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Chemoenzymatic synthesis of the enantiomerically pure 1,2,3,4-tetrahydroquinoline moiety of the antithrombotic (21*R*)- and (21*S*)-argatroban

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ABSTRACT

The synthetic antithrombotic argatroban is a dipeptide between the nonproteogenic (2*R*,4*R*)-4-methyl-2-piperidine carboxylic acid and L-arginine, in turn bonded to a 3-methyltetrahydroquinoline sulfonyl group; the drug is usually prepared and administered as a mixture of C-21-diastereoisomers. By means of a biocatalytic transformation enantiomerically pure (*R*)- and (*S*)-synthons, suitable for the synthesis of separate (21*R*)- and (21*S*)- argatroban, were obtained.

1. Introduction

Thrombin is a serine protease that plays a key role in the blood coagulation and fibrinolysis that induces platelet aggregation and secretion.[1–3] The central role of thrombin in the coagulation cascade has made it a target for antithrombotic agents in the management of cardiovascular diseases.[4] Heparin, a sulfated glycosaminoglycan, has historically been used as the anticoagulant of choice in the management of thrombotic diseases: however limitations due to its chemical heterogeneity, a high variability in dose efficacy, and several adverse events, such as the heparin induced thrombocytopenia (HIT) prompted the development of low molecular weight selective and orally bioavailable direct inhibitors of thrombin.[4]

Argatroban **1** is a synthetic, peptidomimetic, small molecule that binds selectively and reversibly to the catalytic site of thrombin, serving as a competitive inhibitor.[5] It was identified by Okamoto et al.[6–8] and used in Japan (Novastan®, MD-805) since the early 1980s; it was later approved in Europe and in the US for patients with HIT.[9] Three moieties are easily recognized as constituents of **1**, the 4-methyl-2-piperidine carboxylic acid bonded to arginine, in turn bearing a 3-methyl-1,2,3,4-tetrahydroquinoline sulfonyl group. Four stereogenic centers are present in the structure. The (2*R*)-stereocenter of the piperidine moiety is usually introduced over the course of a Diels–Alder reaction, with the stereochemical outcome determined by a chiral auxiliary;[10,11] the (4*R*)-stereocenter is subsequently obtained after hydrogenation of the 4,5-double bond.[10,11] Recently we have identified a complex between a rhodium compound and the Mandiphos ligand that catalyzes the diastereoselective [98:2, (4*R*)/(4*S*)] hydrogenation of the double bond.[12] The use of L-arginine as a starting material leads to the introduction of a third stereocenter with the desired configuration.[13] An additional stereocenter is formed during the last step of the synthesis, that is, the reduction of the heteroaromatic ring of a quinoline that affords the (21*R*)- and (21*S*)-diastereoisomers.[13] The diastereoisomeric mixture is applied as the antithrombotic drug without separation of the (21*R*)- and (21*S*)-epimers, **1a** and **1b**, respectively, provided that the diastereomeric ratio (dr) is 64:36 ± 2. The (21*R*)- and (21*S*)-configurations have been assigned in 1993 by an X-ray crystallographic study after HPLC separation.[14] Compound (21*S*)-**1b** is twice as potent as (21*R*)-**1a** and about five times less soluble in water.[14] Recently, in order to fully characterize the two C-21-epimers of argatroban **1** through crystallography, NMR, and computational techniques, we accomplished their separation by means of fractional crystallization. While the (21*R*)-diastereoisomer **1a** (96.8:3.2 dr, 19% yield) was easily obtained by two crystallizations, the preparation of (21*S*)-**1b** (0.5:99.5 dr, 4.8% yield) required ten crystallizations.[15] These results, in addition to the current regulatory guidelines that for all active pharmaceutical ingredients (API), presenting one or more stereocenters, require that all of the possible isomers undergo a full study from a chemical–physical and pharmacological point of view, prompted us to investigate the synthesis of the (*R*)- and (*S*)-isomers of 3-methyl-1,2,3,4-tetrahydroquinoline **2**, that is, the suitable synthons for the preparation of (21*R*)- and (21*S*)-**1**, giving priority to an enzymatic approach.

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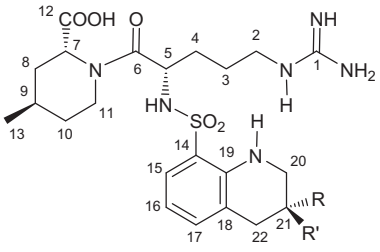
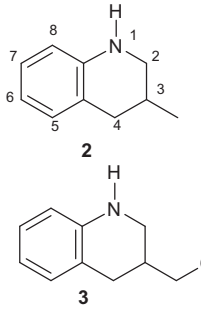


