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# Efficient and rapid determination of the enantiomeric excess of drugs with chiral solvating agents: carvedilol, fluoxetine and a precursor of diarylether lactams

Míriam Pérez-Trujillo[a,*] and Albert Virgili[b]

[a]*Servei de Ressonància Magnètica Nuclear, Facultat de Ciències, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain*
[b]*Departament de Química Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain*

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**Abstract**—The efficient, rapid and easy determination of the enantiomeric excess of two important drugs carvedilol and fluoxetine and precursor of diarylether lactams, using two chiral solvating agents is described.


## 1. Introduction

The determination of the enantiomeric purity of drugs and their intermediates has now become of crucial importance. Most of the methods applied in determining the enantiomeric excess (ee) of chiral compounds require labour-intensive steps, which involve derivatisation and purification of the product, with the possibility of a kinetic resolution as an important drawback. The majority of these methods are chromatographic and mass spectrometry-based techniques;[1] however, NMR methods developed for this purpose have recently undergone remarkable advances.[2] Nevertheless, most of the NMR methodologies still include derivatisation, as is the case for the widely used chiral derivatising reagents,[3] methoxytrifluoromethylphenylacetic acid (MTPA),[4] methoxyphenylacetic acid (MPA)[5] or a large variety of novel auxiliaries.[6]

We have recently developed[7,8] two chiral solvating agents[9] (CSA) capable of enantiodifferentiating a wide variety of compounds. The advantages of these auxiliaries are firstly, that no derivatisation or purification is required (the enantiodifferentiation occurs via noncovalent interactions) and secondly, if required, the sample can be easily recovered by chromatography. Furthermore, the drawbacks of kinetic resolution are avoided. Compounds (*R*,*R*)-α,α′-bis(trifluoromethyl)-9,10-anthracenedimethanol **1**,[7,10] (*R*,*R*)-α,α′-bis(trifluoromethyl)-1,8-anthracenedimethanol **2**[8] and their corresponding enantiomers (Fig. 1) behave as inductors of enantiodifferentiation with a wide range of compounds such as amines, alcohols, epoxides and products with and without aryl groups (some of them are indicated in Fig. 2).

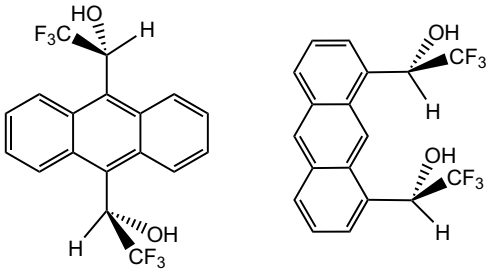


**Figure 1.** (*R*,*R*)-α,α′-Bis(trifluoromethyl)-9,10-anthracenedimethanol **1** and (*R*,*R*)-α,α′-bis(trifluoromethyl)-1,8-anthracenedimethanol **2**.

The best results observed are obtained with amine and/or alcohol-based substrates. The cause of enantiodifferentiation is the formation of diastereomeric complexes between the chiral agent and the substrate, which are not magnetically equivalent. We have demonstrated and described elsewhere,[7a,8a] that the enantiodifferentiation phenomena is mainly due to structural differences in the geometry of these diastereomeric complexes, and that no significant thermodynamic differences exist between them. Important

* Corresponding author. Tel.: +34 935811947; fax: +34 935812291; e-mail: miriam.perez@uab.es

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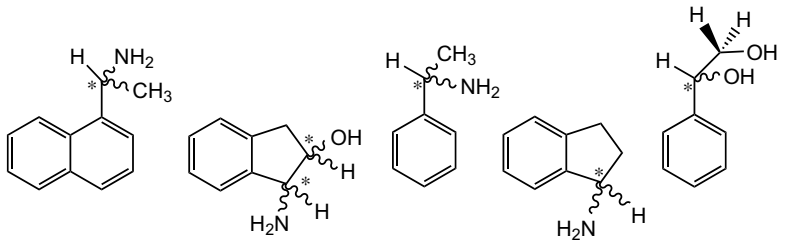

