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Enantioselective biocatalytic synthesis of (*S*)-monastrol

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ABSTRACT

The first enantioselective biocatalytic synthesis of (*S*)-monastrol has been developed via an unexpected and unusual enzymatic pathway as suitable route. Whereas attempts for a direct hydrolysis of racemic monastrol were not successful, formation of racemic *O*-butanoyl monastrol and subsequent enantioselective hydrolysis furnished *O*-butanoyl (*S*)-monastrol with 97% ee. Cleavage of the *O*-butanoyl moiety then gave the desired (*S*)-monastrol with 96% ee.

Monastrol, *rac*-**1**, is the first small molecule inhibitor of the mitotic motor Eg5 (kinesin spindle protein, KSP), and represents a promising current lead structure in anticancer research.[1,2] Several KSP inhibitors are currently being studied in clinical trials and provide new opportunities for the development of novel anticancer drugs alternative from the available microtubule targeting agents.[2] Based on monastrol as a lead structure novel derivatives bearing the 4-aryl-3,4-dihydropyrimidin-2(1*H*)-thione scaffold of *rac*-**1** have been identified recently by Giannis and co-workers, and these compounds turned out to act as very potent cell-permeable inhibitors of Eg5.[3] While both enantiomers of monastrol abolished basal Eg5 ATPase activity, the (*S*)-enantiomer shows a 15 times higher potency compared with the opposite (*R*)-enantiomer.[1b] Thus, synthetic efforts based on different approaches have been made for the stereoselective preparation of the (*S*)-enantiomer of monastrol [(*S*)-monastrol, (*S*)-**1**, Fig. 1]. A route to (*S*)-**1** reported by Dondoni et al. is based on the formation of diastereomeric *N*-3-ribofuranosyl amides from racemic monastrol, separation of both diastereomers and subsequent amide hydrolysis of the desired diastereomer.[4] An enantioselective Biginelli reaction using 10 mol % of a bis-phenyl-substituted H_8-binaphthol-based phosphoric acid as a chiral organocatalyst, leading to TBS-protected monastrol with 91% ee, was developed by Gong and co-workers.[5] Zhu and co-workers reported an enantioselective Biginelli reaction in the presence of a chiral metal catalyst synthesized from ytterbium triflate and a hexadentate ligand. At a catalyst loading of 10 mol % the (*R*)-enantiomer of monastrol was obtained in 80% yield and with excellent 99% ee.[6] Surprisingly, however, to the best of our knowledge enzymatic routes have not been used so far in spite of the known efficiency of biocatalysis for the synthesis of chiral compounds, and their wide use in industrial drug synthesis.[7] In the following we report the first enantioselective biocatalytic synthesis of (*S*)-monastrol, (*S*)-**1**.

Since the racemic compound monastrol, *rac*-**1**, is easily prepared in the Biginelli reaction[8] starting from simple starting materials it appeared attractive to us to use this racemate as a starting material for an enzymatic resolution process.

Inspired by numerous successful examples of enzymatic resolutions via hydrolysis of esters with a stereogenic center in β-position,[9] at the start of our experiments we considered this type of reaction as most promising and straightforward for synthesizing the (*S*)-enantiomer of monastrol (according to the synthetic strategy shown in Scheme 1). To our surprise, however, when screening a set of commercially available hydrolases such as, for example, lipases from *Aspergillus niger*, *Candida antarctica* B (CAL-B), *Candida*

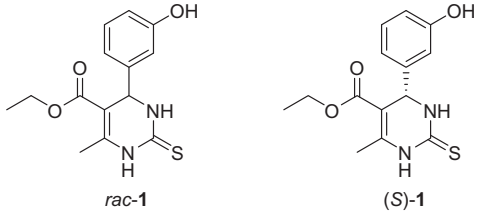

Figure 1. Structures of monastrol (*rac*-**1**) and (*S*)-monastrol ((*S*)-**1**).

