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Baker's yeast catalyzed preparation of a new enantiomerically pure synthon of (*S*)-pramipexole and its enantiomer (dexpramipexole)

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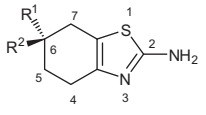
This work is dedicated to Professor Enzo Santaniello in the year of his 70th birthday

ABSTRACT

A biocatalyzed reduction of a prochiral bicyclic ketone afforded enantiomerically pure (*R*)-2-acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole, a synthon of the anti-Parkinson (*S*)-pramipexole and its (*R*)-isomer, which is currently under investigation for the treatment of amyotrophic lateral sclerosis (ALS).

1. Introduction

Parkinson disease is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons of *substantia nigra* in the brain basal glia accompanied by motor symptoms (bradykinesia, muscle rigidity, resting tremor, and equilibrium impairment). Parkinson disease is treated with dopamine replacement agents, such as levodopa or dopaminergic agonists. Pramipexole **1**, approved in 1997 in the USA and in 1998 in most European countries, is the most prescribed dopamine agonist, both as monotherapy and as adjunctive therapy with levodopa.[1–3]



1

R^1 = H, R^2 = $NHCH_2CH_2CH_3$ (*R*)-pramipexole
R^1 = $NHCH_2CH_2CH_3$, R^2 = H (*S*)-pramipexole

Pramipexole **1** (2-amino-6-propylamino-4,5,6,7-tetrahydrobenzothiazole) bears a stereocenter at the 6-position. Only the (*S*)-isomer is active as a dopamine agonist and is marketed, as the dihydrochloride monohydrate (Mirapex brand name) for Parkinson disease treatment. The (*R*)-isomer (dexpramipexole) is currently in clinical development for the treatment of amyotrophic lateral sclerosis (ALS), a rapidly progressive and lethal neurodegenerative disorder of motor neurons. The enantiomeric purity of the (*R*)-isomer drug substance is a critical parameter for accurately interpreting studies conducted with dexpramipexole.[4] The presence of a trace amount of (*S*)-pramipexole has a significant pharmacological effect due to its extremely high dopamine agonist affinity.[4] Therefore the synthesis of both enantiomerically pure (*R*)- and (*S*)-pramipexole is an engaging goal not only in laboratory amounts but also for large scale preparative purposes.

Usually the synthesis of (*S*)-pramipexole is realized via the resolution of racemic pramipexole[5] or of an intermediate[6] by fractional crystallization of a suitable diastereomeric salt. Preparative chiral chromatography has also been applied to the separation of (*R*)- and (*S*)-pramipexole[7,8] or of a racemic precursor.[8]

In order to avoid the long and tedious processes that characterize fractional crystallization and preparative HPLC, an asymmetric synthesis should be performed; we planned to take advantage of the high stereoselectivity of biocatalysts in order to prepare an enantiomerically pure intermediate, that could then be easily converted into (*S*)-pramipexole or dexpramipexole.

2. Results and discussion

Two distinct biocatalytic approaches to the synthesis of (*S*)-pramipexole have been already described starting from (*RS*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole **2**[9] or from the

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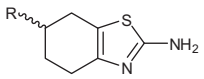
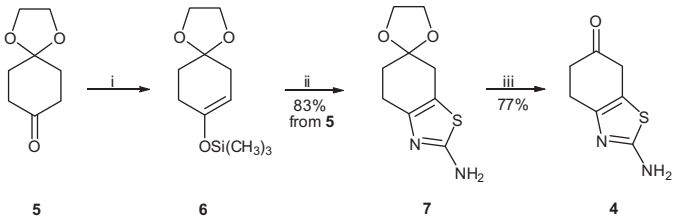
ester of (*RS*)-6-carboxylic acid **3**.[10] In both cases, the (*R*)- and (*S*)-intermediates were obtained via a lipase-catalyzed transformation.



2 R = NH_2
3 R = COOR

The well documented capability of microorganisms[11,12] to perform asymmetric reductions of prochiral carbonyl groups to alcohols prompted us to study this approach. Initially, 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole **4** seemed to be the best substrate for preliminary microorganism screening. Starting from the commercially available monoprotected cyclohexandione **5**, we prepared enolsilyl ether **6**,[13] which was transformed into the 6-acetal of 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole **7** by treatment with *N*-bromosuccinimide[14] followed by cyclization with thiourea. Hydrolysis of acetal with 5% hydrochloric acid afforded the desired ketone **4** in 64% yields from **5** (Scheme 1).



Scheme 1. Reagents and conditions: (i) $(CH_3)_3SiOSO_2CF_3$, $(CH_3CH_2)_3N$, CH_2Cl_2; (ii) NBS, AcONa, thiourea, THF/H_2O; (iii) 5% HCl, THF.

The choice of *Saccharomyces cerevisiae* (the common Baker's yeast), as the first microorganism to be screened was due to its well-known advantages such as simple experimental conditions, easy availability, and efficiency in relation to the yields and stereoselectivity.[11,15–17] The bioreduction was carried out under different conditions as summarized in Table 1, in order to achieve the highest ee.

Commercial lyophilized Baker's yeast[18] catalyzed the complete conversion of ketone **4** into alcohol **8** but with moderate ee (84%) (Scheme 2). The best results, even if not yet completely satisfactory (94% ee and low recovery), were observed by addition to the biotransformation medium of an organic solvent, according to a reported procedure.[19–22] Multiple reducing enzymes are present in yeast with different enantioselectivities. The role of an organic solvent can be explained either by a change in the substrate aqueous concentration, which should favor a single reducing enzyme, or by possible inhibition of one or more reducing enzymes.[11]

In order to obtain enantiomerically pure alcohol **8**, we focused our attention on other microorganisms, listed in Table 2, that are already used for stereoselective reduction of carbonyl compounds.[23] However, with regard to the complete conversion of ketone **4** into alcohol **8**, the results were not satisfactory in terms of the ee (see Table 2) and the low recovery.

