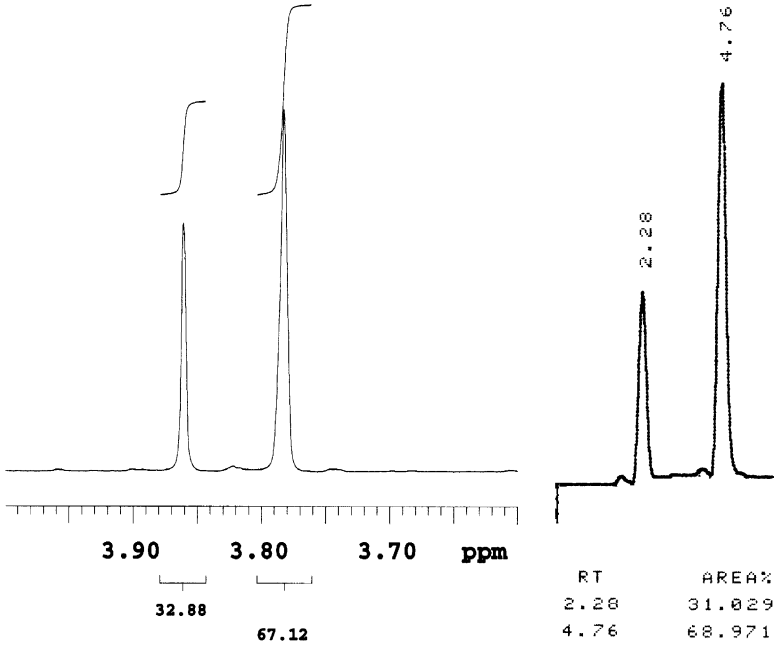


Figure 8. Partial ^{1}H NMR spectrum of enantiomerically enriched **3** in the presence of (*S*)-CSA **2** and the HPLC chromatogram obtained by injection of this same sample on (3*R*,4*S*)-CSP **1**.

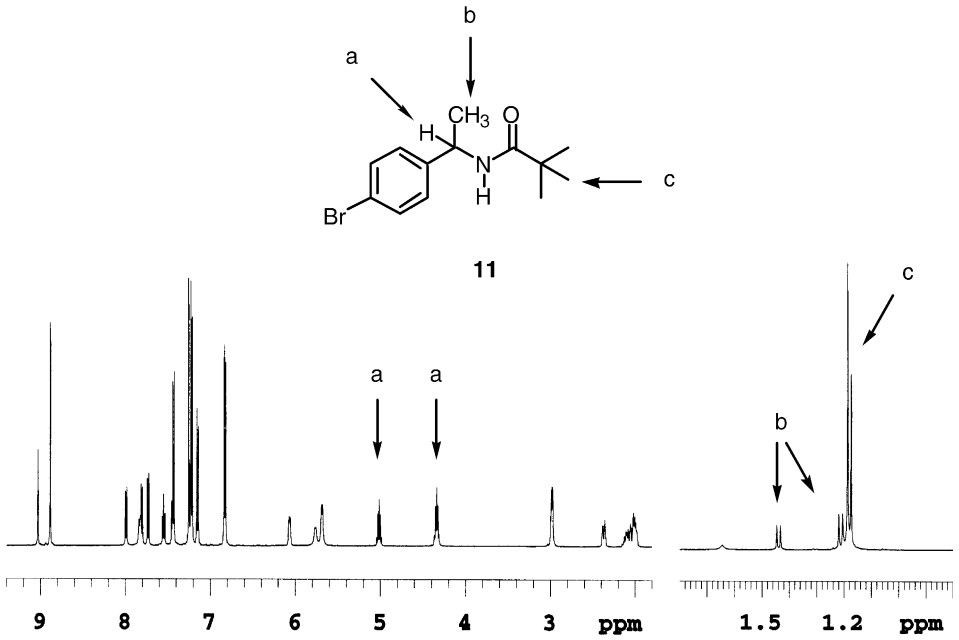


Figure 9. ^{1}H NMR spectrum of enantiomerically enriched **11** in the presence of CSA **2**. The regions between 1.8–9.3 and 0.8–1.8 ppm are shown to different scales for clarity.

quantity of each analyte, **3**–**12**, was resolved on a semi-preparative version of CSP **1**. To the previously prepared NMR sample for each of these analytes (40 mM analyte, 20 mM CSA **2** in deuterochloroform) was added an unweighed quantity of the enantiomer, which was more retained on (3*R*,4*S*)-CSP **1**, and the spectra recorded at 500 MHz. For the enantiomeric signals that displayed non-equivalence in the ^{1}H NMR spectra, with one special exception that the more retained enantiomer on (3*R*,4*S*)-CSP **1** is shifted further upfield than the less retained enantiomer in the presence of (*S*)-CSA **2**. A partial ^{1}H NMR spectrum of enantiomerically enriched **3** in the presence of CSA **2** and a chromatogram obtained from an analytical version of (3*R*,4*S*)-CSP **1** using this same solution is shown in Figure 8.

