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Directed (*R*)- or (*S*)-Selective Dynamic Kinetic Enzymatic Hydrolysis of 1,2,3,4-Tetrahydroisoquinoline-1-carboxylic Esters

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The first synthesis of both enantiomers of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid was accomplished through dynamic kinetic resolution in procedures based on CAL-B- or subtilisin Carlsberg-catalysed enantioselective hydrolysis of the corresponding ethyl esters in aqueous NH_4OAc buffer at pH 8.5. The products were obtained with high enantiopurity (92–93 % *ee*) in good yields (85–92 %). (*R*)-1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid was obtained with high enantiopurity (98 % *ee*) and in good yield (85 %) in a CAL-B-catalysed process, under similar conditions.

Introduction

Isoquinolines of either natural or synthetic origin have wide-ranging therapeutic applications. Some 1,2,3,4-tetrahydroisoquinoline antibiotics exert potent antitumour activity,[1] and trabectedin (formerly ecteinascidin-743) was recently introduced into the therapy of cancer.[2] A few derivatives of 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (1-TIC) occur naturally [e.g., peyoxylic acid,[3] identified in a plant, and (–)-salsolinol-1-carboxylic acid,[4] in the human brain]. As a building block, 1-TIC can be used in the synthesis of promising matrix metalloproteinase inhibitors.[5] 1-TIC is also important as a conformationally constrained analogue of phenylglycine, present in the structures of several biologically active synthetic peptides.[6] The replacement of amino acids with conformationally constrained analogues allows elucidation of the minimum conformational requirements for biological activity and the selectivity for different targets can be increased.[7] Most isoquinoline alkaloids with broad therapeutic applications contain 6,7-dimethoxy or 6,7-methylenedioxy moieties (e.g., papaverine, noscapine, emetine, atracurium and mivacurium),[8] which make the molecules less hydrophobic and therefore more drug-like. 6,7-Dimethoxy-1-TIC has been applied in the synthesis of β-site amyloid precursor protein-cleaving enzyme inhibitors, which are useful in Alzheimer's disease.[9] A small number of chemical methods are available for the preparation of the enantiomers of 1-TIC.[10] 6,7-Dimethoxy-1-TIC enantiomers have not yet been isolated and characterised, but some enantiopure derivatives have been prepared as intermediates in the synthesis of calycotomine.[11] Methods have been reported for the preparation of 1-TIC and 6-hydroxy-1-TIC enantiomers by chemoenzymatic kinetic resolution through ester hydrolysis.[12] When the aim is the synthesis of a single enantiomer, dynamic kinetic resolution (DKR) methods are more effective than simple kinetic resolutions, as they provide 100 % theoretical yield for the desired enantiomer. The importance of DKR methods is demonstrated by the large number of recent publications in this area.[13] General biocatalytic DKR processes have been developed for some groups of acyclic α-aryl-α-amino acids.[14] A CAL-B-catalysed (*R*)-selective DKR of 1-TIC ethyl ester by using enzymatic ester hydrolysis in organic solvents was recently published by our group.[12a] Although the method provided (*R*)-1-TIC in 80 % yield (96 % *ee*) the space-time yield was relatively low (a reaction mixture containing 0.05 M substrate concentration required 6 d to attain conversions ≥99 %). In the present work, we set out to develop an efficient chemoenzymatic DKR method for the preparation of enantiopure 6,7-dimethoxy-1-TIC on the basis of enzymatic hydrolysis in water. A similar DKR process with reversed selectivity has also been studied in the cases of 6,7-dimethoxy-1-TIC and 1-TIC.

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Results and Discussion

Syntheses of Substrates (±)-1b and (±)-2b

The amino ester substrates were synthesised according to literature methods: 1-TIC ethyl ester (±)-**1b** was synthesised

by partial hydrogenation of isoquinoline-1-carboxylic acid and esterification with EtOH/$SOCl_2$.[12a] 6,7-Dimethoxy-1-TIC ethyl ester (±)-**2b** was synthesised by the Bischler–Napieralsky cyclisation of homoveratrylamine hemioxalamide ethyl ester (formed by heating homoveratrylamine and diethyl oxalate at reflux) and subsequent partial hydrogenation.[15]

CAL-B-Catalysed (*R*)-Selective DKR of (±)-2b

In view of the previously reported CAL-B-catalysed kinetic resolution of (±)-**1b**,[12a] we first attempted the hydrolysis of (±)-**2b** in organic solvents. CAL-B provided excellent (*R*) selectivity in diisopropyl ether (DIPE) at 25 °C. However, slow spontaneous racemisation of unreacted (*S*)-**2b** was observed, as indicated by the observed enantiopurities [ee_s = 88%, ee_p ≥99% (ee_s and ee_p stand for the enantiomeric excess of the substrates and the products, respectively)] at 79% conversion after 14 d. Reactions with basic additives were performed in toluene/MeCN (4:1) with added water (1 equiv.) and CAL-B (20 mg mL^{-1}) at 25 °C. The use of mild bases (*i*Pr_2NH or Amberlite IRA-904 resin) resulted in a slow enzymatic reaction and slow racemisation [conv. = 54%, ee_s = 64% and ee_p ≥99% with Amberlite IRA-904 (15 mg mL^{-1}) after 6 d]. Strong organic bases such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1 equiv.) gave almost racemic products (due to chemical hydrolysis or racemisation). After the failure of our attempts to develop a real-time DKR in organic solvent, we turned to the CAL-B-catalysed hydrolysis of (±)-**2b** in aqueous medium (Table 1, Scheme 1).