It is well known that substrate modification can reverse the biotransformation stereochemical outcome and also increase the enantiomeric purity.[11,24] In our case, the functionalization of the 2-amino group could improve the recovery of the alcohol from the aqueous media. We therefore carried out Baker's yeast catalyzed reduction on *N*-acetyl derivative **9** (Scheme 2). In this case, the addition of *n*-heptane improved the enantiomeric excess in pH 7 buffer alone (92%). If the reaction was stopped at complete conversion a 94% ee was obtained; lowering the conversion to 87%, >98% ee was achieved. The easier recovery of the less polar alcohol **10** (compared with **8**) from the reaction medium, by means of a continuous extraction, led to 75–80% yields. The ee was determined by HPLC analysis on a chiral stationary phase (Chiralpak IA) by comparison with a racemic sample. The formation over the course of the bioreduction of a by-product (5%), with a retention time very similar to that of the enantiomer later eluted, required careful purification of the crude alcohol **10**. The enantiomeric purity was unequivocally ascertained through high field ^1H NMR analysis of the diastereomeric esters **11**, which were obtained by reaction with the (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chloride[25] of alcohols (*R*)- and (*R,S*)-**10**. The same analysis also allowed us to assign, according to the modified method of Mosher,[26] the (*R*)-configuration to alcohol **10**, not previously described in the literature (see the NMR section below).

The knowledge of the stereocenter configuration was necessary in order to plan how to accomplish the synthesis of (*S*)-pramipexole **1**. Therefore, (*R*)-**10** was transformed into the corresponding tosylate **12** (74%), which upon treatment with sodium azide in dimethylformamide afforded azide **13** with the desired (*S*)-configuration. Reduction with polymer bonded triphenylphosphine gave (*S*)-2-acetylamino-6-amino-4,5,6,7-tetrahydrobenzothiazole **14** (76% from tosylate **12**). By removing the acetyl group, (*S*)-2,6-diamine **2**, an advanced known intermediate of (*S*)-pramipexole **1**, was obtained [in 51% overall yield from (*R*)-alcohol **10**] (Scheme 3).

The configuration and enantiomeric purity were determined by comparison with obtained **2** with a sample of (*S*)-**2**, [previously prepared by fractional crystallization[6] of (*R,S*)-**2**] via HPLC analysis on chiral stationary phase [Crownpak CR (+)]; the (*S*)-configuration of **2** and the (*R*)-configuration of biosynthesized **10** were confirmed. In addition, the observed >98% ee allowed us to conclude that the enantiomeric purity remained the same over the course of the transformation of **10** into **2**.

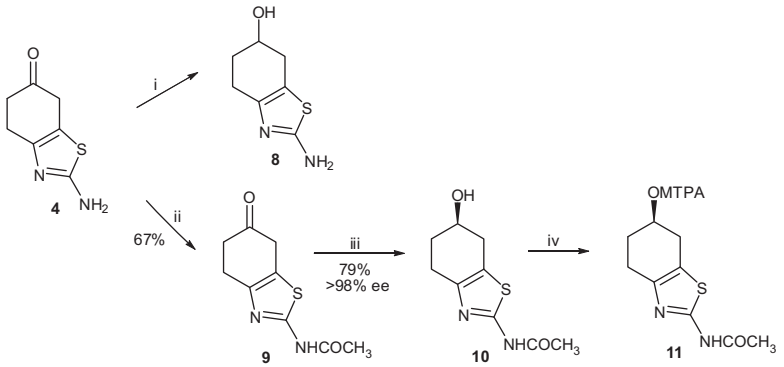
Finally, according to the literature,[27] (*S*)-2,6-diamine **2** was treated with 1-propanol tosylate **15** to afford after suitable work-up the dihydrochloride monohydrate of (*S*)-pramipexole **1** in 42% yields.

Enantiomerically pure alcohol (*R*)-**10** was chosen as the starting material for the synthesis of the intermediate of the (*R*)-isomer of pramipexole **1**, the dexpramipexole, which is undesired in anti-Parkinson preparations, but is currently under investigation for ALS treatment. A Mitsunobu reaction[28] starting from alcohol (*R*)-

Table 1
Baker's yeast-catalyzed reduction of ketones **4** and **9**

Ketone	Alcohol	Experimental conditions	Time (h)	Conversion[a] (%)	ee[a] (%)
4	**8**	Buffer pH 7	15	100	84
4	**8**	Buffer pH 7/*n*-heptane 1:1	10	100	94
4	**8**	Buffer pH 7/toluene 1:1	10	100	93
9	**10**	Buffer pH 7	18	100	92
9	**10**	Buffer pH 7/*n*-heptane 1:1	18	100	94
9	**10**	Buffer pH 7/*n*-heptane 1:1	16	87	>98

[a] Determined by HPLC on chiral stationary phase.



Scheme 2. Reagents: (i) Bioreduction; (ii) Ac_2O; (iii) Baker's yeast; (iv) MTPA-Cl.

Table 2
Bioreduction of ketone **4** to alcohol **8**

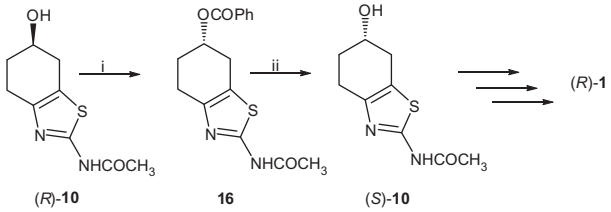
Microorganism[a]	Enantiopurity of alcohol[b] **8** (%)
Torulopsis magnoliae IMAP 4425	0
Torulopsis molischiana CBS 837	0
Sporobolomyces salmonicolor	0
Pichia etchelsii	50
Kluyveromyces marxianus CBS 1553	80
Hansenula polymorpha CBS 4732	50

[a] Resting cells resuspended in 0.1 M phosphate buffer pH 7 containing 50 g/L of glucose. The biotransformations were stopped after 48 h at 100% conversion (by TLC).
[b] Determined by HPLC on chiral stationary phase.

10 afforded enantiomerically pure (by HPLC) benzoate **16**; alcohol **10** was obtained by ester hydrolysis and its the (*S*)-configuration was assigned by comparison with the HPLC chromatograms of (*RS*)- and (*R*)-**10** (Scheme 4).

