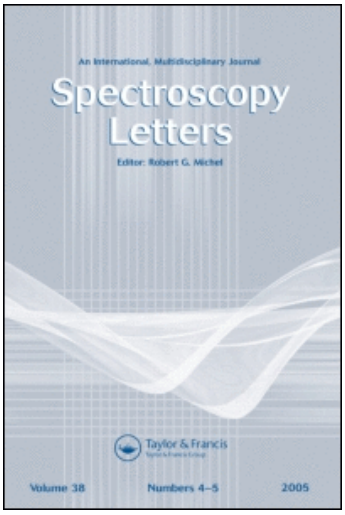

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ABSTRACT Studies of the perturbing effect of the trifluoromethylanthryl carbinol used as chiral solvating agent (CSA) upon the ^{1}H NMR spectra of chiral α-*O*-substituted *β*-hydroxylamides demonstrated the ability of this fluoroalcohol to afford diastereomeric solvates with these solutes. Thus, for all the tested amides, there is at least one possibility to proceed to their enantiomeric discrimination by ^{1}H NMR using CSA. The method was developed to determine (later and indirectly) a possible chiral recognition during *in vitro* enzymatic hydrolysis in locust biological tissues of *N*-acylaziridines conceived as proinsecticides of carboxylic acids, in view to eventually optimize their efficiency.

KEYWORDS β-hydroxylamides, chiral solvating agent, enantiomer, *N*-acylaziridines, optical purity analysis, stereomer

INTRODUCTION

During studies related to proinsecticides of carboxylic acids **3H** activated *via* enzymatic hydrolysis,[1–3] it was demonstrated that *N*-acylaziridine **1** is a particular amide structure quite convenient for reversibly masking an acyl moiety (see Fig. 1). The fluorinated *N*-acyl aziridine **1a** (used as a racemate), which exhibits noticeable biological activities against some insects or pests,[4] is efficiently hydrolyzed in locust fat-body and mesenteron, as are numerous other members of the series, to afford unmasked carboxylate $\mathbf{3}^-$ and protonated aziridine $\mathbf{2H}^+$ (see Fig. 1, pathway a).[3–7] It was also shown that this hydrolysis is catalyzed by enzymes of the α-chymotrypsin type.[5] Nevertheless as enantiomers of *N*-acylaziridines **1** can interact differently with enzymes, as previously observed for the metabolism of some chiral esters[8] in locust tissues, the stereochemical course of their enzymatic hydrolysis remains under interrogation. However, the possibility that an enantiomer of a given *N*-acyl aziridine **1** would be more efficiently activated in insects is a fact of primordial importance in view to optimize its efficiency as a proinsecticide.

This question can be solved using chiral analysis performed after an incomplete enzymatic hydrolysis of *N*-acylaziridine **1** racemates, which

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Figure 1

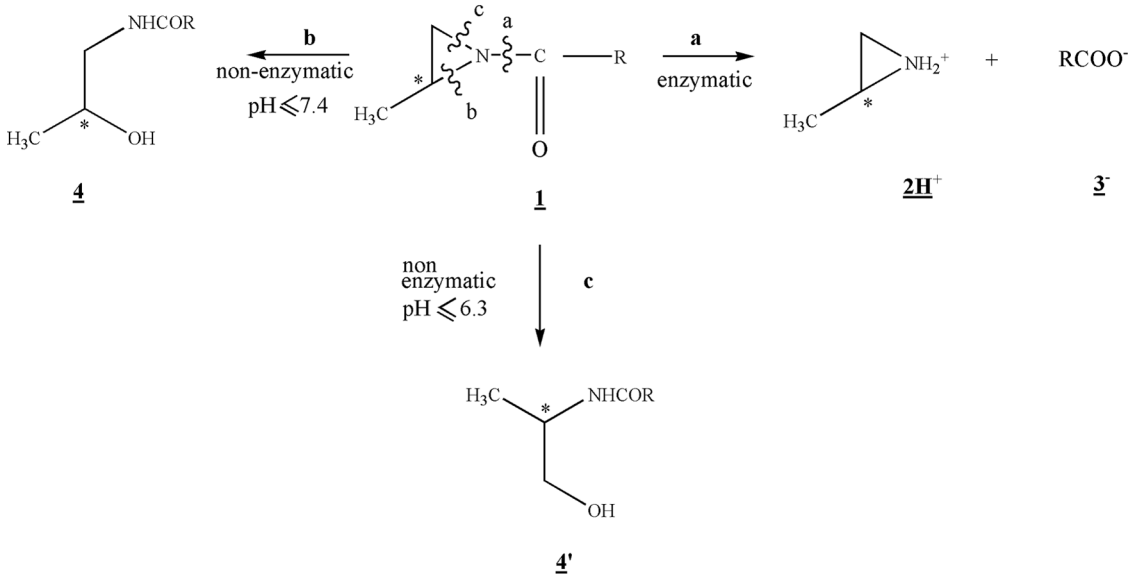

FIGURE 1 **Hydrolysis pathways for *N*-acylaziridines 1 [3–7] (*N*-acylaziridine 1a (R = pF-C_6H_4) exhibits interesting biological activities against some insect species[4];. The asterisk denotes the stereogenic center.) Pathway a: Exclusive enzymatic pathway in the presence of locust tissues (pH > 7.4). This pathway is very minor in a buffered solution at pH 7.8 and becomes unefficient in a buffered solution at pH 7.4. Pathway b: This regiospecific cleavage of the heterocycle that is unequivocal in a buffered solution at pH 7.4 becomes only regioselective in a buffered solution at pH 6.3. Pathway c: This very minor pathway is only observed at the same time as the major pathway b in a buffered solution at pH ≤ 6.3.**

