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# Bioreduction of β-carboline imines to amines employing *Saccharomyces bayanus*

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ABSTRACT

β-Carboline imine reductions mediated by *Saccharomyces bayanus* have been described achieving moderate to good enantiomeric excesses of the amine products. The enantiomeric excesses of the bioreduction showed a dependence on the imine substituents. Compounds presenting $C_1$–$C_{11}$ aliphatic substituent groups afforded amines with an (*S*)-configuration, whereas $C_{15}$ and higher aliphatic, and aromatic substituted β-carboline imines achieved inversion of the configuration in the final (*R*)-**2** amine products. Based on this data, a model for the *Saccharomyces* reduction is proposed.



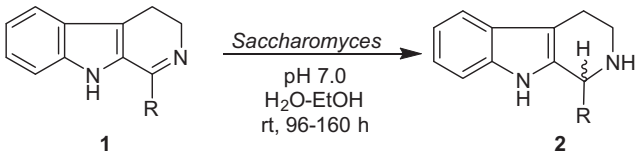
## 1. Introduction

Several stereoselective transformations using biocatalysts are regularly exploited in the preparation of chiral synthons in natural products synthesis.[1] There are two different biotransformation systems, whole cells or isolated enzymes, and both display several advantages. The availability of a certain microorganism is often the deciding factor for an organic chemist as to whether to use biotransformations in synthesis. For example, Baker's yeast (BY), *Saccharomyces cerevisiae*, is a readily available microorganism but obtaining other microorganisms may require help from a microbiologist and access to fermentation facilities. The yeast of the *Saccharomyces sensu stricto* complex includes most of the species related to the fermentation industry and those commonly used in science, namely *S. cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, and *Saccharomyces pastorianus*.[2,3] *S. bayanus*, for instance, is constituted by a number of strains that are highly heterogeneous in their physiology and molecular characteristics.[4] In the wine industry, the commercial strains of *S. cerevisiae* and *S. bayanus* have been selected due to their ability to safely conduct and complete the fermentation of grape sugars.[5,6]

After great developments in synthetic organic chemistry, few methodologies allow the stereoselective construction of predetermined moieties in some classes of compounds. In this context, particular attention towards efficient synthetic routes for novel chemotypes is already pursued when stereoselectivity is required. Furthermore, chirality in molecules plays an important role in areas ranging from medicine to material science. As part of our efforts in the field of biologically relevant β-carbolines, we turned our attention toward an alternative synthetic route for amines **2**, via key intermediate **1**. Several methodologies for the synthesis of optically active alcohols are known based on bio-catalysis. Among the most popular methods is the asymmetric hydrogenation of ketimines or enamides using chiral Rh(I), Ir(I), or Ru(II) complexes.[7]

Thus, we investigated the scope of reduction of imines **1** mediated by different *Saccharomyces*. The methodology is based on the use of aggressive *Saccharomyces* species employed in the fermentation of grape sugars for the reduction of dihydro-β-carbolines (Scheme 1).

There has been a growing interest in bio-catalysis and transformations mediated by yeasts. Reductions of carbonyl and imine compounds have become a valuable strategy in organic synthesis, although the reductions or oxidations of other functionalities have not been investigated. As part of our efforts to unravel applications of *Saccharomyces cerevisae* (Baker's yeast), we studied the reduction of imines to amines (Scheme 1).




**Scheme 1.** Bioreduction of β-carboline imines by *Saccharomyces*.

## 2. Results and discussion

Imine **1** was obtained in 75–83% overall yield from different carboxylic acids and tryptamine by coupling with EDC/HOBt in

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$CH_2Cl_2$ at room temperature, which afforded the corresponding amides **3**. The amides were subjected to Bischler–Napieralsky cyclization to afford imines **1**.[8] Having prepared several imines **1**, the next stage was set to explore asymmetric reduction with different yeasts. In Nature, oxidoreductases catalyze remarkably selective transfer hydrogenations of carbonyl compounds to alcohols using co-factors such as NADH or NADPH.[9] Thus, imines might be reduced by NAD(P)H in a similar manner.