The enzymatic reaction was associated with fast racemisation of the unreacted substrate, leading to conv. ≥99% and ee_p = 75–86% in 1–4 d. Both the nature of the buffer and pH usually have an impact on enzymatic activity and enantioselectivity. CAL-B typically works over a wide pH range in such a way that the hydrolysis rate increases when the pH is raised from 5 to 7 and decreases between pH 7 and 9[16] (the optimum pH = 7 for CAL-B).[17] Although pH profiles of the immobilised CAL-B preparations have not been thoroughly studied, according to Table 1 it can be concluded that the conversions attained after 4 or 8 h decreased persistently as the pH was lowered from 8.0 (in the case of H_2O, the pH decreased during the reaction). The conversion depends on the rate of the enzymatic hydrolysis and indirectly on the rate of racemisation of **2b** (this latter increases with the pH during a base-catalysed process),[14] whereas, the effect of pH on the enantiopurities of (*R*)-**2a** is moderate. The minor variations of ee_p with the pH and implicitly with the overall reaction rate prompted us to investigate the chemical hydrolysis of (±)-**2b** in different buffer systems (Figure 1). It turned out that the chemical hydrolysis of (±)-**2b** is significant except for the hydrolysis in malate buffer at pH 5.0, and the reaction is generally faster at higher pH values (after 12 h, 28–32% hydrolysed in phosphate and Tris buffer at pH = 7–8, whereas only 4% hydrolysed at pH = 5.0).

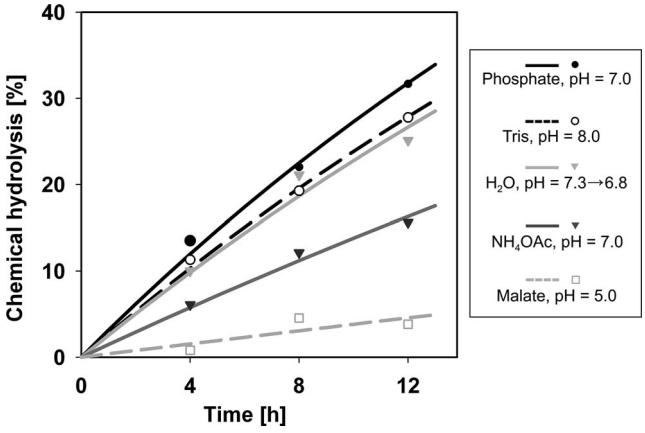


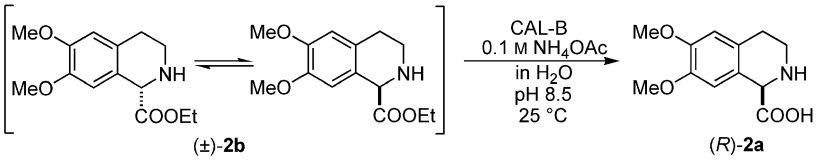
Figure 1. The chemical hydrolysis of (±)-**2b** (0.02 M) in different aqueous buffers (0.1 M) at 25 °C.

In Tris-HCl buffer, under the conditions given in Table 1, ee_p increased along with the amount of enzyme from ee_p = 62% at 10 mg mL^{-1} enzyme to ee_p = 86% at 40 mg mL^{-1} after 24 h. Accordingly, we concluded that the main de-

Table 1. Effects of different aqueous media on the conversion and enantiopurity of (*R*)-**2a** in the CAL-B-catalysed hydrolysis of (±)-**2b**.[a]

	Solvent or buffer salt/pH					
	H_2O/7.3→6.0	Tris/8.0	Phosphate/7.0	Malonate/6.0	Citrate/5.5	Malate/5.0
Conv. after 4 h [%][b]	77	69	57	42	46	54
Conv. after 8 h [%][b]	89	99	93	72	59	61
ee_p [%][c]	79[d]	83[d]	75[d]	82[d]	83[e]	86[e]

[a] 0.02 M (±)-**2b** in the tested solvent (0.1 M buffer salt in H_2O for buffered reactions), with 20 mg mL^{-1} CAL-B at 25 °C. [b] Determined by GC after extraction of the substrate. [c] Determined by HPLC. [d] Samples taken after 24 h (conv. ≥99%). [e] Samples taken after 4 d (conv. ≥99%).



Scheme 1. (*R*)-Selective DKR of (±)-**2b** by CAL-B-catalysed hydrolysis in the aqueous phase.

ficiency of these reactions is the fast chemical hydrolysis, and the enantioselectivity of the enzymatic reaction is otherwise satisfactory. As may be expected from the opposite effects of the enzymatic activity and the chemical hydrolysis upon ee_p, the enantiopurities obtained with different buffers (Table 1) present little variation.

We tested the reactions at different temperatures (3, 25 and 47 °C). Hydrolysis at 25 °C provided the highest ee_p for each tested buffer (malate at pH = 5.0, phosphate at pH = 7.0 and Tris at pH = 8.0).

The chemical natures of the buffer salts significantly influenced the chemical hydrolysis, which is slightly faster in phosphate buffer (pH = 7.0) than in water or NH_4OAc at the same pH (Figure 1).

Accordingly, we compared the ee_p obtained with different buffer salts in the same pH ranges (Table 2). The substrate concentration was increased from 0.02 to 0.1 M.

