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Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2.2241 and synthesis of the β-blocker (*R*)-nifenalol

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Abstract—A broad range of prochiral ketones were efficiently reduced to the corresponding optically active secondary alcohols using resting cells of *Rhodotorula* sp. AS2.2241. The microbial reduction system exhibited high activity and enantioselectivity in the reduction of various aromatic ketones and acetylpyridines (>97% ee), but moderate to high enantioselectivity in the reduction of α- and β-keto esters. (*R*)-Nifenalol, a β-adrenergic blocker, was also synthesized using 2-bromo-1(*R*)-(4-nitrophenyl)ethanol (97% ee) which was prepared through the asymmetric reduction of 2-bromo-1-(4-nitrophenyl)ethanone employing *Rhodotorula* sp. AS2.2241. The simple preparation and the high activity of the biocatalyst turned this system into a versatile tool for organic synthesis.

1. Introduction

The asymmetric reduction of ketones is one of the most important, fundamental and practical reactions for producing chiral alcohols, which can be transformed into various functionalities, without racemization, for the synthesis of many industrially important chemicals such as pharmaceuticals, agrochemicals and natural products.[1,2] Bioreduction catalyzed by isolated dehydrogenases or whole cells provides an attractive approach to selectively reducing a broad range of ketones.[3] The use of microbial whole cells as biocatalysts is particularly advantageous for carrying out the desired reduction, since they contain multiple dehydrogenases, which are able to accept a broad spectrum of nonnatural substrates, all the necessary co-factors and the metabolic pathways for their regeneration. Furthermore, all the enzymes and co-factors are well protected within their natural cellular environment.[4,5]

Saccharomyces cerevisiae (baker's yeast or brewer's yeast) is by far the most widely used microorganism for the asymmetric reduction of ketones, since it is readily available, inexpensive, and possesses a high capacity as a redox biocatalyst in a variety of stereoselective reductions. Nevertheless, in many instances the use of baker's yeast gives a product with an unsatisfactory enantiopurity due to the coexistence of several reductases with different specific activities and stereoseletivities.[6,7] Many other microorganisms, such as *Rhizopus arrhizus*,[8] white-rot fungus *Merulius tremellosus*,[9] *Geotrichum candidum*,[10] *Rhodococcus rubber*[11] and *Trichothecium* sp.,[12] were also used for the preparation of enantiomerically pure alcohols, and even plant cell cultures may be employed for this purpose.[13–15] However, there are still some problems, which remain to be solved, such as moderate enantiomeric purity, narrow substrate spectrum, a long time for fermentation and reaction, and incomplete transformation.

Several attempts have been made to optimize the enantiomeric excess (ee) of the product. In this way, modification of the chemical substitutions, use of different organic media, pretreatment of cells by acetone, and the addition of a selective inhibitor or hydrophobic polymer have been tested, and the enantiomeric excesses were improved.[1,16,17] However, additional steps were needed and the results achieved in some cases were unsatisfactory.[18,19] Exploration of new microorganisms is an alternative approach for addressing this issue.

In our previous report, for the asymmetric reduction of acetophenone and α-bromoacetophenone, a new microbial

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strain, *Rhodotorula* sp. AS2.2241, was successfully isolated from soil samples,[20] which was later shown as being useful in reducing acetytrimethylsilane to (−)-1-trimethylsilyethanol, with 99% yield and 90% ee.[21] Additionally, it has high growth rate under a simple culture condition so that a sufficient amount of catalysts can be supplied. Herein, we extended the use of *Rhodotorula* sp. AS2.2241 to the reduction of some other prochiral ketones, including aromatic ketones, acetylpyridines as well as α- and β-keto esters. We also investigated the synthesis of a β-blocker, (*R*)-nifenalol, using 2-bromo-1(*R*)-(4-nitrophenyl)ethanol as a chiral precursor, which was prepared through the asymmetric reduction of 2-bromo-1-(4-nitrophenyl)ethanone employing *Rhodotorula* sp. AS2.2241. This (*R*)-ethanol can usually be gained by enantioselective kinetic resolution employing either lipase[22] or halohydrin dehalogenase,[23] but the reduction of its corresponding prochiral ketone with a whole-cell biocatalyst has hardly been reported. This will provide a novel route for the synthesis of (*R*)-nifenalol.

