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Asymmetric synthesis of (+)-febrifugine and (+)-isofebrifugine using yeast reduction

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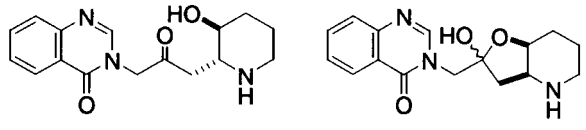
Abstract—The antimalarial agents febrifugine (D-**1**) and isofebrifugine (D-**2**) were synthesized from chiral 3-piperidinol (D-**4**), which was asymmetrically prepared by the yeast reduction of 3-piperidone derivatives (DL-**3**), with dynamic optical resolution.

1. Introduction

Malaria is the world's most important tropical parasitic disease and there are an estimated 300–500 million cases of malaria each year. The emergence of multi-drug resistant strains of the parasite is exacerbating the situation. Febrifugine (D-**1**), which was isolated from *Dichroa febrifuga* and *Hydrangea umbellata* along with isofebrifugine (D-**2**),[1a–c] is a well-known candidate antimalarial agent. The plane structure[2a] of D-1 and D-2 was first proposed in 1950. Subsequently, their relative[2b] and absolute[2c] structures were proposed, based on Baker's synthetic work.[3a–c] The relative configuration[2d] of D-**1** was corrected in 1973 and then the absolute structures[2e] of D-**1** and D-**2** were corrected in 1999, as shown in Fig. 1. These repeated errors and corrections have caused much confusion in the study of the relationship between the structure and antimalarial activity of febrifugine derivatives, and have resulted in sacrifice of many test animals. However, investigation of the antimalarial activity of febrifugine derivatives is beginning anew, since it was reported that D-**1** had higher activity than clinically used antimalarial drugs, and a derivative more potent than D-**1** was found.[4a,b] We previously synthesized the racemates of D-**1** and D-**2** via the unusual Claisen rearrangement and highly diastereoselective reduction.[5] In this paper, we describe the asymmetric synthesis of D-**1** and D-**2** based on our synthetic method.



Febrifugine (D-**1**) Isofebrifugine (D-**2**)

Figure 1. The finally corrected structure of D-**1** and D-**2**.

2. Results and discussion

Considering *green chemistry*, we selected the yeast reduction of 3-piperidone derivatives (DL-**3**)[5] protected by a Cbz group as a key asymmetric reaction. The reaction of DL-**3** with baker's yeast and sucrose in EtOH and water afforded *cis*-3-piperidinol (D-**4**) with high selectivity. We determined that the absolute configuration of L-**3** is certainly 2*R* from the optical rotation of a known compound (L-**6**)[6] via reduction[7] of the tosylhydrazone (L-**5**) with $NaBH_3CN$ and $ZnCl_2$ from L-**3**. The reduction of DL-**3** with $NaBH_4$ is known to give the corresponding *cis* alcohol (DL-**4**) with complete diastereoselectivity in high yield.[5] Reduction of L-**3** with $NaBH_4$ gave L-**4**, which had the same ^{1}H-NMR as D-**4** and the opposite optical rotation. These results showed that the absolute configuration of D-**4** must be 2*S*,3*S* (Scheme 1).

Fortunately, a mixture of DL-**3** and DL-**4** produced four completely separate peaks on HPLC with a chiral column. Using HPLC, we examined the conditions that optimized the yield and enantiomer excess of D-**4** having the same configuration as D-**2** in the yeast reduction of DL-**3**. Under the conditions shown in Fig. 2, DL-**3** afforded the best results for D-**4** on HPLC. This supported the prediction that the yeast reduction of DL-**3** would consume only D-**3** according to Prelog's rule[8] to give D-**4** and L-**3** by optical resolution. We think that the decrease in the enantiomer excess of L-**3** observed with time might be due to the effect of yeast empimerase, since the epimerization of L-**3** was not

Keywords: asymmetric synthesis; optical resolution; reduction; antimalarial compounds.

