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Research Article

Microwave-Assisted Synthesis of (±)-Mandelic Acid-d_5, Optical Resolution, and Absolute Configuration Determination

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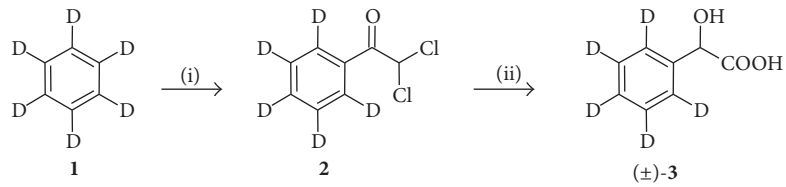
An efficient microwave-assisted synthesis of (±)-mandelic acid-d_5 was developed. The racemic mixture was resolved by diastereomeric salt formation using 1-phenylethylamine enantiomers as resolving agents. At each step, the resolution process was checked by determining mandelic acid-d_5 enantiomer ee values directly on fractional crystallized diastereomeric salts by chiral capillary electrophoresis analysis. Highly enriched (−)- and (+)-mandelic acid-d_5 (95% and 90% ee, resp.) were obtained and their absolute configurations—*R* and *S*, respectively—were determined by correlation of the (−)-mandelic acid-d_5 circular dichroism spectrum to the (*R*)-mandelic acid one.

1. Introduction

Mandelic acid (MA) is one of the major mammal urinary metabolites of styrene [1, 2], a hazardous pollutant widely used as a monomer in plastic industry [3]. The toxicity of styrene is metabolism-dependent [4] and stems from its bioactivation to styrene oxide, which in turn generates a cascade of metabolites including MA [1]. Thus, MA may be considered as a marker of the human exposure to styrene and related hazard [5]. This is why MA is currently the object of studies aimed at exploring the mechanism of its in vivo formation. Interestingly, MA was found to undergo one-directional chiral inversion from (+)-(*S*)- to (−)-(*R*)-enantiomer [6, 7]. The chiral inversion of (+)-(*S*)-MA seems to involve its corresponding CoA thioester but the details of the mechanism remain to be completely understood. It might be supposed that deuterated analogues of (+)-(*S*)-MA could favour further investigation. On the other hand, MA and its *O*-derivatives are well-known chiral solvating agents (CSAs) used for the direct ^{1}H NMR determination of the enantiomeric composition of chiral compounds [8, 9]. The synthesis of new homochiral *O*-aryl and *O*-heteroaryl mandelic acids has been recently reported [10]. Generally, the so-called "mandelic acid effect" [11] induces nonequivalence in ^{1}H NMR signals of the aliphatic protons of the analytes. In several cases, however, also the aromatic protons of the chiral species under observation display good enantiodiscrimination [10, 12, 13]. Thus, it may be hypothesized that MA and derivatives perdeuterated on the phenyl rings could be useful CSAs for bad cases where ee values have to be determined for compounds devoid of singlets or doublets in a free high field region of their ^{1}H NMR spectra, and when the aromatic proton resonances of the CSA overlap the aromatic proton signals of the compound under study. Here we present a convenient and facile route to obtain MA-d_5 enantiomers, possible pharmacological tools for styrene metabolism investigation, and chiral synthons for the synthesis of their corresponding *O*-derivatives, by the conventional diastereomeric salt formation using homochiral 1-phenylethylamine as the resolving base.

2. Results and Discussion

Racemic MA-d_5 [(±)-2-hydroxy-2-(D_5)phenylacetic acid, (±)-**3**] was prepared by modifying a procedure early reported



Scheme 1: Synthesis of (±)-2-hydroxy-2-(D_5)phenyl acetic acid [(±)-MA-d_5, (±)-**3**]. Reagents and conditions: (i) 2,2-dichloroacetyl chloride, $AlCl_3$, CH_2Cl_2, microwave irradiation, 70°C, 8 min; (ii) 1 M NaOH, H_2O, microwave irradiation, 110°C, 11 min; 82% overall yield.