can be focused either on the nonhydrolyzed substrate **1** or on the corresponding unmasked protonated aziridines $\mathbf{2H}^+$ (see Fig. 1, pathway a). A second possibility would be to proceed to comparative assays with each enantiomer of **1**. However, these two solutions present the drawback that they require the availability of enantiomers of *N*-acylaziridines **1** or of aziridines **2**, in order to determine the nonequivalence sense[9] of a possible chiral recognition of the enantiomers. Actually, the synthesis of pure enantiomers of aziridines **2** that are also the evident precursors of *N*-acylaziridine **1** enantiomers is far from easy.[7]

Consequently, we have chosen a third approach consisting of the indirect analysis of the enantiomeric composition of the nonhydrolyzed fraction of *N*-acylaziridines **1** resulting from their incomplete enzymatic hydrolysis in insect tissues *via* their corresponding α-*O*-substituted β-hydroxylamides **4** (see Fig. 2). In fact, a subsequent nonenzymatic hydrolysis of the nonhydrolyzed *N*-acylaziridines **1** performed in a phosphate buffer at pH = 7.4 can unequivocally lead to the corresponding β-hydroxylamides **4**[4–6] (see Fig. 1, pathway b, and Fig. 2, step b). This approach presents the advantage of using β-hydroxylamides **4** where the synthesis of pure enantiomers is straightforward starting from commercially available substituted β-ethanolamines **5** enantiomers and carboxylic acids $\mathbf{3H}$[7] (see Fig. 3).

Thus our concern turned towards the analysis of the enantiomeric composition of chiral α-*O*-substituted β-hydroxylamides **4** related to the *N*-acylaziridines **1** of interest (see Fig. 2). This indirect approach was recently applied in our laboratory with *N*-acylaziridines **1b** and **1d** using chromatographic methods[6] with an achiral column for following the step a in Fig. 2 and a chiral column for the chiral analysis (see step c in Fig. 2).

As recently we succeeded easily in the enantiomeric discrimination of chiral oxazolines[10] and thiazolines[11] using chiral solvating agents (CSAs) of the trifluoromethyl carbinol type (Pirkle's alcohols),[12] we wanted to try also this simple chiral NMR analytical method for the chiral analysis of a greater number of α-*O*-substituted β-hydroxylamides **4** (see step c in Fig. 2).

The principles and techniques for use of CSAs in enantiomeric purity determination have been previously discussed in reviews compiling their