Table 2. Effects of buffers in different pH ranges on the conversion and enantiopurity of (*R*)-**2a** in the CAL-B-catalysed hydrolysis of (±)-**2b**.[a]

Entry	Buffer salt	Starting pH[b]	Final pH	Conv. after 24 h [%][c]	Conv. after 48 h [%][c]	ee_p [%][d]
1	phosphate	7.0	6.7	≥99	≥99	77
2	malonate	6.0	5.6	≥99	≥99	86
3	malate	5.0	4.7	92	94	86
4	malate	6.0	4.9	94	94	81
5	malate	7.0	5.1	96	≥99	90
6	NH_4OAc[e]	5.0	4.9	53	86	83
7	NH_4OAc[e]	6.0	5.3	65	98	92
8	NH_4OAc[e]	7.0	5.6	88	≥99	92
9	NH_4OAc	7.0	5.2	≥99	≥99	92
10	NH_4OAc	8.0	6.4	≥99	≥99	88

[a] 0.1 M (±)-**2b** in the tested buffer (0.1 M buffer salt) with 75 mg mL^{-1} CAL-B at 25 °C. [b] The pH of the reaction mixture was adjusted to the given value before the enzyme was added. [c] Determined by GC after extraction of the unreacted substrate. [d] Determined by HPLC (after conv. ≥99%). [e] The reaction mixture contained NH_4OAc (0.3 M); the initial pH was adjusted with AcOH.

Ions can also act on the rate of the enzymatic hydrolysis by specific binding (allosteric modification) or by stabilising/destabilising the enzyme structure in the aqueous phase.[18] This latter effect (salting-out/salting-in) is the most general; the influence on the enzyme activity follows the Hofmeister series (with a few exceptions).[18a]

For practical reasons, our aim was to identify the circumstances under which the salt effect on the two competing reactions (enzymatic and chemical hydrolysis) has the most favourable influence on ee_p. The reactions performed in solutions containing malate (0.1 M) and especially NH_4OAc (0.1 or 0.3 M) in the pH range of 7.0–5.1 or 7.0–5.6 (Table 2, entries 5, 8 and 9) gave products with enantiopurities superior to those obtained with phosphate and malonate buffers (Table 2, entries 1 and 2). NH_4OAc generally has favourable effects on enzyme activities (compared to other salts), where AcO^- is a kosmotropic anion (preceded in this respect by F^-, SO_4^{2-} or HPO_4^{2-}) and NH_4^+ is a chaotropic cation (preceded by tetraalkyl ammonium).[18a]

One reason for the effectiveness of NH_4OAc is the relatively slow chemical hydrolysis of (±)-**2b** in this medium (relative to H_2O and phosphate buffer at pH = 7.0 in Figure 1). Another important benefit of NH_4OAc is its volatility: it can be removed from the product by evaporation, which simplifies the purification. It is likely that NH_4OAc (at 0.1–0.3 M) inhibits the enzyme activity (Table 2, entry 8 vs. 9); this could also be observed with Tris-HCl and Na-phosphate buffers as compared with H_2O (Table 1, conv. after 4 h). The inhibition potencies of some hydrolases (trypsin, α-chymotrypsin, renal acylase, β-amylase, etc.) in response to the action of anions follow the Hofmeister series ($AcO^- < Cl^- < Br^-$, $NO_3^- < I^- < ClO_4^- < SCN^-$).[19] We subsequently used 0.1 M NH_4OAc as the optimum buffer system and studied further the effects of the pH and the enzyme and substrate concentrations on the conversion and ee_p (Table 3).

Table 3. Optimisation of the amounts of enzyme and solvent in the (*R*)-selective CAL-B-catalysed DKR of (±)-**2b**.[a]

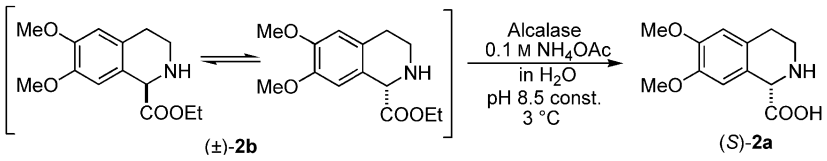
Entry	Initial conc. of (±)-**2b** [M]	Enzyme [mg mL^{-1}]	pH	Time until ≥99% conv. [h][b]	ee_p [%][c]
1	0.1	50	7.0	16	87
2	0.1	50	7.0[d]	16	84
3[e]	0.1	50	8.5[d]	6	87
4	0.1	50	8.5[d]	7	89
5	0.2	100	8.5[d]	7	92

[a] (±)-**2b** in aqueous NH_4OAc (0.1 M) in the given concentration at 25 °C. [b] The conversions were determined by GC after extraction of the unreacted substrate. [c] Determined by HPLC (after conv. ≥99%). [d] The pH of the reaction mixture was adjusted from time to time to the given value with NH_4OH (0.5 M) in H_2O. [e] In H_2O, without added buffer salt.

The decreased enzyme/substrate ratio (2:1, *m*/*m*) furnished a worse ee_p (Table 3, entry 1), as expected because of the marked chemical hydrolysis of (±)-**2b**. For reasons of economy we subsequently used this ratio. Keeping the pH constant at 7.0 decreased the value of ee_p (Table 3, entry 2). The good reactivity and relatively high ee_p obtained at pH = 8.0 (Table 1) encouraged us to test the reaction at pH ≥ 8.0 in NH_4OAc. The reactivity was improved to a noteworthy extent and the value of ee_p was found to be higher at pH = 8.5 (Table 3, entry 4). With a doubled substrate concentration (0.2 M) and an identical enzyme/substrate ratio the reaction required the same time until completion and furnished a better ee_p (Table 3, entry 5 vs. 4). Preparative-scale DKR with (±)-**2b** (0.2 M) under the optimum conditions (in 0.1 M aqueous NH_4OAc solution, with 100 mg mL^{-1} CAL-B at constant pH = 8.5 at 25 °C; Scheme 1) furnished (*R*)-**2a** with 92% enantiopurity in 85% yield after 7 h (Table 6, entry 1).

