Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 1170–1175

Enantiomeric resolution of albuterol sulfate by preferential crystallization

Sara M. Palacios[a,*] and Marcela A. Palacio[b]

[a] *Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Cno. a Alta Gracia Km 10, 5000 Córdoba, Argentina*
[b] *Agencia Córdoba Ciencia SE, Unidad CEPROCOR, 5164, Santa María de Punilla, Córdoba, Argentina*

Received 22 March 2007; accepted 15 May 2007

Abstract—Albuterol is a β_2-adrenoceptor agonist prescribed for the treatment of bronchial asthma; it exists as a racemate and its bronchodilator activity resides in the (*R*)-isomer or levalbuterol. The aim of this study was to determine a methodology that would separate the enantiomers of albuterol by preferential crystallization after a conglomerate is identified within its derivatives. We found that albuterol sulfate behaves as a conglomerate showing the characteristic α_x-value = 2 (mole fraction solubility ratio of racemate vs enantiomer), the V-shaped ternary phase diagram and the preferential crystallization by seeding with the pure enantiomer. On the basis of these characteristics, we separated the enantiomers by entrainment, and crystallizing out a saturated methanolic solution of albuterol sulfate at 15 °C.

1. Introduction

Chirality is important in the context of biological activity because at a molecular level, asymmetry dominates the biological processes.[1,2] As the essential components of life such as proteins, carbohydrates, or DNA are constructed from enantiomerically pure building blocks, great differences are observed in the activity of enantiomeric drugs, and this results in different bioactivity, bioavailability, pharmacokinetics, and pharmacodynamics.[2] Consequently, regulatory authorities increasingly demand that chiral drugs should be administered in an enantiomerically pure form.[3] This has provoked tremendous interest within the pharmaceutical industry in obtaining pure enantiomers, either through enantioselective synthesis or the subsequent enantioseparation of racemic mixtures.[4]

Classical crystallization techniques continue to be one of the most commonly used methods in industrial resolutions of racemic mixtures.[5] In order to achieve an efficient separation of enantiomers by crystallization, the nature of the racemate must be known. Three types of racemic mixtures have been identified on the basis of their solid–liquid phase diagrams: (a) conglomerate, (b) racemic compounds, and (c) pseudoracemate.[6] Only 5–10% of racemates belong to the conglomerate forming group, which is the most favorable group for achieving a certain enantiomeric enrichment by fractional crystallization;[7] the search for or the discovery of new conglomerates among the chiral drugs, synthons, or their precursors has proven to be quite fruitful.[8]

Albuterol **Al**, also known as (±)-2-*tert*-butylamino-1-(4-hydroxy-3-hydroxy-methyl) phenyl ethanol, is a β_2-adrenoceptor agonist prescribed for the treatment of bronchial asthma.[9] **Al** is a racemate and its bronchodilator activity resides in the (*R*)-isomer or levalbuterol.[10,11]

In 1997, under the chiral switch strategy,[12] levalbuterol hydrochloride and levalbuterol sulfate (***R*AS**, Fig. 1) were approved by the FDA.[13] Since 1999 levalbuterol hydrochloride has been marketed as a nebulizer solution and in

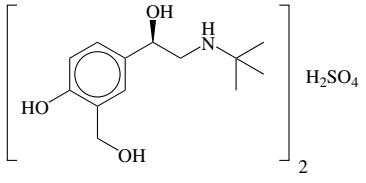

Figure 1. (*R*)-Albuterol sulfate (***R*AS**).

* Corresponding author. Tel.: +54 351 4938000x611; fax: +54 351 4938061; e-mail: sarapalacios@campus1.uccor.edu.ar

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.05.010

metered dose inhalers. Efforts to make a safer albuterol sulfate (**AS**) drug have prompted the need for new methods to obtain ***R*AS**. Herein, we report that albuterol sulfate crystallizes as a conglomerate and its resolution by preferential crystallization.