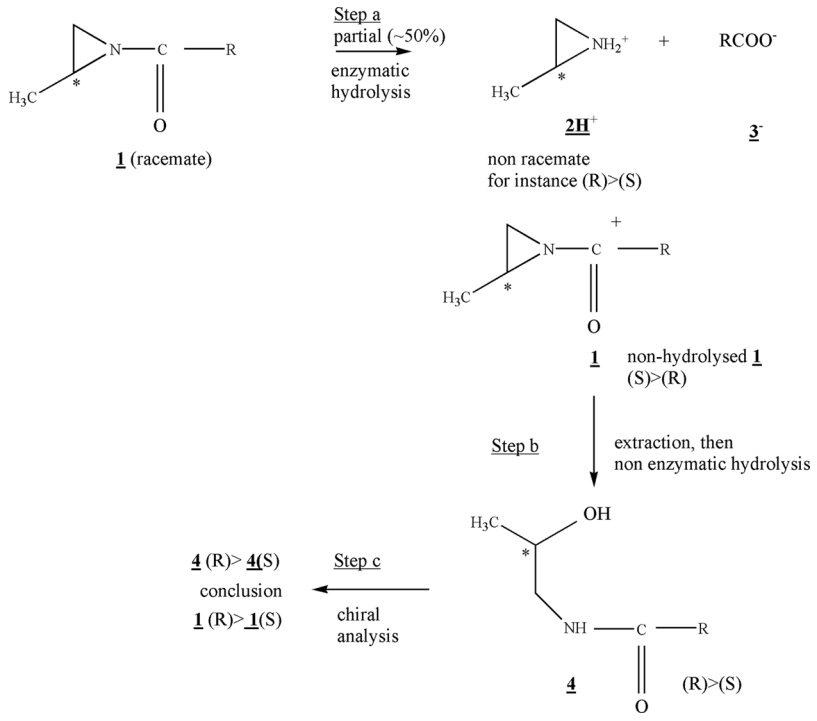

FIGURE 2 **Principle of the research of a possible recognition during the enzymatic hydrolysis of *N*-acylaziridines 1 in biological tissues. Step a: The duration of the incubation with given biological tissues and *N*-acylaziridine 1 is empirically determinated in order to entail approximatively half hydrolysis of 1 according to pathway a of Fig. 1, see Ref. 6. Step b: The nonhydrolyzed *N*-acylaziridines 1 that is recovered by a solid-phase extraction technique[6,7] is then univocally hydrolyzed in a buffer solution at pH 7.4 into the corresponding α-*O* substituted β-hydroxylamides 4 according to pathway b of the Fig. 1. During this SN_2 reaction, there is an inversion of the chiral center. It follows in case of a chiral recognition (in step a), with for example the situation (*S*) 1 > (*R*) 1 observed- for the nonhydrolyzed *N*-acylaziridines 1, that the (*R*) 4 > (*S*) 4 situation will be observed for the corresponding α-*O* substituted β-hydroxylamides 4. Step c: The chiral analysis of the given non-racemate α-*O* substituted β-hydroxylamide 4, conduced either by a chromatographic method[6] or using CSA 6 (in development of this work) will indicate the reversed enantiomeric composition for the given corresponding *N*-acylaziridine 1: that is, that (*R*) *N*-acylaziridine 1 is preferentially hydrolyzed in the given tissues over (*S*) 1 enantiomer.**

applications,[13–15] and important research is still dedicated to this field.[16–21] Ideally, the observed signals are assumed to correspond with the condition of fast-exchange limit (FEL) for the free substrate molecule in equilibrium with a complexed chiral additive leading to short-lived diastereomeric solvates. The

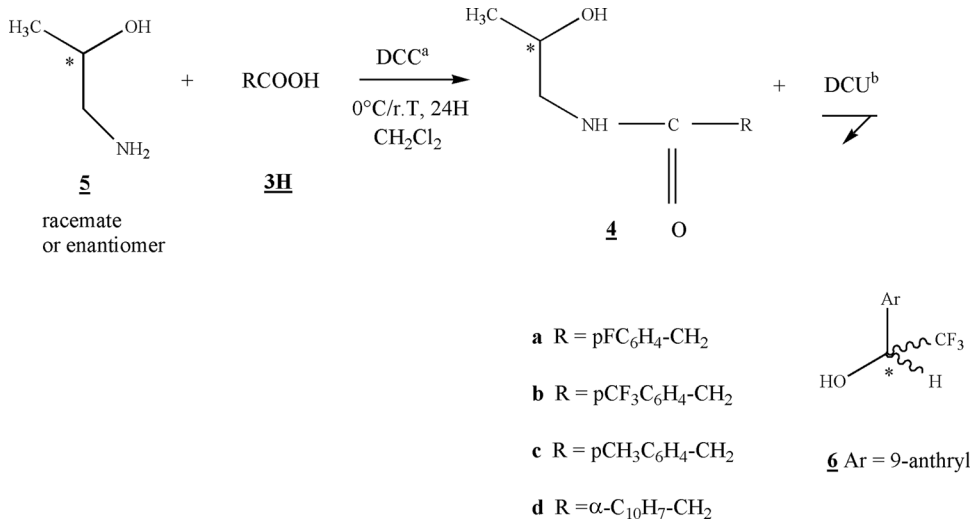

FIGURE 3 **Synthesis of racemates and enantiomers of α-*O*-substituted β-hydroxylamides 4 studied in this work using trifluoromethylanthryl carbinol 6 as CSA. ([a]DCC, dicyclohexylcarbodiimide; [b]DCU, dicyclohexylurea. The asterisk denotes the stereogenic center.)**

assumption of binary association is moreover well justified in many instances.[15]

α-*O*-substituted β-hydroxylamides **4** (see Figs. 1–3) share with the oxa- and thia-zolines previously studied the common feature that they possess in addition to aromatic substituents several potential basic sites. These molecular characteristics suggest that substrates **4** are susceptible to afford a sufficient number of interactions with the acidic centers and the aromatic group of CSA **6**, which are required for an enantiomeric discrimination as stipulated by Dalgliesch[22] and later by Pirkle.[23] Moreover, it was previously made evident from ^{1}H NMR studies dealing with the interaction of various amides with chiral auxiliaries that they actually give diastereomeric labile complexes with 1-arylethylamines,[24] amides,[25] and even with trifluoromethyl carbinol.[12]

Thus all these features allowed us to expect that the enantiomeric composition of α-*O*-substituted β-hydroxylamides **4** could be easily determined using CSA **6**, which is demonstrated in the present work.

MATERIALS AND METHODS

Chemicals

Chiral α-*O*-substituted β-hydroxylamides **4a–d** were obtained as racemates or as pure enantiomers by condensation of 1 equiv. of 1-amino-2-propanol **5** (racemate or pure enantiomer) added at 0°C, with 1 equiv. of carboxylic acid **3H a–d** using 1 equiv. of dicyclohexylcarbodiimide (DCC)[4,5] in CH_2Cl_2 (see Fig. 3). DCC and aminopropanol (racemate or pure enantiomer) were supplied by Aldrich (Sigma-Aldrich Chimie, L'Isle d'Abeau Chesnes, Saint-Quentin-Fallavier, France).

Chiral CSA (*R*)-2,2,2-trifluoro-1-(9-anthryl) ethanol **6** was obtained from Fluka (Saint-Quentin-Favallier, France). Deuterated solvents C_6D_6 and $CDCl_3$ were obtained from Euriso-Top (Gif-Sur-Yvette, France). The solvents were stored over 3 Å molecular sieves. CSA **6** was stored in a desiccator over P_2O_5.