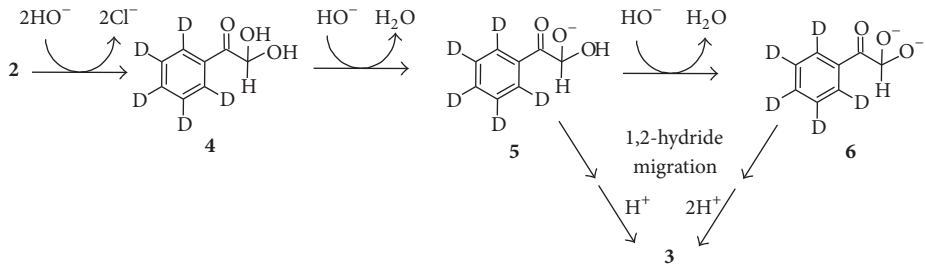
in the literature [14]. Single-mode microwave irradiation was adopted in order to shorten reaction times with respect to the conventional synthetic procedure. The microwave-assisted synthesis of (±)-**3** started from hexadeuterobenzene **1** which was submitted to a Friedel-Crafts acylation with 2,2-dichloroacetyl chloride to give dichloroacetophenone-d_5 (**2**) (Scheme 1). An aliquot of the reaction mixture was analyzed by EIMS and presented the desired ketone **2** as the only product. Treatment of **2** with NaOH gave the desired compound (±)-**3** with 82% overall yield. This procedure is undoubtedly more convenient than the one reported in the literature [14] since it guarantees higher yield (82% versus 63%) as well as shortening of reaction times from 2 hours to 8 minutes and from 3 hours to 11 minutes in the first and in the second step, respectively. Temperature, pressure, and power profiles for both steps are shown in Figures 1(a) and 1(b), respectively. In both steps, the maximum pressure in the reaction vessel did not exceed 80 psi. After the set temperatures of 70°C and 110°C, respectively, were reached, the power regulates itself down to about 0 W for step 1 (Figure 1(a)) while the power profile for the second step presented several oscillations possibly reflecting distinct mechanistic steps (Figure 1(b)). In fact, **2** should be converted into its corresponding dihydroxy derivative **4**—phenylglyoxal-d_5 hydrate (Scheme 2). In the presence of a concentrated strong base such as NaOH, **4** is deprotonated to give the monoanion **5**, which may lose a further proton to give the dianion **6**. Both charged species may undergo intramolecular hydride migration according to the well-known internal Cannizzaro reaction mechanism [15] to give the desired product **3**. The organic layer obtained by extraction of the reaction mixture was analyzed by EIMS and presented, together with high molecular weight byproducts possibly deriving from aldol condensation of dichloromethyl ketones, traces of (D_5)-phenylglycolic aldehyde (**7**, Figure 2). The latter compound, together with traces of (D_5)-phenylglyoxylic acid (**8**, Figure 2) found as a contaminant of crude MA-d_5 (ESIMS analysis), suggests intermolecular hydride migration as a possible concurrent mechanism. Thus, it may be concluded that microwave assistance reduces parasite reactions (aldol condensation and intermolecular hydride reactions) thus reducing by-product formation and improving yields. Optical resolution of MA-d_5 with (*S*)-1-phenylethylamine [(*S*)-PEA] as the resolving agent was performed by adding 1 equivalent of the optically active amine to an ethanol solution of (±)-**3** (Scheme 3). The diastereomeric salt was recrystallized three times from 95% ethanol. At each step, the enantiomeric enrichment was evaluated directly on an aliquot of the salt by capillary zone electrophoresis (CZE), in the presence of 2-hydroxypropyl-β-cyclodextrin as a chiral selector. This technique is advantageous in that, unlike HPLC or gas chromatography, it does not need to liberate the acid from the salt, thus ensuring speed of analysis. The so obtained salt was dissolved in water and, after acidification with 2 M HCl, gave (+)-MA-d_5 as white crystals (16% yield, 90% ee). The enriched (−)-MA-d_5 recovered from mother liquors of the first recrystallization step was used for further resolution by using (*R*)-PEA as the resolving agent. After two recrystallization steps, (−)-MA-d_5 was recovered from the corresponding (*R*)-PEA diastereomeric salt as white crystals (18% yield, 95% ee). The absolute configurations of MA-d_5 enantiomers were determined on the basis of CD analysis. The R configuration was assigned to the levorotatory isomer of **3** by spectroscopic correlation with (−)-(*R*)-MA as both their corresponding CD curves show negative Cotton effects (Figures 3(a) and 3(b)).

