

# <sup>1</sup>H-NMR determination of the enantiomeric excess of the antiarrhythmic drug Mexiletine by using mandelic acid analogues as chiral solvating agents

Giuseppe Carbonara, Alessia Carocci, Giuseppe Fracchiolla, Carlo Franchini, Giovanni Lentini, Fulvio Loiodice,\* and Paolo Tortorella

Dipartimento Farmaco-Chimico, Università degli Studi di Bari, via E. Orabona 4,  
70125 Bari, Italy

E-mail: [floiodice@farmchim.uniba.it](mailto:floiodice@farmchim.uniba.it)

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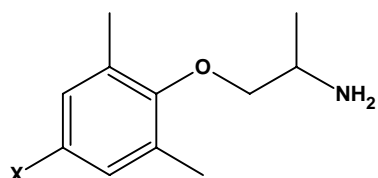
## Abstract

Several optically active mandelic acid analogues were shown to allow a direct <sup>1</sup>H-NMR determination of the enantiomeric composition of Mexiletine [1-(2,6-dimethylphenoxy)-2-propanamine], a chiral, orally effective antiarrhythmic agent, and some of its derivatives. Excellent results were obtained with few milligram samples, at room temperature in CDCl<sub>3</sub>, without the need of deconvolution software, and regardless of the instrument used (200 or 300 MHz).

**Keywords:** Mexiletine, 2-phenoxyphenylacetic acid, <sup>1</sup>H-NMR analyses, chiral solvating agent

## Introduction

Mexiletine (**1a**, Figure 1) is a chiral therapeutically relevant compound, clinically used as an antiarrhythmic,<sup>1</sup> antimyotonic,<sup>2</sup> and analgesic agent,<sup>3</sup> in its racemic form. However, several lines of evidence have shown that the (–)-(R) enantiomer is the eutomer,<sup>4</sup> while the distomer is the major responsible for mexiletine adverse side effects.<sup>4f,5</sup>



**1a.** X = H, Mexiletine

**1b.** X = CH<sub>3</sub>

**1c.** X = Cl

Figure 1

As part of a study aimed at the discovery of new antimyotonic agents,<sup>4d,6</sup> in the last decade we have been developing both classical resolution and 'chiral pool' synthetic methods to obtain optically active, highly enriched **1a** enantiomers.<sup>7</sup> Hence, we needed an efficient and facile way to assess the ee of our products. A plethora of chromatographic and electrophoretic methods have been applied to **1a** enantiomers analysis in the last two decades and the list reported in Table 1, though comprehensive, is far from exhaustive.

**Table 1.** High-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE) based methods applied to **1a** ee evaluation

Entry	Method	Chiral selector <sup>a</sup>	References
1	HPLC	A: D214N <sup>b</sup>	8
2	HPLC	A: Cellulose tris(3,5-dimethylphenylcarbamate)	7b, <sup>c</sup> 9
3	HPLC	B: <i>N</i> -Acetyl-L-Cysteine/ <i>o</i> -phthalaldehyde	10–12
4	HPLC	B: ( <i>S</i> )-1-(1-Naphthyl)ethyl isocyanate	13
5	HPLC	A: Crownpak CR(+) <sup>d</sup>	14
6	HPLC	B: ( <i>S</i> )-2-(6-Methoxy-2-naphthyl)-1-propyl chloroformate	15
7	HPLC	A: Amylose tris(3,5-dimethylcarbamate)	16, 17 <sup>c</sup>
8	HPLC	B: ( <i>R</i> )- $\alpha$ -Methoxy- $\alpha$ -(trifluoromethyl)phenylacetic acid	18
9	HPLC	A: Cellulose tris(4-methylbenzoate)	11 <sup>f</sup>
10	HPLC	B: 4-(6-Methoxy-2-naphthyl)-2-butyl chloroformate	19
11	HPLC	A: Cellobiohydrolase II	20
12	HPLC	A: Phenylcarbamylated $\beta$ -cyclodextrin	21 <sup>g</sup>
13	HPLC	B:(1 <i>S</i> - <i>trans</i> )- <i>N</i> -(2-Isothiocyanatocyclohexyl)-2,2-dimethylpropanamide	22
14	HPLC	A: ( <i>R</i> )-Phenylglycine	23, <sup>h</sup> 24 <sup>f</sup>
15	HPLC	B: 2,3,4,6-Tetra- <i>O</i> -acetyl-1-thio- $\beta$ -D-glucopyranoside/ <i>o</i> -phthalaldehyde	12
16	HPLC	B: (–)-Menthyl chloroformate	25
17	HPLC	B: 2,3,4,6-Tetra- <i>O</i> -acetyl- $\beta$ -D-glucopyranosyl isothiocyanate	4a, 26, 27
18	HPLC	A: ( <i>R</i> )-3,5-Dinitrobenzoylphenylglycine	28 <sup>f</sup> , 29 <sup>l</sup> , 30 <sup>l</sup>
19	HPLC	A: 3,4,6-Tribenzoyl-2-benzoylamino-2-deoxy-D-glucose	31 <sup>i</sup>
20	HPLC	B: <i>N</i> -[(2-Naphthalene)sulphonyl]-L-prolyl isocyanate	32
21	HPLC	B: <i>N</i> -[(2-Naphthalene)sulphonyl]-L-prolyl azide	32
22	HPLC	B: ( <i>R</i> )- $\alpha$ -Methylbenzyl isothiocyanate	27
23	HPLC	B: ( <i>R</i> )-1-(2-Naphthyl)ethyl isothiocyanate	27
24	HPLC	B: ( <i>S</i> )-Flunoxaprofen isocyanate	33
25	HPLC	B: ( <i>S</i> )- $\alpha$ -methoxybenzyl isocyanate	34
26	HPLC	A: <i>N</i> -(2-naphthyl)-D-alanine	35 <sup>m</sup>
27	HPLC	B: <i>N</i> -( <i>p</i> -toluenesulphonyl)prolyl isocyanate	36
28	GC	B: ( <i>S</i> )- <i>N</i> -Trifluoroacetylprolyl chloride	37, 38
29	GC	B: ( <i>S</i> )- <i>N</i> -Heptafluorobutyrylprolyl chloride	37b
30	GC	A: Heptakis(6- <i>O</i> - <i>tert</i> -butyldimethylsilyl-2,3-di- <i>O</i> -methyl)- $\beta$ -cyclodextrin	39

**Table 1.** Continued

31	GC	A: Heptakis(3- <i>O</i> -acetyl-2,6-di- <i>O</i> -pentyl)- $\beta$ -cyclodextrin	40 <sup>n</sup>
32	CE	C: L-Glutamine	41
33	CE	C: L-Aspartic acid	42
34	CE	C: 3- <i>O</i> -Phenylcarbamoyl- $\beta$ -cyclodextrin	43
35	CE	C: 2-Hydroxypropyl- $\beta$ -cyclodextrin	44, 45
36	CE	C: Heptakis(2,3,6-tri- <i>O</i> -methyl)- $\beta$ -cyclodextrin	45, 46
37	CE	C: (+)-18-Crown-6 tetracarboxylic acid	14
38	CE	C: $\alpha_1$ -Acid glycoprotein	47
39	CE	C: Tetrasulphobutyl- <i>O</i> - $\beta$ -cyclodextrin	45
40	CE	C: Sulphated cyclodextrins	48
41	CE	C: L-Leucine	49

<sup>a</sup> A: bonded on the stationary phase; B: used as a chiral derivatizing agent; C: used as a soluble chiral selector. <sup>b</sup> Immobilized mutant form of cellobiohydrolase I. <sup>c</sup> On the *N*-Ac derivatives. <sup>d</sup> Hydrophobic chiral crown ether coated on ODS-silica. <sup>e</sup> On the derivatives obtained by treatment with *o*-phthalaldehyde. <sup>f</sup> On the *N*-(2-naphthoyl) derivatives. <sup>g</sup> On the *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl) derivatives. <sup>h</sup> On the *N*-(2-anthroyl) derivatives. <sup>i</sup> On either the *N*-(3,5-dinitrobenzoyl) or benzoyl derivatives. <sup>j</sup> On the 1-naphthylaminocarbonyl derivatives. <sup>m</sup> On the *N*-(2,4,6-trinitro)benzenesulphonyl derivatives. <sup>n</sup> On the *N*-trifluoroacetyl derivatives.