Spectroscopic Studies

Structural characterization (^{1}H and ^{13}C NMR, IR and MS) of racemates and enantiomers of hydroxylamides **4a–d** that agree well with the proposed structures will be published elsewhere.[26]

Spectra were recorded on a 300 MHz (^{1}H) AC 300 Bruker (Wissembourg, France) equipped with a 5 mm probe and a Bruker Aspect 3000 computer.

Typical conditions for recording one-dimensional spectra were as follows: spectral width 5376 Hz, data points 32,000, flip angle 30°, pulse repetition 1 s, acquisition time 3.047 s. Resolution enhancement was performed by using a Gaussian window. The Fourier transform was carried out with 65,000, after zero filling so that the digital resolution was 0.17 Hz per point.

The different β-hydroxylamides **4a–d** were dissolved either in a mixture of deuterated chloroform/carbon tetrachloride (30/70, v/v), or in deuterated benzene to give concentrations ranging from 3×10^{-3} to 9×10^{-3} mole L^{-1}. Measurements were mostly carried out at a probe temperature of 23 ± 1°C, and the proton chemical shifts were initially referenced to the solvent values of 7.16 and 7.27 ppm (RMN ^{1}H in C_6D_6 and $CDCl_3$, respectively). As recommended,[9] CCl_4 and C_6D_6 were used as achiral and apolar solvents for recording the 300 MHz ^{1}H NMR spectra of -hydroxylamides **4** to avoid competition between the interactions of the achiral solvent and of the chiral agent with the substrate during the chiral analysis.

In runs with CSA **6**, an aliquot of standard solution of β-hydroxylamide **4** was rapidly introduced into a vial containing the solid reagent **6** which had been accurately weighed.

When enantiomeric shift differences were observed for selected resonances in the presence of CSA, the following abbreviations $\Delta\Delta\delta$ and h% represent, respectively, $|\delta_R - \delta_S|$ expressed in ppm and (valley height/average peak height) ×100 of a signal obtained with CSA.

Precision of Enantiomeric Excess (e.e.) Determination

An aliquot of standard solution of racemate **4c** (0.4 mg) was enriched with an aliquot of standard solution of (*S*)-enantiomer (0.6 mg), the samples being weighed on a precision balance (± 0.01 mg). The expected e.e. for **4c** was 60%. Using typical conditions for recording one-dimensional spectra and a Gaussian window for resolution enhancement, the measured e.e. was 56.4% for H_4 ($\Delta = 0.01$ ppm), indicating a precision of about ±4% of the

e.e. determination. Similar results were obtained with **4d** for $\underline{CH_3}(C_5)$ ($\Delta = 0.008$ ppm).

RESULTS AND DISCUSSION

Experiments with Trifluorinated Carbinol as CSA

We chose trifluoromethylanthryl carbinol **6**, which has been shown to be more efficient in terms of chiral discrimination compared with its phenyl analog. In fact, this greater power of trifluoromethylanthryl carbinol has previously been explained on the basis of a more pronounced π-donor character of the anthryl group compared with phenyl and/or a stronger anisotropy effect resulting in a more important shielding of the NMR signals.[9,12]

^{1}H NMR data of racemic hydroxylamides **4a–d** using CSA **6** with [**6**]/[**4**] ratios ranging from 3 to 8 are presented in Table 1. A general shielding of the signals is observed as expected when considering the literature[27] and our results.[10,11]

Concerning the evaluation of the enantiomeric discrimination, we have been using the criterion $\Delta\Delta\delta = |\delta_R - \delta_S|$ expressed as an absolute value (because at this stage of the study, the signals of the enantiomers were not yet identified) and overall the criterion h%, which was more significant with regard to the enantiomeric resolution as it takes into account the peak width (see "Materials and methods"). A general trend can be seen for these α-*O*-substituted *β*-hydroxylamides **4a–d**: the signal of one of the protons H_4 (in α position from N) is enantiomerically well resolved, h% reaching even zero, depending on the [**6**]/[**4**] ratio used (*vide infra* for a comment about this factor).

Moreover the signal $\underline{CH_3}(C_5)$ of **4a** and **4d** is nearly enantiomerically resolved (with a low h% value: 11.4 and 5.8 respectively) when $CCl_4/CDCl_3$ is used as an achiral solvent. This signal constitutes a more interesting NMR marker than do the H_4 protons, giving well isolated doublets instead of multiplets for H_4.

For all the studied α-*O*-substituted *β*-hydroxylamides **4a–d**, the anisochrony of at least one of the ^{1}H NMR signals of the enantiomers is sufficient to allow quantitation without deconvolution of the signals for a [**6**]/[**4**] ratio ≥5 conferring a low value for the criterion of relative valley height in the range: $0 \le h\% \le 11.4\%$. This concerns H_4 and $\underline{CH_3}(C_5)$ for **4a** and **4d** and H_4 for **4b** and **4c** (see Table 1 and Fig. 4 as an illustration).