3. Conclusions

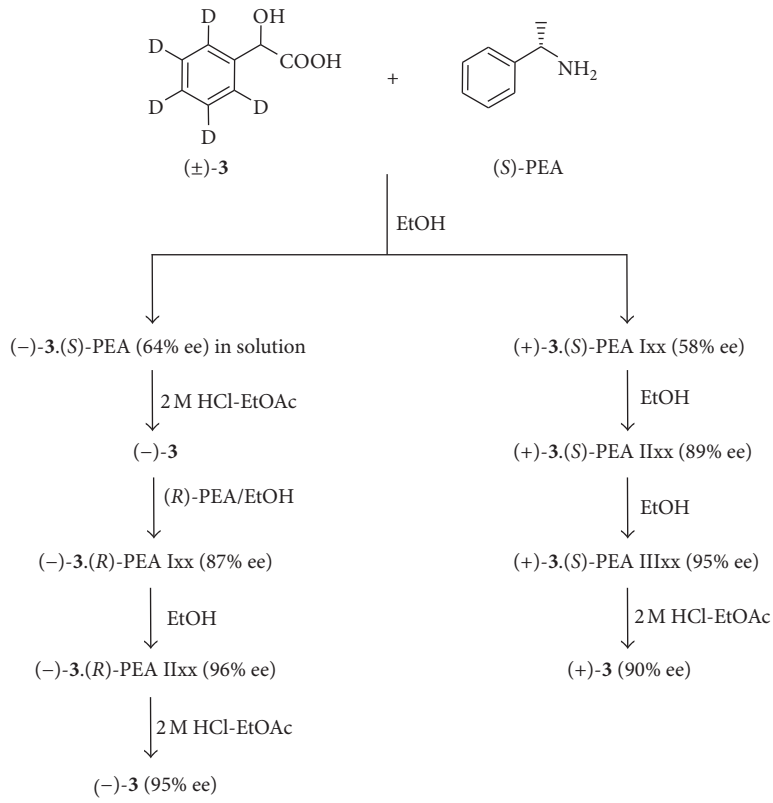
In summary, we have reported a simple procedure to obtain MA-d_5 enantiomers in highly enriched optically active forms. Unambiguous attribution of (*R*)- and (*S*)-absolute configurations to (−)- and (+)-MA-d_5, respectively, was given. (+)-(*S*)-MA-d_5 might be useful as a pharmacological tool to deepen the studies on styrene metabolism while the possibility to use MA-d_5 enantiomers as chiral synthons for the preparation of their corresponding *O*-derivatives is currently under investigation.

4. Experimental

4.1. Chemistry. All chemicals were purchased from Sigma-Aldrich or Lancaster. Solvents were RP grade unless otherwise indicated and 95% ethanol was used. The reactions under microwave irradiation were carried out at constant temperature in a CEM Discover BenchMate microwave reactor, with continuous stirring. The temperature was measured and controlled by a built-in infrared detector. The structures of the compounds were confirmed by routine spectrometric analyses. Melting points were determined on a Gallenkamp melting point apparatus in open glass capillary tubes and



Scheme 2: Intramolecular Cannizzaro disproportionation of phenylglyoxal-d_5 hydrate **4**, possible intermediate of step 2 in Scheme 1.



Scheme 3: Optical resolution of (±)-mandelic acid-d_5 [(±)-(**3**)].

were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-VX spectrometer operating at 300 and 75 MHz for ^{1}H and ^{13}C, respectively, using DMSO-d_6 as a solvent. Chemical shifts are reported in parts per million (ppm) relative to the residual nondeuterated solvent resonance: δ 2.47 (^{1}H NMR) and 39.5 (^{13}C NMR). J values are given in Hz. The ee values for (−)-(*R*)- and (+)-(*S*)-**3** were determined by capillary electrophoresis on a P/ACE MDQ Beckman instrument (Palo Alto, CA, USA), equipped with a diode-array spectrophotometric detector. A fused silica capillary of 60 cm (effective length 50 cm) and 0.05 mm i.d. (Quadrex Corporation, Woodbridge, CT, USA) thermostated at 20°C was used as a separation tube. Diastereomeric salt samples (0.1 mg/mL) were pressure injected (0.5 psi for 5 sec) at the anionic end of the capillary and detected at 214 nm. As a background electrolyte (BGE), 2-hydroxypropyl-β-cyclodextrin (60 mg/mL) in phosphate buffer 0.033 M at pH 6.0 was used. The applied voltage was 30 kV. Migration times were 4.3 min for (*R*)- and (*S*)-1-phenylethylamine, 23.5 min for (*R*)-**3**, and 24.0 min for (*S*)-**3**. EIMS spectra were recorded on a Hewlett-Packard 6890-5973 MSD gas chromatograph/mass spectrometer at low resolution. ESI$^+$/MS/MS analyses were performed with an Agilent 1100 Series LC/MSD Trap System VL Workstation.