We first investigated the scope of the reduction of imine **1a** mediated by different *Saccharomyces*. The *Saccharomyces* tested were four strains of *S. cerevisae*, and four strains of *S. bayanus* as depicted in Table 1. Reductions of **1a** (in ethanol) were carried out using a solution of fructose and different strains of *Saccharomyces* in distilled water, and the mixture stirred until completion of the reaction, as monitored by gas chromatography at 37 °C. After completion of the reaction, the crude was basified with NaOH to pH 10, after which brine was added, the contents were centrifuged and the supernatant and cell pellet were extracted separately. Amine **2a** was obtained in 38–78% yield and enantiomeric excesses between 74% and 94% (Table 1). The best results according to the ee were obtained with *S. bayanus* (Table 1, entries 5–8) and fructose in the reactions. The use of fructose instead of glucose is due to *S. bayanus* has an active transport system for fructose, which is lacking in other *Saccharomyces sensu stricto* species, including *S. cerevisiae*.[10,11] In *Saccharomyces sensu stricto*, the yeast strains of the same species could be highly polymorphic, thus making it difficult to extrapolate the results observed here to other strain of the same species. However, the total uptake capacity of both sugars, glucose and fructose, vary from strain to strain. In general, yeast cells convert glucose more efficiently to ethanol and $CO_2$ than fructose.[12] This situation might be due to the fact that glucose and other sugars are transported in the pyranose rather than the furanose form;[13] the latter being present in a significant proportion in fructose solutions (ca. 30%), thus reducing the actual concentration of transport-competent fructose.[11] However, better enantioselectivities for amine **2a** were obtained when fructose was employed (91–94% ee) instead of glucose (85–90% ee, data not shown) for the reduction, corroborating that *S. bayanus* transport system is more active for fructose than glucose.

**Table 1**
Reduction of imine **1a** by using *S. cerevisae* (Levuline: CHP and ALS; Littolevure; Vitilevure) and *S. bayanus* (Danster Ferment; Oeno France; Fermivin; Nouveaux Ferments) to amine **2a**

| Entry | *Saccharomyces* | % ee | $[\alpha]_D$ | Yield (%) |
|---|---|---|---|---|
| 1 | *S. cerevisae*, CHP 2011 | 80 | 37 | 48 |
| 2 | *S. cerevisae*, ALS | 75 | 33 | 38 |
| 3 | *S. cerevisae*, LP4885 | 80 | 38 | 45 |
| 4 | *S. cerevisae*, 3235SP | 74 | 28 | 52 |
| 5 | *S. bayanus*, AGU2642 | 94 | 48 | 68 |
| 6 | *S. bayanus*, Oeno | 93 | 47 | 78 |
| 7 | *S. bayanus*, SP 8906 | 92 | 47 | 60 |
| 8 | *S. bayanus*, Nouveaux | 91 | 44 | 63 |

[a] Absolute configuration determined by the Noyori model using HPLC employing a ChiralPack-OD column (hexane/2-propanol/diethylamine, 80:20:0.1) after comparing with products obtained by imine reduction with TsDPEN–Ru(II) catalyst. The reactions using *Saccharomyces* were conducted under anoxic conditions.

Next, we tested other imines for the reduction with *S. bayanus*, as depicted in Table 2. Excesses ranging from 50% to 97% ee were observed. For **1** the enzyme has to distinguish between the *Re*- and the *Si*-face of the $\pi$-system to yield chiral **2**. The asymmetric reduction of imine-containing compounds by BY is not a widespread reaction. According to the results obtained in Table 2, the imine reduction suggested hydrogen transfer to the *Re*-face of prochiral imine **1**, when R is a small aliphatic group, with $R_L$ representing a large aliphatic or aromatic substituent and $R_S$ a small aliphatic substituent adjacent to the imine group (Fig. 1) to yield amine **2**. The absolute configurations of the new formed centers were corroborated after Noyori asymmetric reduction of imines **1** and HPLC analyses employing a ChiralPack OD column. On the basis of the examples that have been reported by us and Noyori, the absolute stereochemistry of **2** was expected to be (*R*) when the (*S*,*S*)-TsDPEN–Ru(II) complex was used in the hydrogen transfer. The *Si*-face of imine **1** is expected to be selected for hydrogenation providing (*R*)-**2** when the catalyst is employed. This outcome is consistent with the general model that Noyori had proposed for asymmetric hydrogen transfer reactions with TsDPEN–Ru(II) complexes, and it was probed by comparing the specific rotation of **2** (Table 2). Compound **2n** did not produce the corresponding amine **1n** when employing the Noyori catalyst. The absolute configuration assignment of compound **1n** (R = 2,5-dinitrophenyl) obtained by *S. bayanus* ($[\alpha]_D$ = +34, entry *n*, Table 2) was suggested to be (*R*) due to Noyori asymmetric catalyst did not afforded imine **1n**, based on the proposed model for bioreduction, as depicted in Figure 1.