Subtilisin-Catalysed (*S*)-Selective DKR of (±)-2b

Enzyme-catalysed DKR methods with opposite enantioselectivities for the same substrate allow the directed synthesis of both enantiomers in high yields. Although very useful, there are only a few compounds for which such pro-



Scheme 2. (*S*)-Selective DKR of (±)-**2b** by subtilisin Carlsberg-catalysed hydrolysis in the aqueous phase.

cesses have been developed: the DKRs of some secondary alcohols (1-phenylethanol and related compounds) were accomplished with opposite enantioselectivities by CAL-B- and subtilisin-catalysed acylation, and Ru complexes were used as hydrogen-transfer catalysts for racemisation.[20] Enzyme screening for the hydrolysis of (±)-**2b** (0.05 M) in DIPE saturated with water was performed with 20 mg mL^{-1} enzymes at 25 °C. Of the eight enzymes tested only Alcalase (a cross-linked enzyme aggregate from subtilisin Carlsberg) displayed modest (*S*) selectivity (E = 6). The selectivity was slightly better in *tert*-butyl methyl ether (TBME; E = 13). As to the given E values, they may contain contributions of chemical hydrolysis and spontaneous racemisation, although they were calculated at an early stage of hydrolysis. Alcalase generally preferentially hydrolyses L-amino acid esters.[21] We attempted the hydrolysis by using a basic pH range (Table 4) favourable for subtilisin (pH optimum 6–9).[21] The racemisation of (*R*)-**2b** took place when the Alcalase-catalysed hydrolysis was performed in Tris buffer (pH = 8.0) at 25 °C, furnishing (*S*)-**2a** with ee_p = 61% after total conversion (Table 4, entry 1). It was not possible to enhance the enantiopurity of (*S*)-**2a** by increasing the enzyme content (Table 4, entry 2). NH_4OAc buffer (0.1 M) was once again preferred over Tris-HCl, and it was used during further optimisations (Table 4, entry 3). Acetate salts previously proved to be the most potent activators of lyophilised subtilisin Carlsberg in transesterifications carried out in hexane.[22] Increase of the pH to 8.5 improved the enantiopurity of the product (Table 4, entry 4). When the reaction was performed at 3 °C, ee_p increased significantly (Table 4, entry 6), and it could be improved further by increasing the enzyme amount (Table 4, entries 5, 6 and 8), unlike at 25 °C (Table 4, entries 1 and 2). This demonstrates better enantioselectivity at lower temperature. A doubled substrate concentration (with maintained enzyme/substrate ratio) favoured the space-time yield (the reaction required the same interval to proceed), and the value of ee_p also improved (Table 4, entry 7 vs. 6 and 9 vs. 8). A gram-scale resolution under the optimum conditions [0.2 M (±)-**2b** in 0.1 M aqueous NH_4OAc solution with 80 mg mL^{-1} Alcalase at constant pH = 8.5 at 3 °C; Scheme 2] afforded (*S*)-**2a** with 93%*ee* in 92% yield (Table 6, entry 2).

Table 4. Effects of buffers, pH, temperature and enzyme concentration on the conversion and enantiopurity of (*S*)-**2a** in the (*S*)-selective Alcalase-catalysed DKR of (±)-**2b**.

Entry	Conc. of (±)-**2b** [M]	Enzyme [mg mL^{-1}]	Buffer/ pH[a]	Temp. [°C]	Time until ≥99% conv. [d][b]	ee_p [%][c]
1	0.1	10	Tris-HCl/8.0	25	1	61
2	0.1	20	Tris-HCl/8.0	25	0.5	60
3	0.1	20	NH_4OAc/8.0	25	0.5	64
4	0.1	20	NH_4OAc/8.5	25	0.5	69
5	0.1	10	NH_4OAc/8.5	3	6	70
6	0.1	20	NH_4OAc/8.5	3	4	81
7	0.2	40	NH_4OAc/8.5	3	4	84
8	0.1	40	NH_4OAc/8.5	3	3	86
9	0.2	80	NH_4OAc/8.5	3	3	93

[a] Buffers at 0.1 M. The pH of the reaction mixture was adjusted from time to time to the given value with NH_4OH (0.5 M) in H_2O. [b] The conversions were determined by GC after extraction of the unreacted substrate. [c] Determined by HPLC (after conv. ≥99%).

CAL-B-Catalysed (*R*)-Selective DKR of (±)-1b

The relatively fast racemisation of the enantiomers of **1b** in aprotic polar organic solvents is already known.[12a] Also known is that the CAL-B-catalysed hydrolysis of (±)-**1b** in organic solvents is enantioselective at 25 °C.[12a] After the success with the CAL-B-catalysed DKR of (±)-**2b** in water, (±)-**1b** was subjected to hydrolysis with CAL-B in aqueous media, buffered with NH_4OAc (0.1 M, Table 5). Performance of the reaction at 25 °C provided (*R*)-**1a** with ee_p = 91% after total conversion in less than 20 h (Table 5, entry 1).