Several reported chromatographic methods are indirect (B-type chiral selector entries in Table 1): they require prior derivatization with a chiral reagent of (high) known ee and must avoid both racemization of reagent and substrate, and epimerization of diastereomeric products; the absence of fractionation effects, kinetic resolution, differences in detecting the diastereomeric products, and CDRs optically active contaminants should also be verified.<sup>50</sup> On the other hand, most of the direct methods (chromatographic methods using A-type chiral selectors in Table 1), i. e. methods based on the use of a chiral stationary phase (CSP), require sample derivatizations with achiral reagents (see legend in Table 1). Finally, capillary electrophoresis (CE) methods do not require any prior derivatization step but a number of experimental factors<sup>45,46,51</sup> constitute a *Damoclis gladium* hanging over reproducibility and repeatability. The most is that some of the involved experimental aspects may also be unknown a priori: for example, the composition of the most widely used CE chiral additives, i. e. the commercially available functionalized  $\beta$ -cyclodextrins, may considerably vary depending on the vendor and thus unforeseeably alter the results of the analyses.<sup>52</sup> Consequently, we turned our attention to <sup>1</sup>H-NMR spectroscopy methods based on diastereomeric complex formation between chiral amines and either chiral lanthanide shift reagents (CLSRs)<sup>53</sup> or chiral solvating agents (CSAs):<sup>54</sup> generally differences in the resonance signals from each enantiomer are observed and the relative area may be easily utilized to derive ee, provided that sufficient resolution of signals is obtained. However, only few examples of <sup>1</sup>H-NMR methods devoted to **1a** ee determination are found in the literature, and the

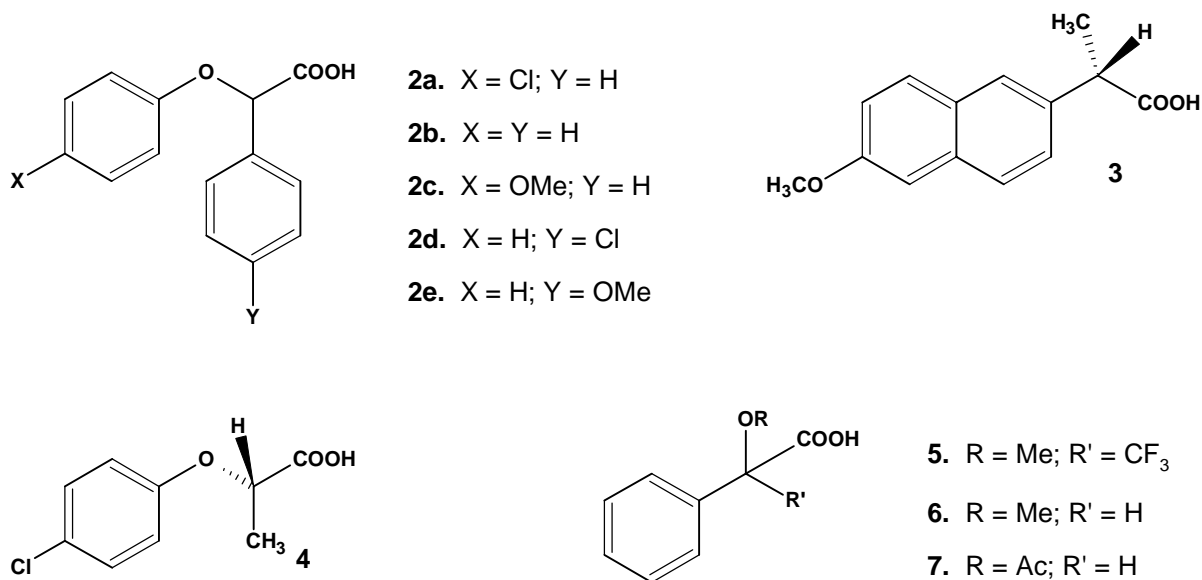
reported applications were tainted with some drawbacks. The use of CLSRs is normally accompanied by broadening of signals, which is generally more severe at high fields and discourages the use of more sensitive high field FT spectrometers.<sup>54a</sup> Indeed, the two methods based on CLSRs found in the literature needed either previous N-acetylation of **1a**<sup>7a</sup> (in order to observe the CH<sub>3</sub>CO singlet non-equivalence in a relatively clean region of the spectrum, on a 200 MHz instrument) or relatively high quantities of the analyte (40–60 mg, on a 60 MHz instrument).<sup>55</sup> On the other hand, the magnitude of the chemical-shift differences ( $\Delta\delta$ ) between corresponding protons of enantiomers observed using CSAs is normally small. In fact, the only published method usefully applied to **1a** ee determination utilized (–)-(R)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) as the CSA but needed the ancillary technique of spectral deconvolution of the CH<sub>3</sub>CH doublet signals, which were not baseline resolved.<sup>56</sup> However, being in our opinion the methods based on CSAs quicker and simpler than all the above mentioned ones, and the ionic interactions occurring in diastereomeric salts stronger than the hydrogen-bonding interactions occurring in TFAE complexes, we focused our attention on homochiral carboxylic acid CSAs. Here we report an efficient method to ascertain the ee of both **1a** and its newly synthesized analogues **1b** and **1c**<sup>7c</sup> (Figure 1), using optically pure (+)-(S)-2-(4-chlorophenoxy)phenylacetic acid<sup>57</sup> (**2a**, Figure 2) as CSA.

The dependency of  $\Delta\delta$  magnitude on solvent, temperature, stoichiometry, and concentration is also reported. In addition, we describe a facile synthesis of both enantiomers of **2a**, which had been previously obtained by crystallization of its diastereomeric salts with (+)-amphetamine,<sup>57a</sup> and its analogues **2b–e** (Figure 2) for which the use as possible CSAs in the <sup>1</sup>H-NMR analysis of **1a** was examined too.

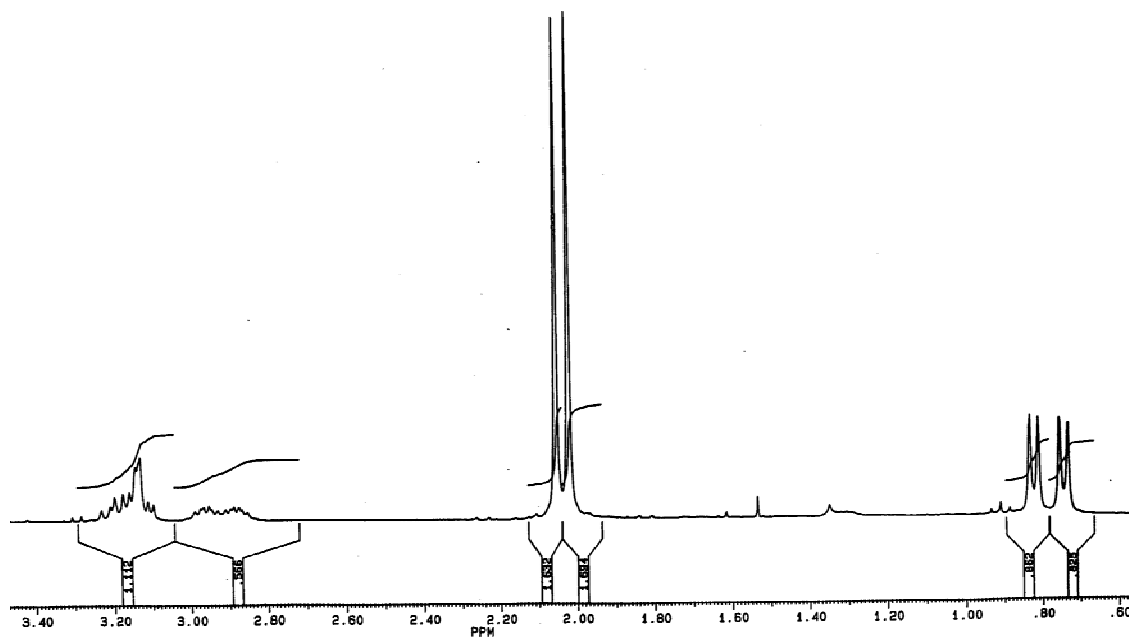
## Results and Discussion

The samples for the <sup>1</sup>H-NMR experiments were prepared by mixing standard CDCl<sub>3</sub> or C<sub>6</sub>D<sub>6</sub> solutions (about 20 mg/mL) of the chiral acid **2** and the amine **1** to give the desired stoichiometry ratio and concentration. The <sup>1</sup>H-NMR spectra were recorded at once.

Our first attempts of direct <sup>1</sup>H-NMR **1a** ee determination used either commercially available (+)-(S)-naproxen (**3**) or (–)-(S)-2-(4-chlorophenoxy)propanoic acid (**4**) (Figure 2), recently synthesized in our laboratory in optically pure form,<sup>58</sup> as CSAs. Their use was suggested by the number of applications of some CDAs structurally related to the former<sup>15,33,59</sup> and by the reported efficacy of 2-phenoxypropionic acids as CSAs,<sup>54c</sup> respectively. Unfortunately, in these experiments a low value of  $\Delta\delta$  for both methyl doublets and methyl singlets ( $\Delta\delta < 0.02$  ppm or completely unresolved peaks) of **1a** was revealed. The observed shift non-equivalence was not sufficient to allow the direct determination of **1a** ee. So we decided to use the chiral mandelic acid derivatives **5–7** (Figure 2) whose direct applicability as CSAs for amines has been intensively studied.<sup>54a,d–f</sup>

**Figure 2**

Even in these experiments, however, no suitable chemical shift non-equivalence was displayed, at r.t., in the most common deuterated solvents. On the contrary, an exploitable  $\Delta\delta$  of the methyl doublets ( $\Delta\delta = 0.089$  ppm) of racemic **1a** was observed using (*S*)-**2a** as the CSA at a 2:1 ratio of acid:amine, in C<sub>6</sub>D<sub>6</sub> at room temperature (Figure 3).



**Figure 3.** 0.50–3.50 ppm width spectrum of (*R,S*)-mexiletine in the presence of (*S*)-**2a** (r.t.; C<sub>6</sub>D<sub>6</sub>; 1:2 stoichiometry; 0.02 M; Bruker FT 300 MHz).

The use of chiral **2a** was suggested by the presence in the molecule of the structural characteristics commonly required for chiral molecules used as CSAs in NMR analyses.<sup>54a,h</sup> This compound, in fact, can be considered as a combination of the mandelic acid scaffold with the typical aryloxy moiety of the 2-phenoxypropionic acids.<sup>54c</sup> On the other hand, we had previously reported the application of (*S*)-**2a** for the direct determination of the enantiomeric excesses of some tocinide analogues<sup>60</sup> and 4-methylpiperidine derivatives with high  $\sigma$  receptors affinity.<sup>58</sup> In the NMR experiment related to racemic **1a** in the presence of (*S*)-**2a**, both methyl doublets and methine multiplets of **1a** were shifted to higher frequencies of 0.09 ppm and 0.15 ppm, respectively; a similar effect was also observed for the methyl singlets (0.12 ppm). This finding was probably related to a high percentage of diastereomeric salt formation, at working stoichiometry. In these conditions, the enantiomeric purity of chiral amine samples **1a–c** was determined by integrating the diastereomeric salt complex separated resonances (Table 2).