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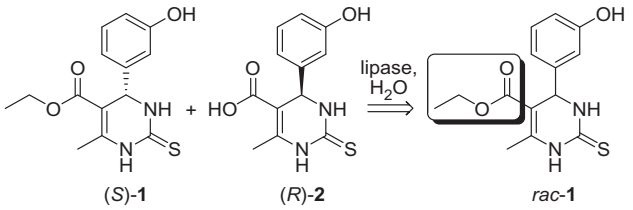
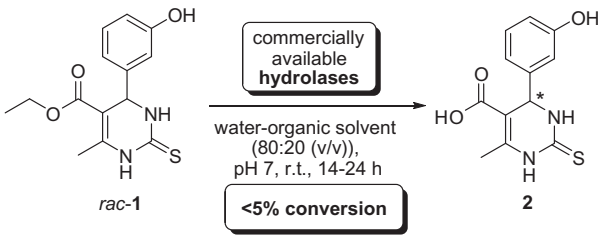


Scheme 1. Planned retrosynthetic concept to (*S*)-**1** via enzymatic hydrolysis of *rac*-**1**.

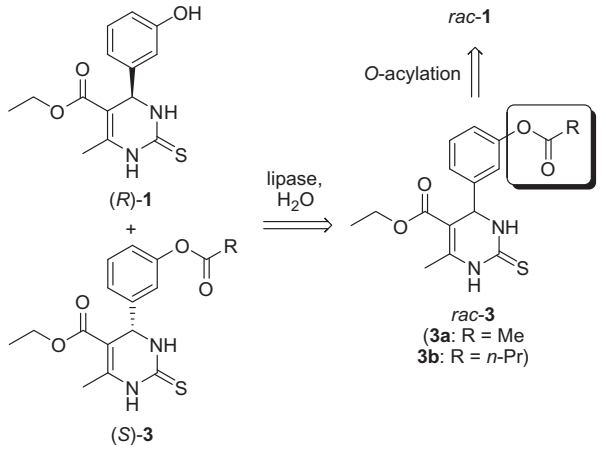


Scheme 2. Enzymatic hydrolysis of monastrol, *rac*-**1** (organic solvents: methylene chloride, DMSO, hexane, MTBE, *i*-PrOH).

rugosa, *Mucor javanicus*, and *Pseudomonas fluorescens* as well as porcine liver esterase we could not identify a suitable biocatalyst (Scheme 2). Independent of the type of lipase conversions were below 5% for the studied biocatalysts (data not shown in detail). Due to the lack of a significant activity of this process, further process development has not been carried out for this route.

As a second option we then focused on the use of racemic O-acylated monastrol derivatives of type *rac*-**3** as substrates. The ester group in *rac*-**3**, which is obtained through derivatization of the phenolic moiety in *rac*-**1**, appeared to us as an interesting functional group for a hydrolytic biotransformation. The retro-synthesis of such an approach to the O-acylated derivative (*S*)-**3** of (*S*)-monastrol, (*S*)-**1**, is shown in Scheme 3. At the same time, however, this synthetic route is challenging since in these substrates of type *rac*-**3** the stereogenic center and the functional group for the biotransformation are separated from each other by a planar aromatic moiety. So far only a few examples are reported for enzymatic resolutions of compounds with such a so-called remote stereogenic center.[10]

In our initial screening for a suitable biocatalyst O-acetylated monastrol, *rac*-**3a**,[11] served as a substrate. This compound was easily prepared via acetylation of monastrol, *rac*-**1**, using acetic anhydride in the presence of DMAP.[11] As a reaction media for the



Scheme 3. Retrosynthetic route to (*S*)-**3** via enzymatic hydrolysis of *rac*-**3**.

enantioselective enzymatic hydrolysis, a two-phase system consisting of water and dichloromethane (80:20 (v/v)) has been used. When screening[12] a set of commercially available lipases we were pleased to find a promising reaction course in the presence of the lipase from *C. antarctica* B (CAL-B). By means of this enzyme a conversion of 52% was achieved under formation of (*R*)-**1** with 71% ee (Table 1, entry 1). An enantiomeric excess of 77% ee has been found for the remaining substrate (*S*)-**3a**, which corresponds to an enantioselectivity of $E = 13$ for this biotransformation. Thus, the desired (*S*)-configuration is obtained for the remaining substrate, **3a**.