a R = CH_3; R' = H (21*R*)-argatroban
b R = H; R' = CH_3 (21*S*)-argatroban



2. Results and discussion

Two syntheses of optically active 3-methyl-1,2,3,4-tetrahydroquinoline **2**, both aimed to the preparation of argatroban **1** or its analogues have been reported in the literature: in one case the starting material is Oppolzer's sultam that, after acylation, in five steps leads to the enantiomerically pure (3*R*)-methyl-6-bromo-1,2,3,4-tetrahydroquinoline.[16] The synthesis of (3*R*)- and (3*S*)-methyl-1,2,3,4-tetrahydroquinoline is described in a Synthelabo patent: the starting material, in this case, is the enantiomerically pure methyl (*S*)- and (*R*)-3-iodo-2-methyl-propanoate, respectively.[17] The use of a chiral hydrogenation catalyst is a well known method[18,19] for the preparation of enantiomerically pure tetrahydroquinolines starting from variously substituted quinolines; nevertheless a chiral rhodium complex, giving optimal results (98% ee and quantitative yield) in the case of 2-substituted quinolines, failed when applied to 3-methylquinoline.[20]

We planned to explore an enzymatic approach to the synthesis of both enantiomers of compound **2** by the resolution of a suitable racemic substrate. It is known[21] that despite the many enzymatic methods available for the preparation of non-racemic chiral primary amines, only a few examples have been reported for the resolution of secondary amines, maybe due to steric hindrance. For this reason our attention was focused on the identification of a compound bearing a functional group convertible by a hydrolytic enzyme and an immediate precursor of the 3-methyl group. The 3-hydroxymethyl group of compound **3** was evaluated as being the most suitable for our purposes. Starting from methyl ester **4** of commercially available 3-quinoline carboxylic acid we prepared 3-carboxymethyl-1,2,3,4-tetrahydroquinoline **5** (78% from 3-carboxylic acid) by selective reduction with sodium cyanoborohydride, as described by Gotor et al.[22] The $LiAlH_4$ reduction of **5** afforded the 3-hydroxymethyl derivative **3** (89%) (Scheme 1).

After protection of the amino group (80% yield), the obtained *tert*-butyl carbamate **6** was used for screening with lipases, selected for their well known stability in organic solvents, and capability of selectively transforming a wide spectrum of substrates, including many 2-substituted primary alcohols.[23] Among the lipases tested under irreversible transesterification conditions,[24,25] the highest but not satisfactory enantiomeric excess (ee) was obtained by *Pseudomonas fluorescens* lipase (PFL); in fact at 32% conversion 76% ee was determined by HPLC analysis on a chiral stationary phase[†] of the obtained (+)-acetate **7** (Scheme 2).

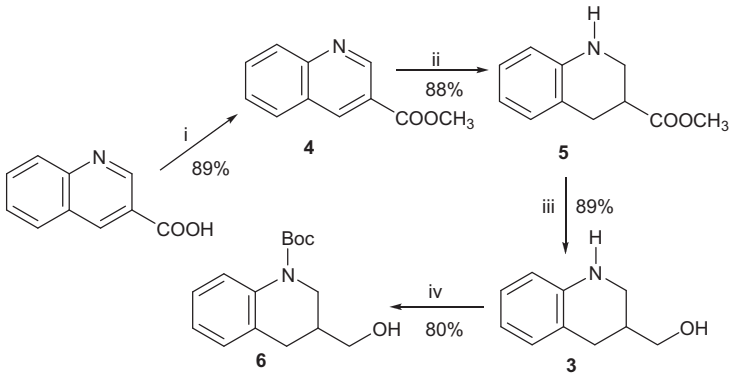
Other lipases, even at a very low extent of conversion, showed lower ee values (Table 1). Also different solvents from the initially employed toluene did not enhance the PFL stereoselectivity (Table 2).

Table 1
Lipase-catalyzed irreversible transesterification of alcohol **6** in toluene

Entry	Enzyme	Time (h)	Conv.[a] (%)	ee[b] (%) (config)	*E*
a	*Candida cylindracea* lipase (CCL)	2.5	39	33 (*S*)[c]	2.4
b	*Candida antarctica* lipase A (CAL A)	36	23	30 (*S*)[c]	2
c	*Candida antarctica* lipase B (CAL B)	2.5	5	30 (*R*)[c]	1.9
d	CAL B CLEA[e]	1	68	45 (*S*)[d]	2.3
e	PFL	24	32	76 (*S*)[c]	10.4

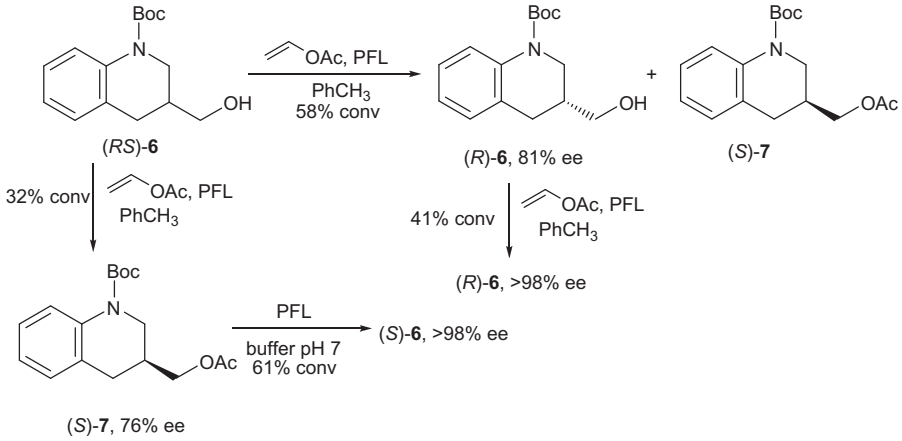
[a] From GLC analysis.
[b] From HPLC on a chiral stationary phase.
[c] Product ee and configuration.
[d] Substrate ee and configuration.
[e] Cross linked enzyme aggregate.