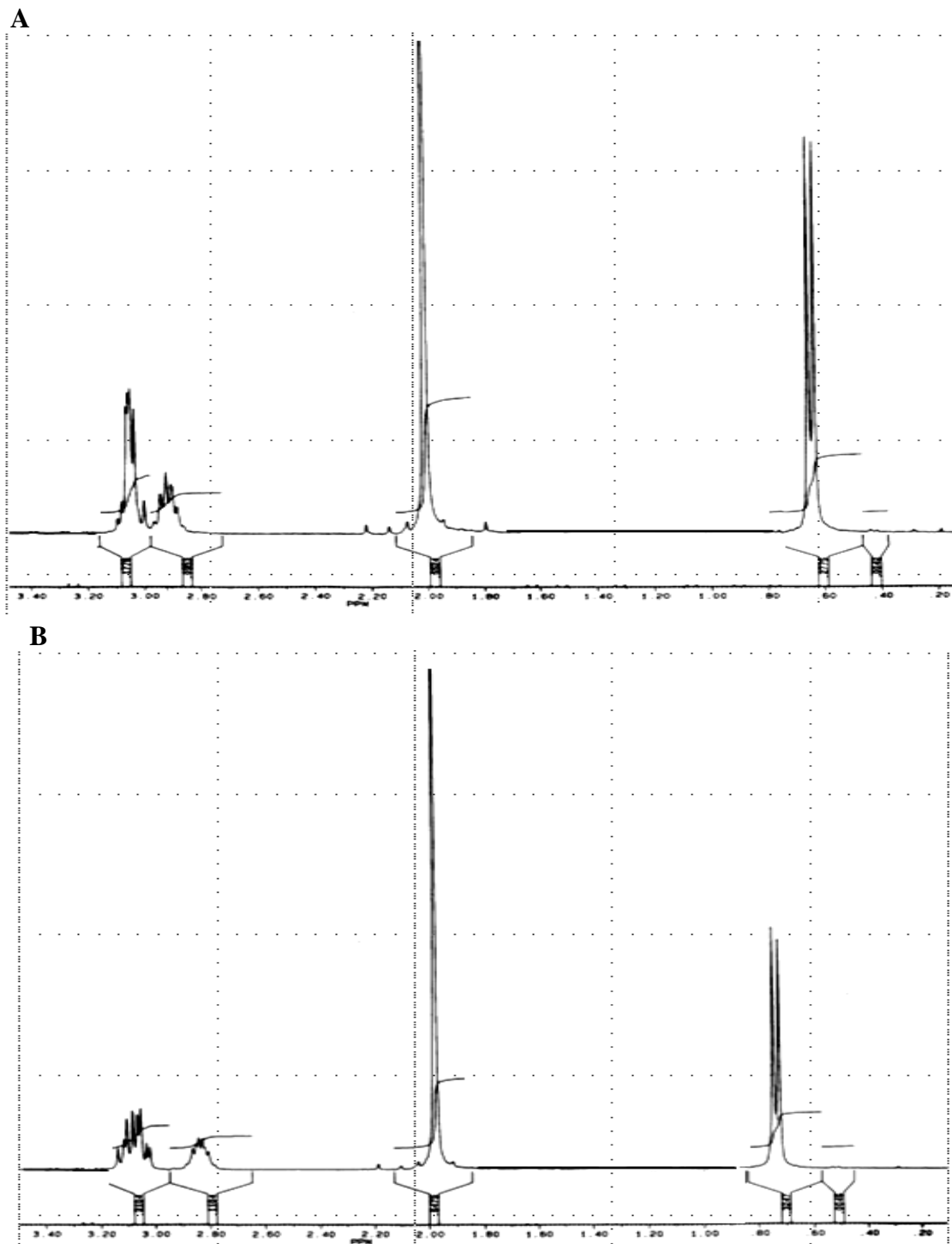
**Table 2.** Analysis of enantiomeric purity of (*R*)- and (*S*)-**1a–c** using (*S*)-**2a**

Entry	Amine	ee (%) <sup>a</sup>
1	1a	98
2	1b	98
3	1c	98

Concentration range 0.05–0.1 M; C<sub>6</sub>D<sub>6</sub>; r.t.; 2:1 acid:amine ratio; Bruker FT 300 MHz;  
<sup>a</sup> Enantiomeric composition was derived by comparing the integrals of the resonances due to the major and the minor diastereomers; estimated error  $\pm 1\%$ .

In Figure 4 the <sup>1</sup>H-NMR spectra (0.50–3.50 ppm width) of (*R*)- and (*S*)-mexiletine in the presence of (*S*)-**2a** are reported. In these spectra, a larger distance between the methyl doublet and the methine multiplet in the (*S*)-acid/(*R*)-amine complex (Figure 4A,  $\delta_{\text{CH}} = 2.92$  ppm and  $\delta_{\text{Me}} = 0.62$  ppm), than in the (*S,S*) complex (Figure 4B,  $\delta_{\text{CH}}$  and  $\delta_{\text{Me}}$  2.85 and 0.73 ppm, respectively) was observed. The same trend was observed also when using (*S*)-**2b**.

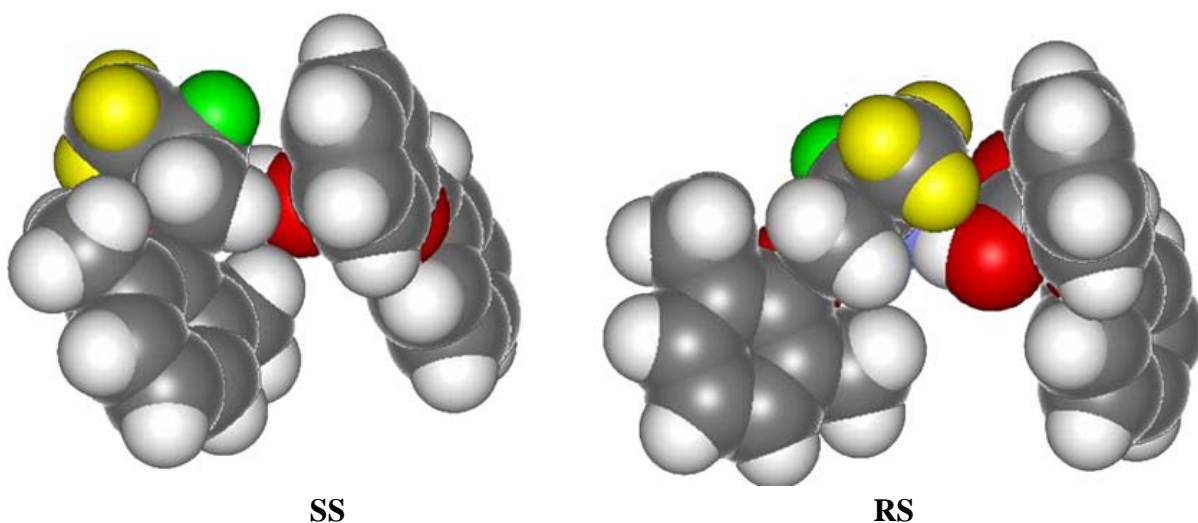
To explain this behaviour, we proposed a working model<sup>54h</sup> for both diastereomeric complexes of (*R*)- and (*S*)-**1a** with (*S*)-**2b** on the basis of the theoretically calculated predominant conformations. The theoretical calculations were accomplished by Wavefunction Spartan 5.1 (unix release). The structures were constructed by fragments and equilibrium geometries were calculated by MMFF starting with a systematic conformer distribution analysis. Conformers were grouped into families on the basis of relevant torsion angle values. The geometry optimization of the best (i. e., more stable) representative of each family was performed at Hartree-Fock 3-21G(\*) level.



**Figure 4.** 0.50–3.50 ppm width spectra of (*S*)-**2a**/*(R)*-mexiletine (**A**), and (*S*)-**2a**/*(S)*-mexiletine (**B**) (2:1 acid:amine ratio; r.t.; C<sub>6</sub>D<sub>6</sub>; 0.02 M; Bruker FT 300 MHz).

Indeed, in the more stable (*S*)-**2b**:(*R*)-**1a** complex conformation, the methyl on the stereogenic center of mexiletine was found closer than methine to the shielding cone of the

phenoxy moiety of the acid, whereas, in the (S,S) complex, an opposite situation would occur (Figure 5).



**Figure 5.** Space-filling model representation of (*S*)-**1a**:(*S*)-**2b** and (*R*)-**1a**:(*S*)-**2b** ionic complexes predominant conformations calculated by Wavefunction Spartan 5.1 (unix release) at HF-3.21G(\*) level (the methine and the methyl protons of **1a** alternately oriented toward the phenoxy ring of (*S*)-**2b** are shown in green and yellow, respectively; the oxygen atoms are shown in red). The distances of the acidic hydrogen from carboxyl oxygen and amine nitrogen in both (S,S) and (R,S) diastereomeric salt complexes were calculated [1.04 Å and 1.57 Å in the (S,S) complex, 1.03 Å and 1.61 Å in the (R,S) complex].