**Table 2**
Reduction of imines **2** by using *S. bayanus* (Danster Ferment AGU2642) to amines **1**

| Entry | R | % ee | $[\alpha]_D$ | Absolute configuration[a] | Yield (%) |
|---|---|---|---|---|---|
| a | Me | 94 | −48 | (*S*) | 68 |
| b | Et | 93 | −60 | (*S*) | 66 |
| c | $CH_2Cl$ | 92 | −12.3 | (*S*) | 68 |
| d | $CH_2I$ | 50 | +16.8 | (*S*) | 45 |
| e | Iso-Pr | 84 | −60.6 | (*S*) | 62 |
| f | 4-Penten | 85 | −20.0 | (*S*) | 53 |
| g | $C_{11}H_{23}$ | 74 | −48.4 | (*S*) | 58 |
| h | $C_{15}H_{31}$ | 65 | +11.0 | (*R*) | 62 |
| i | $C_{17}H_{33}$ | 62 | +12.0 | (*R*) | 60 |
| j | $C_{17}H_{31}$ | 64 | +21.6 | (*R*) | 61 |
| k | Ph | 88 | −4.0 | (*R*) | 52 |
| l | 2-(OH)Ph | 97 | −11.9 | (*R*) | 48 |
| m | Bn | 79 | −57.9 | (*R*) | 62 |
| n | Nicotinic | 75 | +34.0 | (*R*)[b] | 57 |

[a] Absolute configuration was determined by using HPLC employing a ChiralPack-OD column (hexane/2-propanol/diethylamine, 80:20:0.1) after comparing with the products obtained by imine reduction with TsDPEN–Ru(II) catalyst. The reaction using *Saccharomyces* were conducted in anoxic conditions.
[b] Configuration suggested to be (*R*).

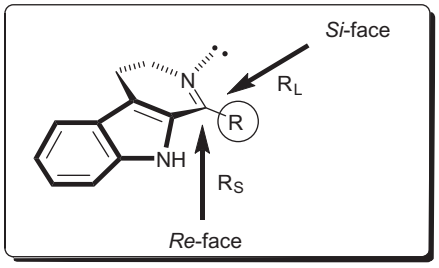


**Figure 1.** Proposed model for bioreduction of β-carboline imines by *Saccharomyces*.

## 3. Conclusion

In conclusion, various NAD(P)H-dependent oxidoreductases may be involved in this type of reduction by Baker's yeast. Especially in redox reactions, the use of whole cells is attractive because of the in vivo regeneration of cofactors that are essential for continuous reaction. The regeneration of NAD(P)H must take place as

yeast cells contain only a catalytic amount of NAD(P)H. This is achieved by the metabolism of an electron donor, in our case fructose or ethanol. The process may also involve NAD-dependent dehydrogenases of the fructose-monophosphate pathway, NADP-dependent isocitrate dehydrogenase, and NADP-dependent acetaldehyde dehydrogenase. Moreover, the pathways, in which these dehydrogenases participate reduce NADP as well as NAD, thus the produced NADH needs to be re-oxidized by mitochondrial respiration. This could explain the apparent paradox of an oxygen requirement for sustaining the overall reduction reaction. Thus, β-carboline imine reductions mediated by *Saccharomyces* have been described with moderate to good enantiomeric excesses of product amines being obtained. The methodology is an attractive alternative to the catalytic asymmetric methods employing Noyori catalysts. The enantiomeric excesses of the bioreduction showed a dependence upon imine substituents. Compounds presenting $C_1$–$C_{11}$ aliphatic substituent groups afforded amines with (*S*)-configuration, as determined by comparing the specific rotations with amines obtained employing Noyori asymmetric hydrogenations. However, $C_{15}$–$C_{22}$ aliphatic and aromatic substituted β-carboline imines achieved inversion of the configuration in the final (*R*)-**2** amine products.

