Journal of Molecular Catalysis B: Enzymatic 121 (2015) 28–31

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Aureobasidium pullulans as a key for the preparation of optical purity (*R*)-2-(anthracen-9-yl)-2-methoxyacetic acid – The chiral auxiliary reagent in determination of absolute configuration

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ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 30 April 2015
Accepted 7 May 2015
Available online 15 May 2015

Keywords:
2-(Anthracen-9-yl)-2-methoxyacetic acid
Microbiological reduction
Stereochemistry
Saccharomyces cerevisiae
Aureobasidium pullulans

ABSTRACT

Optically pure (*R*)-2-(anthracen-9-yl)-2-methoxyacetic acid ((*R*)-9-AMA) was obtained. The most important stage of the synthesis generating chirality and ensuring high enantioselectivity was the stage of desymmetrization of prochiral α-ketoester. Enzymatic biotransformation was used in the presence of *Saccharomyces cerevisiae* and *Aureobasidium pullulans*. Biotransformation using *S. cerevisiae* leads to 65–70% enantiomeric excess (*R*-isomer). The antimycotic agent Boni Protect containing live cells of *A. pullulans* allowed to obtain enantiomerically pure (*R*)-9-AMA.

1. Introduction

2-(Anthracen-9-yl)-2-methoxyacetic acid (9-AMA) is used as a derivatizing agent in the determining of the absolute configuration of chiral organic compounds by NMR [1–4]. This compound enables determining the absolute configuration of α- and β-chiral alcohols. It is a better derivatizing agent than traditionally used Mosher acid, because it generates more intensive chemical shifts [1]. Unfortunately, the main disadvantage of this chiral agent is its lack of availability on the market.

9-AMA is obtained from anthracene in a multistage synthesis [5,6]. The most difficult stage is the reduction of carbonyl group, which results in appearing the chiral centre in the molecule of the obtained ethyl 2-(anthracen-9-yl)-2-hydroxyacetate. In the synthesis of 9-AMA previously conducted the racemic mixture was obtained. Next, 9-AMA racemate was separated chromatographically on a chiral column [6], or by a capillary electrophoresis [5], or by a crystallization method transforming the acid into proper diastereomeric salt [6]. Both electrophoresis and chromatography are expensive and time-consuming methods. The main focus has been a search for a method of obtaining enantiomerically pure 9-AMA.

2. Materials and methods

2.1. Analytical methods

The HPLC experiments were performed on a Shimadzu SCL-10A VP, analytical column Chiralcel OJ 250 mm × 4.6 mm, 10 μm, Daicel (France) and semi-preparative column Lux Cellulose-3, LC Column 100 mm × 10 mm, 5 μm, Phenomenex (Poland).

^{1}H NMR spectra were recorder on Bruker apparatus (TMS as an internal standard).

Enantiomeric separation of **2** was performed using the chiral column and a mixture of *n*-hexane and propan-2-ol 60:40 (v/v) as eluant was used, at 1 mL/min or 0.5 mL/min flow rate (Table 1).

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http://dx.doi.org/10.1016/j.molcatb.2015.05.007
1381-1177/

Table 1
The retention times of the **1** and **2**.

Compound	Retention time [min]
1	20.5[a]/28.6[b]
(*S*)-**2**	17.3[a]/24.7[b]
(*R*)-**2**	26.6[a]/35.9[b]

[a] Chiralcel OJ; *n*-hexane:propan-2-ol 60:40; 0.5 mL/min.
[b] Lux Cellulose-3; *n*-hexane:propan-2-ol 60:40; 1 mL/min.

2.2. Reagents and solvents

The chemical substances of analytical grade were commercially available: sucrose, glucose, allyl alcohol, allyl bromide, ethyl chloroacetate, isopropyl chloroacetate, ethyl acetate, ethanol, NaCl, $MgSO_4$, *n*-hexane for HPLC, propan-2-ol for HPLC from POCH (Poland), baker's yeast (Merck), Boni Protect (Koppert Biological Systems, Poland).

2.3. Synthesis of ***1***, ***3***, ***4***

Compounds **1**, **3**, **4** were obtained according to literature procedure [5].

2.3.1. Ethyl 2-(anthracen-9-yl)-2-oxoacetate (***1***)

Isolated yield: 37%; ^{1}H NMR (400 Hz, $CDCl_3$): δ (ppm) = 1.34 (t, 3H; J = 7.2 Hz), 4.39 (4, 2H; J = 7.2 Hz), 7.56 (m, 4H), 7.89 (ddd, 2H; J = 6.4 Hz, J = 1.2 Hz, J = 0.8 Hz), 8.09 (dd, 2H; J = 6.4 Hz, J = 0.8 Hz), 8.65 (s, 1H); MS-CI (*m*/*z*) 278 $(MH)^+$.

2.3.2. Ethyl 2-(anthracen-9-yl)-2-methoxyacetate (***3***)

Isolated yield: 90%; ^{1}H NMR (400 Hz, $CDCl_3$): δ (ppm) = 1.06 (t, 3H; J = 7.2 Hz), 3.43 (s, 3H), 4.09 (m, 1H), 4.20 (m, 1H), 7.50 (m, 2H), 7.57 (m, 2H), 8.05 (ddd, 2H; J = 8.4 Hz, J = 1.2 Hz, J = 0.8 Hz), 8.51 (s, 1H), 8.58 (d, 2H; J = 8.8 Hz); MS-CI (*m*/*z*) 294 $(MH)^+$.