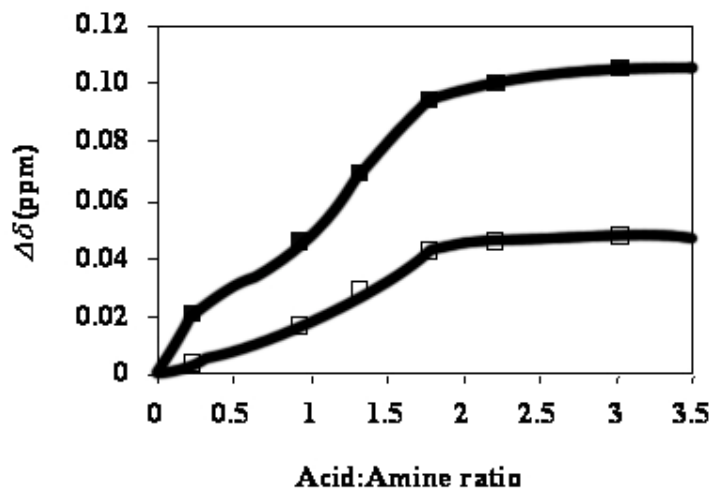
Another conformer, in which the same situation described above was obtained with the acid phenyl moiety taking the place of the phenoxy group, was found not far from the first in terms of energy. Thus, the anisotropic phenoxy and phenyl moieties of (*S*)-**2b** would lead to a larger shift in the position of the closest amine substituent, while the other substituent would be less perturbed, being more distant by both aromatic rings. In order to optimise the value of the chemical shift non-equivalence, the variation of  $\Delta\delta$  with acid:amine stoichiometry, solvent, concentration and temperature was studied. In addition, some modifications of the substituents on both aromatic rings of **2a** were introduced to give the chiral acids **2c–e** whose applicability as CSAs for mexiletine was studied in order to assess if their different electronic properties could affect the  $\Delta\delta$  of the tested amine.

**Effect of solvent.**  $^1\text{H-NMR}$  spectra were recorded in the conditions previously reported, both in  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ . The magnitude of  $\Delta\delta$  for all signals was slightly affected as the solvent changed from  $\text{C}_6\text{D}_6$  to  $\text{CDCl}_3$ . However, for methyl doublets used for ee determination of chiral **1a** in the presence of (*S*)-**2a**, higher values of  $\Delta\delta$  were obtained for spectra in  $\text{CDCl}_3$  ( $\Delta\delta = 0.101$



ppm) compared to spectra recorded in  $C_6D_6$  ( $\Delta\delta = 0.089$  ppm) at a 2:1 acid:amine ratio. Thus the most commonly used and less toxic  $CDCl_3$  appears to be the first choice solvent.

**Effect of stoichiometry.** Standard  $CDCl_3$  solution of tested acids and mexiletine were prepared and mixed to give the desired acid:amine ratios. With (*S*)-**2a**, the shift non-equivalence for the methyl doublet and methyl singlet resonances increased in the range 0.2–3:1 acid to amine ratio (Figure 6).



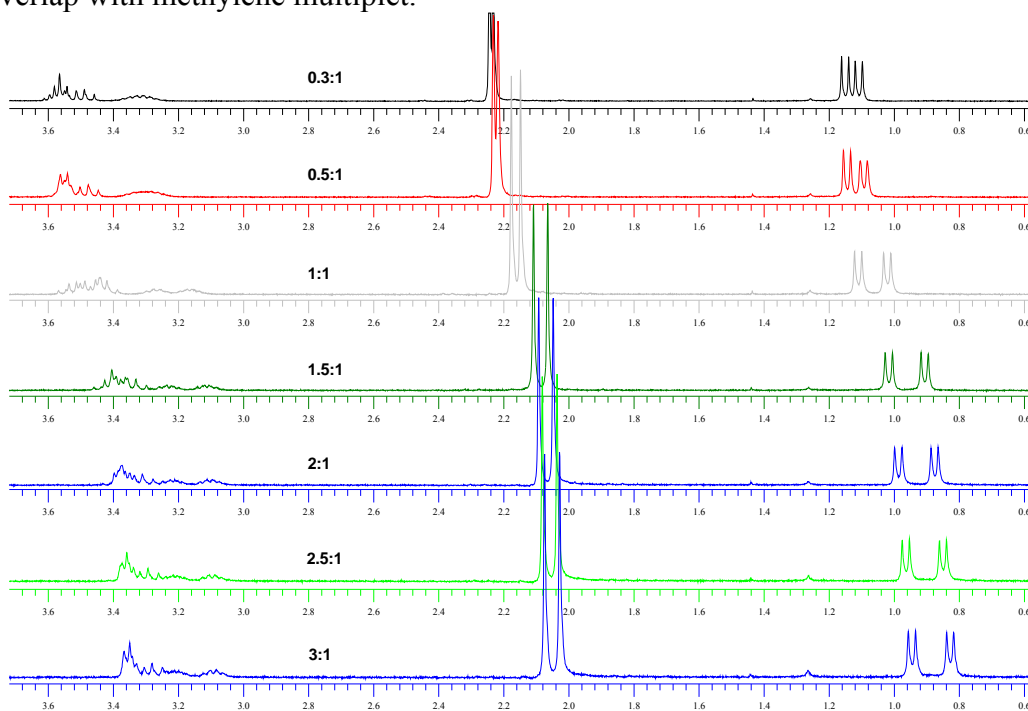
**Figure 6.** Variation of  $\Delta\delta$  with stoichiometry for the methyl doublets (black squares) and the methyl singlets (white squares) of (*R,S*)-mexiletine in the presence of (*S*)-**2a**.

A similar behaviour was observed, also, with the (*S*) isomers of the analogues **2b–e** (Table 3) and it is probably due to complete formation of acid:amine complexes as shown from the concomitant shift to higher frequencies of all signals of **1a** (Figure 7).

**Table 3.** Variation of  $\Delta\delta$  with stoichiometry for (*R,S*)-mexiletine in the presence of acids **2a–e** in the (*S*) configuration

Entry	Acid	Acid:Amine ratio	$\Delta\delta$ CH <sub>3</sub>	$\Delta\delta$ CH	$\Delta\delta$ CH <sub>3</sub> Ar
			ppm	ppm	ppm
1	<b>(S)-2a</b>	1	0.072	0.085	0.031
2		1.5	0.096	0.092	0.044
3		2	0.101	0.090	0.046
4	<b>(S)-2b</b>	1	0.091	0.101	0.029
5		1.5	0.110	0.119	0.045
6		2	0.114	0.110	0.046
7	<b>(S)-2c</b>	1	0.101	0.098	0.034
8		1.5	0.106	0.101	0.038
9		2	0.106	0.101	0.038
10	<b>(S)-2d</b>	1	0.079	a	0.026
11		1.5	0.095	a	0.039
12		2	0.091	a	0.035
13	<b>(S)-2e</b>	1	0.096	a	0.034
14		1.5	0.110	a	0.035
15		2	0.110	a	0.035

Concentration range 0.02–0.05 M; CDCl<sub>3</sub>; r.t.; Varian-Mercury 300 MHz; a) not determinable due to overlap with methylene multiplet.

**Figure 7.** Spectra (0.50–3.50 ppm width) of (*S*)-**2b**/*(R,S)*-mexiletine (0.3–3:1 ratio; r.t.; 0.02–0.05 M; CDCl<sub>3</sub>; Varian-Mercury 300 MHz).

Maximum values of  $\Delta\delta$  were observed in the range 1.5–2:1 acid to amine ratio remaining substantially unchanged at higher acid concentrations. The data reported in Table 3 shows that the substituents introduced on the aromatic rings of these acids exert little influence on **1a**  $\Delta\delta$ .

**Effect of concentration.** Proton NMR spectra for the complexes of (*S*)-**2a** with racemic mexiletine were recorded in  $\text{CDCl}_3$  in the concentration range 0.005–0.1 M at a 1.5:1 acid:amine ratio. For all observed resonances,  $\Delta\delta$  remained unchanged up to 0.01 M (Table 4), but decreased for concentrated solutions.

**Table 4.** Variation of  $\Delta\delta$  with concentration for the diastereomeric complexes of (*S*)-**2a** and racemic mexiletine

Entry	Concentration (M)	$\Delta\delta$ CH <sub>3</sub>	$\Delta\delta$ CH <sub>3</sub> Ar
		ppm	ppm
1	0.100	0.071	0.032
2	0.050	0.080	0.035
3	0.010	0.096	0.041
4	0.005	0.096	0.041

Acid: Amine ratio was 1.5:1;  $\text{CDCl}_3$ ; r.t.; Varian-Mercury 300 MHz spectrometer.

Such behaviour may be attributed to aggregation of the ion-pairs of the diastereomeric salts.<sup>54e,h</sup>

**Effect of temperature.** The temperature dependence of  $\Delta\delta$  for the complexes derived from (*S*)-**2a** and racemic mexiletine in  $\text{CDCl}_3$  was measured in the range from +25°C to –30°C on a 200 MHz spectrometer. As shown in Table 5, only the  $\Delta\delta$  value related to the methyl on the stereogenic center of **1a** was slightly increased as the temperature was lowered to –10°C; slightly lower values of  $\Delta\delta$  were obtained with a further decrease of the temperature.