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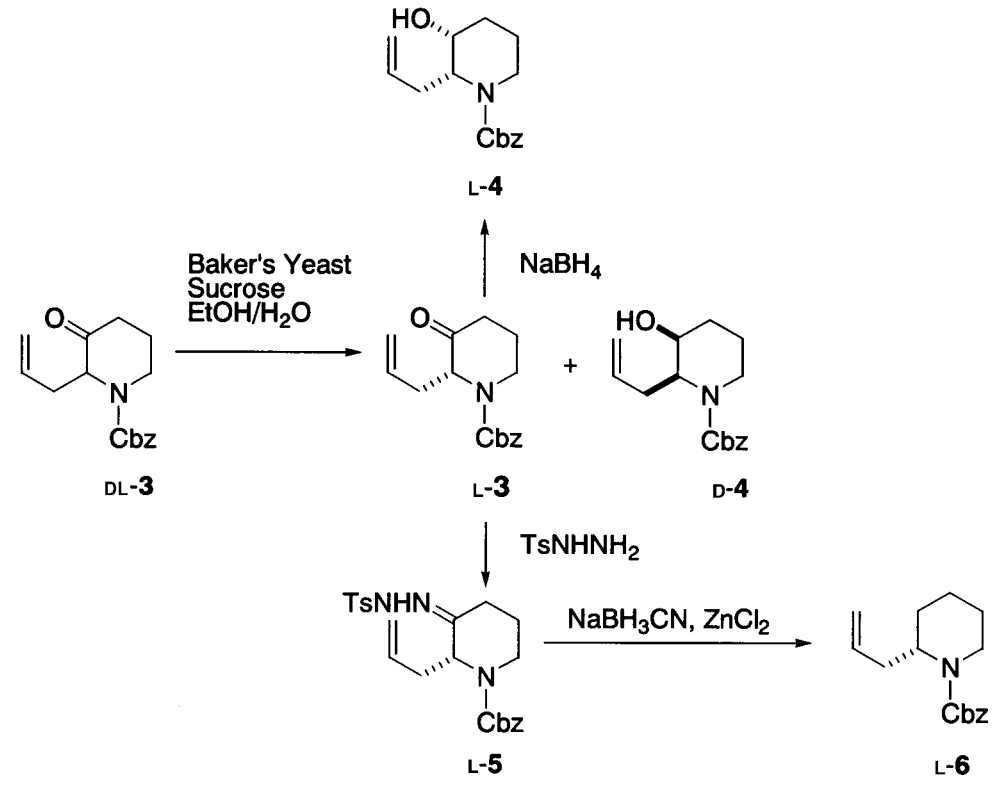


Scheme 1.

observed in the reaction of L-**3**, even after 168 h, unless there was yeast present. Successful isolation of these compounds was achieved by column chromatography on SiO_2 to give L-**3** in 31% yield (93% ee) and D-**4** in 41% yield (97% ee) (method A). From a practical perspective, we estimate that this reaction has an *E* value[9] of 215.

The dynamic optical resolution using a substrate that was enolized easily demonstrated that the product yield in the yeast reduction would exceed 50%, maintaining high enantioselectivity.[10] If we could cause the epimerization of L-**3** quickly enough to maintain the reductive activity of the yeast, we thought that reductive dynamic optical resolution would occur, since epimerization of L-**3** with K_2CO_3 at room temperature for 4 h in MeOH gave DL-**3** in 67% yield. Stirring a mixture of DL-**3**, baker's yeast, sucrose, and K_2CO_3 in ethanol and water at room temperature for 90 h afforded D-**4** in 62% yield (97% ee) along with L-**3** in 14% yield (14% ee) (method B). However, the yield of D-**4** in this reaction varied markedly with small changes in the activity of the yeast, temperature, or K_2CO_3 concentration. We are further investigating the reaction conditions that produce a steady yield. Whereas yield of D-**4** is lower in method A than it is in Method B, repeating the reaction in method A using DL-**3** which was easily obtained by treatment of L-**3** with the base, certainly gave D-**4** in high yield.