It can be noted that an increase in the ratio [**6**]/[**4**] does not always entail a concomitant improvement in enantiomeric discrimination, as the regular increase observed for ΔΔδ can be outmatched by a simultaneous broadening of the signal. For instance for the marker H_4 of **4a** when [**6**]/[**4**] varies from 3 to 8, ΔΔδ increases regularly and h% is improved.

TABLE 1 **Enantiomeric Discrimination of ^{1}H NMR Signals of α-*O*-Substituted *β*-Hydroxylamides 4a–d[a] Induced by Chiral Solvating Agent CSA 6 at 296 K**

			NMR ^{1}H		
	Achiral solvent	[(*R*)-**6**]/[**4**]	Proton	$\Delta\Delta\delta^b \times 10^{-3}$ (ppm)	h%[c]
4a	$CCl_4/CDCl_3$	3/1	**H_4**	19	**3.0**
			$H_{4'}$	6	44.1
			CH_3-(C_5)	5.2	15.0
		5/1	**H_4**	**21**	**0**
			$H_{4'}$	n.u.[d]	—
			CH_3-(C_5)	**6.6**	**11.4**
		8/1	**H_4**	26	**0**
			$H_{4'}$	n.u.	—
			CH_3-(C_5)	8.8	12.8
4b	C_6D_6	5/1	**H_4**	**9.6**	**0**
			$H_{4'}$	0	100
			CH_3-(C_5)	0	100
		8/1	**H_4**	**4.9**	**0**
			$H_{4'}$	0	100
			CH_3-(C_5)	0	100
4c[e]	C_6D_6	5/1	**H_4**	**13.7**	**0**
			$H_{4'}$	6	41.5
			CH_3-(C_5)	2.7	50.0
		8/1	**H_4**	**19.4**	**0**
			$H_{4'}$	7.1	22.7
			CH_3-(C_5)	3.5	52.3
4d	$CCl_4/CDCl_3$	3/1	H_4	3.8	43.0
			CH_3-(C_5)	2.4	67.3
		5/1	H_4	6.4	18.7
			CH_3-(C_5)	4.1	21.3
		8/1	**H_4**	**11.5**	**0**
			CH_3-(C_5)	**7.1**	**5.8**

Note: In bold fare characters are indicated the usable signals for enantiomeric NMR discrimination.

[a]the concentrations of *β*-hydroxylamides **4a–d** were in the range 3×10^{-3} to 9×10^{-3} mole·L^{-1}.

[b]$\Delta\Delta\delta = |\delta_R - \delta_S|$difference in chemical shifts expressed in ppm.

[c]h% = (valley height/average peak height) × 100.

[d]n.u.: discrimination not usable due to overlapping of signals of hydroxylamide 4 with the 6 hydroxyl group.

[e]Experiment performed at 305 K.