2. Results and discussion

During the reduction of various ketones, *Rhodotorula* sp. AS2.2241 was found to be a promising redox biocatalyst with great synthetic potential due to its wide substrate spectrum and high chemical yields in short conversion times. Aromatic alcohols and pyridylethanols were formed with excellent enantioselectivities, while α- and β-keto esters were reduced in high chemical yield and moderate to high ee values. The reductions using *Rhodotorula* sp. AS2.2241 cells obey Prelog's rule.

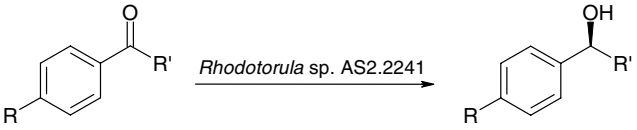
2.1. Reduction of aromatic ketones

An enantioselectivity of more than 99% ee was obtained for the reduction of all the acetophenone derivatives tested, except that for **11a**, which was 97% ee. Although the selectivities of previously reported reduction systems were relatively high (around 90–95% ee) to moderate, enantiomerically pure compounds (>99% ee) could not be obtained except for in a few cases.

The rate of the reduction depended on the electronic effects of the substituents (Table 1). Electron-withdrawing substituents (e.g., $-NO_2$, –Cl, or –Br) at the *p*-position of the phenyl ring were favorable for the *Rhodotorula* sp. AS2.2241 mediated reduction, but electron-donating substituents (–OMe, $-NH_2$) obviously slowed down the reaction rate. The reduction yield of *p*-methoxyacetophenone **5a** was only 50% in 24 h and did not increase even if the reaction time was extended. Poor reduction was observed with *p*-aminoacetophenone **6a**. This result was in line with other reports, which was possibly due to the electron-donating (+I) effect of the substituent.[4]

Asymmetric reduction of substrates, such as α-bromoacetophenone **7a**, α-chloro acetophenone **8a** and β-chloropropiophenone **10a**, gives products that can be converted into valuable synthetic intermediates, such chiral epoxides.[25] Substrates **7a** and **8a** were also efficiently reduced by *Rhodotorula* sp. AS2.2241 within 3 h, with 100% yield and >99% ee. The rate of reduction increased obviously compared with acetophenone. However, when the methyl moiety of acetophenone was replaced by an ethyl group, the reaction rate decreased, although the enantioselectivity remained high (>99% ee). Even longer reaction times (20 h) were needed to reduce **10a** completely.

Table 1. Reduction of aromatic ketones by *Rhodotorula* sp. AS2.2241



1a-11a **1b-11b**

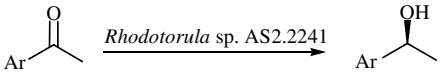
Substrate	R	R′	Time (h)	Yield[a,b] (%)	ee[a] (%)	Config.[f]
1a	H	CH_3	7	100 (65)	99	*S*
2a	NO_2	CH_3	3	100 (84)	>99	*S*
3a	Br	CH_3	3	100 (63)	>99	*S*
4a	Cl	CH_3	4	100 (72)	99	*S*
5a	MeO	CH_3	24	50 (35)	>99	*S*
6a	NH_2	CH_3	24	ND[e] (6)	ND[e]	ND[e]
7a	H	CH_2Br	3	100 (69)	>99	*R*
8a	H	CH_2Cl	3	100 (52)	>99	*R*
9a	H	C_2H_5	9	100 (62)	>99	*S*
10a	H	C_2H_4Cl	20	100 (50)	>99	*S*[g]
11a	NO_2	CH_2Br	5	100[c] (51)	97[d]	*R*

[a] Determined by GC analyses.
[b] Isolated yield in parentheses.
[c] Determined by thin layer chromatography (eluent: petroleum ether–ethyl acetate, 4:1).
[d] Determined by HPLC using Chiralcel AD-H column.
[e] Not determined.
[f] Absolute configurations were assigned by comparison of the specific rotations with the literature values.
[g] Determined by comparison of the GC retention times with authentic sample.