## 4. Experimental

### 4.1. Microorganism

Lyophilized commercial cells of the siblings *S. cerevisiae*: Levuline ALS, Oeno France CHP 2011 (lot number 2181240130821, EUA); Levuline ALS, Oeno France ALS (lot number 2680827085061v, EUA); Littolevure, LP4885 (Germany); Vitilevure 3235SP (France) and stored in a refrigerator (4 °C). Lyophilized commercial cells of the siblings *S. bayanus*: Danster Ferment AG U2642 (Denmark); Oeno France (France); Fermivin PDM SP 8906 (EUA); Nouveaux Fermentes (France) and stored in a refrigerator (4 °C).

### 4.2. Chemicals

Imine compounds **1** were synthesized according to previously described protocols.[8] Ethanol, acetonitrile, tryptamine, $POCl_3$, organic acids for imine preparation were purchased from Merck and used without previous purification.

### 4.3. Reduction conditions

To a stirred solution of fructose (8.0 g) and *Saccharomyces* (13.0 g) in distilled water (50.0 mL) was added an ethanolic solution of compound **1a** (3.33 mmol, 1.0 mL) and the contents stirred until completion of the reaction at 37 °C as monitored by gas chromatography. After completion of the reaction (around 56 h), the crude was basified with NaOH (1.0 M) to pH 10, after which brine was added (20 mL), the contents were centrifuged and the supernatant and cell pellet were extracted separately with $CHCl_3$ (3 × 70 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish the crude product, which was purified by column chromatography on silica gel eluting with hexane–ethyl acetate–MeOH (90:7:3).

#### 4.3.1. (*S*)-1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2a

$[\alpha]_D^{20} = -48.0$ (*c* 1.0, MeOH), {lit.[14] (*R*)-isomer, $[\alpha]_D^{20} = +53.5$ (*c* 2.08, EtOH)}, 94% ee by HPLC analysis (Chiralcel OD, hexane/2-propanol/diethylamine = 80:20:0.1, 1.0 mL/min, 254 nm, major isomer 8.5 min, minor isomer 5.6 min)). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 1.46 (3H, d, *J* 6,7 Hz), 1.80 (1H, br s), 2.88–2.83 (2H, m), 3.05 (1H, ddd, *J* 13.1, 9.2, 5.2 Hz), 3.37 (1H, ddd, *J* 13.1, 5.2, 3.7 Hz), 4.19 (1H, tq, *J* 6.7, 2.0 Hz), 7.09 (1H, dt, *J* 7.3, 0.9 Hz), 7.15 (1H, dt, *J* 7.3, 0.9 Hz), 7.31 (1H, d, *J* 7.3 Hz), 7,48 (1H, d, *J* 7.3 Hz), 7.78 (1H, br s). HRMS (EI): calcd for $C_{12}H_{14}N_2$ *m*/*z* 186,1157, found: 186.1147.

#### 4.3.2. (*S*)-1-Ethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2b

$[\alpha]_D^{20} = -60.0$ (*c* 1.0, MeOH), {lit.[15] (*S*)-isomer, $[\alpha]_D^{20} = -62.6$, acetone)}, 93% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.5 mL/min, 254 nm, minor isomer 8.9 min, major isomer 11.0 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 1.10 (3H, t, *J* 7.1 Hz), 1.67–1.75 (1H, m), 1.85–2.07 (1H, m), 2.72–2.78 (2H, m), 3.00–3.06 (1H, m), 3.34–3.40 (1H, m), 4.00–4.04 (1H, m), 7.06–7.19 (2H, m), 7.32 (1H, d, *J* 7.2 Hz), 7.49 (1H, d, *J* 7.6 Hz), 7.77 (1H, br s). HRMS (ESI) for $[C_{13}H_{16}N_2+H]^+$: calcd 201.13917, found: 201.13910. The spectrometric data is in accordance with the above reference.