The enantiomeric ratio (*E*)[26,27] of the PFL-catalyzed transesterification was moderate (about 10) but adequate, according to the observations of Sih et al.,[26] to achieve a satisfactory final ee after a second enzymatic resolution of the enantiomerically enriched compound. Taking into consideration the results of Davies[28] and Kanerva[29] we chose to submit enantiomerically enriched (+)-ace-



Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH; (ii) $NaBH_3CN$, THF, and MeOH; (iii) $LiAlH_4$, THF; (iv) $(Boc)_2O$, dioxane, and NaOH, water.

[†] The best separation of (*R*)- and (*S*)-**7** was obtained with a Phenomenex Lux 3μ Cellulose-1; (*R*)- and (*S*)-alcohols **6** were not resolved. The Chiralpak IA (Daicell) afforded only a partial separation of the acetates and did not resolve alcohols.



Scheme 2. Double enzymatic resolutions of alcohol **6**.

Table 2
PFL-catalyzed irreversible transesterification of alcohol **6** in different solvents

Entry	Solvent	Time (h)	Conv.[a] (%)	ee[b] (%) (config)	*E*
a	Chloroform	36	/	/	/
b	Heptane	6	33	61 (*S*) [c]	5.5
c	Tetrahydrofuran	24	16	67 (*S*) [c]	5.7
d	Hexane	158	64	82 (*R*) [d]	6.5
e	Cyclohexane	6	26	68 (*S*) [c]	6.6
f	Vinyl acetate	66	58	81 (*R*) [d]	9.2

[a] From GLC analysis.
[b] From HPLC on a chiral stationary phase.
[c] Product ee and configuration.
[d] Substrate ee and configuration.

tate **7** to PFL-catalyzed hydrolysis. The reaction was monitored by GLC and stopped at 61% conversion. Alcohol **6** showed >98% ee (by chiral HPLC). In order to establish the stereochemical outcome of the double resolution, alcohol **6**, reported in the literature only as a racemic mixture,[30] was transformed into a known chiral synthon **2** by esterification to tosylate **8**, lithium aluminum hydride reduction, and removal of the protecting group (59% yield from **6**) (in Scheme 3 the synthetic sequence is described for the (*R*)-enantiomer). By comparison of the specific rotation of **2** with the reported one[17] the (*S*)-configuration was assigned to the 3-methyl-1,2,3,4-tetrahydroquinoline obtained starting from (+)-acetate **7**. The enantiomeric purity of **2**, determined by chiral HPLC, showed that the ee of alcohol **6** remained unaltered over the course of the synthesis.

In order to accomplish our goal we also needed (*R*)-3-methyl-1,2,3,4-tetrahydroquinoline **2**, corresponding to (*R*)-**6**, that is, the slower reacting enantiomer in the course of the PFL-catalyzed transformation. The PFL-catalyzed transesterification was stopped, in this case, at 58% conversion and the ee of obtained (+)-**6** was determined, after acetylation, by HPLC on a chiral column. The expected moderate ee (81%) suggested submitting enriched (*R*)-**6** to a second PFL-catalyzed transesterification, a 'continuation'[29] of the first resolution. The desired >98% ee of (*R*)-**6** was achieved by stopping the second reaction at 41% conversion (Scheme 2). Following the same synthetic pathway previously optimized for (*S*)-**2**, compound (*R*)-**2** (Scheme 3) was also available as a chiral building block for the synthesis of (*R*)- and (*S*)-1,2,3,4-tetrahydroquinoline-8-sulfonic acid chloride according to the reported method.[17]

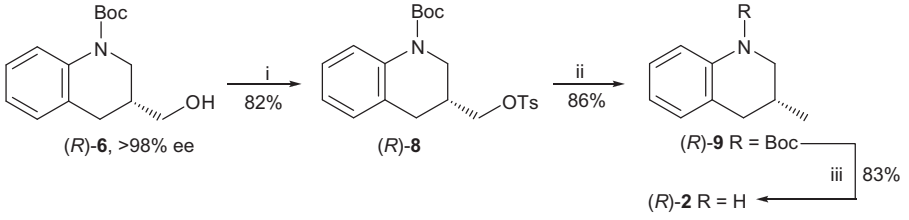
Enantiomerically pure (*R*)-1,2,3,4-tetrahydroquinoline-8-sulfonic acid chloride is the starting material for several analogues modified[16] in the arginine or in the piperidyl moiety, prepared in attempts to develop drugs with increased efficacy and bioavailability.