2.2. Reduction of acetylpyridines

Optically active pyridyl alcohols are useful compounds, not only as pharmaceutical intermediates but also as useful chiral ligands and auxiliaries in asymmetric synthesis.[26] 2-, 3-, and 4-Acetylpyridines **12a**–**14a** were reduced efficiently by *Rhodotorula* sp. AS2.2241 resting cells with high yield and enantiomeric excess. The time for complete conversion depended on the position of the carbonyl group on the pyridine moiety (Table 2). We noticed that 3-acetylpyridine **13a** was reduced more slowly than 2- and 4-acetylpyridines, and the corresponding alcohol **13b** was obtained with only 86% yield within 11 h when the substrate concentration was increased to 100 mM. Moreover 2- and 3-acylpyridines **12a** and **13a** were reduced to afford the corresponding (*S*)-1-pyridyl alkanols **12b** and **13b** with excellent enantioselectivity, although the ee of the reduction product **14b** for 4-acylpyridine **14a** was somewhat decreased when the substrate concentration was increased from 20 to 100 mM. This is probably due to the competition of two or more coexisting dehydrogenases in *Rhodotorula* sp. AS2.2241 cells for reducing 4-acetylpyridine with different stereo-

Table 2. Reduction of acetylpyridines by *Rhodotorula* sp. AS2.2241



12a-14a **12b-14b**

Ar =2-pyridyl (**12**); 3-pyridyl (**13**); 4-pyridyl (**14**)

Substrate	Concn (mM)	Time (h)	Yield[a,b] (%)	ee[a] (%)	Config.
12a	20	2	100 (70)	>99	*S*
	100	8	100	>99	*S*
13a	20	3	100 (81)	99	*S*
	100	11	86	98	*S*
14a	20	2	100 (70)	97	*S*
	100	6	100	89	*S*

[a] Determined by GC analyses.
[b] Isolated yield in parentheses.

chemical preferences. When there are plural enzymes in a cell with overlapping substrate specificities but different enantioselectivities, a change in the substrate concentration may alter the stereochemical preference of the bioreduction because enzymatic reactions follow the Michaelis–Menten equation. Enzymes having lower K_m values catalyze the reduction more effectively under the lower substrate concentrations; whereas, other enzymes having higher K_m and V_{max} values act more effectively under higher substrate concentrations.[1] A different result was reported[27] on the reduction by *Pseudomonas putida* UV4, in which better results were obtained for 4-acetylpyridine, with 83% yield and >99% ee, whereas 2- and 3-acetylpridines were poorly reduced, in 45% and 51% yields, 95% and 71% ees, respectively.

2.3. Reduction of α- and β-keto esters

The reduction of ketoesters is probably the most extensively investigated reaction of carbonyl compounds leading to chiral building blocks for natural products and biologically active compounds. For instance, **16b** is useful for highly potent nonpeptide AI1 receptor antagonists,[28] (*S*)-**19b** for (*R*)-tomoxetin and (*S*)-fluoxetine,[29] (*S*)-**20b** for pheromones and carbapenem antibiotics,[30] and (*S*)-**21b** for HMG-CoA reductase inhibitors.[31]

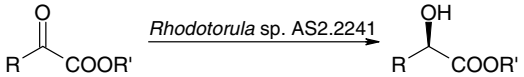
The versatility of the *Rhodotorula* sp. AS2.2241 whole-cell biocatalyst was further shown by using keto esters as substrates. The results for reduction of α-ketoesters (**15a**–**18a**) and β-ketoesters **19a**–**21a** are shown in Table 3. α-Ketoester **15a** was reduced quantitatively in 6 h, to give (*R*)-hydroxyl esters in 86% ee. However, when the methyl group of the ester moiety was replaced by an ethyl **16a**, isopropyl **17a**, or a *tert*-butyl **18a** group, the enantioselectivity decreased dramatically, although the yield remained high. The enantiomeric excess of the products most likely depends upon the size of the substituents adjacent to the ester moiety.

A complete reduction of β-aromatic ketoester **19a** could be carried out in 8 h, to afford (*S*)-**19b** with >99% ee, as determined by the specific rotation and comparison with the literature value.[33] However, the small molecule β-ketoesters **20a** and **21a** were reduced to (*S*)-**20b** or (*S*)-**21b** with only moderate selectivity (64–74% ee).