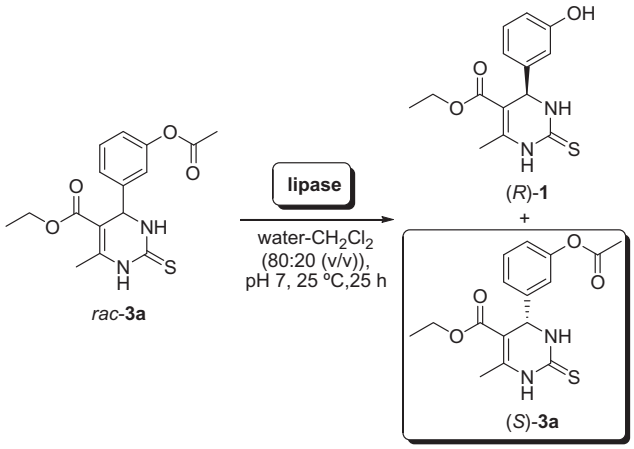


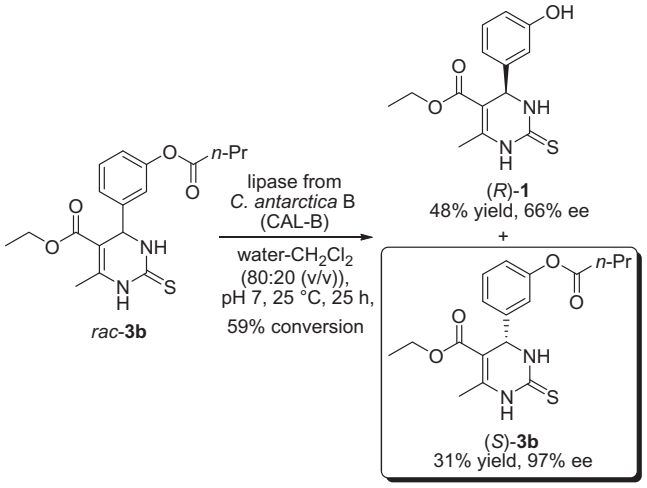
Table 1
Enzymatic hydrolysis of O-acetylated monastrol, *rac*-**3a**

Entry[a]	Lipase	Conv.[g] (%)	ee of (*R*)-**1** (%)	ee of (*S*)-**3a** (%)	*E*[h]
1	*C. antarctica* B[b]	52	71	77	13
2	*C. rugosa*[c]	33	66	15	6
3	*A. niger*[d]	35	17	5	1
4	*P. fluorescens*[e]	51	8	8	1
5	*B. cepacia*[f]	9	6	1	1

[a] For the experimental protocol, see Ref. 12.
[b] *Candida antarctica* B (CAL-B), commercial product: lipase Novozym 435.
[c] *Candida rugosa*, type VII; commercial product from Sigma.
[d] *Aspergillus niger*, commercial product: lipase AS Amano.
[e] *Pseudomonas fluorescens*, commercial product: lipase AK Amano 20.
[f] *Burkholderia cepacia*, commercial product: lipase PS Amano IM.
[g] Conversion was determined from the resulting crude product by means of proton NMR spectroscopy.
[h] The *E* value (enantioselectivity) has been calculated by means of the measured ee values of (*R*)-**1** and (*S*)-**3a**.

With other enzymes the resolutions based on hydrolysis of *rac*-**3a** proceeded less successfully (Table 1, entries 2–5). A low conversion of 9% was obtained when using a lipase from *Burkholderia cepacia* (entry 5). The use of a lipase from *C. rugosa* led to an improved conversion of 33%, but a non-satisfying enantioselectivity with an *E* value of 6 was found in this experiment (entry 2). Good to high conversions of 35% and 51% were observed with lipases from *A. niger* and *P. fluorescens*, respectively. However, both reactions proceeded with very low enantioselectivities, which are indicated by the *E* value of only 1 for both reactions (entries 3,4).