**Figure 2.** Some chiral compounds of diverse structural characteristics studied previously.

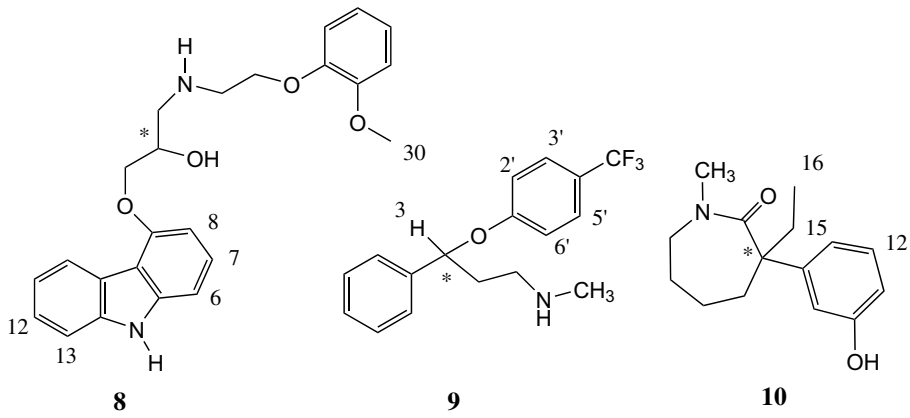

**Figure 3.** Carvedilol **8**, 3-ethyl-3-(3-methoxyphenyl)-1-methylazepan-2-one **9** and fluoxetine **10**.

structural features that favour this agent–substrate complexation are hydroxyl groups, which allow the formation of hydrogen bonds with the substrate and the anthryl ring, which forms π–π stacks with the substrate. Nevertheless, the importance of the acidity of the methynic proton (due to the presence of the trifluoromethyl group), which can favour the formation of hydrogen bonds with the substrate, has been proven.

In previous papers,[7,8a] we have shown the applicability of these agents to small chiral molecules with one or two moieties. However, the applicability of these agents for the determination of the ee of compounds with a more complex structure, highly functionalised, such as some pharmaceutical agents, has not yet been studied.

Chiral drugs are in some cases administered as racemates although the enantiomers of most of them may show varying pharmacological behaviour. This is the case of fluoxetine and carvedilol, two currently relevant biologically active compounds. Herein, we report the application of CSAs (*S*,*S*)-**1** and (*R*,*R*)-**2** to determine the ee of two important drugs and a pharmaceutical precursor: carvedilol **8**, a cardiovascular drug, 3-ethyl-3-(3-methoxyphenyl)-1-methylazepan-2-one **9**, a precursor of diarylether lactams described as cancer chemotherapeutic agents, and the antidepressant fluoxetine **10** (Fig. 3).

## 2. Results and discussion

Carvedilol,[11] 1-[carbazolyl-(4)-oxyl]-3-[(2-methoxyphenoxyethyl)-amino]-2-propanol, is a cardiovascular drug of proven effectiveness in the treatment of hypertension, ischemic heart disease, congestive heart failure and may also have uses in the prevention or slowing down of Alzheimer's disease. It is commercially available as a racemic mixture of both of its enantiomers [(*R*)-(+) and (*S*)-(−)] and represents a situation in which both enantiomers offer beneficial effects to the patient, but show markedly different properties from one another. In many studies it was important to determine the ee and/or have the enantiomers separately so that the similarities and differences between them can be understood.

The methodology used to quantify by NMR the enantiomers of **8** using CSAs **1** or **2** is very simple and easy to carry out. It consists of mixing the sample with the solvating agent directly in the NMR tube and acquiring NMR spectra before and after addition of the complexating agent. For this work (*S*,*S*)-**1** and (*R*,*R*)-**2** are used, although equivalent results would be achieved with their respective enantiomers. NMR spectra were recorded at 400.13 and 500.13 MHz for 1H and at 298 K. Previously $^{1}$H NMR spectra of **8**–**10** were assigned.