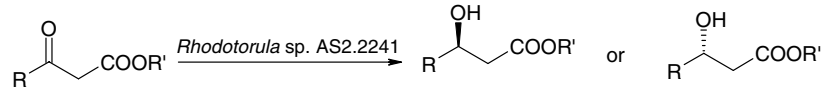
2.4. Synthesis of β-blocker (*R*)-nifenalol

Nifenalol is a molecule endowed with interesting biological properties, since it has been shown to act as a β-adrenergic

Table 3. Reduction of α- and β-ketoesters by *Rhodotorula* sp. AS2.2241



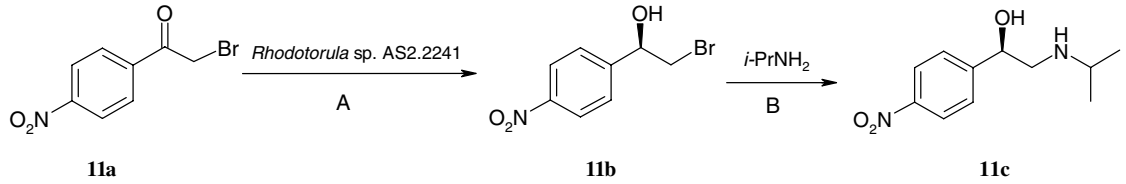
15a-18a **15b-18b**



19a-21a **19b, 21b** **20b**

Entry	R	R′	Time (h)	Yield[c] (%)	ee (%)	Config.
15	Ph	CH_3	6	100[a] (73)	86[d]	*R*
16	*m*-Me–C_6H_4	C_2H_5	2	100[b] (44)	21[d]	*R*
17	Ph	$(CH_3)_2CH$	2	100[b] (55)	27[d]	*R*
18	Ph	$(CH_3)_3C$	3	100[b] (55)	18[d]	*R*
19	Ph	C_2H_5	8	100[a] (50)	>99[e]	*S*
20	CH_3	C_2H_5	4	100[a] (31)	74[f]	*S*
21	$ClCH_2$	C_2H_5	2	100[a] (76)	64[f]	*S*

[a] Determined by GC analyses.
[b] Determined by TLC (eluent 8:1 petroleum ether–ethyl acetate).
[c] Isolated yield in parentheses.
[d] Determined by HPLC.
[e] Determined by specific rotation and comparison with the literature value.[33]
[f] Determined by GC analyses after acetylation of the product.



11a **11b** **11c**

Scheme 1. Synthesis of (*R*)-nifenalol by a chemoenzymatic approach. Step A, asymmetric reduction of **11a** (0.5 mmol) by *Rhodotorula* sp. AS2.2241 (12 g wet cells) in 100 mL potassium phosphate buffer (pH 7.0, 0.1 M) at 30 °C and 160 rpm for 5 h. Step B, chemical synthesis of (*R*)-nifenalol **11c** from **11b** at 40 °C for 40 h in 10 mL *i*-$PrNH_2$.

blocker with antianginal and antiarrhythmic properties. Furthermore, it has been emphasized that only its (*R*)-enantiomer displays any biological activity. The key step in the synthesis of (*R*)-nifenalol is to produce a chiral intermediate, which was previously prepared using an epoxide hydrolase that selectively hydrolyzes the (*S*)-epoxide from its racemic mixture, leaving the (*R*)-epoxide in an enantiomerically pure form.[34] Very few examples were reported regarding the bioreduction of **11a** to the corresponding (*R*)-alcohol, which can be directly transformed into (*R*)-nifenalol (see Scheme 1).

We successfully reduced compound **11a** with 100% conversion (as determined by TLC) and 97% ee by employing the resting cells of *Rhodotorula* sp. AS2.2241. When acetonitrile (5%) was added, the reaction could be finished within 2 h with the same enantiomeric excess. When the isolated (*R*)-**11b** was added to *i*-$PrNH_2$, the reaction was completed within 40 h, affording (*R*)-**11c** with an isolated yield of 47%. When (*R*)-**11c** was dissolved in dry ether and the solution bubbled with dry HCl, (*R*)-nifenalol hydrochloride was immediately formed as a white solid, $[\alpha]_D^{15} = -41.5$ (*c* 0.286, H_2O) {lit.[34] $[\alpha]_D^{15} = -40.3$ (*c* 1.07, H_2O)}.