3. Conclusion

Four stereocenters are present in argatroban **1**, three of which must present a well defined configuration; the fourth is introduced over the course of the hydrogenation of a quinoline ring that leads to a mixture of diastereoisomers.

Compound **1** is endowed with antithrombotic activity and administered in the treatment of cardiovascular diseases as a mixture of (21*R*)-**1a** and (21*S*)-**1b** diastereoisomers (in a ratio 64:36 ± 2), the (*S*)-isomer being twice as potent as the (*R*)-isomer and five times less soluble in aqueous solution. A double PFL-catalyzed resolution of a 2-substituted primary alcohol, easily prepared from commercially available quinoline 3-carboxylic acid, allowed suitable chiral synthons to be obtained for both (21*R*)-**1a** and (21*S*)-**1b** argatroban, required, for example, for deeper pharmacological activity studies. The apparently low yields of synthons **2** [approximately 10% from (±)-**6** for either (*R*)- or (*S*)-enantiomer] obtained by the double enzymatic resolution, necessary in order to reach the desired >98% ee, represents 20% of the theoretical yield that can be achieved by working on a racemic substrate.

The availability of the enantiomerically pure 1,2,3,4-tetrahydroquinoline moiety allows the separate C-21-diastereoisomers to be



Scheme 3. Reagents and conditions: (i) TsCl, py; (ii) $LiAlH_4$, THF; (iii) TFA, CH_2Cl_2.

obtained in a more convenient way than the previous fractional crystallization[15] of a diastereomeric mixture of argatroban [19% and 4.8% yields for (21*R*)-**1a** and (21*S*)-**1b**, respectively]. Indeed over the course of the fractional crystallization of a diastereomeric mixture of **1**, a significant amount of argatroban, obtained from L-arginine and the precious (2*R*,4*R*)-piperidyl moiety, has to be discarded, while 10% yields of the enzymatic method are related to a less expensive intermediate.

The present synthesis is suitable for gram-scale preparations and furnishes the enantiomerically pure compounds that are useful not only for (21R)-**1a** and (21S)-**1b** argatroban synthesis, but also for other analogues[16,17] still under investigation. The developed analytical GLC and HPLC methods allow a careful control of the outcome of the enzymatic reactions (rate of conversion and ee).

Moreover (*R*)- and (*S*)-**6** could be useful in order to obtain the farnesyl protein transferase inhibitors reported in a 2002 patent;[30] indeed, the use of (*RS*)-**6** as the starting material for the preparation of a racemic imidazol-derivative of 1,2,3,4-tetrahydroquinoline, requires separation by preparative HPLC on a chiral stationary phase.

Since argatroban is used for designing antithrombotic surfaces in angioplasty devices,[31] the availability of the separated C-21-diastereoisomers could be very helpful in the enhancement of its water-solubility, required in order to improve its efficiency in coating hemocompatible polymers.

4. Experimental

4.1. General

All of the reagents and enzymes were purchased by Sigma–Aldrich. CAL B CLEA was purchased by CLEA Technologies (The Netherlands). All reactions were monitored by TLC on silica gel 60 F_{254} precoated plates with a fluorescent indicator (Merck) with detection by spraying with 10% phosphomolybdic acid ethanol solution and heating at 110 °C. Column chromatography was performed on silica gel 60 (70–230 mesh) (Merck) with a substrate/silica gel ratio 1:20. HPLC analyses were performed with a Merck–Hitachi L-6200; chiral column: Phenomenex Lux 3 Cellulose-1, 250 × 4.6 mm; UV detector wavelength 254 nm). GLC analyses were performed with a Hewlett–Packard 5890-series II. ^{1}H NMR spectra were recorded on a Bruker-Avance 500 MHz spectrometer. ^{13}C NMR spectra were collected at 125.76 MHz. The values of the optical rotations were registered on a Perkin–Elmer (mod. 343) polarimeter in a 1 dm cell at 20 °C, setting the wavelength at 589 nm. Mass spectra were recorded on a Agilent instrument (mod 6339 Ion trap LC/MS) using the ESI source with positive ion polarity; the samples were dissolved in methanol (0.02 μg μL^{-1}) and were examined utilizing the direct inlet probe technique at an infusion rate of about 0.6 mL min^{-1}; data acquisition and analysis were accomplished with BRUKER DALTONICS DATA ANALYSIS 3.3 software. The infrared spectra were registered on a Perkin Elmer instrument (mod. FT-IR spectrum one) equipped with universal attenuated total reflection (ATR) sampling.