Table 5. Effects of the initial pH, temperature and enzyme concentration on the conversion and enantiopurity of (*R*)-**1a** in the (*R*)-selective CAL-B-catalysed DKR of (±)-**1b**.[a]

Entry	Starting pH[b]	Enzyme [mg mL^{-1}]	Temp. [°C]	Conv. after 20 h [%][c]	Conv. after 48 h [%][c]	ee_p [%][d]
1	8	20	25	≥99	≥99	91
2	8	10	3	86	≥99	93
3	8	20	3	97	≥99	98
4	8	30	3	≥99	≥99	98
5	7	20	3	50	78	93

[a] 0.1 M (±)-**1b** in aqueous NH_4OAc (0.1 M). [b] The pH of the reaction mixture was adjusted to the given value before the enzyme was added. [c] Determined by GC after extraction of the unreacted substrate. [d] Determined by HPLC (after conv. ≥99%).

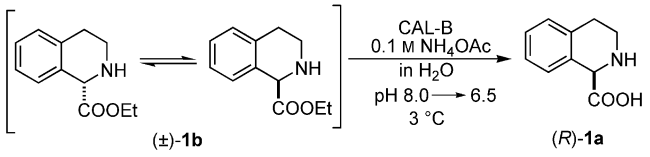
In order to improve the enantioselectivity, the reaction was attempted at 3 °C. The value of ee_p increased to 98% (Table 5, entry 3). Modification of the starting pH to 7.0 decreased the conversion and the enantiopurity of (*R*)-**1a** (Table 5, entry 5).

Enzyme contents over 20 mg mL^{-1} did not influence the value of ee_p. A gram-scale DKR with (±)-**1b** (0.1 M) under the optimum conditions (in 0.1 M aqueous NH_4OAc solution with starting pH = 8.0 at 3 °C; Scheme 3) furnished (*R*)-**1a** in isolated 85% yield (ee_p = 98%) after 24 h (Table 6, entry 3).

Table 6. Preparative scale DKRs on the studied amino ester substrates.

Entry	Substrate/conc. [M][a]	Enzyme [mg mL^{-1}]	pH	Temperature [°C]	Time [h][b]	Product	Yield [%]	ee_p [%][c]
1	(±)-**2b**/0.2	CAL-B/100	Const.8.5	25	7	(*R*)-**2a**	85	92
2	(±)-**2b**/0.2	Alcalase/80	Const.8.5	3	72	(*S*)-**2a**	92	93
3	(±)-**1b**/0.1	CAL-B/20	8.0→6.5	3	24	(*R*)-**1a**	85	98

[a] The substrates were dissolved in aqueous NH_4OAc (0.1 M) solution. [b] The time required until conv. ≥99%. [c] Determined by HPLC.



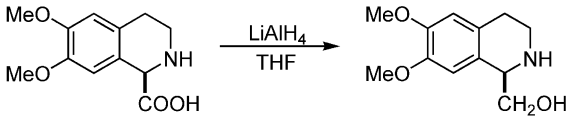
Scheme 3. (*R*)-Selective DKR of (±)-**1b** by CAL-B-catalysed hydrolysis in the aqueous phase.

In comparison to the similar DKR in organic solvent,[12a] the most evident difference is the 12-fold increase in the space-time yield (0.1 M substrate instead of 0.05 M required 1 d until completion, in contrast with 6 d in the organic medium). Moreover, H_2O as solvent is considerably more environmentally friendly than toluene/MeCN (4:1). The process is enzyme economic (1:1 enzyme/substrate mass ratio instead of 4:1) and both the yield (85%) and ee_p (98%) were slightly improved relative to the published method.[12a] The gram-scale DKRs are listed in Table 6.

Unfortunately, the Alcalase-catalysed hydrolysis of (±)-**1b** did not provide the desired (*S*)-**1a** enantiomer. Instead, Alcalase displayed weak (*R*) selectivity. The reaction with (±)-**1b** under the conditions described for (±)-**2b** (Table 4, entry 8) furnished (*R*)-**1a** (ee_p = 20%) after conv. ≥99% achieved in 3 d.

The Absolute Configurations of the Enantiomeric Products

Compound (*R*)-**3**, the (*R*) enantiomer of the alkaloid calycotomine [(*R*)-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methanol] was synthesised by the reduction of (*R*)-**2a** (95%*ee*) with $LiAlH_4$ (Scheme 4). The optical rotation of (*R*)-**3** (91%*ee*) corresponds to that of (*R*)-calycotomine: $[a]_D^{25}$ = –33 (*c* = 0.2, H_2O), in agreement with the literature data $[a]_D^{25}$ = +37.9 (*c* = 0.2, H_2O) for (*S*)-calycotomine.[23]



Scheme 4. The synthesis of (*R*)-calycotomine.

Conclusions

Directed DKR processes were developed for the efficient, independent syntheses of both enantiomers of 6,7-dimethoxy-1-TIC in enantiopure form. To the best of our knowledge, this is the first procedure for the preparation of both enantiomers of an amino acid through enzyme-catalysed DKR. The methods are based on the CAL-B- or subtilisin Carlsberg-catalysed enantioselective hydrolysis of 6,7-dimethoxy-1-TIC ethyl ester in the aqueous phase and the simultaneous base-catalysed racemisation of the unreacted amino ester enantiomer. 0.1 M NH_4OAc proved useful in both cases: it stabilises the pH of the reaction, keeps the chemical hydrolysis at a relatively low level and can be eliminated easily due to its volatility. The gram-scale resolutions provided the enantiomers with good enantiopurities (92–93%) in high yields (85–92%). Followed by the $LiAlH_4$-mediated reduction of the amino acid products, the method serves for the biocatalytic preparation of both enantiomers of calycotomine. The CAL-B-catalysed DKR of 1-TIC ethyl ester was also optimised in an aqueous solution of NH_4OAc (0.1 M): the preparative-scale resolution at 3 °C resulted in (*R*)-1-TIC with high enantiopurity (98%*ee*) and in good yield (85%).