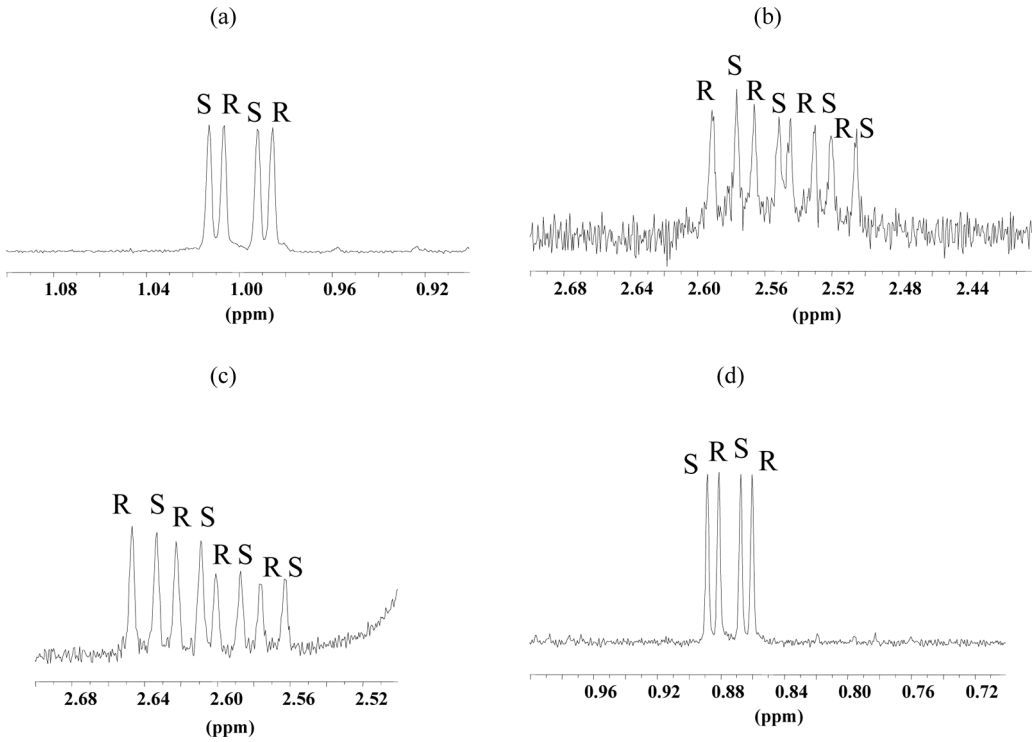

FIGURE 4 Enantiomeric discrimination of the ^{1}H signals of α-*O*-substituted β-hydroxylamides 4 in the presence of the CSA (*R*)-6. Assignments were made using pure enantiomers of substrates 4. The concentrations of β-hydroxylamides 4a–d were in the range 3×10^{-3} to 9×10^{-3} mole.L^{-1}. (a) CH_3-(C_5) ^{1}H NMR signal of 4a, with [6]/[4] = 5/1, in CCl_4 /$CDCl_3$ solutions at 296 K. (b) H_4 ^{1}H NMR signal of 4b, with [6]/[4] = 8/1, in C_6 D_6 solutions at 296 K. (c) H_4 ^{1}H NMR signal of 4c, with [6]/[4] = 5/1, in C_6 D_6 solutions at 305 K. (d) CH_3-(C_5) ^{1}H NMR signal of 4d, with [6]/[4] = 8/1, in CCl_4/$CDCl_3$ solutions at 296 K.

However at the same time for the marker $\underline{CH_3}(C_5)$, despite a regular increase in ΔΔδ, we observed an optimal h% for the value 5 of the ratio [**6**]/[**4**] (see Table 1).

Attempts at chiral analysis have also been performed using ^{19}F NMR for the fluorinated α-*O*-substituted *β*-hydroxylamides **4a–b**. It appears that in the present case the fluorine nucleus is a bad NMR marker. Indeed, even with a high [**6**]/[**4**] ratio = 8, either no discrimination or only a small but not usable effect was observed in the case of **4b** and **4a**, respectively.

The good enantiomeric discrimination afforded by the CSA **6** for α-*O*-substituted *β*-hydroxylamides **4** reveals as expected the existence of significant interactions between these two entities. To evaluate the potency of α-*O*-substituted *β*-hydroxylamides **4** as possible CSAs themselves, we examined the signals of the CSA **6**. During the previous assays, neither ^{1}H NMR nor ^{19}F NMR allowed an enantiomeric discrimination of the signals of the CSA **6** itself to be observed. In fact, the signals of the alcoholic hydrogen and of the tertiary proton of CSA, although enlarged, are not split.

Improvement of Enantiomeric Discrimination

We have previously discussed the effect of the increase in the factor [**6**]/[**4**] ratio on enantiomeric discrimination, and we now examine the temperature factor that is known to influence the anisochrony of the chemical shifts. Thus, for separations presenting a good (low) h% criterion, we attempted to improve the resolution by testing temperature effects. In fact, a decrease in the temperature, by decreasing the total energy of the molecule solvate, can favor peculiar conformations of diastereoisomeric solvates in which the CSA **6** moiety can exercise more pronounced diamagnetic anisotropy effects on the substrate **4** moiety.[9]

For the $\underline{CH_3}(C_5)$ ^{1}H NMR signal of α-*O*-substituted *β*-hydroxylamides **4a** and **4d** that were already almost baseline resolved at 296 K (see Table 1), diminishing the temperature to 276 K for **4a** and 281 K for **4d** provides effectively a ΔΔδ enhancement from 0.0066 to 0.015 ppm for **4a** and from 0.0071 to 0.0136 ppm for **4d** (see Table 2). However, this temperature variation results also in a loss of

TABLE 2 Temperature Effect on Enantiomeric Discrimination of ^{1}H NMR Signals of α-*O*-Substituted β-Hydroxylamides 4[a]–d[a] by Solvation with Chiral Solvating CSA 6

	Temperature (K)	Achiral solvent	[(*R*)-**6**]/[**4**]	Proton	$\Delta\Delta\delta^b \times 10^{-3}$ (ppm)	$\delta^{1/2c} \times 10^{-3}$ (ppm)	h%[b]
4a	**296**	$CCl_4/CDCl_3$	5/1	**H_4**	**21.3**	**3.7**	**0**
				$H_{4'}$	n.u.[d]	—	—
				CH_3-(C_5)	**6.6**	**2.9**	**11.4**
	286			**H_4**	**25.9**	**4.9**	**0**
				$H_{4'}$	10.1	4.9	14.7
				CH_3-(C_5)	8.8	2.5	12.8
	281			H_4	30.7	4.9	72.7
				$H_{4'}$	10.2	3.7	64.2
				CH_3-(C_5)	**11.5**	**3.6**	**11.6**
	276			H_4	36.4	**4.9**	25.2
				$H_{4'}$	**21.2**	**4.9**	**13.6**
				CH_3-(C_5)	15	3.8	49.1
4d	**296**	$CCl_4/CDCl_3$	8/1	**H_4**	**11.5**	**4.2**	**0**
				CH_3-(C_5)	**7.1**	**2.0**	**5.8**
	291			**H_4**	**13.2**	**4.2**	**0**
				CH_3-(C_5)	9	2.4	8.4
	286			**H_4**	**15.3**	**4.2**	**0**
				CH_3-(C_5)	11.1	3.3	10.5
	281			**H_4**	**17.1**	**4.2**	**0**
				CH_3-(C_5)	13.6	3.5	8.3

Note: In bold face characters are indicated the usable signals for enantiomeric NMR discrimination.
[a]The concentrations of **β**-hydroxylamides **4a–d** were in the range 3×10^{-3} to 9×10^{-3} mole·L^{-1}.
[b]See **Table 1** for the signification of **ΔΔδ** and h%.
[c]Width at half-peak height.
[d]n.u.: discrimination not usable due to overlapping of signals of hydroxylamide **4** with the **6** hydroxyl group.

resolution due to the broadening of signals with the observation of an increase for h% values: from 11.4 to 49.1 for **4a** and from 5.8 to 8.3 for **4d** (see Table 2).