4.2. (*RS*)-3-Hydroxymethyl-1-*tert*butyloxycarbonyl-1,2,3,4-tetrahydroquinoline 6

4.2.1. Methyl quinoline-3-carboxylate 4

Quinoline-3-carboxylic acid (8 g, 46.2 mmol) was dissolved in methanol (900 mL), after which thionyl chloride (5 mL, 68.5 mmol) was added at 0 °C. The solution was kept at reflux, under stirring (10 h), monitoring the reaction progress by TLC (dichloromethane/methanol 9:1). An additional amount of thionyl chloride (5 mL) was added and the solution was kept at reflux (20 h). After cooling at room temperature, the solvent was evaporated at reduced pressure. To the residue water (400 mL) and 1 M sodium hydroxide (until pH 8) were added; the mixture was extracted with dichloromethane (4 × 400 mL). The collected organic phases were dried over sodium sulfate; after filtration, the solvent was removed at reduced pressure, to afford title compound **4** (7.78 g, 89%), which was used directly in the next step without any further purification. The chemical and physical properties were in agreement with the reported ones.[22]

4.2.2. (*RS*)-Methyl 1,2,3,4-tetrahydroquinoline-3-carboxylate 5

To a solution of methyl ester **4** (7.68 g, 41 mmol) in dry tetrahydrofuran (150 mL) and methanol (70 mL) sodium cyanoborohydride (10.8 g, 172 mmol) was added under a nitrogen atmosphere. The pH was adjusted to 4, by the addition of 4 M hydrogen chloride in dioxane and kept at this value over the course of the reaction (10 h), by the addition of the same hydrogen chloride solution. The reaction progress was monitored by TLC (dichloromethane/acetone 9:1) until the starting material disappeared. The reaction mixture was cooled in an ice bath, after which water (200 mL) and a saturated sodium hydrogen carbonate aqueous solution (until neutral pH) were added. The organic solvents were removed at reduced pressure. The aqueous phase was extracted with ethyl acetate (3 × 200 mL). The collected organic phases were dried over sodium sulfate and after the usual work-up an oily residue (8.84 g) was obtained; the residue was purified by silica gel column chromatography by elution with hexane/ethyl acetate (9:1) to give pure **5** (6.88 g, 88%). The chemical and physical properties were in agreement with the reported ones.[22]

4.2.3. (*RS*)-3-(1′-Hydroxymethyl)-1,2,3,4-tetrahydroquinoline 3

To a suspension of lithium aluminum hydride (5.3 g, 140 mmol) in dry tetrahydrofuran (125 mL), cooled at 0-5 °C, ester **5** (6.68 g, 35 mmol) dissolved in tetrahydrofuran (125 mL) was added dropwise. The ice bath was then removed and the reaction mixture was kept at room temperature (4 h), with the reaction progress monitored by TLC (dichloromethane/acetone 9:1) until the starting material disappeared. To the reaction mixture, cooled at 0–5 °C, water (5.3 mL), 15% sodium hydroxide aqueous solution (5.3 mL), and water (16 mL) were sequentially added. The white precipitate was removed by suction through a Celite pad. The solvent was evaporated at reduced pressure and the recovered oily **3** (5.02 g, 89%) was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 2.23 (m, 1H, H-3); 2.56 (dd, 1H, J = 16.17 and 8.40 Hz, H-4); 2.88 (dd, 1H, J = 16.17 and 5.18 Hz, H-4); 2.62–2.82 (m, 2H, exchange with D_2O); 3.15 (dd, 1H, J = 8.09 and 11.14 Hz, H-2), 3.46 (ddd, 1H, J = 11.14, 3.51 and 1.52, H-2); 3.64 (dd, 1H, J = 11.14 and 7.62 Hz, H-1′); 3.72 (dd, 1H, 11.14 and 5.95 Hz, H-1′); 6.53 (d, 1H, J = 7.78 Hz, H-5); 6.67 (dd, 1H, J = 7.78 and 8.24 Hz, H-6); 6.97–7.05 (m, 2H, H-7 and H-8). ^{13}C NMR ($CDCl_3$) δ 29.56 (C-4); 34.91 (C-3), 44.03 (C-2); 65.23 (C-1′); 114.16 (C-5); 117.36 (C-6); 120.21 (C-10); 126.84 (C-8); 129.82 (C-7); 144.48 (C-9). IR ν_{max} 3380.86, 3238.16, 2918.32, 2864.52, 2837.38, 1602.37, 1582.56, 1494.88, 1471.35, 1368.95, 1323.05, 1293.83, 1266.81, 1071.36, 1022.97 cm^{-1}; MS (ESI+) m/z 164.1 $[M+1]^+$, 186.0 $[M+Na]^+$.

4.2.4. (*RS*)-3-(1′-Hydroxymethyl)-1-*tert*-butyloxycarbonyl- 1,2,3,4-tetrahydroquinoline 6