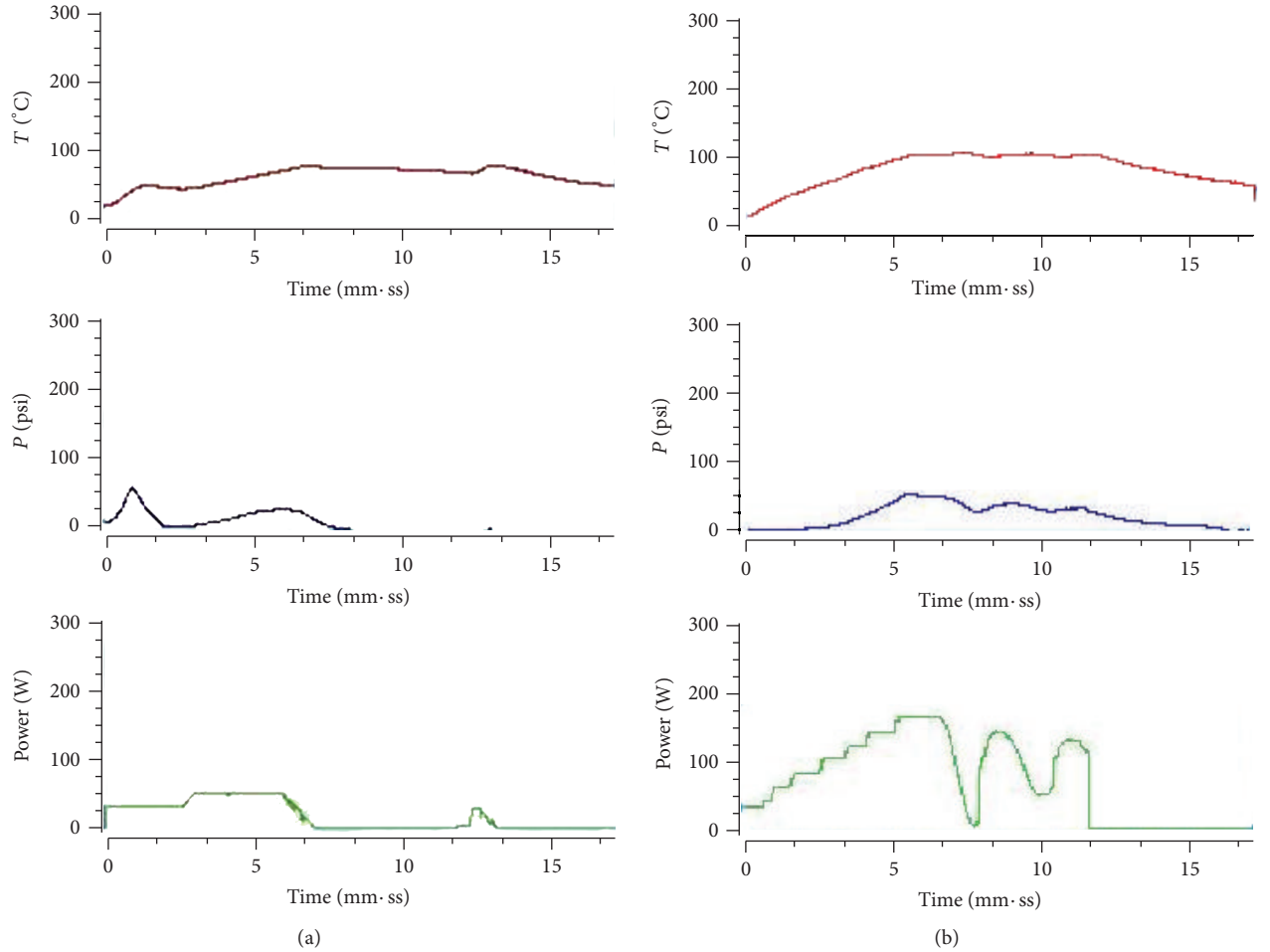
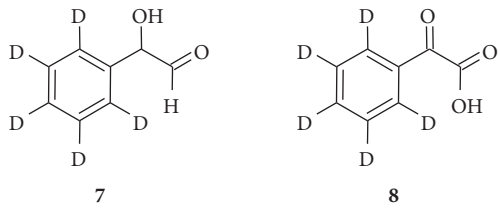


Figure 1: (a) Temperature, pressure, and power profiles for step 1 in Scheme 1; (b) temperature, pressure, and power profiles for step 2 in Scheme 1.