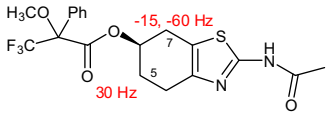
2.1. NMR analysis

In spite of the narrow dimensions of the studied molecules, in order to unequivocally assign the NMR signals, careful analysis was required. Proton and carbon 1D NMR spectra, as well as 2D NMR homocorrelation (COSY) and heterocorrelation (HMQC and HMBC) spectra were employed for complete structural assignments. In compound **4**, the assignment of the signals of the protons at C-4 and at C-5 was made on the basis of the long-range coupling constants and splitting patterns observed in the ^{1}H NMR. The resonance of the protons at δ 2.95 was a triple triplet (J = 6.9 and J = 1.8 Hz) which was correlated in the COSY experiment with both protons at δ 2.71 (t, J = 6.9 Hz) and δ 3.43 (7-CH_2, t, J = 1.8 Hz). These data revealed that these hydrogens belonged to 4-CH_2 since they have a homoallylic coupling constant ^5J with the 7-CH_2 protons due to the presence of π-electron system. On the basis of these observations, the spectra of other compounds were assigned.

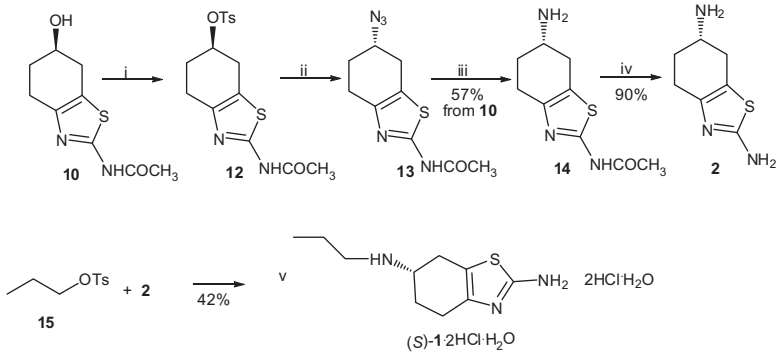


Scheme 4. Reagents and conditions: (i) PhCOOH, DEAD, Ph_3P, DMF; (ii) 1% NaOH, MeOH.

In order to assign the configuration of the C(6) of secondary alcohol **10**, we used a modified Mosher's method[26] to establish the absolute configuration of the secondary alcohols. Treatment of **10** with (*S*)- and (*R*)-2-methoxy-2-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl)[25] gave the 6-*O*-(*R*)-MTPA and 6-*O*-(*S*)-MTPA esters, respectively, whose ^{1}H NMR spectra at 500 MHz were acquired and assigned. The $\Delta\delta$ values ($\delta_S - \delta_R$) are expressed in Hertz and are shown in Scheme 5. The chemical shifts of the protons at the 5-position of the (*R*)-MTPA esters appear shielded with respect to those of the (*S*)-MTPA diastereomer. Conversely, the chemical shifts of the protons at the 7-position appear deshielded



Scheme 5. Configuration assignment to alcohol **10** based on the ^{1}H NMR $\Delta\delta$ values obtained for their (*S*)- and (*R*)-MTPA esters. $\Delta\delta$ values ($\delta_S - \delta_R$) are expressed in Hz (500 MHz).



Scheme 3. Reagents and conditions: (i) TsCl, py; (ii) NaN_3, DMF; (iii) Ph_3P polymer bonded, THF/H_2O; (iv) 5% HCl, THF; (v) NEt(*i*Pr)$_2$, DMF, 1 M NaOH, 12 M HCl.

in (*R*)-MTPA esters relative to the (*S*)-MPTA ones, thus allowing us to assign the (*R*)-configuration at C-6.

The NMR analysis of (*R*)-MTPA esters **11** of (*R*)- and (*R*,*S*)-**10** also allowed us to confirm the ee of alcohol **10** previously determined by HPLC. One of protons at the 7-position showed two double doublets centered at 3.16 and 3.14 ppm in the case of (*R*,*S*)-**10**. The signal centered at 3.14 ppm was not detectable in the spectrum of MTPA ester of (*R*)-**10**.

3. Conclusion

The synthesis of alcohols via Baker's yeast-catalyzed reduction of carbonyl compounds represents a classic approach of organic synthesis;[15–17] when a prochiral ketone is reduced a chiral alcohol is formed. Nevertheless several reductases with different stereoselectivities are present in the *Saccharomyces cerevisiae*, but enantiomerically pure compounds are obtained in most cases. These considerations prompted us to plan a biocatalyzed reduction of a suitable ketone in order to obtain an enantiomerically pure advanced intermediate of the synthesis of (*S*)-pramipexole, a known anti-Parkinson drug. Modification of the starting ketone and of the biotransformation medium allowed us to obtain enantiomerically pure (*R*)-alcohol **10**, thus showing the Baker's yeast efficiency. This synthon, not yet described in the literature, was transformed through very simple steps into the desired dihydrochloride monohydrate derivative of (*S*)-pramipexole **1** in 21% overall yield.

The limitation usually ascribed to the biocatalyzed reduction of a ketone, namely that only one enantiomer is achievable, was overcome by the complete inversion of the configuration realized under the Mitsunobu conditions.[28] The (*S*)-alcohol **10** obtained is a suitable substrate for dexpramipexole, the (*R*)-isomer of pramipexole, which is currently under investigation in the treatment of ALS.

The use of readily available and inexpensive *Saccharomyces cerevisiae*, the easy preparation of the biotransformation substrate, and the simple steps required to accomplish the synthesis, make the method applicable to a preparative scale. The optimized HPLC analysis conditions (on a chiral stationary phase) and careful NMR studies allow us to monitor the identity of the compounds and the stereochemical outcome of the whole process.