To a solution of **3** (4.90 g, 30 mmol) in dioxane (230 mL) and water (290 mL), sodium hydroxide (14 g, 0.35 mol) and di-*tert*butyl carbonate (72 mL, 313 mmol) were added sequentially. The reaction mixture was kept, under stirring, at room temperature (24 h). The reaction progress was monitored by TLC (dichloromethane/methanol 9:1). Dioxane was removed under reduced pressure and the remaining aqueous phase was extracted with dichloromethane (4 × 70 mL). The collected organic phases were washed

with water (2 × 100 mL) until pH 7. After the usual work-up, a yellow oil was obtained, which was purified by silica gel column chromatography. The desired Boc derivative **6** (6.3 g, 80%) was recovered by elution with hexane/ethyl acetate 8:2. ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 9H, CH_3); 1.89 (br s, 1H, exchange with D_2O); 2.31 (m, 1H, H-3); 2.53 (dd, 1H, J = 16.9 and 5.6 Hz, H-4); 2.99 (dd, 1H, J = 16.9 and 6.99 Hz, H-4); 3.50 (dd, 1H, J = 11.7 and 8.9 Hz, H-2); 3.57–3.65 (m, 2H, H-2 and H-1′); 3.92 (dd, 1H, J = 13.5 and 5.9 Hz, H-1′); 7.04 (dd, 1H, J = 7.87 and 7.35 Hz, H-6); 7.11 (d, 1H, J = 7.35 Hz, H-5); 7.16 (dd, 1H, J = 8.22 and 7.87 Hz, H-7); 7.59 (d, 1H, J = 8.22 Hz, H-8). ^{13}C NMR δ 28.35 ((CH_3)$_3$C); 29.50 (C-4); 36.15 (C-3); 45.19 (C-1′); 63.67 (C-2); 81.42 ((CH_3)$_3$*C*); 123.91 (C-6); 124.57 (C-8); 125.58 (C-7); 128.28 (C-10); 129.13 (C-5); 138.68 (C-9); 154.63 (N-COO). IR ν_{max} 3430.76, 2975.98, 2929.84, 1690.36, 1673.95, 1492.20, 1367.09, 1159.36 cm^{-1}; MS (ESI+) m/z 286.1 [M+Na]$^+$, 549.1 [2M+Na]$^+$.

4.2.5. (*RS*)-3-(1′-Hydroxymethyl)-1-*tert*-butyloxycarbonyl-1,2,3,4-tetrahydroquinoline, 1′-acetate 7

To a solution of (*RS*)-**6** (0.200 g, 0.76 mmol) in dry pyridine (2 mL) acetic anhydride (0.25 mL, 2.64 mmol) was added. The reaction mixture was kept at room temperature overnight, after which TLC analysis (hexane/ethyl acetate 7:3) showed complete conversion. The solution was poured in ice cooled water (40 mL) and the product was recovered by extraction with dichloromethane (3 × 40 mL). The collected organic phases were washed with water (3 × 40 mL). After the usual work-up crude acetate **7** was recovered. Purification on silica gel column chromatography (hexane/ethyl acetate 9:1 as eluant) afforded pure **7** (0.204 g, 88%). ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 9H, (CH_3)$_3$C); 2.11 (s, 3H, CH_3CO); 2.37 (m, 1H, H-3); 2.58 (dd, 1H, J = 16.02 and 8.70 Hz, H-4); 2.93 (dd, 1H, J = 16.02 and 5.65 Hz, H-4); 3.37 (dd, 1H, J = 12.8 and 8.85 Hz, H-2); 3.96-4.16 (m, 3H, 2H-1′ and H-2); 7.02 (dd, 1H, J = 7.56 and 7.02 Hz, H-6); 7.11 (d, 1H, J = 7.02 Hz, H-5); 7.17 (dd, 1H, J = 7.95 and 7.56 Hz, H-7); 7.65 (d, 1H, J = 7.95 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ 20.90 (*C*H_3CO); 28.37 ((CH_3)$_3$C); 30.09 (C-4); 33.77 (C-3); 46.28 (C-2); 65.71 (C-1′); 81.06 ((CH_3)$_3$*C*); 123.61 (C-6); 124.08 (C-8); 125.95 (C-7); 127.87 (C-10); 128.88 (C-5); 138.44 (C-9); 153.75 (NCOO); 171.03 (CO*C*H$_3$); IR ν_{max} 2976.11, 2932.21, 1741.73, 1697.36, 1492.67, 1367.59, 1239.61, 1161.70 cm^{-1}; MS (ESI+) m/z 328.2 [M+Na]$^+$, 344 [M+K]$^+$.

4.3. (*R*)-3-(1′-Hydroxymethyl)-1-*tert*-butyloxycarbonyl-1,2,3,4-tetrahydroquinoline 6

4.3.1. First resolution

To a solution of (*RS*)-**6** (1.95 g, 7.4 mmol) in toluene (168 mL), vinyl acetate (2.93 mL, 31.4 mmol) and PFL (36 mg, 40.2 U/mg) were added sequentially. The reaction mixture was kept at room temperature with vigorous stirring in a screw cap flask. The reaction progress was monitored by GLC (column: HP-5 WB, 30 m, 0.88 μm, ID 0.53 mm; oven temperature: 160 °C, isothermal; carrier N_2; 140 kPa). R_t Alcohol **6** 9.8 min; acetate **7** 15.5 min. The reaction was stopped at 58% conversion; the enzyme was removed by filtration and the solvent was evaporated at reduced pressure. The residue (1.91 g) was purified by silica gel column chromatography. By elution with hexane/ethyl acetate 9:1, acetate **7** was recovered (1.02 g, 45%). Elution with hexane/ethyl acetate 7:3 afforded alcohol **6** (0.69 g, 35%). The ee of (*R*)-**6** (81%) was determined after acetylation (acetic anhydride in pyridine) by HPLC analysis on a chiral stationary phase (eluant: *n*-hexane/2-propanol 100:2; flow rate 0.250 mL min^{-1}), by comparison with the chromatogram of racemic acetate **7**. R_t (*S*)-**7** 48.72; (*R*)-**7** 52.34. The acetylation was required in order to suitably separate (*R*)- and (*S*)-isomers (see footnote in the Results and discussion section).