2. Results and discussion

In order to find a conglomerate within the **Al** derivatives, we made a series of determinations and experiments to support the presence of a conglomerate. First, we determined the eutectic composition of albuterol (**Al**), albuterol HCl (**AHC**), and albuterol sulfate (**AS**). The eutectic composition of **Al** was $R/S = 75/35$, indicating that it is a racemic compound. **AHC** did not crystallize or separate from the mother liquor as a gel material and so we were unable to determine its eutectic composition. **AS**, which has good crystal behavior, showed a eutectic point equal to $R/S = 50/50$. This result indicated that we were possibly in the presence of a conglomerate; a series of experiments were therefore carried out.

Second, the solubility, determined in methanol at 25 °C, of **AS** and ***R*AS** was 8.77 and 4.00 mg/ml, respectively. As this was consistent with the presence of a conglomerate,[7] we were able to determine a 2.22 ratio α_x between the solubility of **AS** and ***R*AS** (both expressed as mole fractions) while similar α_x-values were found at 30 and 40 °C (Table 1).

Table 1. Experimental solubility (molar fraction) of ***R*AS**, **AS** and α_x ratio

Temperature (°C)	***R*AS** ($\times 10^5$)	**AS** ($\times 10^5$)	α_x Ratio
25	2.80	6.23	2.22
30	33.08	73.58	2.22
40	52.41	108.58	2.07

As a third confirmation, a typical preferential crystallization experiment was performed. Thus, 0.236 g of **AS** was dissolved in 20 ml of MeOH at reflux, then allowed to cool down, and seeded (1% w/w) with pure ***R*AS**. After 12 h, we decanted the mother liquors, isolating a precipitate with an enantiomeric composition of $R/S = 95/5$ in a 5% yield.

Prompted by the successful resolution of **AS** through preferential crystallization, we determined the ternary phase diagram (TPD) of **AS** instead of the binary phase diagram (BPD), because **AS** decomposes before it melts, thus preventing the determination of melting points.[14]

The solubility of **AS**, ***R*AS**, and mixtures of both compounds determined in methanol at 25, 30, and 40 °C are graphically represented in Figure 2. The V-shaped curve of the TPD confirmed the finding of a conglomerate.[6,7]

The eutectic composition of **AS**, the α-value, the V-shaped TPD, and the preferential crystallization, indicated that **AS** shows conglomerate characteristics and that separation by entrainment is a suitable methodology for separating its enantiomers.

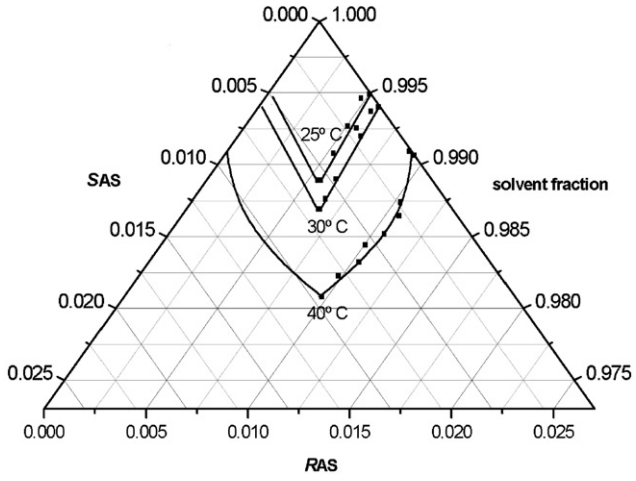

Figure 2. TPD of **AS**.

2.1. Preferential crystallization by entrainment of (*R*)-albuterol sulfate

Before performing the crystallization experiments, the optimum values for the different crystallization variables were evaluated and determined at a concentration of 16–18 g/l, stirring at 450–500 rpm, and a crystallization temperature of 15 °C. Seeding was also evaluated and was considered unnecessary, since an enriched solution of **AS** (17 g/l, $R/S = 55/45$) seeded with 0.5% of ***R*AS** gave a 24% yield of ***R*AS** with an enantiomeric excess of $R/S = 76.5/23.5$, while the same solution without seeding, yielded 29% of ***R*AS** and $R/S = 80/20$.