Isofebrifugine (D-**2**) was prepared by improving our

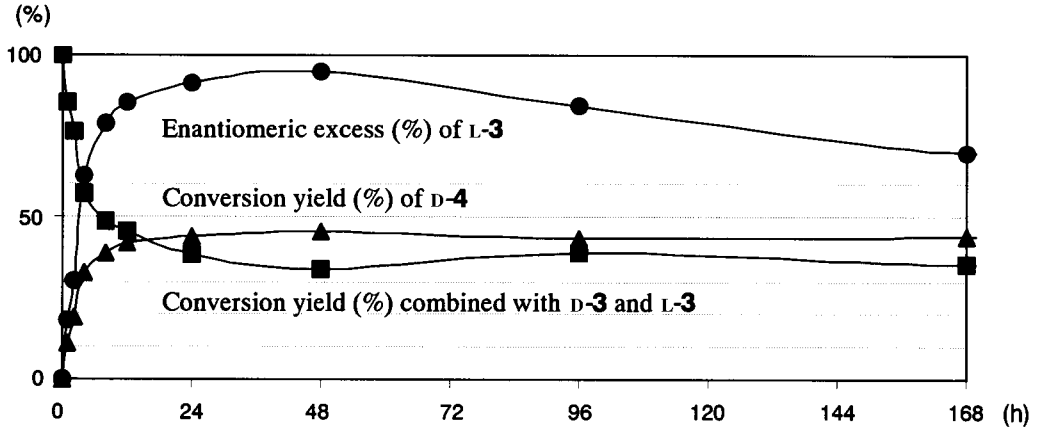
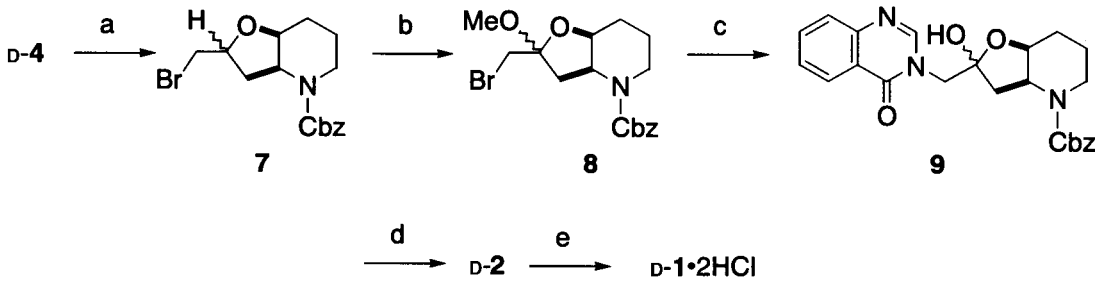


Figure 2. Yeast reduction of DL-**3**. A stirring mixture of DL-**3** (0.4 mmol), yeast (1.0 g), and sucrose (1.0 g) in EtOH (1 ml) and water (20 ml) at 25°C was measured by HPLC with a chiral column.



Scheme 2. *Reagents and conditions*: (a) NBS, MeCN, rt, 0.5 h, 87%; (b) (i) ^{t}BuOK, THF, 0°C, 0.25 h; (ii) NBS, MeOH, rt, 1 h, 90%; (c) (i) H^+, MeCN, rt, 1 h; (ii) 4(3*H*)-quinazolinone, K_2CO_3, DMF, rt, 1 h, 75%; (d) H_2, 20%-Pd(OH)$_2$/C, MeOH, rt, 4 h, 62%; (e) (i) H_2O, 80°C, 15 min; (ii) H^+, 73%.

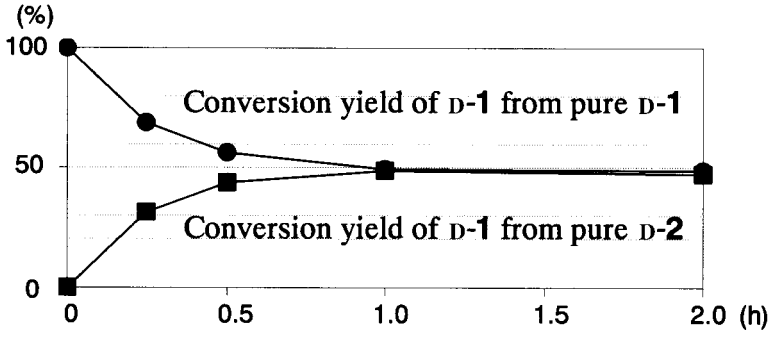


Figure 3. Isomerization of D-**1** and D-**2**. A mixture of D-**1** or D-**2** in EtOH at 80°C was measured by HPLC.