#### 4.3.3. (*S*)-1-Chloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2c

$[\alpha]_D^{20} = -12.3$ (*c* 1.0, MeOH), 92% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, minor isomer 6.7 min, major isomer 13.2 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 2.71–2.78 (2H, m), 3.00–3.07 (1H, m), 3.50–3.60 (1H, m), 3.78–3.81 (1H, m), 4.45 (1H, m), 7.07–7.18 (2H, m), 7.33 (1H, d, *J* 7.2 Hz), 7.50 (1H, d, *J* 7.6 Hz), 7.76 (1H, br s). $^1$H and $^{13}$C NMR data are in accordance with Julia et al.[16]

#### 4.3.4. (*S*)-1-Iodomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2d

$[\alpha]_D^{20} = +16.8$ (*c* 1.0, MeOH), 50% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 4.7 min, minor isomer 7.7 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 2.72–2.78 (2H, m), 3.02–3.09 (1H, m), 3.33–3.46 (1H, m), 3.58–3.61 (1H, m), 4.25 (1H, m), 7.04–7.18 (2H, m), 7.32 (1H, d, *J* 7.2 Hz), 7.48 (1H, d, *J* 7.6 Hz), 7.75 (1H, br s). $^1$H and $^{13}$C NMR data are in accordance with Julia et al.[16]

#### 4.3.5. (*S*)-1-Isopropyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2e

$[\alpha]_D^{20} = -60.6$ (*c* 1.0, MeOH), {lit.[15] $[\alpha]_D^{20} = -58.3$ in acetone}, 84% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, minor isomer 4.7 min, major isomer 8.6 min). FT-IR (KBr film), $cm^{-1}$: 3471, 1466. $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 0.91 (3H, d, *J* 7.2 Hz), 1.14 (3H, d, *J* 7.2 Hz), 2.16–2.28 (1H, m), 2.72–2.77 (2H, m), 2.93–3.03 (1H, m), 3.42–3.45 (1H, m), 4.00–4.04 (1H, m), 7.10–7.15 (2H, m), 7.31 (1H, dd, *J* 1.4, 7.3 Hz), 7.50 (1H, dd, *J* 1.4, 7.3 Hz), 7.85 (1H, br s). HRMS (EI): calcd for $C_{14}H_{18}N_2$ *m*/*z* 214.1466, found: 214.1458. The spectrometric data is in accordance with Shankaraiah et al.[17]

#### 4.3.6. (*S*)-1-(4-Pentenyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2f

$[\alpha]_D^{20} = -20.0$ (*c* 1.0, $CHCl_3$), 85% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, minor isomer 6.1 min, major isomer 7.2 min). FT-IR (KBr film), $cm^{-1}$: 3409, 3218, 3062, 1641, 1562. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.52–1.70 (3H, m), 1.84–1.91 (1H, m), 2.09–2.19 (2H, m), 2.68–2.80 (1H, m), 2.75 (1H, dq, *J* 8.0, 1.9 Hz), 3.03 (1H, ddd, J 15.5, 8.0, 5.5), 3.34 (1H, dt, *J* 14.5, 4.5 Hz), 4.07 (1H, br s), 4.98 (1H, br d, *J* 10.2 Hz), 5.03 (1H, dd, *J* 17.1, 1.6 Hz), 5.80 (1H, ddt, *J* 17.1, 10.2, 6.7 Hz), 7.09 (1H, dt, *J* 7.6, 0.7 Hz), 7.14 (1H, dt, *J* 7.6, 0.7 Hz), 7.30 (1H, d, *J* 7.8 Hz), 7,47 (1H, d, *J* 7.8 Hz), 7.84 (1H, br s, NH). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 22.6, 25.0, 33.7, 34.3, 42.5,

52.5, 109.0, 110.7, 115.0, 118.0, 119.3, 121.5, 127.5, 135.6, 136.1, 138.3. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{16}H_{20}N_2+H]^+$ 241.1705, found 241.1701.