In the first cycle of successive crystallizations, a highly supersaturated solution of **AS** (4.04 g in 230 ml of methanol), enriched with an excess of ***R*AS** (0.46 g, $R/S = 55.1/44.9$) was stirred for 1 h at 15 °C. Afterwards, we isolated 1.11 g of ***R*AS** ($R/S = 82.7/17.3$) (Table 2). According to Amaird's method,[7] the optimum yield of enantiomeric crystals in each cycle will be double the amount of the excess ***R*AS** mass. In our case, we found 0.72 g of ***R*AS** (according to the optical purity) was obtained, which is nearly 79% of the theoretical yield (0.92 g). Then 1.00 g of **AS** was added to the resulting (*S*)-enriched mother liquor to restore the initial supersaturation and the procedure was repeated again to obtain the (*S*)-enantiomer (1.34 g, $R/S = 22.2/77.8$). After four cycles for each enan-

Table 2. Resolution of **AS** by entrainment

Cycle no.	Mass added (g)		Recovery of resolved **AS** in g (R/S percentage)	
	AS	***R*AS**	***R*AS** crystals	***S*AS** crystals
1	4.04	0.46	1.11 (82.7/17.3)	
	1			1.34 (22.2/77.8)
2	1		1.5 (60.3/39.7)	
	1.5			0.74 (19.6/80.4)
3	0.70		0.80 (72.9/27.1)	
	0.80			0.99 (31.6/68.4)
4	1		0.80 (71.7/28.3)	
	0.80			0.99 (32.2/67.8)
Total	10.84	0.46	4.21 (70.8/29.2)	4.06 (26.5/73.5)

tiomer, we obtained 4.21 g with $R/S \approx 70/30$ and 4.06 g $R/S \approx 30/70$ (Table 2). Finally, this ***R*AS** quantity was twice recrystallized from methanol, yielding 1.65 g of ***R*AS** ($R/S = 99.8/0.2$).

The ***R*AS** crystals were characterized spectroscopically, as shown in Figures 3–5. The analysis of the liquid NMR spectra (Fig. 3) and DSC thermogram[14] indicated that the ***R*AS** crystals did not correspond to solvates or hydrates; therefore, the conglomerate crystallization corresponded to pure ***R*AS**.

The XRPD pattern of ***R*AS** obtained in the preferential crystallization cycles, showed characteristic peaks at 8.7; 9.6 and 15.2 ($2\theta \pm 0.2°$ 2θ) (Fig. 4c), corresponding to the ***R*AS** polymorphic **Form II** as reported by Palacio et al.[14] A characteristic of the conglomerates is that the XRPD pattern of the pure enantiomer is identical to that of the racemate.[6] However in this case, we observed different patterns in the **AS** (racemate) (Fig. 4a) and the ***R*AS** (enantiomer). ***R*AS** presented two polymorphic forms and neither the ***R*AS Form II** nor ***R*AS Form I** (Fig. 4b) were similar to the racemate pattern.[15] In spite of the fact that there has been no report of the occurrence of polymorphism in **AS**, we looked for other crystal modifications for the racemate but found none. Clear differences were observed between the IR spectra of **AS**,[16] ***R*AS Form II** and ***R*AS Form I**[14] (Fig. 5a–c), confirming the difference in their crystal structure. In light of this evidence, we concluded that ***R*AS Form II**, ***R*AS Form I** and **AS** crystallize in different crystal forms.

These findings (XRPD and FT-IR spectra for **AS** being different from those for ***R*AS**) indicate that **AS** does not crystallize as the classical mixture of enantiomeric crystals expected for a conglomerate.[7] However, its solubility behavior in methanol (eutectic at $R/S = 50/50$, α_x-value ≈ 2, preferential crystallization, V-shape TPD) allows us to separate it through preferential crystallization. As far as we know, this situation in which the characteristic solubility of the conglomerate racemate was not confirmed by crystal spectroscopy is unusual. Thus, we are attempting to follow up this experiment with a study of the single crystal structure in order to elucidate this discrepancy.