**Table 5.** Variation of  $\Delta\delta$  with temperature for the diastereomeric complexes of (*S*)-**2a** and racemic mexiletine

Entry	Temperature (°C)	$\Delta\delta$ CH <sub>3</sub>	$\Delta\delta$ CH <sub>3</sub> Ar
		ppm	ppm
1	r.t.	0.101	0.050
2	0	0.113	0.051
3	–10 <sup>a</sup>	0.113	0.053
4	–20 <sup>a</sup>	0.110	0.053
5	–30 <sup>a</sup>	0.095	0.053

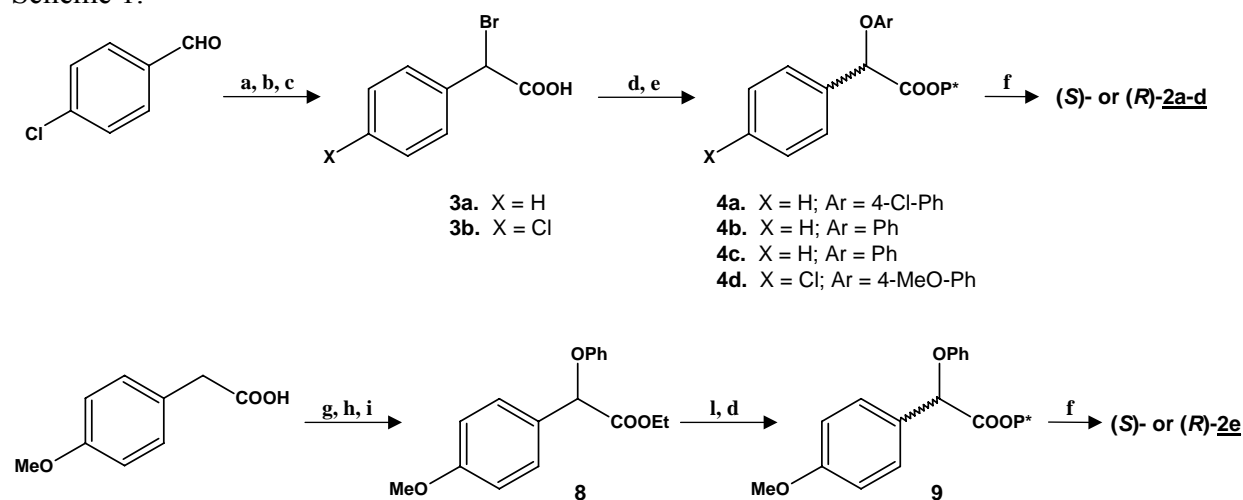
Acid:Amine ratio was 2:1; concentration range 0.02–0.03 M;  $\text{CDCl}_3$ ; Varian XL 200 MHz.

<sup>a</sup> Crystallization occurred.

This behaviour can be correlated to the stabilization of specific conformations of the diastereomeric complexes. For temperatures lower than  $-10^{\circ}\text{C}$ , however, badly resolved signals and crystallization of salts made the observed values less reliable.

## Chemistry

Optically active compounds **2a–e** were prepared according to the procedure described in Scheme 1.



P\* = (*R*)- or (*S*)-pantolactone

**Scheme 1.** (a) TMSCN,  $\text{ZnI}_2$ ,  $\text{CH}_2\text{Cl}_2$ . (b) concd. HCl, glacial AcOH. (c) 48% HBr, concd.  $\text{H}_2\text{SO}_4$ . (d) (*R*)- or (*S*)-pantolactone, DCC, DMAP, dry THF. (e) ArOH, NaH, *n*-tetrapentylammonium iodide, dry THF,  $-10^{\circ}\text{C}$ . (f)  $\text{H}_2\text{O}_2$ , LiOH, THF/ $\text{H}_2\text{O}$  4:1. (g) abs EtOH,  $\text{H}_2\text{SO}_4$ . (h) NBS, 33% HBr in AcOH,  $\text{CCl}_4$ . (i) PhONa, abs EtOH. (l) 1 N NaOH/THF.

Commercially available 2-bromo-2-phenylacetic acid (**3a**, X = H) was condensed with (*R*)- or (*S*)-pantolactone by a 1,3-dicyclohexylcarbodiimide (DCC) coupling in the presence of catalytic amounts of dimethylaminopyridine (DMAP) and the esters so obtained reacted, under Durst conditions,<sup>61</sup> with the suitable aryloxides to give compounds **4a–c** with high diastereoselectivity. After purification by column chromatography and crystallization, the esters were hydrolyzed in mild basic conditions using 35%  $\text{H}_2\text{O}_2$  and LiOH to afford the desired acids **2a–c** with high ee (>95%) after recrystallization. The synthesis of **2d** from **4d** followed the same pathways, but needed the additional preparation of the starting compound 2-bromo-2-(4-chlorophenyl)acetic acid (**3b**, X = Cl) which was obtained by a  $\text{ZnI}_2$ -catalyzed condensation of 4-chlorobenzaldehyde and trimethylsilylcyanide (TMSCN), followed by acidic hydrolysis and  $\alpha$ -bromination with 48% HBr in concentrated  $\text{H}_2\text{SO}_4$ . The acid **2e** was synthesized as a racemic mixture starting from commercially available 4-methoxyphenylacetic acid which was esterified with absolute EtOH and  $\alpha$ -brominated with NBS in the presence of catalytic amounts of 33%

HBr in acetic acid. The resulting  $\alpha$ -bromo ethyl ester was condensed with preformed sodium phenate in absolute EtOH to give ethyl 2-(4-methoxyphenyl)-2-phenoxyacetate (**8**), which was hydrolyzed under basic conditions to afford (*R,S*)-**2e**. The condensation with (*R*)- or (*S*)-pantolactone, carried out in the same conditions reported above, yielded the corresponding diastereomeric esters **9**. A single crystallization of this mixture was sufficient to separate one of the two diastereomers which was hydrolyzed under mild basic conditions to give the desired acid **2e** with high ee (99%) after recrystallization.

The absolute configuration of **2b** was established by chemical correlation with **2a** whose absolute configuration had been previously determined.<sup>57b</sup> For this purpose, a Pd-catalyzed dehalogenation of (*S*)-**2a** was carried out obtaining (+)-**2b** with the same (*S*) configuration. For analogues **2c–e**, the configuration assessment was accomplished by chiroptical properties determination. As for (*S*)-**2a**, in fact, the CD curves of the dextroisomers show a small negative fine structure Cotton effect in the aromatic absorption region and a strong positive Cotton effect around 230 nm, allowing to assign the (*S*) configuration to all of them.

## Conclusions

The chiral 2-aryloxy-2-arylacetic acids **2a–e** induce high <sup>1</sup>H-NMR chemical shift non-equivalence in their 1.5–2:1 diastereomeric salt complexes with mexiletine **1a** and its analogues **1b,c** in the most common deuterated solvent (CDCl<sub>3</sub>) at room temperature. This permits the direct analysis of the enantiomeric purity of these amines in a quick and simple method which does not require any deconvolution software, and allows a ready recovery of the CSA sample by an acid/base wash. In addition, these acids are easy to be synthesized in high yields and enantiomeric purity. Their applicability as effective chiral solvating agents in the <sup>1</sup>H-NMR analysis of the ee of other pharmaceutically relevant chiral amines is now in progress.

## Experimental Section

**General Procedures.** Column chromatography was performed on ICN silica gel 60Å (63–200  $\mu$ m) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Mass spectra were recorded with a HP GC-MS 6890-5973 MSD spectrometer, electron impact 70 eV, equipped with HP chemstation. For acid samples, a prior derivatization to methyl esters was carried out by reaction with an ethereal solution of diazomethane. <sup>1</sup>H-NMR spectra were recorded in C<sub>6</sub>D<sub>6</sub> or in CDCl<sub>3</sub> either on Varian EM-390 (when 90 MHz is indicated), using tetramethylsilane as internal standard, or on Varian-Mercury 300 (300 MHz) and Bruker AM 300 WB (300 MHz) spectrometers. The low temperature NMR experiments were recorded on a Varian XL 200 (200 MHz) spectrometer. Chemical shifts are reported in parts per million ( $\delta$ ). Microanalyses of **2a–e** were carried out with

a Eurovector Euro EA 3000 model analyzer; the analytical results are within  $\pm 0.4\%$  of theoretical values. Optical rotations were measured with a Perkin-Elmer 341 polarimeter at room temperature (20°C): concentrations are expressed as g/100 ml. Analytical liquid chromatography was performed on a PE chromatograph equipped with a Rheodyne 7725i model injector, a 785A model UV/Vis detector, a series 200 model pump and NCI 900 model interface. The enantiomeric excesses of the optically active **2a–e** were determined by HPLC analysis of the acids as such or after derivatization to methyl esters by reaction with an ethereal solution of diazomethane, on Chiralcel AD, AS or OD-R columns (4.6 mm i.d. x 250 mm, Daicel Chemical Industries, Ltd., Tokio, Japan). The CD curves were measured on a J-810 model JASCO spectropolarimeter. Chemicals were from Aldrich or Acros and were used without any further purification. The theoretical calculations were accomplished by Wavefunction Spartan 5.1 (unix release). The structures were constructed by fragments and equilibrium geometries were calculated by MMFF starting with a systematic conformer distribution analysis. Conformers were grouped into families on the basis of relevant torsion angle values. The geometry optimization of the best (i. e., more stable) representative of each family was performed at the Hartree-Fock 3-21G(\*) level.

**2-Bromo-2-(4-chlorophenyl)acetic acid (3b).** Trimethylsilylcyanide (12 mmol) was added dropwise to a stirred solution of 4-chlorobenzaldehyde (10 mmol) and  $\text{ZnI}_2$  (0.2 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL). The reaction mixture was stirred at room temperature for 2 h. The organic phase was washed with 5%  $\text{NaHCO}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$  and evaporated to dryness affording the desired cyanohydrin as a pale yellow oil in quantitative yields.

To a stirred solution of this cyanohydrin (5.10 g; 21.3 mmol) in glacial acetic acid (40 mL), concentrated HCl (75 mL) was added and the reaction mixture refluxed for 2 h. Afterwards, it was quenched with a NaCl saturated solution and extracted with  $\text{Et}_2\text{O}$ . The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$  and evaporated to dryness affording a viscous yellow oil (3.88 g) that, by crystallization from  $\text{CHCl}_3$ /hexane, afforded the pure hydroxyacid as a white solid (2.35 g; 12.6 mmol; 59% yield). GC/MS (methyl ester),  $m/z$ : 202 ( $\text{M}^+ + 2$ , 3), 200 ( $\text{M}^+$ , 9), 141 (100);  $^1\text{H-NMR}$  (90 MHz;  $\text{CDCl}_3$ ): d 5.2 (s, 1H, CH), 5.9–7.0 (bs, 2H, OH + COOH,  $\text{D}_2\text{O}$  exchanged), 7.1–7.8 (m, 4H, aromatic).