previous method (Scheme 2). The intramolecular bromoetherification of D-**4** using NBS afforded hexahydrofuro[3,2-*b*]pyridine (**7**) in 87% yield. The HPLC data indicated that this was a 3:1 mixture of the diastereomeric isomers. To improve the yield and reproducibility of preparing *Z*-protected isofebrifugine (**9**) from **7**, we designed a 2-methoxy derivative (**8**). The new intermediate (**8**) could be prepared by a 2-step reaction in high yield (90%) as a 4:1 mixture of the diastereomeric isomers. The steps consisted of dehydrobromination using potassium *tert*-butoxide and bromoetherification using NBS and methanol. Deacetalization of **8** followed by a coupling reaction with 4(3*H*)-quinazolinone afforded **9** in 75% yield. The hydrogenolysis of **9** gave isofebrifugine (D-**2**) in 62% yield as a crystalline solid. The melting point,[1a] ^{1}H NMR data,[4a] and optical rotation[1a] of D-**2** agreed with reported values for the natural product.

In our previous method of synthesizing DL-febrifugine (DL-**1**), the large differences in the melting point and solubility of DL-**1** and DL-**2** facilitated the isolation of DL-**1**.[5] Isomerization was naturally observed in the reaction of D-**1** at 80°C in EtOH, and the reaction reached equilibrium within 1 h to give a 1:1 mixture of D-**1** and D-**2** (Fig. 3). However, we could not isolate pure D-**1** in a satisfactory yield. Although isomerization of the congeners of D-**2** is known,[11,12] there are no reports on D-**2** itself. We examined the transformation of D-**2** to D-**1** in various solvents, including toluene, AcOEt, EtOH, DMSO, water, and 10% HCl aq. An equilibrium mixture of D-**1** and D-**2** was observed in HPLC by heating D-**2** at 80°C in most solvents. The time to reach equilibrium and the ratio of D-**1** and D-**2** changed with the solvent. Of the solvents tested, heating in water produced the largest ratio (2:1) of D-**1** to D-**2**. However, even after 8 h, no isomerization of D-**2** was observed in 10% HCl aq[4b] (Table 1). Based on these results, we isolated pure D-**1** as the hydrochloride salt, and its physicochemical properties and spectral data were identical to those reported for the natural product.[1c] Herein, we prepared D-**1** asymmetrically from DL-**3** with a clearly determined absolute configuration. Our results strongly support the corrected structures of D-**1** and D-**2**.

Although both D-**1** and D-**2** exhibited potent antimalarial activities in vitro as high as that of drugs in clinical use, the hydrochloride of D-**1** was half as potent as the free base (D-**1**) and the activity of D-**2** was 1/10 that of D-**1** (Table 2). While screening the antimalarial activity of febrifugine derivatives, we have been troubled by the decrease in activity with time. We are certain that this results from

Table 1. Isomerization of D-**1** and D-**2** in heating solvent at 80°C

Solvent	Time until reaching equilibrium (h)	Product ratio (D-**1**:D-**2**)[a]
Toluene	4	1:5
AcOEt	4	1:1
EtOH	1	1:1
DMSO	4	2:1
H_2O	0.25	2:1
10% HCl aq.	–[b]	D-**2** only

[a] Determined by HPLC.
[b] Even after 8 h, no isomerization was observed.

Table 2. Antimalarial activity and toxic selectivity of D-**1**

Compound	*FM*3A EC_{50}, μM	*P. falciparum* EC_{50}, *n*M	Toxic selctivity
Febrifugine (D-**1**)	0.18	0.91	198
Febrifugine·2HCl (D-**1**·2HCl)	0.14	1.8	78
Isofebrifugine (D-**2**)	0.74	9.0	82
Quinine	100	110	909
Chloroquine	32	18	1778
Pyrimethamine	0.12	1.0	120
Artemisinin	10	7.9	1266

isomerization. We propose that the hydrochloric salt be administered to screen the antimalarial activity of febrifugine derivatives.