3. Conclusions

Rhodotorula sp. AS2.2241 has already been shown to be a promising asymmetric reduction system as a biocatalyst. This strain of pink yeast grows quickly under simple culture conditions and the cells can reduce a wide variety of ketones (except for ketoesters) in high yields and ee within a short period. We also investigated the reduction of 2-bromo-1-(4-nitrophenyl)ethanone to the corresponding (*R*)-alcohol using *Rhodotorula* sp. AS2.2241, which provides an alternative method for the synthesis of (*R*)-nifenalol.

4. Experimental

α-Ketoesters **16a**, **17a**, and **18a** were donated by Mr. Guang-Yin Wang from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. All the other ketones were obtained from commercial suppliers. Gas chromatographic analyses were performed using a chiral GC-column (Betadex-120). HPLC analyses were performed using Chiralcel AD-H, OB or OD columns (∅0.46 cm × 25 cm).

4.1. Cultivation of *Rhodotorula* sp. AS2.2241

Glucose (1.5 g), yeast extract (0.5 g), peptone (0.5 g), KH_2PO_4 (0.05 g), K_2HPO_4 (0.05 g), NaCl (0.1 g) and $MgSO_4{\cdot}7H_2O$ (0.05 g) were mixed with water and the volume was adjusted to 100 mL. A portion of the resulting solution (3 mL) was placed in a 10 mL test tube, and the rest placed in a 500 mL Erlenmeyer flask and sterilized at 121 °C for 20 min. The solution in the test tube was inoculated with the stored microbe of *Rhodotorula* sp. AS2.2241 and shaken at 30 °C and 160 rpm for 12 h. The resultant culture in the test tube was transferred to the flask and shaken for 20 h at 30 °C and 160 rpm. The resulting culture was centrifuged and washed twice with a physiological saline (0.85% NaCl), giving 4 g of wet cells.

4.2. Reduction of ketones with *Rhodotorula* sp. AS2.2241

To a suspension of *Rhodotorula* sp. AS2.2241 cells (12 g, wet weight) in 100 mL of 50 mM potassium phosphate buffer (pH 7.0), was added one of the aromatic ketones (1.0 mmol), acylpyridines (2.0 mmol), ketoesters **15a**, **19a**, **20a**, and **21a** (5.0 mmol) or **16a**, **17a**, and **18a** (1.0 mmol). The reaction mixtures were incubated in an orbital shaker (160 rpm) at 30 °C for the time necessary to obtain an appropriate conversion. After centrifugation at 8000*g* for 8 min, the supernatant was saturated with NaCl and then extracted three times with ethyl acetate. Chemical yield and ee of the product were determined by TLC, GC, or HPLC analysis. The organic solution was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The crude product was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate, 8:1–1:1, v/v), followed by evaporation under vacuum. The product was identified by ^1H NMR analysis. The absolute configuration was determined by the specific rotation and comparison with the literature or by chiral GC or HPLC analysis and comparison with known racemates.

4.3. Preparation of (*R*)-nifenalol

To 10 mL of *i*-$PrNH_2$ was added 150 mg of (*R*)-**11b**, and the reaction mixture incubated in an orbital shaker (160 rpm) at 40 °C for 40 h. After excessive *i*-$PrNH_2$ was evaporated, 20 mL of water was added and product **11c** extracted with CH_2Cl_2 (20 mL × 3). The crude product was further purified by silica gel column chromatography with CH_2Cl_2/MeOH (5/1) as eluent. (*R*)-Nifenalol was obtained as a flaxen solid with an isolated yield of 47%. Finally, (*R*)-**11c** was dissolved in dry ether and the solution bubbled

with HCl. As a result, (*R*)-nifenalol hydrochloride was produced as a white solid.

4.4. Spectral data for the biosynthesized compounds

Compound **1b**. $[\alpha]_D^{25} = -59.2$ (*c* 0.658, $CHCl_3$) {lit.[15] $[\alpha]_D^{25} = -55.1$ (*c* 1.63, $CHCl_3$), *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.53 (d, 3H, $J = 6.4$ Hz), 4.85 (dd, 1H, $J_1 = 12.9$ Hz, $J_2 = 6.5$ Hz), 7.24–7.36 (m, 5H).