Experimental Section

Materials and Methods: CAL-B (lipase B from *Candida antarctica*), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was purchased from Sigma–Aldrich. Alcalase, a cross-linked enzyme aggregate from subtilisin Carlsberg (a serine-protease from *Bacillus licheniformis*) was a product of Fluka. Isoquinoline-1-carboxylic acid, applied in the synthesis of (±)-**1b**, was purchased from Sigma–Aldrich, whereas homoveratrylamine [2-(3,4-dimethoxyphenyl)ethylamine, used in the synthesis of (±)-**2b**] was purchased from Chemos. Octadecane (used as a standard for GC) was obtained from Fluka. Solvents were of the highest analytical grade. DIPE was a product of Fluka, and TBME was purchased from Sigma–Aldrich.

In small-scale experiments carried out in organic solvents with CAL-B (20, 40 or 50 mg mL^{-1}), the enzyme was added to (±)-**2b** (0.05 M) in DIPE or toluene/MeCN (4:1). Additives such as H_2O (0 or 1 equiv.), organic bases [*i*Pr_2NH, 1,8-bis(dimethylamino)-naphthalene or DBU, 0 or 1 equiv.] or a basic resin (Amberlite IRA-904, 0 or 15 mg mL^{-1}), and an internal standard (octadecane, 0.012 M) were applied. The Alcalase (10 mg mL^{-1}) reaction mixtures contained (±)-**2b** (0.05 M) in DIPE or TBME saturated with H_2O, with octadecane (0.012 M) as internal standard.

Small-scale reactions in buffered aqueous media contained (±)-**1b** (0.1 M) or (±)-**2b** (0.02, 0.1 or 0.2 M) as substrate. Compound (±)-**2b** was dissolved in liquid (oily) form. The pH of the reaction mixtures was measured with a Metrohm digital pH meter with a glass membrane electrode. Various buffer systems were used: Tris buffer

[tris(hydroxymethyl)aminomethane hydrochloride (0.1 M) + NaOH, pH = 8.0], phosphate buffer [NaH_2PO_4 (0.1 M) + NaOH, pH = 7.0], malonate buffer [malonic acid (0.1 M) + NaOH, pH = 6.0], citrate buffer [citric acid (0.1 M) + NaOH, pH = 5.5], malate buffer [malic acid (0.1 M) + NaOH, tried under many pH conditions], and ammonium acetate buffer [NH_4OAc (0.1 or 0.3 M) + acetic acid or NH_4OH, tried under many pH conditions]. The pH of the reaction mixtures was adjusted appropriately (with NH_4OH in the case of NH_4OAc buffer; otherwise with NaOH) to the desired value before the enzyme was added. In the case of reactions performed at constant pH, the pH was adjusted manually (with aqueous NH_4OH or NaOH), from time to time.

Unreacted substrates **1b** and **2b** were analysed by GC on a CP Chirasil-DEX CB-coated chiral capillary column (25 m × 0.25 mm) containing permethylated β-cyclodextrin as chiral selector (manufactured by Varian). Compound **1b** {145 °C constant, 140 kPa; carrier gas: N_2; retention times [min]: 16.26 for (*R*)-**1b** and 17.32 for (*S*)-**1b**} and **2b** {152 °C constant, 140 kPa; carrier gas: N_2; retention times [min]: 67.96 for (*R*)-**2b** and 70.75 for (*S*)-**2b**}.

Samples from reactions in organic solvents were injected directly. Samples from aqueous phases were extracted with DIPE [50 μL sample was extracted with 2 mL of DIPE after the addition of NH_4OH (0.5 M, 10 μL)], the organic phase was separated, the solvents evaporated under vacuum, and the residue was dissolved in toluene (100 μL) and thereafter injected. The ee_s values obtained from reactions in the aqueous phase may not be accurate (the evaporation under heating might lead to partial racemisation) and are therefore not reported (we found $ee_s \leq 30\%$ in each case).

Conversions were determined with the aid of a standard, based on a calibration, by GC. Octadecane (0.012 M) served as internal standard for samples in organic solvents. For samples in aqueous media, octadecane was used as external standard: the samples were extracted as described previously; the organic phase was evaporated under vacuum and the residue was dissolved in 100 μL solution of octadecane (0.02 M) in toluene, and thereafter injected for GC.

Amino acid products (*R*)-**1a**, (*R*)-**2a** and (*S*)-**2a** were analysed by HPLC by using a Chirobiotic TAG (250 × 4.6 mm) column with teicoplanin aglycon as chiral selector (manufactured by Supelco); eluent: MeOH/H_2O (1:1), flow: 0.8 mL min^{-1}, at 25 °C. **1a** {retention times [min]: 17.80 for (*S*)-**1a** and 25.12 for (*R*)-**1a**} and **2a** {retention times [min]: 21.81 for (*S*)-**2a** and 27.84 for (*R*)-**2a**} were detected at 220 and 232 nm, respectively.