Moreover, we have used $CCl_4/CDCl_3$ as achiral solvent, thereby avoiding competition for the diamagnetic effect exercised by aromatic CSA **6** on substrate **4**, which would be the case for an achiral aromatic solvent such as C_6D_6.

The *nonequivalence sense*,[9] which were determined using enantiomerically enriched mixtures of each hydroxylamide **4** and (*R*)-**6** CSA, are presented in Table 3. It appeared that the most shielded H_4 signals correspond with (*S*)-enantiomers and the most shielded signals for $\underline{CH_3}$-(C_5) correspond with (*R*)-enantiomers. For the hydroxylamide **4c**, the most shielded signal of $H_{4'}$

TABLE 3 Nonequivalence Sense for the NMR Enantiomeric Discrimination of α-*O*-Substituted β-Hydroxylamides 4[a] induced by solvation with CSA (R)-6

					^{1}H NMR	
	Achiral solvent	[(*R*)-**4**]/[(S)-**4**]	[(R)-**6**]/[**4**]	Temperature (K)	Proton	$\delta_R - \delta_S \times 10^{-3}$ (ppm)
4a	$CCl_4/CDCl_3$	4/1	5/1	296	H_4	+24.3
					CH_3-(C_5)	−7.9
4b	C_6D_6	1/3	5/1	296	H_4	+9.8
4c	C_6 D_6	1/4	5/1	305	H_4	+11.9
					$H_{4'}$	−4.5
					CH_3-(C_5)	−2.4
4d	$CCl_4/CDCl_3$	1/4	8/1	296	H_4	+11.1
					CH_3-(C_5)	−7.6

Note: The determination is obtained by overloading with one enantiomer of **4**.
[a]The concentrations of **β**-hydroxylamides **4a—d** were in the range 3×10^{-3} to 9×10^{-3} mole · L^{-1}.

(another usable NMR marker) corresponds also with the (*R*)-enantiomer.

Concerning the *precision of the enantiomeric excess*, we observed a difference of about 3% to 4% between the determinations obtained by NMR and those resulting from the enantiomer weights. This level of precision is sufficient for a preliminary screening, which is done to obtain a clear answer with regard to the interest of using an enantiomer instead of the racemate for a given compound.

CONCLUSIONS

We have obtained a good enantiomeric discrimination for all the α-*O*-substituted β-hydroxylamides **4** tested with the CSA **6**. This confirms the existence of efficient interactions between this CSA and the substrates **4** as initially expected when considering the complementarity between the functionalities of these two entities. This simple, rapid, and inexpensive method will soon be applied as foreseen to extracts of locust tissues incubated with racemates of *N*-acylaziridine **1** to determine the occurrence of chiral recognition during their enzymatic hydrolysis conducted either during *in vitro*, or *ex vivo* assays in this tissues.[1,5]