48% HBr (3.5 mL) was carefully added to a stirred and cooled to 0°C suspension of this hydroxyacid (2.46 g; 13.2 mmol) in concentrated  $\text{H}_2\text{SO}_4$  (3 mL). The reaction mixture was stirred at reflux for 4 h, then it was poured into ice and extracted with  $\text{Et}_2\text{O}$ . The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$  and evaporated to dryness affording a yellow solid (3.11 g) which was crystallized from  $\text{CHCl}_3$ /hexane affording the pure acid as a pale yellow solid (2.82 g; 11.4 mmol; 86% yield). GC/MS (methyl ester),  $m/z$ : 262 ( $\text{M}^+$ , 6), 183 (100);  $^1\text{H-NMR}$  (90 MHz;  $\text{CDCl}_3$ ): d 5.3 (s, 1H, CH), 7.2–7.6 (m, 4H, aromatic), 9.2 (bs, 1H, COOH,  $\text{D}_2\text{O}$  exchanged).

**General procedure for the preparation of the (*R*)-pantolactone esters of **3a** and **3b****

(*R*)-pantolactone (10 mmol), dimethylaminopyridine (DMAP; 0.1 mmol) and 1,3-dicyclohexylcarbodiimide (DCC; 10 mmol) were added, under N<sub>2</sub> atmosphere, to a stirred solution of the acid **3a** or **3b** (10 mmol) in anhydrous THF (40 mL). The reaction mixture was stirred at room temperature for 24 h, afterwards the precipitate was filtered off and the organic phase was evaporated to dryness, dissolved in ethyl acetate (50 mL) and washed two times with H<sub>2</sub>O, 3 N HCl and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness affording a yellow oil. The desired esters were obtained, as pale yellow oils, by column chromatography on silica gel (petroleum ether/ethyl acetate 9:1 as eluent).

**(*R*)-Pantolactone ester of 3a.** 75% yield; GC/MS, *m/z*: 326 (M<sup>+</sup>, 3), 247 (100).

**(*R*)-Pantolactone ester of 3b.** 81% yield; GC/MS, *m/z*: 360 (M<sup>+</sup>, 9), 152 (100).

**General procedure for the preparation of 4a–d**

The appropriate phenol (13 mmol) was added, under N<sub>2</sub> atmosphere, to a stirred and cooled to 0°C suspension of 95% NaH powder (11 mmol) in anhydrous THF (50 mL). Stirring continued until evolution of hydrogen ceased. The resulting solution was then added dropwise, under N<sub>2</sub> atmosphere, to a stirred and cooled to –10°C anhydrous THF solution (110 mL) of the (*R*)-pantolactone esters of racemic **3a** or **3b** (10 mmol) and *n*-tetrapentylammonium iodide (2 mmol). The reaction mixture was stirred at –10°C for 7 h and then it was quenched with a saturated solution of NaCl (30 mL). The organic layer was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed two times with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness affording the crude esters as viscous yellow oils. Diastereomeric excesses were in the range 80–95% as determined by GC/MS or <sup>1</sup>H-NMR (300 MHz) of the crude reaction mixture. The crude products were then purified by column chromatography on silica gel using petroleum ether/ethyl acetate 8:2 as eluant. Compounds **4b–d** were white solid (**4d** solidified on standing) and were further enriched by recrystallization from hexane/CHCl<sub>3</sub> to give the major isomer. Compound **4a** was a colorless oil which was used in the next step without any further purification.

**(*S*)-2-(4-Chlorophenoxy)-2-phenylacetic acid (*R*)-pantolactone ester (**4a**).** 67% yield; GC/MS, *m/z*: 374 (M<sup>+</sup>, 18), 247 (100).

**(*S*)-2-Phenoxy-2-phenylacetic acid (*R*)-pantolactone ester (**4b**).** 49% yield; GC/MS, *m/z*: 340 (M<sup>+</sup>, 21), 247 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>): δ 0.69 (s, 3H, CH<sub>3</sub>), 0.91 (s, 3H, CH<sub>3</sub>), 3.93 (s, 2H, CH<sub>2</sub>), 5.39 (s, 1H, CHOCO), 5.81 (s, 1H, CHPh), 6.88–7.92 (m, 10H, aromatic).

**(*S*)-2-(4-Methoxyphenoxy)-2-phenylacetic acid (*R*)-pantolactone ester (**4c**).** 70% yield; <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>): δ 0.71 (s, 3H, CH<sub>3</sub>), 0.93 (s, 3H, CH<sub>3</sub>), 3.74 (s, 3H, CH<sub>3</sub>O), 3.94 (s, 2H, CH<sub>2</sub>), 5.38 (s, 1H, CHOCO), 5.76 (s, 1H, CHPh), 6.78–7.64 (m, 9H, aromatic).

**(*S*)-2-(4-Chlorophenyl)-2-phenoxyacetic acid (*R*)-pantolactone ester (**4d**).** 36% yield; GC/MS, *m/z*: 374 (M<sup>+</sup>, 7), 281 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>): δ 0.74 (s, 3H, CH<sub>3</sub>), 0.93 (s,

3H, CH<sub>3</sub>), 3.95 (s, 2H, CH<sub>2</sub>), 5.37 (s, 1H, CHOCO), 5.78 (s, 1H, CHPh), 6.96–7.65 (m, 9H, aromatic).

**Ethyl 2-(4-methoxyphenyl)-2-phenoxyacetate (8).** A solution of 4-methoxyphenylacetic acid (5.0 g; 30 mmol) in absolute EtOH (150 mL) and 0.2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was refluxed under stirring for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The organic layer was washed with NaHCO<sub>3</sub> saturated solution and brine, then dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness affording the ethyl 4-methoxyphenylacetate (4.55 g; 78% yield) as a pale yellow oil.

To a suspension of this ester in CCl<sub>4</sub> (60 mL), *N*-bromosuccinimide (NBS; 4.14 g; 23.4 mmol) and one drop of 33% HBr in acetic acid (Sigma-Aldrich) were added. The resulting mixture was stirred with reflux for 2.5 h, afterwards the precipitate was filtered off and the solvent was evaporated to dryness affording the desired ethyl 2-bromo-2-(4-methoxyphenyl)acetate as a yellow oil in quantitative yield (6.43 g). GC/MS, *m/z*: 272 (M<sup>+</sup>, 3), 193 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>): δ 1.28 (t, 3H, CH<sub>3</sub>), 3.80 (s, 3H, CH<sub>3</sub>O), 4.11–4.42 (m, 2H, CH<sub>2</sub>), 5.33 (s, 1H, CH), 6.82–7.52 (m, 4H, aromatic).

To a stirred solution of this bromoester (6.43 g; 23.2 mmol) in absolute EtOH (85 mL), was added dropwise sodium phenate, prepared by adding phenol (2.23 g; 23.6 mmol) to a solution of metallic Na (0.54 g; 23.6 mmol) in absolute EtOH (40 mL). The reaction mixture was stirred with reflux for 2 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the organic phase was washed with 1 N NaOH and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated to dryness affording the desired ester as a colorless oil which was chromatographed on a silica gel column using petroleum ether/ethyl acetate 9:1 as eluant (3.0 g; 44 % yield). GC/MS, *m/z*: 286 (M<sup>+</sup>, 1), 193 (100).

**(S)-2-(4-Methoxyphenyl)-2-phenoxyacetic acid (R)-pantolactone ester (9).** A solution of the ethyl ester **5** (3.0 g; 10.4 mmol) in THF (90 mL) and 1 N NaOH (90 mL) was stirred at room temperature for 4 h. The organic layer was removed under reduced pressure and the aqueous phase was acidified with 6 N HCl and extracted with Et<sub>2</sub>O. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness affording the 2-(4-methoxyphenyl)-2-phenoxyacetic acid as a white solid (2.60 g; 97% yield) which was used in the next step without any further purification. GC/MS (methyl ester with diazomethane), *m/z*: 272 (M<sup>+</sup>, 1), 179 (100).

The corresponding (*R*)-pantolactone ester was obtained as reported above starting from 2.60 g (10.1 mmol) of the acid, (*R*)-pantolactone (1.86 g; 14.3 mmol), DCC (2.80 g; 13.57 mmol) and DMAP (0.165 g; 1.36 mmol) in anhydrous THF (50 mL). The crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 8:2 as eluant obtaining a white solid (2.72 g; 72% yield) which, after a single crystallization from hexane/CHCl<sub>3</sub>, afforded the desired isomer **6** (0.48 g). <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>): δ 0.72 (s, 3H, CH<sub>3</sub>), 0.93 (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, CH<sub>3</sub>O), 3.95 (s, 2H, CH<sub>2</sub>), 5.38 (s, 1H, CHOCO), 5.74 (s, 1H, CHPh), 6.90–7.58 (m, 9H, aromatic).



**(S)-2-Aryloxy-2-arylacetic acids (2a–e).** To a stirred and cooled to 0°C suspension of the 2-aryloxy-2-arylacetic acids pantolactone esters (2.8 mmol) in THF/H<sub>2</sub>O (4:1, 40 mL) were added 35% v/v H<sub>2</sub>O<sub>2</sub> (1.1 mL) and a solution of LiOH·H<sub>2</sub>O (5.6 mmol) in H<sub>2</sub>O (15 mL). The reaction mixture was stirred at 0°C for 6 h. THF was evaporated in vacuo and the aqueous phase was acidified with 6 N HCl and extracted with Et<sub>2</sub>O. The combined organic layers were washed two times with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness affording the desired acids as white solids which were purified by recrystallization from suitable solvents.