3. Experimental

3.1. General

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. Mass spectra (MS) were recorded on a VG-70SE spectrometer. ^1H and ^{13}C NMR spectra were run on a Hitachi R-1500, a JASCO MY 60FT or a Varian VXR-500 spectrometer. Optical rotations were measured on a JASCO DIP-1000 spectrometer. Analytical HPLC was performed with a Shimadzu SPD-6A instrument on a chiral phase column, Chiralcel OJ (Daicel) or a silica gel column, Chemcosorb 5Si-U (Chemco). Merck silica gel 60 (230–400 mesh) and Wako activated alumina (300 mesh) were employed for column chromatography. Extracts were dried over anhydrous $MgSO_4$. The yeast was used a dried yeast (super camellia, Nisshin flour milling).

3.1.1. Yeast reduction (method A). A mixture of benzyl 2-allyl-3-oxopiperidinecarboxylate (DL-**3**,[5] 1.64 g, 6.00 mmol), yeast (15 g), and sucrose (15 g) in EtOH (15 ml) and water (300 ml) was stirred at 25°C for 24 h. AcOEt (800 ml) was added to the mixture and stirred at room temperature for 10 min. The AcOEt layer separated by centrifugation (×1000*g*, 5 min) was dried, filtered, and concentrated. MeCN (50 ml) was added to the residue and the precipitates were filtered through a membrane filter (0.5 μm). The filtrate was concentrated and the residue was subjected to column chromatography (silica gel). The first eluant (AcOEt/hexane=1:7) gave (*R*)-benzyl 2-allyl-3-oxopiperidinecarboxylate (L-**3**, 0.51 g, 31%, 93% ee based on HPLC) as a colorless oil, $[\alpha]^{28}_D=-40.2°$ (*c* 1.00, EtOH). HPLC: column, Chiralcel OJ; column temperature, room temperature; eluent, hexane/isopropyl alcohol=37:3; flow rate=1.5 ml/min; wavelength, 254 nm; t_R=9.1 and 11.9 min. The ^1H NMR spectrum agreed with DL-**3**. ^1H NMR (60 MHz, $CDCl_3$, rotomers) δ 1.71–2.17 (2H, m), 2.37–2.60 (4H, m), 2.98–3.44 (1H, m), 3.96–4.27 (1H, m), 4.53–5.80 (4H, m), 5.14 (2H, s), 7.35 (5H, s). HRMS (FAB): *m*/*z* calcd for $C_{16}H_{20}NO_3$ ($M+1^+$) 274.1443, found 274.1423. The second eluant (AcOEt/hexane=1:3) gave (2*S*,3*S*)-benzyl 2-allyl-3-hydroxypiperidinecarboxylate (D-**4**, 0.68 g, 41%, 97% ee based on HPLC with a chiral column) as a viscous colorless oil, $[\alpha]^{20}_D=+78.5°$ (*c* 1.00, EtOH). HPLC: column, Chiralcel OJ; column temperature, room temperature; eluent, hexane/isopropyl alcohol=37:3; flow rate=1.5 ml/min; wavelength, 254 nm; t_R=6.6 and 8.2 min. The ^1H NMR spectrum agreed with that reported in the literature.[5] ^1H NMR (60 MHz, $CDCl_3$, rotomers) δ 1.40–1.90 (4H, m), 2.20–2.80 (3H, m), 2.41 (1H, s), 3.64–4.09 (2H, m), 4.34–4.68 (1H, m), 4.87–5.81 (3H, m), 5.10 (2H, s), 7.33 (5H, s). HRMS (FAB): *m*/*z* calcd for $C_{16}H_{22}NO_3$ ($M+1^+$) 276.1599, found 276.1571.