Compound **2b**. $[\alpha]_D^{25} = -30.5$ (*c* 1.083, EtOH) {lit.[3] $[\alpha]_D^{25} = -29.7$ (*c* 2.59, EtOH), *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (d, 3H, $J = 6.5$ Hz), 5.02 (dd, 1H, $J_1 = 12.9$ Hz, $J_2 = 6.5$ Hz), 7.59 (d, 2H, $J = 8.6$ Hz), 8.18 (d, 2H, $J = 8.6$ Hz).

Compound **3b**. $[\alpha]_D^{25} = -36.0$ (*c* 0.775, $CHCl_3$) {lit.[15] $[\alpha]_D^{25} = -37.9$ (*c* 1.13, $CHCl_3$), *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (d, 3H, $J = 6.2$ Hz), 4.86 (dd, 1H, $J_1 = 12.9$ Hz, $J_2 = 6.4$ Hz), 7.23 (d, 2H, $J = 8.4$ Hz), 7.46 (d, 2H, $J = 8.3$ Hz).

Compound **4b**. $[\alpha]_D^{25} = -47.8$ (*c* 0.758, Et_2O) {lit.[15] $[\alpha]_D^{25} = -49.0$ (*c* 1.84, Et_2O), *S*} ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (d, 3H, $J = 6.5$ Hz), 4.82 (d, 1H, $J = 6.5$ Hz), 7.28 (dd, 4H, $J_1 = 16.2$ Hz, $J_2 = 8.4$ Hz).

Compound **5b**. $[\alpha]_D^{25} = -56.4$ (*c* 0.442, $CHCl_3$) {lit.[15] $[\alpha]_D^{25} = -51.9$ (*c* 0.718, $CHCl_3$), *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (d, 3H, $J = 5.5$ Hz), 3.75 (s, 3H), 4.75 (d, 1H, $J = 5.6$ Hz), 6.83 (d, 2H, $J = 7.4$ Hz), 7.25 (d, 2H, $J = 7.3$ Hz).

Compound **6b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (d, 3H, 6.5 Hz), 2.16 (br, 3H), 4.87 (q, 1H, $J_1 = 12.9$, $J_2 = 6.4$ Hz), 7.31 (d, 2H, 8.4 Hz), 7.46 (d, 2H, 8.4 Hz).

Compound **7b**. $[\alpha]_D^{25} = -39.5$ (*c* 0.592, $CHCl_3$) {lit.[24] $[\alpha]_D^{25} = +34.9$ (*c* 1.0, $CHCl_3$), 89% ee, *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.52 (t, 1H, $J = 9.6$ Hz), 3.62 (dd, 1H, $J_1 = 10.5$ Hz, $J_2 = 3.3$ Hz), 4.89 (dd, 1H, $J_1 = 8.9$ Hz, $J_2 = 3.2$ Hz), 7.30–7.39 (m, 5H).

Compound **8b**. $[\alpha]_D^{25} = -50.7$ (*c* 0.225, cyclohexane) {lit.[15] $[\alpha]_D^{25} = -50.4$ (*c* 1.78, cyclohexane), 98% ee, *R*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.62–3.67 (m, 1H), 3.71–3.77 (m, 1H), 4.87–4.92 (m, 1H), 7.32–7.40 (m, 5H).

Compound **9b**. $[\alpha]_D^{25} = -50.8$ (*c* 1.03, $CHCl_3$) {lit.[15] $[\alpha]_D^{25} = -47.2$ (*c* 0.643, $CHCl_3$), *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (t, 3H, $J = 7.5$ Hz), 1.70–1.87 (m, 2H), 4.59 (t, 1H, $J = 6.6$ Hz), 7.23–7.37 (m, 5H).

Compound **11b**. $[\alpha]_D^{15} = -35.4$ (*c* 0.942, $CHCl_3$) {lit.[24] $[\alpha]_D^{25} = +32.1$ (*c* 1.0, $CHCl_3$), 91% ee, *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.82 (d, 1H, $J = 3.0$ Hz), 3.47–3.72 (m, 2H), 5.03 (dd, 1H, $J_1 = 5.3$ Hz, $J_2 = 3.1$ Hz), 7.58 (d, 2H, $J = 8.6$ Hz), 8.24 (d, 2H, 8.6 Hz).