4.3.2. Second resolution

To a solution of **6** (81% ee, 0.600 g, 2.28 mmol) in toluene (60 mL), vinyl acetate (0.9 mL, 9.65 mmol) and PFL (13 mg) were added. The mixture was kept under stirring at room temperature until 41% conversion. The residue, obtained after filtration and evaporation of the solvent, was purified by silica gel column chromatography. By elution with hexane/ethyl acetate 9:1, pure acetate **7** (0.278 g, 40%) was obtained. (*R*)-Alcohol **6** (0.300 g, 50%) was obtained by elution with hexane/ethyl acetate 7:3. (*R*)-**6** $[\alpha]_D^{20} = +11.8$ (*c* 1, chloroform). Ee >98% (from HPLC); (*R*)-**7** obtained by acetylation of (*R*)-**6** $[\alpha]_D^{20} = -28.7$ (*c* 1, chloroform).

4.4. (*S*)-3-(1′-Hydroxy-methyl)-1-*tert*-butyloxycarbonyl- 1,2,3,4-tetrahydroquinoline 6

4.4.1. First resolution

The irreversible transterification of (*RS*)-**6** (1.21 g, 4.6 mmol) was carried out under the same conditions as described for the preparation of (*R*)-**6**, but the reaction was stopped at 32% conversion. The residue (1.29 g), obtained after the usual work-up, was purified by silica gel column chromatography. By elution with hexane/ethyl acetate 9:1, (*S*)-acetate **7** was recovered as an oil (0.40 g, 29%, 76% ee from HPLC); unreacted (*R*)-**6** was recovered by elution with hexane/ethyl acetate 8:2 as an oil (0.80 g, 65%).

4.4.2. Second resolution

To a suspension of acetate **7** (76% ee, 0.310 g, 1 mmol) in phosphate buffer (pH 7, 18 mL), PFL (6 mg) was added. The pH of the mixture was kept at 7 over the course of the reaction (5 h) by the addition of 0.1 M sodium hydroxide aqueous solution until a calculated 70% conversion. The reaction progress (61%) was verified by GLC (see above for analysis conditions); the aqueous phase was extracted with dichloromethane (3 × 15 mL). The collected organic phases were washed with water (2 × 50 mL) and after the usual work-up, an oily residue was recovered (0.28 g); purification by silica gel column chromatography afforded pure acetate **7** (0.085 g, 28%, hexane/ethyl acetate 9:1 as eluant) and (*S*)-alcohol **6** (0.153 g, 58%, hexane/ethyl acetate 8:2 as eluant. Compound (*S*)-**6** showed a >98% ee (by HPLC, eluant: *n*-hexane/2-propanol 100:2; flow rate 0.250 mL min^{-1}). $[\alpha]_D^{20} = -12.4$ (*c* 1, chloroform).

4.5. (*R*)-3-Methyl-1,2,3,4-tetrahydroquinoline 2

4.5.1. (*R*)-3-(1′-Hydroxymethyl)-1-*tert*-butyloxycarbonyl-1,2,3,4-tetrahydroquinoline, 1′-tosylate 8

To a solution of (*R*)-**6** (>98% ee, 0.27 g, 1.03 mmol) in pyridine (0.7 mL) cooled in an ice bath, tosyl chloride (0.38 g, 2 mmol) was added slowly. The reaction mixture was kept at room temperature until the starting material disappeared (4 h, by TLC hexane/ethyl acetate 7:3). The solution was then poured into ice cooled water (5 mL). The precipitate was recovered by suction, washed with water (3 × 5 mL), and dried under reduced pressure. The recovered tosylate **8** (0.35 g, 82%) was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 9H, CH_3); 2.39 (m, 1H, H-3), 2.48 (s, 3H, *C*H_3Ar); 2.56 (dd, 1H, J = 16.7 and 8.74 Hz, H-4), 2.89 (dd, 1H, J = 16.7 and 5.96 Hz, H-4); 3.34 (dd, 1H, J = 12.9 and 8.54 Hz, H-2); 3.94 (dd, 1H, J = 12.9 and 4.17 Hz, H-2); 4.01 (m, 2H, H-4); 7.00 (dd, 1H, J = 8.34 and 6.95 Hz, H-6); 7.05 (d, 1H, J = 6.95 Hz; H-5); 7.16 (dd, 1H, J = 8.34 and 7.75 Hz, H-7); 7.37 (d, 2H, J = 8.15 Hz, Ar-CH_3); 7.63 (d, 1H, J = 8.34 Hz, H-8); 7.81 (d, 2H, J = 8.15 Hz, ArSO_2).