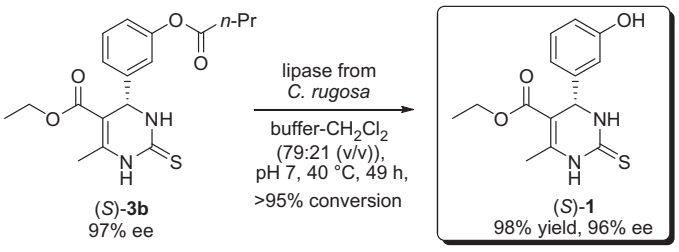
After having identified a suitable biocatalyst (with CAL-B), we focused on the study of the impact of the acyl moiety on conversion and enantioselectivity in order to optimize the 'leaving group' in the hydrolytic process. When investigating the influence of several aliphatic acyl moieties, O-butanoylated monastrol, *rac*-**3b**,[13] turned out as the most suitable substrate. In the enzymatic hydrolysis of this substrate *rac*-**3b** an improved enantioselectivity with an *E* value of 20 was obtained (Scheme 4). When this resolution was stopped at a conversion of 59%, subsequent work-up led to



Scheme 4. Enzymatic hydrolysis of *O*-butanoyl monastrol, *rac*-**3b**.

the hydrolyzed product (*R*)-**1** in 48% yield and with 66% ee. The remaining desired (*S*)-enantiomer, (*S*)-**3b**, was isolated after column chromatography in 31% yield and with a high enantiomeric excess of 97% ee (Scheme 4).[14] When starting from substrates of type **3** bearing acyl moieties such as propanoyl (R = Et) and hexanoyl (R = *n*-pentyl) reactions also proceeded, but gave less satisfactory results with *E* values of 8 and 6, respectively.[15] The conversions of these reactions were 30% and 11%. Notably, a dramatic drop of reactivity was observed when the substituent at the acyl moiety was an isopropyl group (R = *i*-Pr) as a representative for an α-branched substituent (<5% conversion).[15]

Based on the encouraging result in the enzymatic resolution of *rac*-**3b** we then focused on the subsequent cleavage of the *O*-butanoyl moiety in (*S*)-**3b** in order to obtain (*S*)-monastrol, (*S*)-**1**, as the desired final product. Although for such a non-enantioselective hydrolysis 'standard' chemical hydrolytic methods might be also conceivable, the presence of the second ester moiety (ethyl ester) in the molecule (*S*)-**3b**, which should not undergo hydrolysis, makes this step challenging. After a preliminary screening of base-catalyzed methods (using aluminum oxide or NaOH as a base) was not successful (data not shown) we identified a non-selective biocatalytic hydrolysis as the method of choice. This method is based on the use of a lipase from *C. rugosa*, which showed a high activity but low enantioselectivity (*E* value of 7) for the resolution of *rac*-**3b**. At the same time, this enzyme does not possess a (significant) activity for the hydrolysis of the ethyl ester moiety of *rac*-**1** and *rac*-**3** (data not shown), thus making it attractive for the desired non-enantioselective but chemoselective hydrolysis of (*S*)-**3b** under formation of (*S*)-**1**. We were pleased to find that, as expected, in the presence of the lipase from *C. rugosa* the desired (*S*)-enantiomer of monastrol, (*S*)-**1**, was then formed with a high conversion of >95%. After subsequent work-up (*S*)-monastrol, (*S*)-**1**, was obtained in 98% yield and with a high enantiomeric excess of 96% ee (Scheme 5).[16]



Scheme 5. Synthesis of (*S*)-monastrol, (*S*)-**1**, via enzymatic hydrolysis of (*S*)-**3b**.

In summary, we reported the first enantioselective biocatalytic synthesis of (*S*)-monastrol, (*S*)-**1**, which has been realized by means of an unexpected and unusual enzymatic resolution of a substrate with a remote stereogenic center as the preferred route. Notably, an easily available commercial biocatalyst can be used for this resolution as a key step. Currently extension of this new methodology towards a technology platform for the enantioselective synthesis of a broad range of derivatives of (*S*)-monastrol is in progress. In addition, based on this methodology enzymatic resolutions of other types of racemic phenol esters bearing a remote stereogenic center are also planned.