4. Experimental

General: The reagents were purchased from Sigma–Aldrich (Italy). The solvents were purchased from Panreac (Novachimica, Italy). Dry Baker's yeast was supplied by Paneangeli, Italy. Racemic 2,6-diamino-4,5,6,7-tetrahydrobenzothiazole was from Pen Tsao Chemical & Pharmaceutical Industry Co., Ltd (China). All reactions were monitored by TLC on silica gel 60 F_{254} precoated plates with a fluorescent indicator (Merck) with detection by spraying with a 10% phosphomolybdic acid ethanol solution and heating at 110 °C. Column chromatography was performed on silica gel 60 (70–230 mesh) (Merck). HPLC analyses were performed with a Merck-Hitachi L-6200; RP column: Waters μBondapak C18 (0.39 cm × 30 cm, 10 μm); chiral columns: Daicel Crownpak CR(+) (0.4 cm × 15 cm, 5 μm); Daicel Chiralpak IA (0.46 cm × 25 cm, 5 μm); UV detector wavelength 254 nm. Nuclear magnetic resonance (NMR) spectra were recorded at 300 K on a Bruker-Avance 500 MHz spectrometer operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C acquisitions, respectively. Chemical shifts (δ) of the ^{1}H NMR and ^{13}C NMR spectra are reported in ppm using the signal for residual solvent proton resonance as the internal standard (^{1}H NMR: $CDCl_3$ 7.26, DMSO-d_6 2.49, CD_3OD 3.31 ppm; ^{13}C NMR: $CDCl_3$ 77.0 (central line), DMSO-d_6 39.50 (central line), CD_3OD 49.00 (central line) ppm). Optical rotations were registered on a Perkin–Elmer (mod. 241) polarimeter in a 1 dm cell at 20 °C, setting the wavelength at 589 nm or at 546 nm. Mass spectra were recorded on a Agilent instrument (mod 6339 Ion trap LC/MS) using the ESI source with positive and/or negative ion polarity; the samples were dissolved in methanol (0.05 μg/μL) and were examined utilizing the direct inlet probe technique at an infusion rate of about 0.6 mL/min; data acquisition and analysis were accomplished with Bruker Daltonics Data Analysis 3.3 software. The infrared spectra were registered on a Perkin Elmer instrument (mod. FT-IR spectrum one) equipped with universal attenuated total reflection (ATR) sampling. Differential scanning calorimetry (DSC) analyses were performed on a Perkin Elmer DSC-7 instrument, heating 10 °C/min. Melting points were performed on SMP3 Stuart Scientific apparatus, 2.5 °C/min.

4.1. 1,4-Cyclohexanedione-1-ethyleneacetal-4-trimethylsilyl-enolether 6

To a solution of 1,4-cyclohexanedione monoethylene acetal **5** (7 g, 44.8 mmol) in dichloromethane (400 mL) and triethylamine (18.5 mL, 0.133 mol) at 0 °C, a solution of trimethylsilyl trifluoromethanesulfonate (10 mL, 0.055 mol) in dichloromethane (120 mL) was added dropwise. The reaction mixture was kept under stirring at 0 °C under an N_2 atmosphere until the starting material disappeared (1 h), monitoring the reaction progress by TLC (hexane/ethyl acetate/triethyl amine 9.5:0.45:0.05). Saturated sodium hydrogen carbonate aqueous solution (30 mL) and water (200 mL) were then added. The organic phase was washed with brine (4 × 100 mL). After drying over sodium sulfate, filtration, and removal of the solvent under reduced pressure, enol ether **6** (9.9 g, 96%) was recovered as an oil, which was used in the next step, without further purification. The chemical and physical properties were in agreement with the literature values.[13]

4.2. 2-Amino-6-(ethylenedioxy)-4,5,6,7-tetrahydrobenzothiazole 7

To a solution of silyl enol ether **6** (9.9 g, 43.4 mmol) in tetrahydrofuran/water 1:1 (380 mL), *N*-bromosuccinimide (9.6 g, 53.9 mmol) and sodium acetate (0.500 g, 6.1 mmol) were added. The reaction mixture was kept at room temperature with stirring until the starting material disappeared (2 h) (TLC hexane/ethyl acetate 9:1). Thiourea (3.46 g, 45.5 mmol) was added and the reaction mixture was kept at 80 °C (6 h) and then at room temperature overnight, under stirring. The reaction was monitored by TLC (dichloromethane/methanol 9:1). Tetrahydrofuran was removed at reduced pressure and the aqueous phase was extracted with dichloromethane (4 × 50 mL). To the aqueous phase, 2 M sodium hydroxide was added until pH 9. Extraction with dichloromethane (5 × 50 mL), and usual work-up afforded **7** as a solid (7.9 g, 83% from **5**), which was used in the next step without further purification. The chemical and physical properties are in agreement with the literature values.[29]

4.3. 2-Amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole 4

To a solution of acetal **7** (6.6 g, 0.031 mol) in tetrahydrofuran (120 mL), 5% hydrogen chloride aqueous solution (120 mL) was added. The reaction mixture was kept at reflux with stirring while monitoring the reaction progress by HPLC (C18 μBondapak column, water/acetonitrile 7:3 as eluent, flow rate: 0.5 mL/min). After the disappearance of the starting material (4 h), the work-up was carried out as reported in the literature.[30] A saturated aqueous potassium carbonate solution (75 mL) was then added until pH 7. Solvents were removed by evaporation at reduced pressure. To the obtained solid residue, diethyl ether (60 mL) and saturated aqueous potassium carbonate solution were added. The red precip-

itate was recovered by suction and washed with diethyl ether (3 × 20 mL) to afford a crude product (6.2 g) which was purified by silica gel column chromatography (1:10); elution with ethyl acetate/methanol/triethylamine (84:8:8) to give pure **4** (4.0 g, 77%). R_t compound **4** 8.96 min; compound **7** 15.44 min. TLC (dichloromethane/methanol/triethylamine 95:4:1) R_f 0.38. Mp 175 °C (ethyl acetate). DSC endothermic peak at 194 °C. IR ν_{max} 3368.74, 3280.27, 3087.31, 2904.41, 1693.43, 1637.37, 1595.85, 1540.22, 1493.29 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.43 (2H, t, J = 1.8 Hz, 7-H), 2.95 (2H, tt, J = 6.9, 1.8 Hz, 4-H), 2.71 (2H, t, J = 6.9 Hz, 5-H). ^{13}C NMR ($CDCl_3$): δ 207.2 (6-C), 167.4 (2-C), 144.3 (N-C), 113.7 (S-C), 38.7 (5-C), 37.4 (7-C), 25.3 (4-C). ^1H NMR (DMSO-d_6): δ 3.36 (2H, t, J = 0.9 Hz, 7-H), 2.77 (2H, tt, J = 7.0, 0.9 Hz, 4-H), 2.60 (2H, t, J = 7.0 Hz, 5-H). ^{13}C NMR (DMSO-d_6): δ 207.6 (6-C), 167.6 (2-C), 144.6 (N-C), 111.5 (S-C), 38.6 (4-C), 37.6 (7-C), 25.6 (5-C). MS (m/z) (ESI-) 167.3 $[M-1]^+$. (ESI+) 201.1 $[M+CH_3OH]^+$ 223 $[M+Na+CH_3OH]^+$.