4.5.2. (*R*)-3-Methyl-1-*tert*-butyloxycarbonyl-1,2,3,4-tetrahydroquinoline 9

To a solution of tosylate **8** (0.35 g, 0.84 mmol) in dry tetrahydrofuran (7 mL) lithium aluminum hydride (0.143 g, 3.77 mmol) was added. The reaction was kept under stirring at room temperature (2 h) until the starting material disappeared (by TLC hexane/ethyl acetate 9:1). Water (0.14 mL), 15% sodium hydroxide aqueous solution (0.14 mL) and water (0.42 mL) were added sequentially. The white precipitate was removed by suction through a Celite pad and the filtrate evaporated at reduced pressure affording an oily residue (0.178 g, 86%), which was used in the next step without further purification. For analytical purposes a sample (50 mg) was purified by silica gel column chromatography. Elution with hexane/ethyl acetate 99:1 afforded pure **9**. ^{1}H NMR ($CDCl_3$) δ 1.08 (d, 3H, J = 7.02 Hz, CH_3-1′); 1.55 (s, 9H, CH_3); 2.07 (m, 1H, H-3); 2.44 (dd, 1H, J = 16.1 and 9.76 Hz, H-4); 2.89 (dd, 1H, J = 16.1 and 5.34 Hz, H-4); 3.12 (dd, 1H, 12.8 and 9.3 Hz, H-2); 3.99 (dd, 1H, 12.8 and 4.27 Hz, H-2); 7.00 (dd, 1H, J = 8.08 and 7.32 Hz, H-6); 7.08 (d, J = 7.32 Hz, H-5); 7.15 (dd, 1H, 8.08 and 7.32 Hz, H-7); 7.69 (d, 1H, J = 8.08 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ 18.98 (C-1′); 28.43 (C(CH_3)$_3$); 29.10 (C-3); 36.03 (C-4); 50.99 (C-2); 80.75 (C(CH_3)$_3$); 123.22 (C-6); 123.95 (C-8); 125.64 (C-7); 128.75 (C-5); 129.20 (C-9); 138.28 (C-9); 154.00 (CO). $[\alpha]_D^{20} = -11.6$ (c 1, chloroform). IR ν_{max} 2973.54, 2928.69, 2873.75, 1694.36, 1491.85, 1366.44, 1152.17 cm^{-1}; MS (ESI+) m/z 192.2 [M-C(CH_3)$_3$]$^+$, 270.2 [M+Na]$^+$, 517.2 [2M+Na]$^+$.

4.5.3. (*R*)-3-Methyl-1,2,3,4-tetrahydroquinoline 2

To a solution of **9** (0.160 g, 0.65 mmol) in dichloromethane (1.5 mL), trifluoroacetic acid (0.26 mL) was added. The solution was kept at room temperature overnight after which TLC analysis (hexane/ethyl acetate 8:2) showed complete conversion. The organic phase was treated with a saturated sodium hydrogen carbonate aqueous solution (2 × 5 mL) and washed with water (3 × 5 mL). After the usual work-up the oily residue was purified by silica gel column chromatography. Elution with hexane/ethyl acetate 99:1 afforded pure (*R*)-**2** (0.080 g, 83%) as an oil. The ee (>98%) was determined by HPLC (hexane/2-propanol 9:1 as eluant; flow rate 0.5 mL min^{-1}). R_t (*R*)-**2** 15.33, (*S*)-**2** 12.99 min. ^{1}H NMR ($CDCl_3$) δ 1.08 (d, 3H, J = 6.60 Hz, H-1′); 2.11 (m, 1H, H-3); 2.46 (dd, 1H, J = 15.96 and 10.45 Hz, H-4); 2.81 (ddd, 1H, J = 15.96, 4.95 and 1.65 Hz, H-4); 2.93 (dd, 1H, J = 10.45 and 9.9 Hz, H-2); 3.31 (ddd, 1H, J = 11.00, 3.85 and 2.00 Hz, H-2); 6.56 (d, 1H, J = 7.70, H-5); 6.66 (dd, 1H, J = 8.07 and 7.34 Hz, H-7); 6.98 (d, 1H, J = 7.70 Hz, H-8); 7.01 (dd, 1H, J = 8.07 and 7.70 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ 19.07 (C-1′); 27.17 (C-3); 35.43 (C-4); 48.84 (C-2); 114.32 (C-5); 117.49 (C-7); 121.57 (C-10); 126.76 (C-6); 129.59 (C-8); 143.67 (C-9). IR ν_{max} 3318.07, 2943.52, 2831.70, 1448.90, 1415.85, 1114.99, 1022.01 cm^{-1}; MS (ESI+) m/z 148.0; [M+1]$^+$. ms/ms 106.0 [M-CH_3CHCH_2]$^+$; $[\alpha]_D^{20} = -73.4$ (c 3, methanol) (lit.[17] (*S*)-**2** = +79).

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