Compound **11c**. ESI-MS *m/z*: 226 (M+2), 225 (M+1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.12 (t, 6H, $J = 6.2$ Hz), 2.60 (dd, 1H, $J_1 = 12.4$ Hz, $J_2 = 9.0$ Hz), 2.83–2.90 (m, 1H), 3.02 (dd, 1H, $J_1 = 12.2$ Hz, $J_2 = 3.7$ Hz), 4.76 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 3.6$ Hz), 7.55 (d, 2H, $J = 8.6$ Hz), 8.21 (d, 2H, $J = 8.7$ Hz).

(*R*)-Nifenalol hydrochloride $[\alpha]_D^{15} = -41.5$ (*c* 0.286, H_2O) {lit.[34] $[\alpha]_D^{15} = -40.3$ (*c* 1.07, H_2O)}.

Compound **12b**. $[\alpha]_D^{25} = -25.0$ (*c* 1.39, $CHCl_3$) {lit.[26] $[\alpha]_D^{25} = -27.6$ (*c* 0.712, $CHCl_3$), 95% ee, *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (d, 3H, $J = 6.7$ Hz), 4.89 (dd, 1H, $J_1 = 13.1$ Hz, $J_2 = 6.5$ Hz), 7.18 (dd, 1H, $J_1 = 7.3$ Hz, $J_2 = 5.2$ Hz), 7.28 (d, 1H, $J = 7.9$ Hz), 7.65–7.72 (m, 1H), 8.54 (d, 1H, $J = 4.7$ Hz).

Compound **13b**. $[\alpha]_D^{20} = -47.0$ (*c* 0.625, MeOH) {lit.[26] $[\alpha]_D^{25} = -37.2$ (*c* 1.01, MeOH), 89% ee, *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (d, 3H, $J = 6.3$ Hz), 4.93 (m, 1H), 7.27 (t, 1H, $J = 6.2$ Hz), 7.74 (d, 1H, $J = 7.8$ Hz), 8.42 (d, 1H, $J = 4.6$ Hz), 8.53 (s, 1H).

Compound **14b**. $[\alpha]_D^{20} = -44.0$ (*c* 1.03, MeOH) {lit.[26] $[\alpha]_D^{25} = -39.0$ (*c* 0.82, MeOH), 92% ee, *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (d, 3H, $J = 6.7$ Hz), 4.88 (dd, 1H, $J_1 = 13.1$ Hz, $J_2 = 6.5$ Hz), 7.31 (d, 2H, $J = 5.9$ Hz), 8.49 (d, 2H, $J = 4.4$ Hz).

Compound **15b**. $[\alpha]_D^{20} = -124.8$ (*c* 0.8, MeOH) {lit.[32] $[\alpha]_D^{25} = -144.0$ (*c* 1, MeOH), *R*}.

Compound **16b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (t, 3H, $J = 7.3$ Hz), 2.36 (s, 3H), 4.09–4.32 (m, 2H), 5.13 (s, 1H), 7.20 (d, 4H, 5.9 Hz).

Compound **17b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10 (d, 3H, $J = 6$ Hz), 1.27 (d, 3H, $J = 6.6$ Hz), 3.48 (br d, 1H, $J = 6.9$ Hz), 5.05–5.20 (m, 2H), 7.23–7.48 (m, 5H).

Compound **18b**. ^{1}H NMR (500 MHz, $CDCl_3$): δ (s, 9H), 5.03 (s, 1H), 7.28–7.45 (m, 5H).

Compound **19b**. $[\alpha]_D^{15} = -52.7$ (*c* 1.0, $CHCl_3$) {lit.[33] $[\alpha]_D^{27} + 36.7$ (*c* 1.38, $CHCl_3$), 72% ee *R*}.

Compound **20b**. $[\alpha]_D^{25} = +22.2$ (*c* 0.442, $CHCl_3$) {lit.[14] $[\alpha]_D^{25} + 32.8$ (*c* 3.0, $CHCl_3$), 95% ee *S*}.

Compound **21b**. $[\alpha]_D^{25} = -11.4$ (*c* 1.59, $CHCl_3$) {lit.[14] $[\alpha]_D^{25} - 19.9$ (*c* 3.8, $CHCl_3$), 90% ee *S*}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (t, 3H, $J = 7.1$ Hz), 2.58–2.68 (m, 2H), 3.58–3.65 (m, 2H), 4.19 (dd, 2H, $J_1 = 14.3$ Hz, $J_2 = 7.2$ Hz), 4.27 (m, 1H).

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