4.4. 2-Acetylamino-6-oxo-4,5,6,7-tetrahydrobenzothiazole 9

A solution of **4** (3.12 g, 18.6 mmol) in acetic anhydride (7 mL) was kept under stirring at 100 °C.[31] Monitoring the reaction by TLC (dichloromethane/methanol 95:5), complete conversion was observed after 30 min. The reaction mixture, after cooling to room temperature, was poured into water (130 mL). The aqueous phase was extracted with ethyl acetate (5 × 50 mL). The organic phases were washed with water (3 × 50 mL), dried over sodium sulfate and, after filtration, evaporated at reduced pressure. The crude product (5.6 g) was purified by silica gel column chromatography (1:10). Elution with ethyl acetate/hexane 9:1 afforded pure **9** (2.6 g, 67%). TLC R_f 0.38. Mp 220 °C (ethyl acetate). DSC endothermic peak 235.83 °C. IR ν_{max} 3371.99, 3234.12, 3148.90, 3023.23, 2935.27, 2894.40, 2765.87, 1705.59, 1682.65, 1539.23 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.59 (2H, br s, 7-H), 3.12 (2H, br t, J = 7.0 Hz, 4-H), 2.79 (2H, t, J = 7.0 Hz, 5-H), 2.32 (3H, s, CH_3).^{13}C NMR ($CDCl_3$): δ 205.5 (6-C), 167.7 ($NHCOCH_3$), 158.1 (2-C), 141.1 (N-C), 119.4 (S-C), 38.4 (4-C), 37.0 (7-C), 24.7 (5-C), 23.3 (CH_3). MS (m/z) (ESI−) 209.7 $[M-1]^+$. (ESI+) 211.0 $[M+1]^+$, 233.0 $[M+Na]^+$, 265.1 $[M+Na+CH_3OH]^+$.

4.5. (*RS*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole 10

To a solution of ketone **9** (0.365 g, 1.74 mmol) in methanol (20 mL), sodium borohydride (0.250 g, 6.60 mmol) was slowly added. The solution was kept under stirring at room temperature while monitoring the reaction progress until the starting material disappeared (1 h) (TLC dichloromethane/methanol 95:5). Next, 3 M hydrochloric acid was added until pH 7; the solvents were removed at reduced pressure and the solid residue was washed with ethyl acetate (2 × 10 mL). After filtration, the filtrate was dried over sodium sulfate and the usual work-up afforded pure (*RS*)-**10** (0.353 g, 95%).

4.6. (*R*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole 10

To a solution of sucrose (55 g) in a phosphate buffer (pH 7, 1 L) dry Baker's yeast (16 g) was added. The suspension was kept for 1 h at 30 °C under vigorous mechanical stirring. *n*-Heptane (1 L) and ketone **9** (2.5 g, 11.9 mmol) were then added and the mixture was kept at 30 °C, under vigorous stirring. The reaction progress and the ee of the alcohol were monitored by HPLC on chiral stationary phase (Chiralpak IA, hexane/2-propanol 8:2 as eluent, flow rate: 0.7 mL/min). At 87% conversion (16 h), the suspension was filtered through a Celite pad. The organic phase was separated, dried on sodium sulfate and, after filtration, the solvent was removed under reduced pressure to give an oily residue (80 mg). The aqueous phase was extracted in a continuous extraction apparatus with the ethyl acetate previously used in order to wash the Celite pad (5 × 200 mL). The organic phase was separated, dried over sodium sulfate, and filtered. Evaporation of the solvent under reduced pressure afforded a solid residue (2.2 g) which was purified by silica gel column chromatography (1:20). Pure **10** (1.99 g, 79%) was recovered by elution with ethyl acetate. The ee (>98%) was established by HPLC on chiral stationary phase by comparison with the previously prepared (*R,S*)-**10**. R_t (*R*)-isomer 10.90 min; (*S*)-isomer 14.85 min. TLC (dichloromethane/methanol 95:5) R_f 0.25. $[\alpha]_D^{20}$ = +25.5 (*c* 1, methanol); $[\alpha]_{546}^{20}$ = +30.7 (*c* 1, methanol). Mp 179 °C (ethyl acetate). DSC endothermic peak 187 °C. IR ν_{max} 3421.46, 3234.12, 3151.18, 3031.33, 2930.41, 2853.17, 2767.79, 1687.99, 1667.98, 1537.67, 1435.72, 1361.70 cm^{-1}. ^1H NMR (CD_3OD): δ 4.15 (1H, dddd, J = 8.6, 6.9, 4.7, 2.9 Hz, 6-H), 2.99 (1H, ddd, J = 15.7, 4.7, 1.8 Hz, 7a-H), 2.79 (1H, dddd, J = 16.5, 5.9, 5.9, 1.8 Hz, 4a-H), 2.71–2.61 (2H, m, 4b-H and 7b-H), 2.26 (3H, s, CH_3), 2.04 (1H, dddd, J = 14.2, 6.2, 5.9, 2.9 Hz, 5a-H), 1.91 (1H, dddd, J = 14.2, 8.6, 7.7, 5.9 Hz, 5b-H), ^{13}C NMR (CD_3OD): δ 169.1 ($NHCOCH_3$), 156.4 (2-C), 143.2 (N-C), 120.9 (S-C), 66.2 (6-C), 30.7 (7-C), 30.5 (5-C), 23.2 (4-C), 21.2 (CH_3). MS (m/z) (ESI−) 212.1 $[M]^+$. (ESI+) 235.0 $[M+Na]^+$.