The enantiomers of a racemic sample of carvedilol **8** are enantiodifferentiated with both CSAs **1** and **2**. Table 1 show the values of the chemical shift differences ($|\Delta\delta^{RS}|$) obtained after every addition of compounds (*S*,*S*)-**1** or (*R*,*R*)-**2**. In both cases, multiple proton NMR signals of compound **8** are resolved. The best results were obtained with (*S*,*S*)-**1**, which enantiodifferentiates signals corresponding to protons H6, H7, H8, H12, H13 and H30. Protons H30 and H13 are the most convenient to use for the enantiomeric determination when using agent **1**, due to

**Table 1.** Chemical shift differences ($|\Delta\delta^{RS}|$) in $^1$H NMR spectra of compounds **8**–**10** after the addition of *n* equiv of agents (*S*,*S*)-**1** or (*R*,*R*)-**2**

| Sample | Nuclei | equiv | $\|\Delta\delta^{RS}\|$ (ppm) | |
|---|---|---|---|---|
| | | | (*S*,*S*)-**1** | (*R*,*R*)-**2** |
| **8** | H30 | 0.5 | [a] | — |
| | | 1.0 | 0.006 | — |
| | | 1.5 | 0.010 | — |
| | | 2.0 | 0.014 | — |
| | H13 | 0.5 | 0.005 | 0.004 |
| | | 1.0 | 0.013 | 0.009 |
| | | 1.5 | 0.019 | 0.010 |
| | | 2.0 | 0.028 | 0.010 |
| | H12 | 0.5 | 0.003 | 0.000 |
| | | 1.0 | 0.006 | 0.005 |
| | | 1.5 | 0.007 | 0.007 |
| | | 2.0 | 0.007 | 0.010 |
| | H8 | 0.5 | 0.000 | — |
| | | 1.0 | 0.006 | — |
| | | 1.5 | 0.009 | — |
| | | 2.0 | 0.009 | — |
| | H7 | 0.5 | 0.000 | 0.004 |
| | | 1.0 | 0.002 | 0.006 |
| | | 1.5 | 0.003 | 0.007 |
| | | 2.0 | 0.003 | 0.008 |
| | H6 | 0.5 | 0.001 | 0.008 |
| | | 1.0 | 0.004 | 0.012 |
| | | 1.5 | 0.006 | 0.016 |
| | | 2.0 | 0.006 | 0.018 |
| **9** | H16 | 0.5 | — | 0.009 |
| | | 1.0 | — | 0.023 |
| | | 1.5 | — | 0.035 |
| | | 2.5 | — | 0.057 |
| | | 2.8 | — | 0.058 |
| | H15 | 0.5 | 0.006 | 0.008 |
| | | 1.0 | 0.0015 | 0.017 |
| | | 1.5 | [b] | 0.025 |
| | | 2.5 | [b] | 0.036 |
| | | 2.8 | [b] | 0.037 |
| | H12 | 0.5 | 0.012 | 0.001 |
| | | 1.0 | 0.029 | 0.003 |
| | | 1.5 | [b] | 0.006 |
| | | 2.5 | [b] | 0.009 |
| | | 2.8 | [b] | 0.010 |
| **10** | H3 | 0.5 | 0.022 | — |
| | | 1.0 | 0.033 | — |
| | | 1.5 | 0.040 | — |
| | | 2.0 | 0.042 | — |
| | H2′/6′ | 0.5 | 0.021 | [a] |
| | | 1.0 | 0.028 | [a] |
| | | 1.5 | 0.033 | 0.005 |
| | | 2.0 | 0.034 | 0.006 |
| | H3′/5′ | 0.5 | 0.018 | 0.010 |
| | | 1.0 | 0.025 | 0.013 |
| | | 1.5 | 0.029 | 0.017 |
| | | 2.0 | 0.031 | 0.022 |

[a] Measurement of the $|\Delta\delta^{RS}|$ is not possible because of a poor resolution of the signal.