3. Conclusion

Herein, we have reported a continuous cycle process for converting racemic **AS** into the chiral anti-asthmatic drug ***R*AS**, on the basis of the discovery of its conglomerate characteristics. The resolution of **AS** by preferential crystallization according to the entrainment procedure was tried and successfully developed by preventing the seeding of the saturated solution before each crystallization. A clear advantage of this methodology is to produce ***R*AS** from the widely available racemic **AS** without chiral auxiliaries, except for the minimum initial ***R*AS** enrichment.

4. Experimental

4.1. Materials

***R*AS** was prepared as described in a previous report[17] and the purity of this compound was 99.57% assayed against Albuterol Sulfate USP standard, with an enantiomeric excess of 99.8% by HPLC.[18] **AS** was provided by Laboratorio Pablo Cassara, S.R.L. (Buenos Aires, Argentina). All solvents used in this study were of HPLC grade (Fisher Scientific, New Jersey).

4.2. Methods

4.2.1. Determination of eutectic composition. In a 25 ml erlenmeyer flask, 200 mg of solid material ($R/S = 60/40$ and $R/S = 70/30$) was left to be solubilized in 10 ml of methanol at 25 °C. After 24 h, an aliquot of the superna-

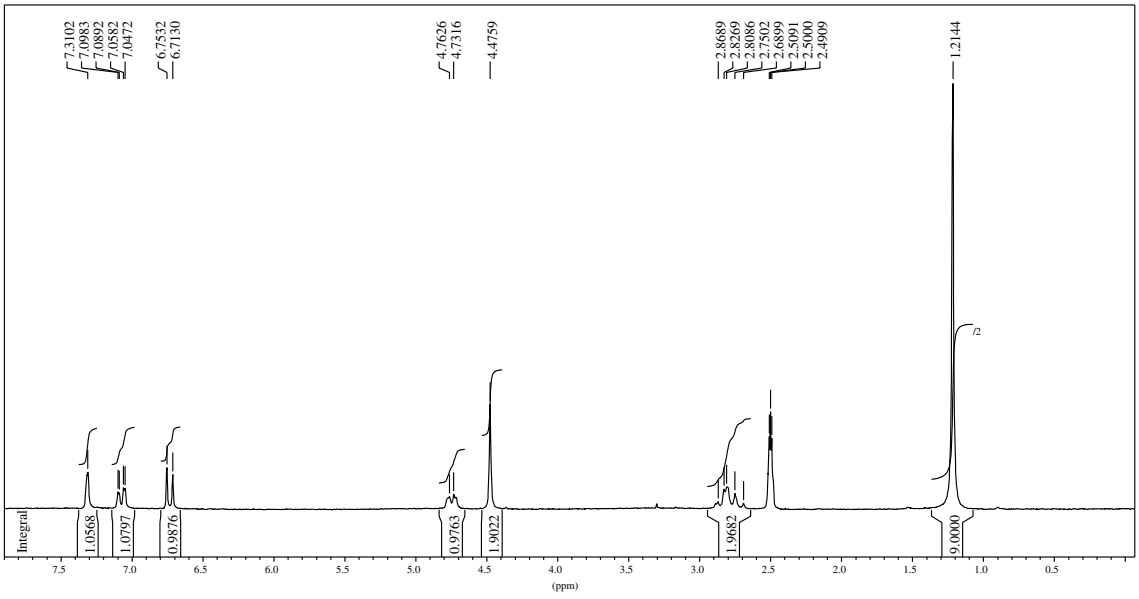

Figure 3. NMR spectra of ***R*AS** and **AS**.