4.7. General procedure for the preparation of Mosher's ester 11

To a stirred solution of alcohol (*R*)-**10** or (*R,S*)-**10** (10 mg, 0.047 mmol) in carbon tetrachloride (0.2 mL) and pyridine (0.2 mL), (*S*)-MTPA-chloride (12 μL, 0.061 mmol) was added. The reaction mixture was allowed to stand at room temperature for 12 h. Next, dichloromethane (3 mL) and 3-dimethylaminopropan-1-amine (13 μL) were added, the organic phase was washed with 1 M hydrogen chloride aqueous solution (2 × 5 mL), a saturated sodium hydrogen carbonate aqueous solution (2 × 5 mL) and with brine (5 mL). After drying over anhydrous sodium sulfate and filtration, the solvents were evaporated under reduced pressure.

4.7.1. (*R*)-Mosher's ester 11 of alcohol (*R*)-10

Colorless oil; ^1H NMR ($CDCl_3$) representative signals δ 5.60 (1H, m, 6-H), 3.17 (1H, dd, J = 16.5, 4.5, 7a-H), 3.01 (1H, dd, J = 16.5, 6.1 Hz, 7b-H), 2.73 (2H, m, 4-CH_2).

4.7.2. (*R*)-Mosher's ester 11 of alcohol (*RS*)-10

Colorless oil; ^1H NMR ($CDCl_3$) representative signals δ 5.60 (1H, m, 6-H), 5.55 (1H, m, 6-H), 3.17 (1H, dd, J = 16.5, 4.5, 7a-H), 3.13 (1H, dd, J = 16.5, 4.5, 7a-H), 3.01 (1H, dd, J = 16.5, 6.1 Hz, 7b-H), 2.90 (1H, dd, J = 16.5, 6.1 Hz, 7b-H), 2.78 (2H, m, 4-CH_2), 2.73 (2H, m, 4-CH_2).

4.8. (*R*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole, 6-tosylate 12

To a solution of (*R*)-**10** (1.5 g, 7.1 mmol) in dry pyridine (5 mL) cooled at 0 °C, tosyl chloride (1.9 g, 9.9 mmol) was added. The solution was kept under stirring at room temperature until TLC analysis (dichloromethane/methanol 95:5) showed complete conversion (2 h). The reaction mixture was then poured into water and ice (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL). The organic phase was washed with water (3 × 10 mL). The oily residue (1.92 g) was used in the next step without further purification. TLC R_f 0.49. ^1H NMR ($CDCl_3$): δ 7.82 (2H, d, J = 8.0 Hz, 2′ and 6′), 7.38 (2H, d, J = 8.0 Hz, 3′ and 5′), 5.04 (1H, dddd, J = 7.2, 5.2, 4.2, 2.4 Hz, 6-H), 2.98 (1H, ddd, J = 16.7, 4.2, 1.6 Hz, 7a-H), 2.92 (1H, dd, J = 16.7, 5.2 Hz, 7b-H), 2.82 (1H, ddd, J = 16.5, 8.4, 5.7 Hz, 4a-H), 2.73 (1H, dddd, J = 16.5, 6.2, 5.7,

1.6 Hz, 4b-H), 2.49 (3H, s, PhCH_3), 2.32 (3H, s, CH_3CO), 2.18 (1H, dddd, J = 14.0, 7.2, 5.7, 5.7 Hz, 5a-H), 2.00 (1H, dddd, J = 14.0, 8.4, 6.2, 2.4 Hz, 5b-H). ^{13}C NMR ($CDCl_3$): δ = 167.9 (NHCOCH$_3$), 158.1 (2-C), 145.1 (N-C), 138.8 (1′-C), 133.9 (4′-C), 130.0 (3′C and 5′-C), 127.7 (2′-C and 6′-C), 118.4 (S-C), 75.7 (6-C), 29.1 (7-C), 27.6 (5-C), 23.3 ($COCH_3$), 21.7 (4-C), 21.3 (Ph-CH_3).

4.9. (*S*)-2-Acetylamino-6-amino-4,5,6,7-tetrahydrobenzothiazole 14

4.9.1. (*S*)-2-Acetylamino-6-azido-4,5,6,7-tetrahydrobenzothiazole 13

To a solution of (*R*)-tosylate **12** (1.9 g, 5.2 mmol) in dimethylformamide (75 mL) under a nitrogen atmosphere at room temperature, sodium azide (1.35 g, 20.8 mmol) was added. The reaction mixture was heated at 70 °C until the starting material disappeared (TLC toluene/ethanol 9:1, two elutions) (6 h). After cooling at room temperature, the reaction mixture was poured into water (350 mL). The aqueous phase was extracted with ethyl acetate (5 × 100 mL); the collected organic phases were washed with brine (2 × 100 mL) and water (100 mL). The usual work-up afforded crude azide **13** (1.07 g), which was directly used in the next step. For analytical purposes, a sample (0.2 g) was purified by silica gel column chromatography (1:10); elution with hexane/ethyl acetate 3:7 afforded pure **13**. TLC R_f (TLC toluene/ethanol 9:1, two elutions) 0.46. $[\alpha]_D^{20}$ = −18.0 (*c* 1, $CHCl_3$). $[\alpha]_{546}^{20}$ = −21.9 (*c* 1, $CHCl_3$). DSC endothermic peak at 193.17 °C. IR ν_{max} 3242.06, 3158.10, 3031.88, 2946.32, 2923.19, 2895.91, 2836.91, 2761.90, 2534.94, 2108.71, 2079.59, 1688.98, 1576.56, 1533.22 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.02 (1H, m, 6-H), 3.06 (1H, ddd, J = 16.0, 4.7, <1 Hz, 7a-H), 2.89–2.72 (3H, m, 4a-H, 4b-H and 7b-H), 2.30 (3H, s, CH_3), 2.15 (1H, dddd, J = 13.0, 6.2, 5.9, 2.9 Hz, 5a-H), 2.06 (1H, dddd, J = 13.0, 10.2, 6.7, 4.9 Hz, 5b-H). ^{13}C NMR ($CDCl_3$): δ 167.9 (NHCOCH$_3$), 157.9 (2-C), 140.1 (N-C), 119.3 (S-C), 56.2 (6-C), 28.3 (5-C), 27.4 (7-C), 23.3 (4-C), 22.8 (CH_3). MS (*m*/*z*) (ESI−) 235.9 [M−1]$^+$. (ESI+) 238.1 [M+1]$^+$, 260.1 [M+Na]$^+$.