7 **8**

Figure 2: Byproducts deriving from intermolecular hydride migration in step 2 in Scheme 1.

Elemental analyses were performed on a EuroVector Euro EA 3000 analyzer. Optical rotations were measured on a Perkin Elmer (Norwalk, CT) Model 341 spectropolarimeter; concentrations are expressed in g/100 mL, and the cell length was 1 dm, thus $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. TLC analyses were performed on precoated silica gel on aluminium sheets (Kieselgel 60F_{254}, Merck). CD and UV curves were registered on a J-810 model JASCO spectropolarimeter and the concentration of the solution was 10^{-5} M. CD and UV measurements were performed at room temperature and are baseline corrected and smoothed.

*4.2. Synthesis of (±)-***3**. A mixture of hexadeuterobenzene **1** (2.63 mL, 29.8 mmol), 2,2-dichloroacetyl chloride (2.86 mL, 29.7 mmol), and $AlCl_3$ (3.97 g, 29.7 mmol) in CH_2Cl_2 (10 mL) was stirred for 8 min at 70°C in a microwave reactor. Then, the mixture was poured on ice and 20 mL of concentrated HCl were added. The organic phase was washed with 1 M NaOH, and then with water. The crude product obtained after solvent evaporation (**2**, 5.30 g) was reacted, without further purification, with NaOH (5.30 g, 133 mmol) in H_2O (44 mL). The solution was stirred for 11 min at 110°C in a microwave reactor under continuous stirring. Concentrated HCl (5.5 mL) was then added and the aqueous phase was extracted three times with EtOAc. The combined organic phases were extracted with 1 M NaOH and then acidified with 6 M HCl. The aqueous phase was extracted three times with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give 1.70 g (82%) of the desired product as a white solid which was recrystallized