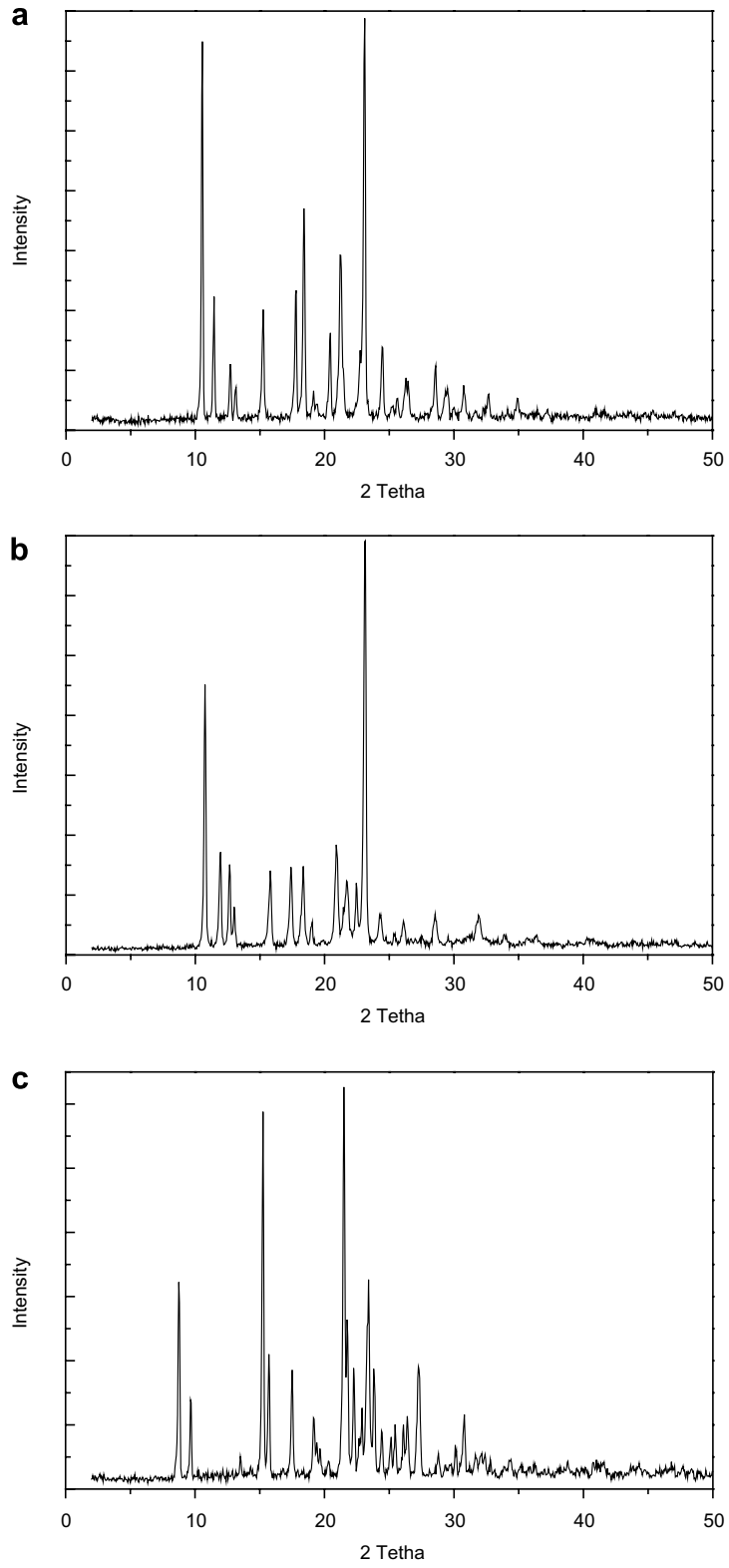

Figure 4. XRPD patterns of (a) **AS**, (b) ***R*AS Form I** and (c) **Form II**.

tant was filtered in a HPLC filter. The composition of the dissolved *R*/*S* was then analyzed by chiral HPLC.[18]