REFERENCES

1. Cherton, J-C.; Cavellec, A.; Correia, I.; Lepotier, I.; Cassier, P. I. Metabolism of fluorinated enol esters in locust organs or hemolymph studied by ^{19}F NMR. *Analusis* **1996**, *24*, 240–245.
2. Cherton, J-C.; Hamm, S.; Halle, J-C.; Menguy, L.; Beaufour, M. Potential proinsecticides of fluorinated carboxylic acids. IV Evaluation of the Δ^2-oxazoline-1,3 structure by ^{19}F NMR monitoring of the *in vitro* behaviour in locust tissues. *J. Fluorine Chemistry* **2001**, *107*, 387–395.
3. Beaufour, M.; Cherton, J.-C.; Carlin-Sinclair, A.; Hamm, S. Potential proinsecticides of fluorinated carboxylic acids and β-ethanolamines. V.Modern TLC for monitoring the metabolism directly in insect tissues. *J. Chromatogr. B* **2001**, *761*, 35–45.
4. Hamm, S. Développement de proinsecticides potentiels d'acides carboxyliques, bases sur les structures ^{2}-oxazolines, ^{2} -thiazolines et N-acylaziridines. Suivis directs dans les milieux biologiques d'insectes par RMN 19 F et HPLC. Réactivité des ^{2} -oxazolines et des ^{2} -thiazolines avec le 4,6-dinitrobenzofuroxane, Ph. D. thesis, University of Versailles, 1999.
5. Hamm, S.; Cherton, J-C.; Menguy, L.; Delorme, R.; Louveaux, A. Potential proinsecticides of fluorinated carboxylic acids. III Evaluation of the N-acylaziridine structure by ^{19}F NMR monitoring of the *in vitro* behaviour in insect tissues. *New J. Chem.* **1999**, *23*, 1239–1944.
6. Merelli, B.; Hamm, S.; Carlin-Sinclair, A.; Cherton, J.-C. N-acylaziridines as potential proinsecticides of fluorinated carboxylic acids. Part VI. Direct HPLC monitoring of the metabolism in insect tissues. *J. Chromatogr. B* **2005**, *826*, 129–138.
7. Merelli, B. Proinsecticides chiraux d'acides carboxyliques et de -éthanolamines. Effet de la chiralité sur la métabolisation et les activités biologiques chez l'insecte, Ph. D. thesis, University of Versailles, 2004.
8. Menguy, L.; Hamm, S.; Cherton, J.-C. Catalysed asymmetric hydrolysis of fluorinated chiral esters in locust hemolymph. *In vitro* ^{19}F NMR monitoring. *Spectroscopy* **1997**, *13*, 137–143.
9. Parker, D. NMR determination of enantiomeric purity. *Chem. Rev.* **1991**, *91*, 1441–1457.
10. Beaufour, M.; Cherton, J.-C.; Menguy, L.; Merelli, B. Determination of the enantiomeric composition of substituted Delta-2-oxazolines-1,3 by NMR spectroscopy using chiral solvating agents. *Chirality* **2003**, *15*, 382–390.
11. Merelli, B.; Menguy, L.; Soubeyrand-Lenoir, E.; Cherton, J.-C. Determination of the enantiomeric composition of chiral delta-2-thiazolines-1,3 by ^{1}H and ^{19}F NMR spectroscopy using chiral solvating agents. *Spectroscopy* **2006**, *20*, 95–107.
12. Pirkle, W. H.; Hoover, D. J. NMR chiral solvating agents. In *Topics in Stereochemistry*; Allinger, N.L.; Eliel, E.L.; Wilen, S.H. Eds;, John Wiley & Sons: New York, 1982; 13, 263–331.
13. Wiesmann, G. R. Nuclear magnetic resonance analysis using chiral solvating agents. In *Asymmetric Synthesis*; Academic Press: New York, 1983; 1, 153–171.
14. Eliel, E.L.; Mander, L.N.; Wilen, S.H. *Stereochemistry of organic compounds*, Wiley Interscience: New York, 1994; 231–236.
15. Rothchild, R. NMR determination of enantiomeric excess. *Enantiomer* **2000**, *5*, 457–472.
16. Blitz, A.; Stork, T.; Helmuchen, G. New chiral agents for carboxylic acids: discrimination of enantiopic nuclei and binding properties. *Tetrahedron: Asymmetry* **1997**, *8*(24), 3999–4002.
17. Ucello Baretta, G.; Bardoni, S.; Balzano, F.; Salvadori, P. Versatile chiral auxiliaries for NMR spectroscopy based on carbamoyl derivatives of dihydroquinine. *Tetrahedron: Asymmetry* **2001**, *12*(12), 2019–2023.
18. Muñoz, A.; Virgili, A. Preparation and behaviour of (R) and (S)-2,2,2-trifluoro-1-(1pyrenyl)ethanol as chiral solvating agents: study of the diastereomeric association by Job's plots, intermolecular NOE and binding constants. *Tetrahedron: Asymmetry* **2002**, *13*, 1529–1534.
19. Koscho, M.; Pirkle, W. Investigation of a broadlyapplicable chiral selector used in enantioselective chromatography (Welk-0 1) as a chiral solvating agent for NMR determination of enantiomeric composition. *Tetrahedron: Asymmetry* **2005**, *16*(20), 3345–3351.
20. Uccello-Barretta, G.; Balzano, F.; Salvadori, P. Rationalization of the multireceptorial character of chiral solvating agents based on quinine and its derivatives: overview of selected NMR investigations. *Chirality* **2005**, *17*, S243–S248.
21. Yang, X.; Wang, G.; Zhong, C.; Wu, X.; Fu, E. Novel NMR chiral solvating agents derived from (1*R*, 2*R*)-diaminocyclohexane: synthesis and enantiodiscrimination for chiral carboxylic acids. *Tetrahedron: Asymmetry* **2006**, *17*, 916–921.
22. Dalgliesch, C.E. Optical resolution of aromatic acids on paper chromatograms. *J. Chem. Soc.* **1952**, *137*, 3940–3942.
23. Pirkle, W.H.; House, D.W.; Finn, J.M. Broad spectrum resolution of optical isomers using chiral high-performance liquid chromatographic bonded phases. *J. Chromatogr.* **1980**, *192*, 143–158.
24. Pirkle, W.H.; Burlingame, T.G.; Beare, S.D. Optically active NMR solvents. VI. The determination of optical purity and absolute configuration of amines. *Tetrahedron Lett.* **1968**, *56*, 5849–5852.
25. Snatzke, G.; Fox, J.E.; El-Abadelah, M. Determination of the optical purity of some substituted N-phenyl propianamides by NMR spectroscopy. *Org. Magn. Reson.* **1973**, *5*, 413–417.
26. Menguy, L.; Merelli, B.; Cherton, J.-C. Manuscript in preparation.
27. Pirkle, W.H.; Popchasky, T.C. Chiral molecular recognition in small bimolecular systems : a spectroscopic investigation into the nature of diastereomeric complexes. *J. Amer. Chem. Soc.* **1987**, *109*, 5975–5982.