4.9.2. (*S*)-2-Acetylamino-6-amino-4,5,6,7-tetrahydrobenzothiazole 14

Crude azide **13** (0.8 g) was dissolved in tetrahydrofuran (30 mL), after which water (1.5 mL) and polymer bonded triphenyl phosphine (2.3 g, 3 mmol/g) were sequentially added. The reaction mixture was kept under stirring at 40 °C for 12 h. The reaction progress was monitored by TLC until complete conversion. The TLC eluant was prepared by mixing water, *n*-butanol, and triethylamine (5:4:1), and separating the organic phase, after vigorous stirring. The reaction mixture was filtered and the solid was washed with methanol (3 × 10 mL). The collected organic phases were evaporated under reduced pressure. The solid residue (0.710 g) was purified by silica gel column chromatography (1:10). Elution with dichloromethane/methanol 9:1 afforded (*S*)-**14** (0.620 g, 76% from tosylate **12**). The ee (>98%) was determined by HPLC on chiral stationary phase (Chiralpak IA; eluant hexane/2-propanol/diethylamine 7:2.95:0.05; flow rate 0.7 mL/min). R_t (*R*)-isomer 11.59 min; (*S*)-isomer 16.25 min. R_f 0.47. $[\alpha]_D^{20}$ = −59.1 (*c* 1, methanol). $[\alpha]_{546}^{20}$ = −70.4 (*c* 1, methanol). DSC endothermic peak at 192.50 °C. IR ν_{max} 3334.13, 3272.90, 3165.16, 3050.47, 2921.44, 2843.81, 2634.92, 2504.97, 1667.67, 1567.19 cm^{-1}. ^{1}H NMR (CD_3OD): δ 3.20 (1H, dddd, J = 10.0, 8.3, 5.3, 2.9 Hz, 6-H), 2.97 (1H, ddd, J = 15.7, 5.3, <1 Hz, 7a-H), 2.76 (1H, dddd, J = 16.5, 5.9, 4.9, <1 Hz, 4a-H), 2.68 (1H, dddd, J = 16.5, 7.7, 6.2, 1.8 Hz, 4b-H), 2.49 (1H, ddd, J = 15.7, 8.3, 1.8 Hz, 7b-H), 2.20 (3H, s, CH_3), 2.05 (1H, dddd, J = 13.0, 6.2, 5.9, 2.9 Hz, 5a-H), 1.74 (1H, dddd, J = 13.0, 10.0, 7.7, 4.9 Hz, 5b-H).^{13}C NMR ($CDCl_3$): δ 169.1 (NHCOCH$_3$), 156.4 (2-C), 143.2 (N-C), 120.3 (S-C), 56.2 (6-C), 31.1 (5-C), 30.8 (7-C), 24.2 (4-C), 21.2 (CH_3). MS (*m*/*z*) (ESI−) 210.1 [M−1]$^+$. (ESI+) 212.0 [M+1]$^+$, 445.0 [2M+Na]$^+$.

4.10. (*S*)-2,6-Diamino-4,5,6,7-tetrahydrobenzothiazole 2

To a solution of **14** (0.520 g, 2.47 mmol) in tetrahydrofuran (10 mL), 5% hydrogen chloride aqueous solution (10 mL) was added. The solution was kept under stirring at 80 °C (12 h) until the starting material disappeared (TLC dichloromethane/methanol/triethylamine 8:1.5:0.5). Saturated potassium carbonate aqueous solution was then added until pH 7. The solvents were removed under reduced pressure and the solid brown residue was suspended in dichloromethane/methanol 7:3 (10 mL). After filtration, the solvents were removed under reduced pressure. The residue (0.580 g) was suspended twice in 0.9 M hydrogen chloride dioxane solution (10 mL) and recovered by filtration. The residue (0.650 g) was dissolved in water (2 mL); the precipitate obtained after addition of 85% potassium hydroxide aqueous solution (3.4 mL) was recovered by suction and washed with cool water (2 × 1 mL) to afford pure **2** (0.375 g, 90%). TLC R_f 0.37. The ee (>98%) and the (*S*)-configuration were determined by HPLC (Crownpak CR(+)). Eluant: perchloric acid aqueous solution at pH 2; flow rate 0.3 mL/min). R_t (*R*)-isomer 11.97 (*S*)-isomer 14.87 min. DSC endothermic peak at 240.00 °C. IR ν_{max} 3369.03, 3353.54, 3255.58, 3085.39, 3007.93, 2911.77, 2835.29, 2757.84, 2240.12, 1632.72, 1587.66, 1573.68, 1532.80, 1440.83 cm^{-1}. $[\alpha]_D^{20}$ = −91.0 (*c* 1, methanol) (lit.[6] −94.2, *c* 1 in methanol). $[\alpha]_{546}^{20}$ = −110.1 (*c* 1, methanol). ^{1}H NMR (CD_3OD): δ 3.15 (1H, dddd, J = 8.5, 8.5, 5.0, 3.0 Hz, 6-H), 2.81 (1H, dd, J = 15.6, 6.2 Hz, 7a-H), 2.62–2.52 (2H, m, 4a-H and 4b-H), 2.35 (1H, ddd, J = 15.6, 8.8, 2.3 Hz, 7b-H), 2.02 (1H, m, 5a-H), 1.68 (1H, dddd, J = 13.9, 8.5, 6.1, 2.4 Hz, 5b-H). ^{13}C NMR (CD_3OD): δ 168.2 (2-C), 143.3 (N-C), 113.9 (S-C), 47.4 (6-C), 31.4 (7-C), 31.3 (5-C), 24.3 (4-C). MS (*m*/*z*) (ESI+) 118 [M−SC(NH$_2$)N+Na]$^+$, 170.1 [M+1]$^+$, 361.1 [2M+Na]$^+$, 401.1 [2M+2CH$_3$OH]$^+$.