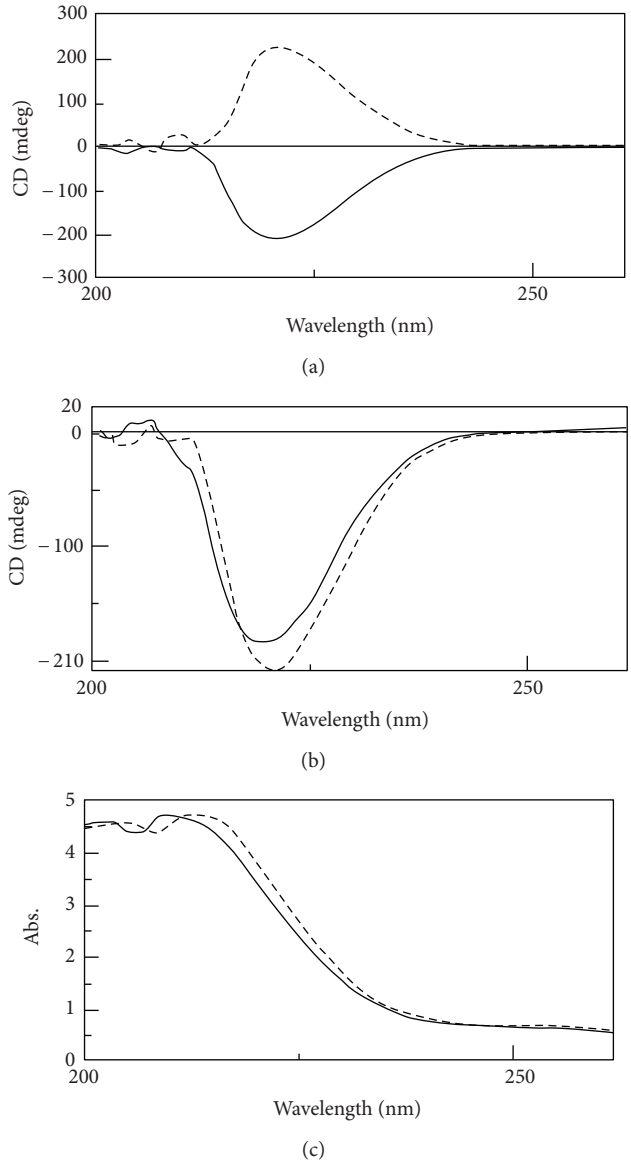


Figure 3: (a) Observed (−)-(*R*)- and (+)-(*S*)-MA CD spectra (solid and dashed lines, resp.) in water; (b) observed (−)-(*R*)-MA and (−)-(*R*)-**3** CD spectra (dashed and solid lines, resp.) in water; (c) observed (−)-(*R*)-MA and (−)-(*R*)-**3** UV absorption spectra (dashed and solid lines, resp.) in water.

from $CHCl_3$ to obtain 1.10 g (52%) of crystalline product: mp 117-118°C; ^{1}H NMR: δ 5.00 (s, 1H), 5.83 (br s, 1H), 12.56 (br s, 1H); ^{13}C NMR: δ.73.0 (1C), 126.9 (t, J = 96.2 Hz, 2C), 127.8 (t, J = 93.3 Hz, 2C), 128.3 (t, J = 96.9 Hz, 1C), 140.7 (1C), 174.8 (1C).

*4.3. Optical Resolution of (±)-***3**. To a solution of racemic mandelic acid-d_5 (6.0 g, 0.038 mol) in 40 mL of ethanol, (*S*)-PEA (4.9 mL, 0.038 mol) was added and a crude solid formed (Scheme 3). The mixture was heated to 70°C by means of a water bath to dissolve the solid. The solution was kept at 70°C for 15 min and then slowly cooled to room temperature. After standing overnight, the crystalline salt was collected by filtration and washed with cold ethanol (25 mL) to give (+)-**3**·(*S*)-PEA Ixx diastereomeric salt (4.9 g, mp: 167–172°C, 58% ee, CZE). This diastereomeric salt was recrystallized from ethanol (32 mL) to afford 2.2 g of (+)-**3**·(*S*)-PEA IIxx as white crystals (mp: 175–176°C, 89% ee, CZE). This salt was recrystallized from 95% ethanol (15 mL) to give 1.4 g of (+)-**3**·(*S*)-PEA IIIxx {mp: 171–173°C, $[\alpha]_D^{20}$ = +44 (*c*2, MeOH), 95% ee, CZE}. This salt was dissolved in H_2O and the resulting solution was acidified with 2 M HCl. The liberated acid was extracted with EtOAc (20 mL × 3). The combined organic phases were dried over Na_2SO_4 and, upon removal of solvent under reduced pressure, gave (+)-MA-d_5 [(+)-**3**] as a white solid {0.48 g, 16% yield based on (+)-MA-d_5 in the starting racemic mixture; mp: 131–132°C; $[\alpha]_D^{20}$ = +141 (*c*1, MeOH), 90% ee, CZE. Anal. calcd. for $C_8H_3D_5O_3$: C, 61.13; H, 5.30. Found: C, 61.20; H, 5.13}.

On the other hand, enriched (−)-MA-d_5 [(−)-**3**] was easily recovered from the mother liquor (−)-**3**·(*S*)-PEA (64% ee, CZE) obtained from the above first recrystallization step and used for further resolution. Thus, 3.2 g of (−)-**3** were treated with (*R*)-PEA as the resolving agent. After the first recrystallization from ethanol (21 mL), 1.93 g of diastereomeric salt were obtained (mp: 168–170°C, 87% ee, CZE). This salt was recrystallized from ethanol (12 mL) to give (−)-**3**·(*R*)-PEA IIxx {1.1 g, mp: 165–167°C; $[\alpha]_D^{20}$ = −44 (*c*2, MeOH), 96% ee, CZE}. Finally, applying the above extraction procedure to the so-obtained salt, (−)-**3** was obtained as a white solid {0.55 g, 18% yield based on (−)-MA-d_5 in the starting racemic mixture; mp: 129–131°C; $[\alpha]_D^{20}$ = −135 (*c*1, MeOH), 95% ee, CZE. Anal. calcd. for $C_8H_3D_5O_3$: C, 61.13; H, 5.30. Found: C, 61.51; H, 5.17}.

Conflict of Interests

The authors did not report any conflict of interests.

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