Compound (*R*)-**3** was analysed after derivatisation (*O*-trimethylsilyl derivative) by HPLC by using a Chiralcel OD-H chiral HPLC column (manufactured by Daicel); eluent: *n*-hexane (+0.1% Et_2NH)/EtOH (49:1); flow: 1.0 mL min^{-1}, at 25 °C {retention times [min]: 14.17 for derivatised (*R*)-**3** and 16.77 for derivatised (*S*)-**3**}. Compound (*R*)-**3** (2 mg) was dissolved in CH_2Cl_2 (200 μL). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, 20 μL) was added, the mixture was kept at 25 °C for 1 h, MeOH (200 μL) was added, and the mixture was incubated at 60 °C for 30 min. After evaporation, the residue was dissolved in the eluent (1 mL) and injected.

The chemical hydrolysis of (±)-**2b** (0.02 M in aqueous buffers) was determined under the reported pH conditions (Figure 1), and the pH was maintained constant with different buffer systems (0.1 M). The pH was set at the start of the test [immediately after the addition of (±)-**2b**]. The test reactions (V = 5.0 mL) were run at 25 °C. Samples (1.0 mL) taken at the specified intervals were extracted with DIPE (3 × 2 mL) and the aqueous phase was partially evaporated under vacuum. Compound (±)-**2a** (formed by chemical hydrolysis) was quantified by HPLC on the basis of a calibration with salicylic acid (used as external standard).

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Bruker Avance DRX 400 spectrometer. Melting points were determined with a Kofler apparatus.

(±)-Ethyl 1,2,3,4-Tetrahydroisoquinoline-1-carboxylate [(±)-1b]: Compound (±)-**1b** was prepared by a method similar to that described previously for this compound.[12a] Starting from isoquinoline-1-carboxylic acid (5.65 g, 32.63 mmol), the procedure yielded (±)-**1b·HCl** (4.81 g, 19.90 mmol, 61% overall yield). Compound (±)-**1b** was released from (±)-**1b·HCl** with an aqueous solution of KOH under cooling with ice. Compound (±)-**1b·HCl** (2.00 g, 8.27 mmol) resulted in (±)-**1b** (1.51 g, 7.36 mmol, 89% yield) after extraction with Et_2O, drying and evaporation under vacuum. Compound (±)-**1b** was obtained as a pale-orange oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.37 (t, J = 7.13 Hz, 3 H, CH_2–CH_3), 1.70–2.35 (br. s, 1 H, NH), 2.61–2.80 (m, 2 H, CH_2–CH_2–NH), 2.97–3.08 (m, 1 H, CH_2–C*H*H–NH), 3.22–3.35 (m, 1 H, CH_2–C*H*H–NH), 4.12–4.33 (m, 2 H, CH_2–CH_3), 4.64–4.80 (s, 1 H, Ar–C*H*–NH), 7.02–7.43 (m, 4 H, Ar) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 14.8, 29.8, 41.5, 59.7, 62.0, 126.4, 127.7, 128.3, 130.0, 132.7, 136.1, 173.7 ppm. $C_{12}H_{15}NO_2$ (205.25): calcd. C 70.22, H 7.37, N 6.82; found C 70.08, H 7.45, N 6.74.

(±)-Ethyl 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylate [(±)-2b]: The synthesis of (±)-**2b** was based on a literature method.[15] Starting from 2-(3,4-dimethoxyphenyl)ethylamine (24.21 g, 133.59 mmol), the procedure yielded (±)-**2b·HCl** (24.86 g, 82.38 mmol, 62% overall yield) as a slightly-yellowish crystalline powder. Compound (±)-**2b** was liberated from (±)-**2b·HCl** with an aqueous solution of KOH under ice cooling. Compound (±)-**2b·HCl** (2.00 g, 6.63 mmol) resulted in (±)-**2b** (1.57 g, 5.92 mmol, 89% yield) after extraction with Et_2O, drying and evaporation under vacuum. Compound (±)-**2b** was obtained as a dense, slightly yellow oil, which solidified with difficulty into a white crystalline mass (m.p. 67–71 °C). Compound (±)-**2b** was applied in enzymatic reactions in liquid form. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.38 (t, J = 7.12 Hz, 3 H, CH_2–CH_3), 1.70–2.35 (br. s, 1 H, NH), 2.59–2.81 (m, 2 H, CH_2–CH_2–NH), 2.95–3.08 (m, 1 H, CH_2–C*H*H–NH), 3.20–3.35 (m, 1 H, CH_2–C*H*H–NH), 3.73–3.85 (s, 3 H, CH_3O–Ar), 3.85–3.93 (s, 3 H, CH_3O–Ar), 4.14–4.30 (m, 2 H, CH_2–CH_3), 4.56–4.67 (s, 1 H, Ar–C*H*–NH), 6.51–6.62 (s, 1 H, Ar), 6.83–6.92 (s, 1 H, Ar) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 14.9, 29.4, 41.5, 56.4, 56.5, 59.2, 61.9, 111.2, 112.4, 124.3, 128.4, 147.8, 148.9, 173.8 ppm. $C_{14}H_{19}NO_4$ (265.31): calcd. C 63.38, H 7.22, N 5.28; found C 63.24, H 7.31, N 5.22.