For the analytes that resolve on CSP **1**, one resonance from the enantiomer which is less retained on (3*R*,4*S*)-CSP **1** was shifted further upfield in the ^{1}H NMR spectrum than the more retained enantiomer in the presence of (*S*)-CSA **2**. This is the signal corresponding to the *tert*-butyl group on analyte **11** (Fig. 9). Based on our mechanistic rationale for the manner in which this chiral selector is capable of producing non-equivalent NMR spectra for enantiomers, this was not completely unexpected. Nuclei that are held in the cleft of CSA **2** are shifted upfield owing to the high diamagnetic anisotropy of the aromatic groups. In general, for analytes that resolve on CSP **1**, the nuclei which are held in this cleft are the ones which are part of, or between, the main interaction sites of the analyte, the π-base and the hydrogen bond acceptor. For the more retained enantiomer of **11**, the *tert*-butyl group is not expected to be held in the cleft of CSA **2**. For the less retained enantiomer of **11**, it is expected that the primary mode of association with the chiral selector occurs from the opposite face of the dinitrobenzoyl group, the 'back face' of the selector. Interaction in this manner does place the *tert*-butyl group of **11** near the cleft of CSA **2**. Interaction in this manner was also observed in the crystal structures previously obtained using analyte **11** and an analogous selector derived from CSP **1**.[13] These observations give support to the hypothesis that the primary selector–analyte modes of association are very similar in solution as was observed in the obtained co-crystals. Similar structures were also obtained recently by Del Rio et al., using computational methods.[14]

3. Conclusions

Chiral solvating agent CSA **2** based on the chiral selector used in the Whelk-O 1 chiral stationary phase CSP **1** was prepared and evaluated. On the basis of our chiral recognition model of this selector, which is supported by chromatographic, NMR and crystallographic evidence, we expected this selector would be capable of producing non-equivalent NMR spectra for the enantiomers of a variety of analytes. Analytes for which the enantiomers resolve on CSP **1** displayed non-equivalent ^{1}H NMR spectra in the presence of a single enantiomer of CSA **2**, the sense of non-equivalence being predictable. For analytes for which the enantiomers do not resolve on CSP **1**, a hydrogen bond acceptor appears to be necessary for differentiation by the chiral selector. For analytes which have a poor hydrogen bond acceptor (e.g., alcohols), high CSA to analyte ratios and/or recording the spectra at lower temperatures may be required in order to observe adequate resolution of the signals arising from the enantiomers of the analyte.

The scope of CSA **2** and the extents of the non-equivalences observed with this set of analytes compares favorably with other CSAs that have been previously

reported. Typically, when using a CSA, the chemical shift differences of enantiomeric (or enantiotopic) nuclei are small (<0.1 ppm),[3] as has been observed with CSA **2** (analyte **11** being a notable exception). Since the extent of the observed non-equivalence is dependent on the concentrations of the CSA and the analyte, the temperature, and the solvent, one must be cautious when making direct comparisons between the data herein and the literature data.

A few of the analytes used herein have been reported to afford spectral non-equivalence in the presence of other CSAs. For example, analyte **3** afforded a chemical shift difference of 0.011 ppm in the presence of 2,2,2-trifluoro-1-(9-anthryl)ethanol, and 0.012 ppm in the presence of 9,10-bis-(trifluoromethylcarbinol)anthracene (CSA-analyte ratio of 2:1 in both cases).[15] Greater chemical shift differences were observed for the enantiomers of analyte **3** in the presence of CSA **2**, at a CSA–analyte ratio of 1:10. A derivative of quinine ($\Delta\Delta\delta = 0.022$ ppm, CSA–analyte ratio of 1:1 in $CDCl_3$),[16] and a tris-1-(1-naphthyl)ethylamino substituted 1,3,5-triazine derivative ($\Delta\Delta\delta = 0.030$ ppm, CSA–analyte ratio of 1:1 in $CDCl_3$)[17] have been used as a CSA for analyte **9** (compare to Fig. 6). *O*-Acetyl mandelic acid ($\Delta\Delta\delta =$ 0.075 ppm, CSA–analyte ratio of 1.2:1 in benzene-d_6),[18] and α-methoxy-α-(trifluoromethyl)phenyl acetic acid ($\Delta\Delta\delta = 0.035$ ppm, CSA–analyte ratio of 1:1 in $CDCl_3$; $\Delta\Delta\delta = 0.061$ ppm, CSA–analyte ratio of 1:1 in pyridine-d_5)[19] have been used as a CSA for analyte **13** (compare to Fig. 7). A bis-1-(1-naphthyl)ethylamino substituted 1,3,5-triazine derivative CSA was also used as a CSA for analyte **14** ($\Delta\Delta\delta = 0.030$ ppm, CSA–analyte ratio of 1.2:1 in $CDCl_3$; compare to $\Delta\Delta\delta = 0.111$ ppm, CSA **2**-analyte **14** ratio of 1:2 in $CDCl_3$).[17]

Undoubtedly, there are specific chiral compounds that are capable of affording greater spectral non-equivalence for each of the analytes reported herein. The utility of CSA **2** is derived from the scope of analytes that can successfully be assayed by this CSA (i.e., analytes with a hydrogen bond acceptor). Ultimately, for the determination of enantiomeric composition, the extent of non-equivalence is irrelevant, as long as the resonances of the enantiomeric nuclei are sufficiently resolved to allow accurate integration. Of course if any non-equivalence is observed, one can always increase the concentration of the CSA, lower the probe temperature, or use a different solvent or solvent combination that will allow an increase in the extent of non-equivalence.