4.2.2. Solubility determination of albuterol sulfate and its enantiomer in methanol. In a 50 ml erlenmeyer flask, an excess amount of the corresponding solid material was allowed to solubilize in 10 ml of dried methanol at 25, 30, and 40 °C. The erlenmeyers were kept in a thermostatic bath regulated at the desired temperature ±1 °C. After 24 h, 1 ml of the supernatant was filtered in an HPLC filter. Then, the amount dissolved and the *R*/*S* composition of solid material were quantified by chiral HPLC.[18]

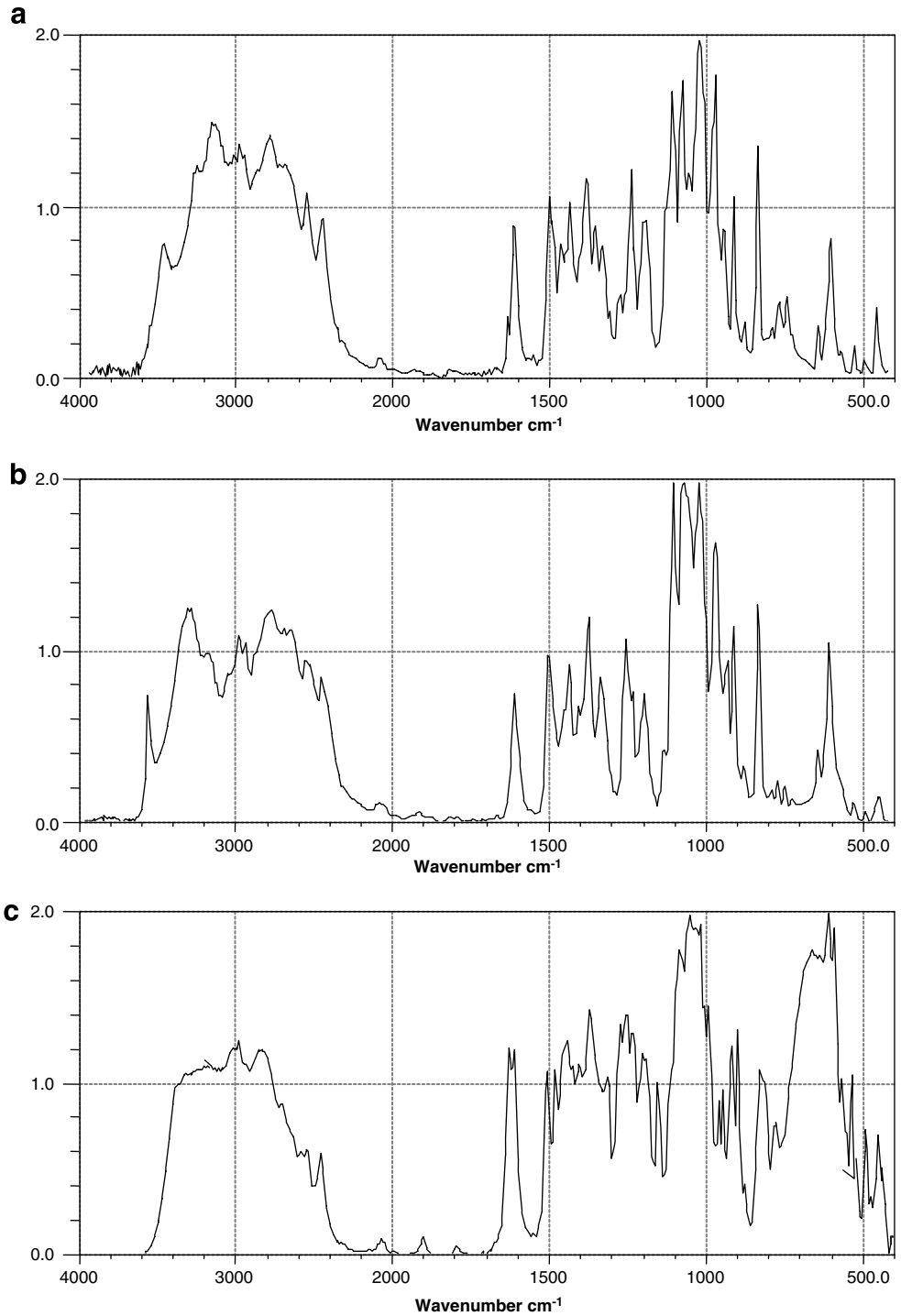

Figure 5. FT-IR spectra of (a) **AS**, (b) ***R*AS Form I** and (c) **Form II**.

4.2.3. Preferential crystallization by entrainment of (*R*)-albuterol sulfate. A reflux solution of **AS** (4.04 g) and ***R*AS** (0.46 g) in methanol as solvent (230 ml) was cooled down to 15 °C to give a supersaturated solution, which was stirred at 500 rpm for 1 h. A precipitate then appeared and was collected by filtration, washed with cool methanol, and dried under vacuum, to give 1.11 g of ***R*AS** ($R/S = 82.7/17.3$). Next, 1 g of **AS** was added to the filtrate and dissolved at 65 °C. The solution was similarly cooled to 15 °C and stirred gently for 1 h, to yield 1.34 g of ***S*AS** ($R/S = 22.2/77.8$). This cycle was repeated four times, with the addition of 0.8–1 g of racemate after each crystallization in order to restore the concentration of the solution.

After several stages of the preferential crystallization, the crystals with the same specific rotation were combined. Recrystallization of ***R*AS** (4.21 g, $R/S = 71/29$) from methanol gave 49% of highly pure ***R*AS** (2.06 g, $R/S = 93/7$). In a similar manner, ***S*AS** (4.06 g, $R/S = 26/74$) was crystallized from methanol giving 2.07 g (51%) of ***S*AS** ($R/S = 8/92$). In