#### 4.3.7. (*S*)-1-Undecyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2g

Compound **2g** was not previously described: $[\alpha]_D^{20} = -48.4$ ($c$ 1.0, MeOH), 74% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, minor isomer 7.35 min, major isomer 9.9 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 0.80 (3H, t, $J$ 7.0 Hz), 1.10–1.89 (16H, m), 1.30–1.45 (2H, m), 1.59–1.69 (1H, m), 1.72–1.82 (1H, m), 2.62–2.73 (2H, m), 2.90–3.00 (1H, m), 3.20–3.30 (1H, m), 4.00 (1H, br s), 7.03 (1H, t, $J$ 7.0 Hz), 7.09 (1H, t, $J$ 7.0 Hz), 7.24 (1H, d, $J$ 7.9 Hz), 7.39 (1H, d, $J$ 7.9 Hz), 8.00 (1H, br s, NH). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 14.1, 22.7, 25.9, 29.4, 29.7, 29.73, 29.75, 29.79, 29.9, 32.0, 34.6, 36.0, 41.7, 52.4, 108.4, 110.9, 118.1, 119.4, 121.7, 127.3, 135.0, 135.8. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{22}H_{34}N_2+H]^+$ 327.2802, found 327.2800.

#### 4.3.8. (*R*)-1-Pentadecyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2h

Compound **2h** was not previously described: $[\alpha]_D^{20} = +11.0$ ($c$ 1.0, MeOH), 65% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 6.7 min, minor isomer 13.2 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 0.66 (3H, t, $J$ 7.0 Hz), 0.96–1.17 (24H, m), 1.20–1.35 (2H, m), 1.46–1.55 (1H, m), 1.70–1.78 (1H, m), 2.48–2.60 (2H, m), 2.77–2.87 (1H, m), 3.10–3.17 (1H, m), 3.35–3.50 (1H, m), 3.90 (1H, br s), 6.82 (1H, t, $J$ 7.0 Hz), 6.88 (1H, t, $J$ 7.0 Hz), 7.10 (1H, d, $J$ 7.9 Hz), 7.20 (1H, d, $J$ 7.9 Hz), 9.40 (1H, br s, NH). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 14.1, 22.7, 25.9, 29.4, 29.59, 29.6, 29.7, 29.72, 29.73, 29.75, 29.78, 29.79, 29.9, 32.0, 34.6, 36.0, 41.7, 52.4, 108.4, 110.9, 118.1, 119.4, 121.7, 127.3, 135.0, 135.8. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{26}H_{42}N_2+H]^+$ 383.3426, found 383.3429.

#### 4.3.9. (*R*)-1-(8-Heptendecyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indo 2i

Compound **2i** was not previously described: $[\alpha]_D^{20} = +12.0$ ($c$ 1.0, MeOH), 62% ee by HPLC analysis (ChiralCell OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 4.6 min, minor isomer 6.3 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 0.90 (3H, t, $J$ 7.0 Hz), 1.20–1.41 (20H, m), 1.41–1.58 (2H, m), 1.64–1.76 (1H, m), 1.81–1.92 (1H, m), 1.93–2.02 (5H, m), 2.72–2.78 (2H, m), 3.02–3.07 (1H, m), 3.33–3.37 (1H, m), 4.05–4.09 (1H, m), 5.29–5.40 (2H, m), 7.09–7.15 (2H, m), 7.31 (1H, d, $J$ 8.0 Hz), 7.49 (1H, d, $J$ 8.0 Hz), 7.92 (1H, s). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 14.1, 22.3, 22.6, 25.8, 27.2, 27.25, 29.2, 29.3, 29.4, 29.5, 29.55, 29.7, 29.8, 29.85, 32.0, 34.9, 42.3, 52.8, 108.7, 110.8, 118.0, 119.4, 121.6, 127.3, 129.7, 130.0, 135.6, 135.70. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{28}H_{44}N_2+H]^+$ 409.3583, found 409.3578.