2.3.3. 2-(Anthracen-9-yl)-2-methoxyacetic acid (***4***)

Isolated yield: 31%; ^{1}H NMR (700 Hz, DMSO-d_6): δ (ppm) = 3.35 (s, 3H), 6.32 (s, 1H), 7.52 (m, 4H), 8.09 (dd, 2H; J = 8.4 Hz, J = 1.4 Hz), 8.60 (d, 2H; J = 9.1 Hz), 8.62 (s, 1H), 12.8 (s, 1H); MS-CI (*m*/*z*) 266 $(MH)^+$; CD ($c = 1.5 \times 10^{-4}$ M, AcCN) λ/nm ($\Delta\varepsilon$/cm^2 mol^{-1}): 260 (−23.8), 243 (15.8).

2.4. Synthesis of ethyl (R)-2-(anthracen-9-yl)-2-hydroxyacetate (***2***)

2.4.1. Baker's yeast catalyzed asymmetric reduction of ***1***

A suspension of baker's yeast (3.75 g) in 20 mL water and 0.9 g (2.6×10^{-3}) sucrose was put into a 250 mL Erlenmeyer flask. The resulting mixture was stirred at 37 °C. After the step of fermenting yeast (30 min), additive compound: allyl alcohol, allyl bromide, ethyl chloroacetate, isopropyl chloroacetate respectively (0.04 g) and solution of **1** (25.8 mg, 1.25×10^{-4} mol in 0.5 mL EtOH) were added. The reaction progress was monitored by TLC (the solvent system used *n*-hexane:ethyl acetate – 4:1). After 48 h the yeast cells were removed by filtration of celit filter aid. The yeast cells were washed with 2× 25 mL water. The filtrate was saturated with NaCl and then extracted with 5× 20 mL portions of ethyl acetate. The combined organic layers were dried over $MgSO_4$ and the solvent evaporated under reduced pressure to leave a residue of the crude product, which was purified by PLC using *n*-hexane:ethyl acetate (4:1) as eluent. The enantiomeric ratios were determined on an HPLC system using a chiral column. The mobile phase consisted of *n*-hexane:propan-2-ol (60:40, v/v; 0.5 mL/min).

Compound **2** with 11.5–88.7% yield and with 65–70% ee was obtained (see Table 2).

Table 2
Reduction of **1** by *Saccharomyces cerevisiae*.

Additive compound	**2** [%]	ee[a] [%]	E[b]
None	88.7	65	2.8
Allyl alcohol	–	–	–
Allyl bromide	11.5	66	2.9
Ethyl chloroacetate	55.4	70	5.7
Isopropyl chloroacetae	54.3	67	5.1

[a] The ee was determined by HPLC.
[b] $E = (1 + ee)/(1 - ee)$.

2.4.2. Asymmetric reduction of ***1*** by Aureobasidium pullulans

The 50 g Boni Protect (Boni Protect containing strains of *A. pullulans*) were suspended in 750 mL potassium phosphate buffer (pH 7.0) and 3.5 g (1.9×10^{-2} mol) glucose was added. The mixture was stirred at 37 °C (1 h). The reduction started with addition solution of **1** (0.5 g, 1.9×10^{-3} mol) in 50 mL EtOH. The reaction was carried out for 48 h at 30 °C. Next celit and ethyl acetate were added and the mixture was filtered. The celit was washed with ethyl acetate. The filtrate was extracted with ethyl acetate (5× 50 mL). The organic portion was washed with water and dried with $MgSO_4$. The solvent evaporated under reduced pressure. The crude product was purified by PLC using *n*-hexane:ethyl acetate (4:1) as eluent. The enantiomeric ratios were determined on an HPLC system using a chiral column. The mobile phase consisted of *n*-hexane:propan-2-ol (60:40, v/v; 0.5 mL/min at 1 mL/min). Compound **2** with 87% yield and with 99% ee (E = 100) was obtained.

2.4.3. Ethyl 2-(anthracen-9-yl)-2-hydroxyacetate (***2***)

^{1}H NMR (400 Hz, $CDCl_3$): δ (ppm) = 1.03 (t, 3H; J = 6.8 Hz), 3.70 (s, 1H), 4.18 (m, 2H), 7.52 (m, 4H), 8.05 (ddd, 2H; J = 6.8 Hz, J = 1.6 Hz, J = 0.8 Hz), 8.35 (d, 2H, J = 8.4 Hz), 8.51 (s, 1H); MS-CI (*m*/*z*) 280 $(MH)^+$.

3. Results and discussion

9-AMA is generally obtained from anthracene in four stages (Scheme 1) [5].

Earlier this compound was obtained as a racemic mixture. There is no information in scientific publications about obtaining enantiomerically pure 9-AMA. In order to avoid time-consuming and expensive resolution of racemate we became interested in obtaining enantiomerically pure 9-AMA. To achieve it, we conducted the enantioselective reduction of compound **1**. The racemic standard of product **2** was obtained in a reduction reaction in the presence of sodium borohydride. In order to conduct enantioselective reduction baker's yeast *Saccharomyces cerevisiae* was initially used, because it is commonly used in such reactions [7–9].

Biotransformation with the use of baker's yeast led to the product **2** with a moderate 65% enantiomeric excess. Baker's yeast contains four basic oxidoreductase differing in molecular weight, activity and specificity. Competitive stereospecificity of dehydrogenases has a big impact on enantiomeric purity of products. The enantioselectivity control of microbiological reduction with baker's yeast is connected with the inhibition of dehydrogenases which is characterized by specific stereopreference (inhibition of (*R*)- or (*S*)-stereospecific dehydrogenases).

Therefore, in order to improve the optical purity of compound **2**, apart from *S. cerevisiae* some other substances, acting as inhibitors, such as allyl alcohol, allyl bromide, ethyl chloroacetate and isopropyl chloroacetate were added respectively [10,11] to this reaction. The highest enantiomeric excess (70% ee) was obtained in the reduction with ethyl chloroacetate. The results are shown in Table 2.