a third process of crystallization from methanol, 2.06 g of ***R*AS** ($R/S = 93/7$) gave 1.65 g of ***R*AS** ($R/S = 99.8/0.2$), representing an 80% yield. The overall yield of ***R*AS** was 15%, calculated on the basis of 50% theoretical yield.

4.2.4. HPLC analysis. A Waters 2690 HPLC system with quaternary pump and autosampler and a Waters 996 photodiode array detector were used. For data acquisition, MILLENNIUM 3.20 software was used. All separations were achieved using a 25×4.6 mm Chirobiotic T column (amphoteric glycopeptide Teicoplanin bonded to a 5 μm silica gel) from ASTEC (Whippany, New Jersey).

All samples and standard solutions were chromatographed at ambient temperature (22 ± 2 °C) using an acetonitrile/methanol/acetic acid/triethylamine mixture (60/40/0.3/0.2 v/v/v/v) as the mobile phase (flow rate of 1.5 ml min^{-1}), with detection at 276 nm and an injection volume of 10 μl.[18]

4.2.5. Solution-state NMR. NMR spectra were obtained in a Bruker NMR with a Bruker AC 200 console (Bruker, Germany). The spectra were processed with WinNMR software (Bruker, Germany). The samples were prepared by dissolving 5 mg of each form in 0.5 ml of DMSO-d_6 with 0.03% of tetramethylsilane (TMS, Sigma–Aldrich Chemical Co., Wisconsin) used as reference for $\delta_H = 0$.

4.2.6. Infrared spectroscopy. Fourier transform infrared (FTIR) spectra were acquired on a Shimadzu spectrometer (Shimadzu, Kyoto, Japan). Spectra over a range of 4000–500 cm^{-1} with a resolution of 2 cm^{-1} (50 scans) were recorded using KBr pellets. For diffuse reflectance analysis, samples weighing approximately 2 mg were mixed with 200 mg KBr using an agate mortar and a pestle, and were placed in sample cups for fast sampling.