[b] No more equivalents of CSA are added because of solubility problems.

their higher $|\Delta\delta^{RS}|$ values: 0.028 ppm for H30, which corresponds to 14 Hz in a 500 MHz spectrometer, and 0.014 ppm for H13, which corresponds to 7 Hz in a 500 MHz spectrometer. The $^1$H NMR spectrum after the addition of 1.0 equiv of (*S*,*S*)-**1** (Fig. 4) reveals a 50:50

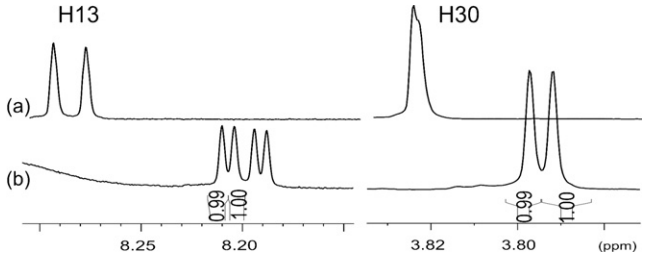


**Figure 4.** (a) $^1$H NMR spectrum of racemic carvedilol **8** in $CDCl_3$ at 298 K. (b) Quantification of the enantiomers of carvedilol **8** by integration of the split signals of protons H13 and H30 after the addition of 1.0 equiv of (*S*,*S*)-**1**.

mixture of diastereoisomeric complexes, corresponding to a racemic mixture of carvedilol **8**. Figure 5 shows the splitting of the signals of protons H6, H7, H8 and H12 of **8** after the addition of 0.5 and 1.0 equiv of (*S*,*S*)-**1**.

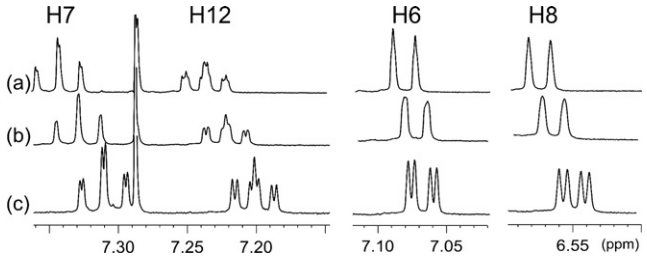


**Figure 5.** $^1$H NMR spectra of racemic carvedilol **8** (a) in $CDCl_3$ at 298 K, (b) after addition of 0.5 equiv of (*S*,*S*)-**1** and (c) after addition of 1.0 equiv of (*S*,*S*)-(**1**).

Compound **9**,[12] 3-ethyl-3-(3-methoxyphenyl)-1-methylazepan-2-one, is a precursor of novel chiral diarylether lactams identified as very potent inhibitors of protein farnesyltransferase and protein geranylgeranyltransferase I, enzymes involved in the prenylation of oncogen Ras, and are described as potential cancer chemotherapeutic agents.

Both CSAs studied (*S*,*S*)-**1** and (*R*,*R*)-**2** differentiate the enantiomers of a racemic sample of **9** (Table 1). Using (*S*,*S*)-**1**, signals of protons H12 and H15 are split and values of $|\Delta\delta^{RS}|$ observed are adequate to allow the

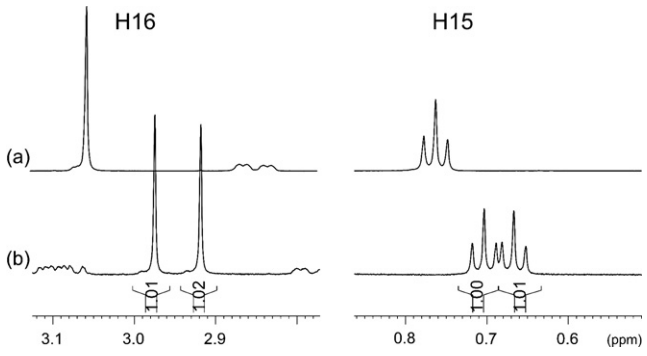


**Figure 6.** (a) $^1$H NMR spectrum of racemate **9** in $CDCl_3$ at 298 K. (b) Quantification of the enantiomers of **9** by integration of the split signals of protons H16 and H15 after the addition of 2.5 equiv of (*R*,*R*)-**2**.