**(+)-(S)-2-(4-Chlorophenoxy)-2-phenylacetic acid (2a).** 50% yield; mp = 117–8°C (CHCl<sub>3</sub>/hexane); [ $\alpha$ ]<sub>D</sub> = +122 (*c* 1, methanol); GC/MS (methyl ester), *m/z*: 276 (M<sup>+</sup>, 21), 149 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>):  $\delta$  5.59 (s, 1H, CH); 6.81–7.61 (m, 10H, 9 aromatic + 1 COOH D<sub>2</sub>O exchanged); ee = 99% (HPLC: Chiralcel AD column; hexane/*i*-propanol/TFA 90:9.5:0.5; flow rate 0.5 mL/min; detection 280 nm). Anal. Calcd. for C<sub>14</sub>H<sub>11</sub>ClO<sub>3</sub> (262.69): C, 64.01; H, 4.22. Found: C, 64.31; H, 4.18.

**(+)-(S)-2-Phenoxy-2-phenylacetic acid (2b).** 68% yield; mp = 117–8°C (hexane); [ $\alpha$ ]<sub>D</sub> = +120 (*c* 1.1, methanol); GC/MS (methyl ester), *m/z*: 242 (M<sup>+</sup>, 20), 121 (100); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  5.66 (s, 1H, CH); 6.95–7.61 (m, 10H, aromatic); 9.12 (bs, 1H, COOH, D<sub>2</sub>O exchanged); ee = 97% (HPLC, methyl ester: Chiralcel OD-R column; CH<sub>3</sub>CN/H<sub>2</sub>O 80:20; flow rate 0.4 mL/min; detection 254 nm). Anal. Calcd. for C<sub>14</sub>H<sub>12</sub>O<sub>3</sub> (228.25): C, 73.67; H, 5.30. Found: C, 74.01; H, 5.48.

**(+)-(S)-2-(4-Methoxyphenoxy)-2-phenylacetic acid (2c).** 45% yield; mp = 108–9°C (CHCl<sub>3</sub>/hexane); [ $\alpha$ ]<sub>D</sub> = +123 (*c* 1, methanol); GC/MS, (methyl ester) *m/z*: 272 (M<sup>+</sup>, 47, 1), 123 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>):  $\delta$  3.74 (s, 3H, CH<sub>3</sub>O); 5.54 (s, 1H, CH); 6.62 (bs, 1H, COOH D<sub>2</sub>O exchanged); 6.70–7.74 (m, 9H, aromatic); ee = 97% (HPLC, methyl ester: Chiralcel AS column; hexane/*i*-propanol 98:2; flow rate 0.5 mL/min; detection 280 nm). Anal. Calcd. for C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> (258.28): C, 69.76; H, 5.46. Found: C, 70.11; H, 5.58.

**(+)-(S)-2-(4-Chlorophenyl)-2-phenoxyacetic acid (2d).** 57% yield; mp = 127–8°C (CHCl<sub>3</sub>/hexane); [ $\alpha$ ]<sub>D</sub> = +119 (*c* 1, methanol); GC/MS, (methyl ester) *m/z*: 276 (M<sup>+</sup>, 15), 183 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>):  $\delta$  5.60 (s, 1H, CH); 6.90–7.58 (m, 9H, aromatic); 8.01 (bs, 1H, COOH D<sub>2</sub>O exchanged); ee = 96% (HPLC, methyl ester: Chiralcel OD-R column; CH<sub>3</sub>CN/H<sub>2</sub>O 80:20; flow rate 0.4 mL/min; detection 254 nm). Anal. Calcd. for C<sub>14</sub>H<sub>11</sub>ClO<sub>3</sub> (262.69): C, 64.01; H, 4.22. Found: C, 64.18; H, 4.28.

**(+)-(S)-2-(4-Methoxyphenyl)-2-phenoxyacetic acid (2e).** 49% yield; mp = 113–4°C (Ethyl acetate/hexane); [ $\alpha$ ]<sub>D</sub> = +162 (*c* 1, methanol); GC/MS, (methyl ester) *m/z*: 272 (M<sup>+</sup>, 1), 179 (100); <sup>1</sup>H-NMR (300 MHz; CDCl<sub>3</sub>):  $\delta$  3.80 (s, 3H, CH<sub>3</sub>O); 5.59 (s, 1H, CH); 6.88–7.54 (m, 9H, aromatic); 8.11 (bs, 1H, COOH D<sub>2</sub>O exchanged). ee = 99% (HPLC, methyl ester: Chiralcel AD column; hexane/*i*-propanol 98:2; flow rate 0.5 mL/min; detection 280 nm). Anal. Calcd. for C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> (258.28): C, 69.76; H, 5.46. Found: C, 69.56; H, 5.38.

**Assignment of the absolute configuration of 2b by dehalogenation of (+)-(S)-2-(4-chlorophenoxy)-2-phenylacetic acid.** Acid (+)-(S)-2a (0.602 g; 2.3 mmol) was dissolved in

95% C<sub>2</sub>H<sub>5</sub>OH (20 mL) and hydrogenated at atmospheric pressure and room temperature in the presence of 5% Pd/C (0.1 gr) during 36 h. The catalyst was filtered off through a celite pad, and the solvent was evaporated in vacuo to give a crude residue, which was dissolved in CHCl<sub>3</sub> and extracted with NaHCO<sub>3</sub>. The aqueous phase was acidified with 1 N HCl and extracted with CHCl<sub>3</sub>, the organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure to give the desired acid as white solid, which was recrystallized by CHCl<sub>3</sub>/hexane (0.110 g; 21% yield). GC/MS, *m/z*: (methyl ester) 242 (M<sup>+</sup>, 20), 121 (100); [α]<sub>D</sub> = +118 (*c* 1, methanol).

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## References

1. Fenster, P. E.; Comess, K. A. *Pharmacotherapy* **1986**, *6*, 1.
2. Rüdell, R.; Lehmann-Horn, F. *Physiol. Rev.* **1985**, *65*, 310.
3. Kalso, E.; Tramer, M. R.; McQuay, H. J.; Moore, R. A. *Eur. J. Pain* **1998**, *2*, 3.
4. (a) Turgeon, J.; Uprichard, A. C. G.; Bélanger, P. M.; Harron, D. W. G.; Grech-Bélanger, O. *J. Pharm. Pharmacol.* **1991**, *43*, 630. (b) Hill, R. J.; Duff, H. J.; Sheldon, R. S. *Mol. Pharmacol.* **1988**, *34*, 659. (c) De Luca, A.; Natuzzi, F.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1995**, *352*, 653. (d) De Luca, A.; Natuzzi, F.; Falcone, G.; Duranti, A.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1997**, *356*, 777. (e) Clarke, D. E.; Lyles, G. A.; Calligham, B. A. *Biochem. Pharmacol.* **1982**, *31*, 27. (f) Sinnott, C. J.; Garfield, J. M.; Zeitlin, A.; Teo, S.; Wu, M. D.; Chen, J. H.; Shafer, S. L.; Strichartz, G. R. *J. Pain* **2000**, *1*, 128.
5. Wu, W. P.; Nordmark, J.; Wiesenfeld-Hallin, Z. *Eur. J. Pain* **2000**, *4*, 409.
6. (a) De Luca, A.; Pierno, S.; Natuzzi, F.; Franchini, C.; Duranti, A.; Lentini, G.; Tortorella, V.; Jockusch, H.; Conte Camerino, D. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 93. (b) Desaphy, J.-F.; Conte Camerino, D.; Franchini, C.; Lentini, G.; Tortorella, V.; De Luca, A. *Br. J. Pharmacol.* **1999**, *128*, 1165. (c) De Luca, A.; Natuzzi, F.; Desaphy, J.-F.; Loni, G.; Lentini, G.; Franchini, C.; Tortorella, V.; Conte Camerino, D. *Mol. Pharmacol.* **2000**, *57*, 268.
7. (a) Franchini, C.; Cellucci, C.; Corbo, F.; Lentini, G.; Scilimati, A.; Tortorella, V.; Stasi, F. *Chirality* **1994**, *6*, 590. (b) Carocci, A.; Franchini, C.; Lentini, G.; Loiodice, F.; Tortorella, V. *Chirality* **2000**, *12*, 103. (c) Carocci, A.; Catalano, A.; Corbo, F.; Duranti, A.; Amoroso, R.; Franchini, C.; Lentini, G.; Tortorella, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3619.