3.1.2. Yeast reduction (method B). A mixture of DL-**3** (1.00 g, 3.66 mmol), K_2CO_3 (3.00 g), yeast (10 g), and sucrose (30 g) in EtOH (30 ml) and water (300 ml) was stirred at 15°C for 90 h. The reaction mixture was treated in the same way as described above. The eluant (AcOEt/hexane=1:7) gave L-**3** (0.14 g, 14%, 14% ee based on HPLC with a chiral column) as a colorless oil. The second eluant (AcOEt/ hexane=1:3) gave D-**4** (0.62 g, 62%, 97% ee based on HPLC with a chiral column) as a viscous colorless oil.

3.1.3. (2*R*,3*R*)-Benzyl 2-allyl-3-hydroxypiperidinecarboxylate (L-4). To solution of L-**3** (0.42 g, 1.54 mmol, 93% ee) in MeOH (4 ml), $NaBH_4$ (0.02 g, 0.53 mmol) was portionwised at 0°C with stirring. The mixture was stirred at the same temperature for 1 h, made acidic with aqueous 10% HCl solution (10 ml), extracted with AcOEt (2×20 ml). The AcOEt layer was washed with saturated aqueous $KHCO_3$ solution (20 ml) and brine (30 ml), dried, and concentrated. The residue was subjected to column chromatography (AcOEt/hexane=1:3) to give L-**4** (0.31 g, 73%, 91% ee based on HPLC) as viscous oil, $[\alpha]^{27}_D=-72.8°$ (*c* 1.00, EtOH). The ^1H NMR spectrum of this compound agreed with D-**4**.

3.1.4. (*R*)-Benzyl 2-allyl-3-[(4-methylphenylsulfonyl)-hydrazono]piperidinecarboxylate (L-5). A mixture of L-**3** (0.98 g, 3.59 mmol, 90% ee) and $TsNHNH_2$ (0.80 g, 4.30 mmol) in EtOH (12 ml) was stirred at the room temperature for 24 h. The resulting precipitates were filtered off, washed with EtOH, recrystallized from a mixture of AcOEt and hexane to give L-**5** (1.01 g, 64%), mp 179–181°C. $[\alpha]^{29}_D=-14.6°$ (*c* 1.01, $CHCl_3$). IR (KBr): 3220, 1700 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, rotomers) δ 1.64–1.67 (1H, m), 1.83 (1H, brs), 2.08–2.55 (4H, m), 2.42 (3H, s), 3.10–3.20 (1H, m), 3.80–3.95 (1H, m), 4.70–4.86 (2H, m), 4.99–5.23 (3H, m), 5.35–5.47 (1H, m), 7.29–7.35 (7H, m), 7.82 (2H, d, *J*=6.5 Hz), 7.80–8.00 (1H, m). Anal Calcd for $C_{23}H_{27}N_3O_4S$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.45; H, 6.24; N, 9.38.

3.1.5. (*S*)-Benzyl 2-allylpiperidinecarboxylate (L-6). To a suspension of L-**5** (0.31 g, 0.70 mmol) in MeOH (4 ml)

added to the solution of $NaBH_3CN$ (0.09 g, 1.43 mmol) and $ZnCl_2$ (0.10 g, 0.73 mmol) in MeOH (3 ml). The mixture was heated at reflux for 3 h under Ar, poured into aqueous 0.1N NaOH solution (50 ml), and extracted with AcOEt (2×50 ml). The AcOEt layer was washed with brine (50 ml), dried, and concentrated. The residue was subjected to column chromatography (AcOET/hexane=1:7) to give L-**6** (0.12 g, 66%, 97% ee based on HPLC) as colorless oil, $[\alpha]^{27}_D=-51.5°$ (*c* 0.82, $CHCl_3$) {lit[6] $[\alpha]^{25}_D=-43.0°$ (*c* 4.1, $CHCl_3$)}. The ^1H NMR spectrum agreed with that reported in the literature.[6] ^1H NMR (500 MHz, $CDCl_3$, rotomers) δ 1.40–1.42 (1H, m), 1.53–1.64 (5H, m), 2.22–2.28 (1H, m), 2.39–2.45 (1H, m), 2.85 (1H, t, *J*=13.3 Hz), 4.05 (1H, br d, *J*=12.5 Hz), 4.37 (1H, br s), 5.01 (2H, dd, *J*=17.0, 10.0 Hz), 5.12 (2H, Abq, *J*=12.5, 10.5 Hz), 5.70 (1H, br s), 7.29–7.35 (5H, m). HRMS (FAB): *m/z* calcd for $C_{16}H_{22}NO_2$ ($M+1^+$) 260.1651, found 260.1648.