4. Experimental

4.1. General

The solvents used were of HPLC grade or distilled prior to use. Analytes were either commercially available from previous studies or prepared by the literature procedures. The preparation of CSA **2** has been previously reported.[10] Analytical chromatography was carried out with a commercial version of (3*S*,4*R*)-CSP **1** (250 × 4.6 mm, Regis Technologies, Morton Grove, IL), with tri-*tert*-butylbenzene being used as a void volume marker. Semi-preparative chromatography was carried out using a commercial (3*R*,4*S*)-CSP **1** (250 × 10 mm). The preparative resolution of CSA **2** was carried out using a CSP derived from *N*,*N*-diallyl-(*S*)-Naproxen (900 × 25 mm).[11] All ^{1}H NMR spectra were recorded on a Varian U400 spectrometer operating at 400 MHz in the deuterium lock mode at ambient temperature (20 ± 2 °C), unless otherwise stated, with residual solvent signal used as an internal standard.

4.2. Enantioresolution of CSA 2

Injection of 1.249 g of CSA **2**, onto a CSP derived from *N*,*N*-diallyl-(*S*)-Naproxen (900 × 25 mm)[11] afforded 592 mg of (*S*)-CSA **2** (>99% ee) followed by 621 mg (*R*)-CSA **2** (94.1% ee) eluting with 15% THF/hexanes in a single pass. The assignment of the absolute configuration from the elution order is based on previous work.[12]

Acknowledgements

This work has been funded by grants from the National Science Foundation and from Eli Lilly and Company.

References

1. Pirkle, W. H. *J. Am. Chem. Soc.* **1966**, *88*, 1837.
2. Pirkle, W. H.; Hoover, D. J. *Top. Stereochem.* **1982**, *13*, 263–331.
3. Parker, D. *Chem. Rev.* **1991**, *91*, 1441–1457.
4. Uccello-Barretta, G.; Balzano, F.; Menicagli, R.; Salvadori, P. J. *J. Org. Chem.* **1996**, *61*, 363–365.
5. Pirkle, W. H.; Pochapsky, T. C. *Chem. Rev.* **1989**, *89*, 347–361.
6. Welch, C. J. *J. Chromatogr. A* **1994**, *666*, 3–26.
7. Pirkle, W. H.; Welch, C. J. *J. Chromatogr. A* **1994**, *683*, 347–353.
8. Pirkle, W. H.; Selness, S. R. *J. Org. Chem.* **1995**, *60*, 3252–3256.
9. Job, G. E.; Shvets, A.; Pirkle, W. H.; Kuwahara, S.; Kosaka, M.; Kasai, Y.; Taji, H.; Fujita, K.; Watanabe, M.; Harada, N. *J. Chromatogr. A* **2004**, *1055*, 41–53.
10. Wolf, C.; Pirkle, W. H. *Tetrahedron* **2002**, *58*, 3597–3603.
11. Pirkle, W. H.; Spence, P. L.; Lamm, B.; Welch, C. J. *J. Chromatogr. A* **1994**, *659*, 69–74.
12. Pirkle, W. H.; Welch, C. J.; Wilson, S. R. *Chirality* **1994**, *6*, 615–622.
13. Koscho, M. E.; Spence, P. L.; Pirkle, W. H. *Tetrahedron: Asymmetry* **2005**, *16*, 3147–3153.
14. Del Rio, A.; Mayes, J. M.; Stein, M.; Piras, P.; Roussel, C. *Chirality* **2004**, *16*, S1–S11.
15. Pomares, M.; Sanchez-Ferrando, F.; Virgili, A.; Alvarez-Larenza, A.; Piniella, J. F. *J. Org. Chem.* **2002**, *67*, 753–758.
16. Uccello-Barretta, G.; Mirabella, F.; Balzano, F.; Salvadori, P. *Tetrahedron: Asymmetry* **2003**, *14*, 1511–1516.
17. Uccello-Barretta, G.; Iuliano, A.; Franchi, E.; Balzano, F.; Salvadori, P. *J. Org. Chem.* **1998**, *63*, 9197–9203.
18. Parker, D.; Taylor, R. J. *Tetrahedron* **1987**, *43*, 5451–5456.
19. Villani, F. J.; Costanzo, M. J.; Inners, R. R.; Mutter, M. S.; McClure, D. E. *J. Org. Chem.* **1986**, *51*, 3715–3718.