8. Hedeland, M.; Holmin, S.; Nygård, M.; Pettersson, C. *J. Chromatogr. A* **1999**, *864*, 1.
9. Loughhead, D. G.; Flippin, L. A.; Weikert, R. J. *J. Org. Chem.* **1999**, *64*, 3373.
10. (a) Lanchote, V. L.; Cesarino, E. J.; Santos, V. J.; Mere, Y. A. Jr.; Santos, S. R. C. *J. Chirality* **1999**, *11*, 29. (b) Lanchote, V. L.; Bonato, P. S.; Cesarino, E. J.; Mere, Y. A. Jr.; Santos, S. R. C. J.; Bertucci, C. *Chirality* **1997**, *9*, 732. (c) Santos, V. J.; Dreossi, S. A. C.; Cesarino, E. J.; Santos, S. R. C. J.; Lanchote, V. L. *Boll. Chim. Farm.* **1997**, *136*, 130. (d) Abolfathi, Z.; Bélanger, P. M.; Gilbert, M.; Rouleau, J. R.; Turgeon, J. *J. Chromatogr. B* **1992**, *579*, 366. (e) Labbé, L.; Abolfathi, Z.; Robitaille, N. M.; St-Maurice, F.; Gilbert, M.; Turgeon, J. *Ther. Drug Monit.* **1999**, *21*, 191. (f) Abolfathi, Z.; Fiset, C.; Gilbert, M.; Moerike, K.; Bélanger, P. M.; Turgeon, J. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1196. (g) Lanchote, V. L.; Cesarino, E. J.; Santos, V. J.; Moraes Junior, A. V.; Zanardi, A. M. T.; Santos, S. R. C. *J. Eur. J. Drug Metab. Pharmacokinet.* **1998**, *23*, 259.
11. Lanchote, V. L.; Bonato, P. S.; Dreossi, S. A. C.; Gonçalves, P. V. B.; Cesarino, E. J.; Bertucci, C. *J. Chromatogr. B* **1996**, *685*, 281.
12. Desai, D. M.; Gal, J. *J. Chromatogr.* **1993**, *629*, 215.
13. (a) Freitag, D. G.; Foster, R. T.; Coutts, R. T.; Pickard, M. A.; Pasutto, F. M. *Drug Metab. Dispos.* **1997**, *25*, 685. (b) Freitag, D. G.; Foster, R. T.; Coutts, R. T.; Pasutto, F. J. *J. Chromatogr. B* **1993**, *616*, 253.
14. Nishi, H.; Nakamura, K.; Nakai, H.; Sato, T. *J. Chromatogr. A* **1997**, *757*, 225.
15. Büschges, R.; Linde, H.; Mutschler, E.; Spahn-Langguth, H. *J. Chromatogr. A* **1996**, *725*, 323.
16. Booth, T. D.; Wainer, I. W. *J. Chromatogr. A* **1996**, *741*, 205.
17. Fieger, H.; Wainer, I. W. *J. Pharm. Biomed. Anal.* **1993**, *11*, 1173.
18. Aav, R.; Parve, O.; Pehk, T.; Claesson, A.; Martin, I. *Tetrahedron: Asymmetry* **1999**, *10*, 3033.
19. Büschges, R.; Devant, R.; Mutschler, E.; Spahn-Langguth, H. *J. Pharm. Biomed. Anal.* **1996**, *15*, 201.
20. Henriksson, H.; Jonsson, S.; Isaksson, R.; Pettersson, G. *Chirality* **1995**, *7*, 415.
21. Fukushima, T.; Kato, M.; Santa, T.; Imai, K. *Analyst* **1995**, *120*, 381.
22. Kleidernigg, O. P.; Lindner, W. *Chromatographia* **1997**, *44*, 465.
23. (a) Kwok, D. K. W.; Igwemezie, L.; Kerr, C. R.; McErlane, K. M. *J. Chromatogr. B* **1994**, *661*, 271. (b) Kwok, D. W.; Kerr, C. R.; McErlane, K. M. *Xenobiotica* **1995**, *25*, 1127.
24. Igwemezie, L.; Beatch, G. N.; Walker, M. J. A.; McErlane, K. M. *Xenobiotica* **1991**, *21*, 1153.
25. Josefsson, M.; Carlsson, B.; Norlander, B. *Chromatographia* **1993**, *37*, 129.
26. Grech-Bélanger, O.; Turgeon, J.; Gilbert, M. *J. Chromatogr. B* **1985**, *337*, 172.
27. Gal, J.; Desai, D. M.; Meyer-Lehnert, S. *Chirality* **1990**, *2*, 43.
28. (a) Igwemezie, L.; Kerr, C. R.; McErlane, K. M. *Xenobiotica* **1989**, *19*, 677. (b) McErlane, K. M.; Igwemezie, L.; Kerr, C. R. *J. Chromatogr. B* **1987**, *415*, 335. (c) McErlane, K. M.; Igwemezie, L.; Kerr, C. R. *Res. Commun. Chem. Pathol. Pharmacol.* **1987**, *56*, 141.
29. Uzunov, D.; Stoev, G. *J. Chromatogr.* **1993**, *645*, 233.
30. Yang, Q.; Sun, Z. P. *Yaoxue Xuebao* **1988**, *23*, 921; *Chem. Abstr.* **1989**, *110*, 219195.
31. Weller, O.; Schulze, J.; König, W. A. *J. Chromatogr. A* **1987**, *403*, 263.

32. Zhou, Y.; Sun, Z.-P. *J. Chromatogr. A* **1990**, *508*, 220.
33. Martin, E.; Quinke, K.; Spahn, H.; Mutschler, E. *Chirality* **1989**, *1*, 223.
34. Zhou, Y.; Sun, Z.-P. *Yaoxue Xuebao* **1991**, *26*, 701; *Chem. Abstr.* **1992**, *116*, 91538.
35. Wilson, J. M.; Hoch, K.; Blake, J.; Lindsay, W.; Smith, E. *Clin. Chem.* **1989**, *35*, 1178.
36. Zhou, Y.; Sun, Z. P.; Lin, K. *J. Liq. Chromatogr.* **1990**, *13*, 875.
37. (a) Wang, S.-J.; Mao, H.-Q.; Zeng, S. *J. Chin. Pharm. Sci.* **1998**, *7*, 201. (b) Wang, S.-J.; Mao, H.-Q.; Zeng, S. *Zhongguo Yaoxue Zazhi* **1997**, *32* (Suppl.), 6; *Chem. Abstr.* **1998**, *130*, 118998.
38. Grech-Bélanger, O.; Turgeon, J.; Gilbert, M. *Br. J. Clin. Pharmacol.* **1986**, *21*, 481.
39. Knoche, B.; Gehrcke, B.; König, W. A.; Wainer, I. W. *Chirality* **1996**, *8*, 30.
40. König, W. A.; Lutz, S.; Wenz, G.; Von der Bey, E. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988**, *11*, 506.
41. Li, G.; Lin, X.; Zhu, C.; Wu, P.; Guan, Y. *Fenxi Huaxue* **2000**, *28*, 1287; *Chem. Abstr.* **2000**, *134*, 9443.
42. Lin, X.; Li, G.; Zhu, C.; Wu, P.; Guang, Y. *Fenxi Ceshi Xuebao* **2000**, *19*, 20; *Chem. Abstr.* **2000**, *133*, 256916.
43. Li, G.; Lin, X.; Zhu, C.; Hao, A.; Guan, Y. *Anal. Chim. Acta* **2000**, *421*, 27.
44. Proksa, B.; Proksová, A. *Pharmazie* **2000**, *55*, 393.
45. Bjørnsdottir, I.; Hansen, S. H. *Chirality* **1995**, *7*, 219.
46. Kang, J.; Ou, Q. *J. Chromatogr. A* **1998**, *795*, 394.
47. Tanaka, Y.; Terabe, S. *Chromatographia* **1997**, *44*, 119.
48. Stalcup, A. M.; Gahm, K. H. *Anal. Chem.* **1996**, *68*, 1360.
49. Lin, X.; Li, G.; Zhu, C.; Wu, P.; Guan, Y. *Sepu* **2001**, *19*, 109; *Chem. Abstr.* **2001**, *135*, 127319.
50. Srinivas, N. R.; Igwemezie, L. N. *Biomed. Chromatogr.* **1992**, *6*, 163.
51. Weinberger, R. *Practical Capillary Electrophoresis*, Academic: New York, 2000; Chapters 2 and 10.
52. Otsuka, K.; Honda, S.; Kato, J.; Terabe, S.; Kimata, K.; Tanaka, N. *J. Pharm. Biomed. Anal.* **1998**, *17*, 1177.
53. Whitesides, G. M.; Lewis, D. W. *J. Am. Chem. Soc.* **1970**, *92*, 6979.
54. (a) Parker, D. *Chem. Rev.* **1991**, *91*, 1441. (b) Prestat, G.; Marchand, A.; Lebreton, J.; Guingant, A.; Pradère, J.-P. *Tetrahedron: Asymmetry* **1995**, *9*, 197. (c) Chinchilla, R.; Foubelo, F.; Nájera, C.; Yus, M. *Tetrahedron: Asymmetry* **1995**, *6*, 1877. (d) Parker, D.; Taylor, R. J. *Tetrahedron* **1987**, *43*, 5451. (e) Villani, F. J., Jr.; Costanzo, M. J.; Inners, R. R.; Mutter, M. S.; McClure, D. E. *J. Org. Chem.* **1986**, *51*, 3715. (f) Benson, S. C.; Cai, P.; Colon, M.; Haiza, M. A.; Tokles, M.; Snyder, J. K. *J. Org. Chem.* **1988**, *53*, 5335. (g) Baxter, C. A. R.; Richards, H. C. *Tetrahedron Lett.* **1972**, *32*, 3357. (h) Fulwood, R.; Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1994**, *1*, 57.
55. Aboul-Enein, H.-Y.; Médonné, S.; Rotchild, R. *Spectrosc. Lett.* **1991**, *24*, 909.
56. Aboul-Enein, H.-Y.; Rotchild, R.; Sinnema, A. *Spectrosc. Lett.* **1992**, *25*, 1367.

57. (a) Bettoni, G.; Ferorelli, S.; Loiodice, F.; Tangari, N.; Tortorella, V.; Gasparrini, F.; Misiti, D.; Villani, C. *Chirality* **1992**, *4*, 193. (b) Rangwala, S. M.; O'Brien, M. L.; Tortorella, V.; Longo, A.; Loiodice, F.; Noonan, D. J.; Feller, D. R. *Chirality* **1997**, *9*, 37.
58. Berardi, F.; Loiodice, F.; Fracchiolla, G.; Colabufo, N. A.; Perrone, R.; Tortorella, V. *J. Med. Chem.* **2003**, *46*, 2117.
59. Görög, S.; Gazdag, M. *J. Chromatogr. B* **1994**, *659*, 51.
60. Franchini, C.; Corbo, F.; Lentini, G.; Bruno, G.; Scilimati A.; Tortorella, V.; Conte-Camerino, D.; De Luca, A. *J. Med. Chem.* **2000**, *43*, 3792.
61. Koh, K.; Durst, T. *J. Org. Chem.* **1994**, *59*, 4683.