#### 4.3.10. (*R*)-1-(8,11-Heptendecyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2j

Compound **2j** was not previously described: $[\alpha]_D^{20} = +21.6$ ($c$ 1.0, MeOH), 64% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 4.5 min, minor isomer 5.5 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 0.88 (3H, t, $J$ 7.0 Hz), 1.26–1.35 (6H, m), 1.52–1.66 (2H, m), 1.71–1.79 (1H, m), 1.86–1.94 (1H, m), 2.03–2.16 (5H, m), 2.75–2.88 (8H, m), 3.05–3.12 (1H, m), 3.34– 3.39 (1H, m), 4.18 (1H, br s), 5.31–5.39 (8H, m), 7.07–7.14 (2H, m), 7.31 (1H, d, $J$ 8.0 Hz), 7.47 (1H, d, $J$ 8.0 Hz), 7.48 (1H, d, $J$ 8.0 Hz), 7.93 (1H, br s). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 14.1, 21.8, 22.7, 25.6, 25.7, 25.75, 25.76, 27.1, 27.2, 29.5, 31.5, 34.0, 42.1, 52.6, 108.5, 110.8, 118.1, 119.7, 121.7, 127.3, 127.5, 127.9, 128.1, 128.3, 128.6, 128.7, 129.4, 130.6, 135.7, 136.0. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{28}H_{42}N_2+H]^+$ $m/z$ 407.3426, found 407.3430.

#### 4.3.11. (*R*)-1-Phenyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2k

$[\alpha]_D^{20} = -4.0$ ($c$ 1.0, $CHCl_3$), {lit.[14] (*R*)-isomer, $[\alpha]_D = -3.9$, ($c$ 1.03, $CHCl_3$}, 88% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 1.0 mL/min, 254 nm, minor isomer 15.5 min, major isomer 19.7 min). Mp 159–160 °C. $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 2.83–2.95 (2H, m), 3.07–3.16 (1H, m), 3.33–3.39 (1H, m), 5.17 (1H, s), 7.10–7.19 (3H, m), 7.30–7.37 (5H, m), 7.56–7.60 (1H, m), 7.66 (1H, br s). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 22.6, 42.8, 58.1, 110.2, 110.9, 118.3, 119.4, 121.8, 127.3, 128.3, 128.6, 128.9, 134.4, 136.0, 141.9. HRMS (EI): calcd for $C_{17}H_{16}N_2$ $m/z$ 248.1314, found: 248.1320.

#### 4.3.12. (*R*)-1-(2-Hydroxyphenyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2l

$[\alpha]_D^{20} = -11.9$ ($c$ 1.0, $CHCl_3$), 97% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 5.7 min, minor isomer 7.5 min). Mp 189–190 °C. FT-IR (KBr film), cm$^{-1}$: 3445, 1600, HRMS. $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 2.76 (1H, br s), 2.83–2.89 (1H, m), 2.90–3.23 (2H, m), 3.46–3.40 (1H, m), 5.26 (1H, s), 6.89–6.80 (2H, m), 7.05–7.25 (5H, m), 7.42–7.50 (2H, m). ESI(+)-MS: $m/z$ calcd for $[C_{17}H_{16}N_2O+H]^+$ $m/z$ 265.1341, found 265.1355.

#### 4.3.13. (*R*)-1-Benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (2m)

$[\alpha]_D^{20} = -57.9$ ($c$ 1.0, MeOH), {lit.[18] (*S*)-isomer, ee 87%, $[\alpha]_D^{20} = +53.0$, ($c$ 0.9, acetone)}; 79% ee by HPLC analysis (ChiralCell OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 10.2 min, minor isomer 16.6 min). $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 2.76–2.79 (2H, m), 3.02–3.17 (3H, m), 3.39 (1H, ddd, $J$ 4.9, 4.9 and 12.0 Hz), 4.43 (1H, t, $J$ 7.0 Hz), 7.10–7.14 (2H, m), 7.25 (1H, d, $J$ 7.5 Hz), 7.32–7.40 (5H, m), 7.49 (1H, s), 7.51 (1H, d, $J$ 7.7 Hz), 7.53 (1H, d, $J$ 7.5 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta$: 22.3, 41.6, 42.5, 54.0, 109.3, 110.7, 118.1, 119.3, 121.6, 126.9, 127.3, 128.8, 129.3, 135.4, 135.5, 138.2. HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{18}H_{18}N_2+H]^+$ 263.1548, found 263.1550.

#### 4.3.14. (*R*)-1-Nicotinic-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2n

$[\alpha]_D^{20} = +34.0$ ($c$ 1.0, MeOH), 75% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 4.6 min, minor isomer 8.9 min). $^1$H and $^{13}$C NMR data are in accordance with Misztal et al.[19] HRMS, ESI(+)-MS: $m/z$ calcd for $[C_{16}H_{15}N_3+H]^+$ 250.1344, found 250.1338.


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