4.11. (*S*)-Pramipexole 1 dihydrochloride monohydrate

The *p*-toluenesulfonic acid salt of (*S*)-pramipexole **1** was prepared in 54% yield, according to the literature,[27] by reaction of diamine (*S*)-**2** with *n*-propyl tosylate **15**, which was also prepared according to the literature.[32] ^{1}H NMR (CD_3OD): δ 7.72 (2H, d, J = 8.0 Hz, 2′ and 6′), 7.25 (2H, d, J = 8.0 Hz, 3′ and 5′), 3.55 (1H, dddd, J = 11.6, 8.8, 5.3, 2.90 Hz, 6-H), 3.09–3.05 (3H, m, 7a-H and $CH_2CH_2CH_3$), 2.71–2.60 (3H, m, 4a-H, 4b-H and 7b-H), 2.39 (3H, s, PhCH_3), 2.27 (1H, m, 5a-H), 1.93 (1H, dddd, J = 12.9, 10.6, 9.6, 6.2 Hz, 5b-H), 1.75 (2H, tq, J = 8.0, 7.4 Hz, $CH_2CH_2CH_3$), 1.06 (3H, t, J = 7.4 Hz, $CH_2CH_2CH_3$). (*S*)-Pramipexole *p*-toluenesulfonic salt (0.350 g, 0.914 mmol) was suspended in water (1.5 mL) and, after cooling at 0 °C, 12 M hydrogen chloride aqueous solution (76 μL) was added. The mixture was kept under stirring for 15 min. after which 1 M sodium hydroxide aqueous solution (0.914 mL) was added while keeping the temperature at 0 °C over 3 h. The precipitate pramipexole **1** was recovered by filtration and following the reported method,[27] was transformed into the corresponding dihydrochloride monohydrate (0.245 g, 78%). The ee was established by HPLC analysis (Chiralpak IA, hexane/ethanol/diethylamine 70:30:0.1) R_t (*R*)-isomer 5.53 min; (*S*)-isomer 7.39 min. ^{1}H NMR (CD_3OD): δ 3.69 (1H, dddd, J = 11.4, 8.6, 5.3, 3.0 Hz, 6-H), 3.17 (1H, dd, J = 16.0, 6.2 Hz, 7a-H), 3.12 (2H, t, J = 8.0 Hz, $CH_2CH_2CH_3$), 2.85–2.72 (3H, m, 4a-H, 4b-H and 7b-H), 2.41 (1H, dddd, J = 13.9, 5.3, 3.0, 1.4 Hz, 5a-H), 2.08 (1H, dddd, J = 13.9, 8.6, 6.3, 2.4 Hz, 5b-H), 1.82 (2H, tq, J = 8.0, 7.4 Hz, $CH_2CH_2CH_3$), 1.08 (3H, t, J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (CD_3OD): δ 170.1 (2-C), 133.0 (N-C), 111.6 (S-C), 53.2 (6-C), 46.9 ($CH_2CH_2CH_3$), 24.9 (7-C), 23.9 (5-C), 20.6 (4-C), 19.5 ($CH_2CH_2CH_3$), 9.9 ($CH_2CH_2CH_3$). Other chem-

ical–physical properties are in agreement with the literature values.[6,27,33,34]

4.12. (*S*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole 10

4.12.1. (*S*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole, 6-benzoate 16

To a solution of benzoic acid (0.215 g, 1.76 mmol) and diethyl azadicarboxylate (0.80 mL of a 40% solution in toluene, 1.66 mmol) in dry dimethylformamide (4 mL), kept under nitrogen at 0 °C with stirring, a solution of (*R*)-**10** (0.390 g, 1.84 mmol) and triphenylphosphine (0.450 g; 1.72 mmol) in dry dimethylformamide (4 mL) was added dropwise (30 min). After the addition, the reaction mixture was kept at room temperature under nitrogen. The reaction progress was monitored by TLC (ethyl acetate); after 20 h, near complete conversion was observed. The reaction mixture was poured into water (40 mL) and the aqueous phase was extracted with ethyl acetate (5 × 15 mL). The oily residue (1.8 g) recovered after the usual work-up was purified by silica gel column chromatography (1:30). Elution with hexane/ethyl acetate 6:4 afforded pure (*S*)-benzoate **16** (0.210 g, 36%). The ee (>98%) and (*S*)-configuration of the obtained benzoate were established by HPLC analysis (Chiralpak IA, hexane/2-propanol 8:2 as eluant, flow rate 0.7 mL/min) by comparison with the chromatogram of (*R*)- and (*R,S*)-**16**. R_t (*R*)-isomer 14.32 min; (*S*)-isomer 16.25 min. ^{1}H NMR ($CDCl_3$): δ 8.02 (2H, d, J = 8.0 Hz, *o*-Ph H), 7.58 (1H, t, J = 8.0 Hz, *p*-Ph H), 7.46 (2H, dd, J = 8.0, 8.0 Hz, *m*-Ph H), 5.58 (1H, dddd, J = 7.6, 5.5, 4.8, 2.5 Hz, 6-H), 3.20 (1H, ddd, J = 16.5, 4.8, 1.2 Hz, 7a-H), 3.02 (1H, dd, J = 16.5, 5.5 Hz, 7b-H), 2.93 (1H, ddd, J = 16.8, 8.4, 5.9 Hz, 4a-H), 2.86 (1H, dddd, J = 16.8, 6.2, 5.8, 1.4 Hz, 4b-H), 2.30 (3H, s, CH_3), 2.30 (1H, dddd, J = 13.5, 7.6, 5.9, 5.8 Hz, 5a-H), 2.00 (1H, dddd, J = 13.5, 8.4, 6.2, 2.5 Hz, 5b-H).

4.12.2. (*S*)-2-Acetylamino-6-hydroxy-4,5,6,7-tetrahydrobenzothiazole 10

(*S*)-Benzoate **16** (0.050 g, 0.158 mmol) was dissolved in a 1% sodium hydroxide solution in methanol (6.2 mL). The solution was kept at room temperature overnight. After complete conversion (TLC dichloromethane/methanol 95:5) 1 M hydrochloric acid was added until pH 7. The solvents were removed and to the residue (0.115 g) dichloromethane (0.5 mL) was added. The obtained suspension was purified by silica gel (1 g) column chromatography. Elution with hexane/ethyl acetate 3:7 afforded pure (*S*)-**10** (0.025 g, 75%, >98% ee by HPLC). $[\alpha]_D^{20} = -22.6$ (c 1, methanol). $[\alpha]_{546}^{20} = -26.8$. Other chemical–physical properties are in agreement with those of (*R*)-**10**.

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