3.1.6. (3a*S*,7a*S*)-Benzyl 2-(bromomethyl)hexahydrofuro-[3,2-b]pyridine-4(2*H*)-carboxylate (D-7). To a solution of D-**4** (1.64 g, 5.96 mmol, 97% ee) in MeCN (20 ml) was added NBS (1.17 g, 6.57 mmol). The mixture was stirred at room temperature for 0.5 h. The mixture was poured into 10% aqueous $Na_2S_2O_3$ solution (100 ml) and extracted with AcOEt (2×100 ml). The combined AcOEt layers were washed with saturated aqueous $KHCO_3$ solution (100 ml) and brine (100 ml), dried, filtered and concentrated. The residue was subjected to column chromatography (SiO_2; hexane/AcOEt=3:1) to give D-**7** (1.83 g, 87%, 54% de, 96% ee from HPLC) as light yellow oil, $[\alpha]^{27}_D=+56.3°$ (*c* 1.00, EtOH). HPLC: column, Chiralcel OJ; column temperature, room temperature; eluent, hexane/isopropyl alcohol=37:3; flow rate=1.5 ml/min; wavelength, 254 nm; t_R=9.0, 9.9, 13.5 min. The IR and ^1H NMR spectrum agreed with DL-**7**.[5b] ^1H NMR (60 MHz, $CDCl_3$, rotomers) δ 1.25–2.60 (6H, m), 2.70–3.20 (1H, m), 3.30–3.55 (2H, m), 3.80–5.00 (4H, m), 5.15 (2H, s), 7.36 (5H, s). HRMS (FAB): *m/z* calcd for $C_{16}H_{21}BrNO_3$ ($M+1^+$) 354.0705, found 354.0662.

3.1.7. (3a*S*,7a*S*)-Benzyl 2-bromomethyl-2-methoxyhexahydrofuro[3,2-b]pyridine-4(2*H*)-carboxylate (D-8). To a solution of D-**7** (2.23 g, 6.30 mmol) in dry THF (10 ml) was added $KOBu^t$ (1.41 g, 12.6 mmol). The mixture was stirred at room temperature for 15 min. To the mixture was added a solution of NBS (1.34 g, 7.53 mmol) in absolute MeOH (20 ml). The mixture was stirred at room temperature for 1 h. The mixture was poured into 10% aqueous $Na_2S_2O_3$ solution (100 ml) and extracted with AcOEt (2×100 ml). The combined AcOEt layers were washed with saturated aqueous $KHCO_3$ solution (100 ml) and brine (100 ml), dried, filtered and concentrated. The residue was subjected to column chromatography (SiO_2; AcOEt/hexane=1:3) to give D-**8** (2.17 g, 90%, 60% de from HPLC) as colorless oil, $[\alpha]^{24}_D=+13.8°$ (*c* 1.00, EtOH). HPLC: column, Chiralcel OJ; column temperature, room temperature; eluent, hexane/isopropyl alcohol=37:3; flow rate=1.5 ml/min; wavelength, 254 nm; t_R=5.8 and 7.8 min. ^1H NMR (60 MHz, $CDCl_3$, rotomers): δ=1.37–2.45 (7H, m), 3.25 and 3.30 (total 3H, each s), 3.43–3.54 (2H, m), 3.74–5.00 (3H, m), 5.15 (2H, s), 7.35 (5H, s). MS (FAB, positive ion mode) *m/z* 352 (M^+−MeOH, 88), 354 (M^+−MeOH+2, 100), 384 (M^+, 50), 386 (M^++2, 28). HRMS (FAB): *m/z* calcd for $C_{16}H_{19}BrNO_3$ (M^+−MeOH) 352.0548, found 352.0531.