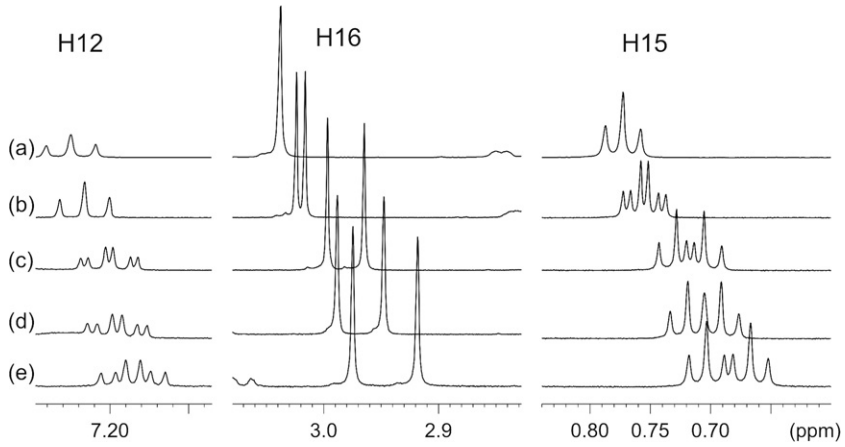


**Figure 7.** Evolution of the $^1$H NMR spectra of racemate **9** when (a) 0 equiv, (b) 0.5 equiv, (c) 1.5 equiv, (d) 2.0 equiv and (e) 2.5 equiv of (*R*,*R*)-**2** are added. Samples dissolved in $CDCl_3$ and at 298 K.

measurement of the ee However, excellent results are given with (*R*,*R*)-**2**. It enantiodifferentiates the NMR peaks of protons H16, H15 and H12, achieving very high values of $|\Delta\delta^{RS}|$ for a CSA. The singlet and triplet of methyl protons H16 and H15 present chemical shift differences of 0.058 ppm and 0.037 ppm respectively (corresponding to 29 Hz and 19 Hz in a 500 MHz spectrometer) after adding 2.8 equiv of (*R*,*R*)-**2** (Figs. 6 and 7). The integration of the split signals confirms the 50:50 enantiomeric mixture of **9**.

Fluoxetine,[13] *N*-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine is widely prescribed for the treatment of depression. It is also marketed as a racemate [(*R*)-(+) and (*S*)-(−)], but the individual enantiomers exhibit different therapeutic effects and metabolic rates. Considerable efforts have been focused on the development of methods to differentiate their enantiomers, most of them based on chromatographic and mass spectrometry techniques.[14]

In a previous paper[7b] we have shown that (*S*,*S*)-**1** is capable of discriminating the enantiomers of fluoxetine **10** by NMR, obtaining high values of $|\Delta\delta^{RS}|$ (0.042 ppm, 0.034 ppm and 0.031 ppm for protons signals of H3, H2′/6′ and H3′/5′, respectively) (Table 1). In this work (*R*,*R*)-**2** is assayed as CSA with racemic fluoxetine **10** and the results are compared with those obtained previously with (*S*,*S*)-**1**.

As shown in Table 1, (*R*,*R*)-**2** differentiates the enantiomers of **10**, signals corresponding to protons H2′/6′ and H3′/5′ are resolved to some extent. A higher chemical shift difference is achieved with protons H3′/5′, with a difference of 0.022 ppm (corresponding to 11 Hz in a 500 MHz spectrometer). Nevertheless, better results are obtained with (*S*,*S*)-**1**.

## 3. Conclusion

In conclusion, we have proved that anthracene derivatives (*S*,*S*)-**1** and (*R*,*R*)-**2** behave as efficient chiral solvating agents with amine, alcohol and amino-alcohol-based substrates, which present a high level of functionality and which are important pharmaceutical products. Furthermore, the ease and rapidity of the methodology, avoiding derivatisation steps and kinetic resolution, together with the possibility of recovering the substrate, makes it an excellent choice for the measurement of the ee of these compounds.

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