4.2.7. X-ray powder diffractometry (XRPD). The diffraction patterns were collected using a Bruker D8-Advance powder diffractometer, in θ–θ geometry, using Cu Kα radiation and working at 40 kV and 30 mA. A Sol-X® solid-state Si(Li) detector was used. C/Ni Goebel mirrors in the incident beam were used as a monochromator; 1.0 mm divergence and scatter slits and a 0.1 mm receiving slit were used, taking care to avoid introducing preferred orientation of the crystallites.

4.2.8. Thermal analysis. DSC thermograms were recorded with a DSC 2920 modulated Differential Scanning Calorimeter (TA Instruments, New Castle, Delaware). Samples weighing 5–8 mg (Precisa 262SMA-FR Balance) were heated in crimped aluminum pans from 30 to 300 °C at a rate of 10 °C min^{-1} under static air.

Acknowledgments

The authors would like to thank Universidad Católica de Córdoba and Agencia Córdoba Ciencia for their financial support.

References

1. For examples, see the following: (a) Bentley, R. *Chem. Rev.* **2006**, *106*, 4099–4112; (b) Rouhi, A. M. *Chem. Eng. News* **2003**, *81*, 45–55; (c) Eliel, E.; Wilen, S. *Stereochemistry of Organic Compounds*; John Wiley & Sons: New York, 1994; pp 201–214.
2. Sheldon, R. *Chirotechnology, Industrial Synthesis of Optically Active Compounds*; Marcel Dekker: New York, 1993; pp 39–72.
3. Lorenz, H.; Perlberg, A.; Sapoundjiev, D.; Elsner, M. P.; Seidel-Morgentern, A. *Chem. Eng. Proc.* **2006**, *45*, 863–873.
4. Stinson, S. C. *Chem. Eng. News* **1995**, 44.
5. Wood, W. M. L. Crystal Science Techniques in the Manufacture of Chiral Compounds. In *Chirality in Industry II*; Collins, A. N., Sheldrake, G. N., Crosby, J., Eds.; John Wiley: New York, 1997; pp 119–156.
6. Jacques, J.; Collet, A.; Wilen, S. In *Enantiomers, Racemates and Resolution*; Krieger: Malabar, 1994; pp 32–57.
7. Jacques, J.; Collet, A.; Brienne, M. J. *Chem. Rev.* **1980**, *80*, 215–230.
8. Perez-Garcia, L.; Amabilino, D. B. *Chem. Soc. Rev.* **2002**, *31*, 342–356.
9. Hartley, D.; Middlemiss, D. J. *J. Med. Chem.* **1971**, *14*, 895–896.
10. Penn, R. B.; Frielle, T. *Clin. Rev. Allerg. Immunol.* **1996**, *14*, 37–45.
11. Slattery, D.; Wong, S. W.; Colin, A. A. *Pediatric Pulmonol.* **2002**, *33*, 151–157.
12. Agranat, I.; Caner, H.; Caldwell, J. *Nat. Rev. Drug Disc.* **2002**, *1*, 753–768.
13. http://www.fda.gov/cder/foi/appletter/1999/20837ltr.pdf.
14. Palacio, M. A.; Cuffini, S.; Badini, R.; Karlsson, A.; Palacios, S. M. *J. Pharm. Biomed. Anal.* **2007**, *43*, 1531–1534.
15. Leger, P. M.; Goursolle, M.; Gradet, M. N. *Acta Crystallogr., Sect. B* **1978**, *34*, 1203–1208.
16. Jashnani, R. N.; Dalby, R.; Byron, P. *J. Pharm. Sci.* **1993**, *82*, 613–616.
17. Ferrayoli, C. G.; Palacio, M. A.; Bresina, M. F.; Palacios, S. M. *Enantiomer* **2000**, *5*, 289–291.
18. Halabi, A.; Palacio, M. A.; Ferrayoli, C. G.; Palacios, S. M. *J. Pharm. Biomed. Anal.* **2004**, *34*, 45–51.