3.1.8. (3a*S*,7a*S*)-Benzyl 2-hydroxy-2-[4-oxo-3(4*H*)-quinazolinyl]-methyl]hexahydrofuro-[3,2-b]pyridine-4(2*H*)-carboxylate (D-9). To a solution of D-**8** (1.86 g, 4.84 mmol) in MeCN (10 ml) added aqueous 10% HCl solution (10 ml) and the mixture was stirred at room temperature for 1 h. The mixture was poured into water (50 ml) and extracted with AcOEt (2×100 ml). The combined organic layers were washed with saturated $KHCO_3$ solution (100 ml) and brine (100 ml), dried, filtered and concentrated. A mixture of the residue, 4(3*H*)-quinazolinone (0.71 g, 4.86 mmol), anhydrous K_2CO_3 (0.80 g, 5.79 mmol) in dry DMF (10 ml) was stirred at room temperature for 1 h. The mixture was poured into brine (100 ml) and extracted with AcOEt (2×100 ml). The combined organic layers were washed with brine (2×100 ml), dried, filtered and concentrated. The residue was subjected to column chromatography (Al_2O_3; AcOEt/isopropyl alcohol=4:1) to give D-**9** (1.58 g, 75%) as amorphous solid. $[\alpha]^{26}_D=+40.6°$ (*c* 1.01, EtOH). The IR and ^1H NMR spectrum agreed with DL-**9**.[5] ^1H NMR (60 MHz, $CDCl_3$, rotomers) δ 1.37–3.12 (7H, m), 3.66–4.50 (5H, m), 5.09 (2H, s), 7.32 (5H, s), 7.46–7.90 (3H, m), 8.13–8.34 (2H, m). HRMS (FAB): *m/z* calcd for $C_{24}H_{26}N_3O_5$ 436.1872, found 436.1893.

3.1.9. D-Isofebrifugine (D-2). A mixture of D-**9** (1.70 g, 3.90 mmol), 20% $Pd(OH)_2$/C (0.09 g) in absolute MeOH (20 ml) was stirred at room temperature for 4 h under H_2 gas. After filtration the solvent was removed. The residue was crystallized from MeOH and recrystallization from toluene to give D-**2** (0.73 g, 62%) as colorless needles, mp 129–130°C (lit[1a] mp 129–130°C) $[\alpha]^{27}_D=+124.3°$ (*c* 0.50, $CHCl_3$) {lit[1a] $[\alpha]^{25}_D=+131°$ (*c* 0.35, $CHCl_3$)}. Anal. Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.48; H, 6.43; N, 13.65. The IR and ^1H NMR spectrum agreed with the natural compound[4a,11] and DL-**2**.[5]

3.1.10. D-Febrifugine dihydrochloride (D-1·2HCl). A solution of D-**2** (0.34 g, 1.13 mmol) in H_2O (10 ml) was heated at 80°C. To the mixture added aqueous 10% HCl solution (2 ml) and the solvent was removed. The azeotropic treatment and the recrystallization of the residue from EtOH gave the dihydrochloride of D-**1** (0.31 g, 73%) as colorless powder, mp 218–219°C (dec) (lit[1c] mp 223–225°C (dec)). $[\alpha]^{29}_D=+13.3°$ (*c* 1.01, H_2O) {lit[1c] $[\alpha]^{31}_D=+12.8°$ (*c* 0.85, H_2O)}. Anal. Calcd for $C_{16}H_{19}N_3O_3\cdot 2HCl$: C, 51.35; H, 5.66; N, 11.23. Found: C, 51.07; H, 5.77; N, 11.12. Free base: mp 140–141°C (from AcOEt) (lit[1a] mp 139–140°C). $[\alpha]^{26}_D=+15.7°$ (*c* 0.30, MeOH) {lit[4a] $[\alpha]^{27}_D=+13.0°$ (*c* 0.65, MeOH)}. Anal. Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.57; H, 6.64; N, 13.91. The IR and ^1H NMR spectrum of free base agreed with the natural compound[4a,11] and DL-**1**.[5]

3.2. Antimalarial activity

Assays and evaluation of antimalarial activities were carried out according to the methods described previously.[13]

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