Acknowledgments

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8. *Synthesis of rac*-**1**: A solution of 3-hydroxybenzaldehyde (0.15 mol, 18.32 g), thiourea (0.15 mol, 11.42 g), ethyl acetoacetate (0.23 mol, 29.28 g, 28.46 mL) and concd HCl (2 mL) in ethanol (85 mL) was heated under reflux for 6 h. The reaction mixture was filtered and the solid residue was recrystallized from ethanol twice to afford *rac*-**1** in 69% yield (30.21 g). The synthesis was carried out according to a protocol reported in: Lewandowski, K. *J. Combinat. Chem.* **1999**, *1*, 105.
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11. *Synthesis of rac*-**3a**: monastrol *rac*-**1** (10 mmol, 2.92 g), acetic anhydride (10 mmol, 1.02 g, 0.95 mL) and DMAP (0.20 mmol, 25 mg) were dissolved in dried pyridine (5 mL). The reaction mixture was stirred for 3 h under reflux. After cooling down the reaction mixture to room temperature and adding ice-water, the mixture was stirred for 1 h at room temperature. Then diluted HCl (0.1 M) was added until a pH of 2 was achieved. The mixture was agitated overnight at room temperature and the resulting precipitate was filtered, washed with water and recrystallized from ethanol to furnish *rac*-**3a** in 72% yield (2.42 g).
12. *General protocol for the screening of lipases for the hydrolysis of rac*-**3a**: The screening was carried out at 25 °C and using a Methrom titrino apparatus (with an automatic titration unit). After dissolving *rac*-**3a** (1 mmol, 334 mg) in CH_2Cl_2 (20 mL) and addition of water (80 mL) under formation of a two-phase system, the corresponding lipase (100 mg) was added. The pH was kept constant at 7 (by dosage of a solution of NaOH (0.21 M)). After a reaction time of 25 h the reaction mixture was filtered and then extracted with CH_2Cl_2 (3 × 60 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The resulting crude product was used for the determination of the conversion (via ^{1}H NMR spectroscopy) and enantiomeric excess (via chiral HPLC chromatography).
13. *Synthesis of rac*-**3b** has been carried out in analogy to the synthesis of *rac*-**3a** (see Ref. 11) using butyric anhydride (15 mmol, 2.37 g), a modified amount of DMAP (0.21 mmol, 26 mg) and dried pyridine (8 mL). After recrystallization from ethanol *rac*-**3b** was obtained in 42% yield (1.53 g).

14. *Synthesis of* (*S*)-**3b**: In a Methrom titrino reaction apparatus *rac*-**3b** (1 mmol, 0.369 g) was dissolved in CH_2Cl_2 (20 mL). After subsequent addition of water (80 mL), which results in the formation of a two-phase system, lipase from *Candida antarctica* B (Novozym 435, 200 mg) was added. Then the reaction mixture was stirred for 25 h at 25 °C, and the pH was kept constant at a pH of 7 (by dosage of a solution of NaOH (0.21 M)). Subsequently, the reaction mixture was filtered and washed with water (5 mL) and CH_2Cl_2 (10 mL). The filtrate was extracted with CH_2Cl_2 (3 × 60 mL), and the combined organic layers were dried over $MgSO_4$. Evaporation of the solvent in vacuo furnished a mixture of (*S*)-**3b** and (*R*)-**1** as a crude product, which was then separated and purified by column chromatography [CH_2Cl_2/ethyl acetate, 5:1 (v/v)] to give (*S*)-**3b** (31% yield, 97% ee) and (*R*)-**1** (48% yield, 66% ee) in isolated form.
15. These reactions were carried out in analogy to the synthesis described in Ref. 12 at a substrate loading of 0.83 mmol and with an amount of the lipase from *Candida antarctica* B (Novozym 435) of 83 mg.
16. *Synthesis of (S)-monastrol*, (*S*)-**1**: After dissolving (*S*)-**3b** (0.284 mmol, 0.103 g, 97% ee; for preparation, see Ref. 14 and Scheme 4) in CH_2Cl_2 (4 mL), phosphate buffer (15 mL, pH 7, 50 mM) and lipase from *Candida rugosa* (type VII, commercial product from Sigma; 150 mg) were added and the reaction mixture was stirred for 49 h at 40 °C. Subsequently, the reaction mixture was filtered and the filtrate was extracted with CH_2Cl_2 (4 × 30 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated in vacuo. The desired (*S*)-monastrol, (*S*)-**1**, was formed with >95% conversion and obtained in 98% yield (0.277 mmol, 0.081 g) and with an enantiomeric excess of 96% ee.