Preparative-Scale DKRs of Substrates (±)-1b and (±)-2b

(*R*)-Selective Gram-Scale DKR of (±)-1b: Compound (±)-**1b** (0.50 g, 2.44 mmol) was dissolved in an aqueous solution of NH_4OAc (0.19 g, 2.45 mmol, 23.6 mL) with the aid of EtOH (0.48 mL) as cosolvent at 3 °C. CAL-B (0.49 g, ca. 20 mg mL^{-1}) was added. The pH was set to 8.0 (with NH_4OH 0.5 M) at the start of the reaction. The mixture was shaken at 3 °C for 24 h, during which conv. ≥99%, and (*R*)-**1a** partly crystallised as a white solid. The enzyme was filtered off and washed with warm H_2O. The solvent and NH_4OAc were evaporated off under vacuum, and the residue was washed with Me_2CO. Compound (*R*)-**1a** (0.37 g, 2.09 mmol, 86% yield) was isolated with 98%*ee*; it was recrystallised from H_2O/Me_2CO. $[\alpha]_D^{25}$ = –65.5 (c = 1, 1 M HCl), ref.[12a] $[\alpha]_D^{25}$ = –63.1 (c = 1, 1 M HCl). M.p. 241–244 °C, ref.[12a] m.p. 239–240 °C.

>99%*ee*. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.98–3.19 (m, 2 H, C*H*$_2$–CH$_2$–NH), 3.35–3.53 (m, 1 H, CH$_2$–C*H*H–NH), 3.53–3.70 (m, 1 H, CH$_2$–C*H*H–NH), 4.88–5.05 (s, 1 H, Ar–C*H*–NH), 7.21–7.63 (m, 4 H, Ar) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 25.0, 40.3, 59.0, 127.4, 128.3, 128.6, 128.8, 129.1, 132.2, 172.4 ppm. $C_{10}H_{11}NO_2$ (177.20): calcd. C 67.78, H 6.26, N 7.90; found C 67.71, H 6.38, N 7.72.

(*R*)-Selective Gram-Scale DKR of (±)-2b: Compound (±)-**2b** (0.50 g, 1.88 mmol) was transformed by the procedure described above in an aqueous solution of NH_4OAc (0.072 g, 0.94 mmol, 8.7 mL) with EtOH (0.19 mL), at 25 °C, with the aid of CAL-B (1.00 g, ca. 100 mg mL^{-1}) at constant pH 8.5 (adjusted from time to time with NH_4OH 0.5 M). After 7 h (conv. ≥99%), the reaction was worked up. Compound (*R*)-**2a** (0.38 g, 1.60 mmol, 85% yield) was isolated with 92%*ee*; it was recrystallised from H_2O/EtOH (1:1). $[\alpha]_D^{25}$ = –63 (*c* = 0.30, H_2O). M.p. 266–268 °C. ≥99%*ee*. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.95–3.15 (m, 2 H, C*H*$_2$–CH$_2$–NH), 3.42–3.57 (m, 1 H, CH$_2$–C*H*H–NH), 3.57–3.71 (m, 1 H, CH$_2$–C*H*H–NH), 3.77–3.92 (s, 3 H, C*H*$_3$O–Ar), 3.92–4.03 (s, 3 H, C*H*$_3$O–Ar), 4.88–4.99 (s, 1 H, Ar–C*H*–NH), 6.88–7.01 (s, 1 H, Ar), 7.17–7.28 (s, 1 H, Ar) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 24.5, 40.3, 56.1, 56.2, 58.6, 111.1, 112.0, 120.3, 124.9, 146.9, 148.0, 172.1 ppm. $C_{12}H_{15}NO_4$ (237.25): calcd. C 60.75, H 6.37, N 5.90; found C 60.58, H 6.51, N 5.81.

(*S*)-Selective Gram-Scale DKR of (±)-2b: Compound (±)-**2b** (0.50 g, 1.88 mmol) was transformed by the procedure described above in an aqueous solution of NH_4OAc (0.072 g, 0.94 mmol, 8.7 mL) with EtOH (0.19 mL) at 3 °C, with Alcalase (0.76 g, ca. 80 mg mL^{-1}) at constant pH 8.5. After 3 d (conv. ≥99%), the reaction was worked up. Compound (*S*)-**2a** (0.41 g, 1.73 mmol, 92% yield) was isolated with 93%*ee*; it was recrystallised from H_2O/EtOH (1:1). $[\alpha]_D^{25}$ = +62 (*c* = 0.30, H_2O). M.p. 265–267 °C. ≥99%*ee*. The ^{1}H NMR (400 MHz, H_2O) data are similar to those for (*R*)-**2a**. $C_{12}H_{15}NO_4$ (237.25): calcd. C 60.75, H 6.37, N 5.90; found C 60.62, H 6.46, N 5.83.

Assignment of the Absolute Configurations of (*R*)-2a and (*S*)-2a

(*R*)-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methanol [(*R*)-Calycotomine, (*R*)-3]: To a suspension of $LiAlH_4$ (0.19 g, 5.01 mmol) in dry THF (4 mL) at 25 °C was added (*R*)-**2a** (0.20 g, 0.84 mmol, 95%*ee*) in portions. The mixture was heated at reflux for 6 h under vigorous stirring and thereafter cooled to 25 °C. The excess amount of $LiAlH_4$ was decomposed by adding H_2O (0.36 mL) in THF (5.0 mL) in portions, and stirring the mixture for 30 min. The insoluble residue was filtered off and washed repeatedly with THF. The filtrate was dried with Na_2SO_4, and the solvent was evaporated. The residue was recrystallised from toluene. Compound (*R*)-**3** (0.075 g, 0.34 mmol, 40% yield) was obtained as white crystalline powder after filtration. $[\alpha]_D^{25}$ = –33 (*c* = 0.2, H_2O), ref.[23] $[\alpha]_D^{25}$ = +37.9 (*c* = 0.2, H_2O) (for (*S*)-calycotomine). M.p. 146–148 °C, ref.[11c] m.p. 138 °C, ref.[23] m.p. 140 °C. 91%*ee*. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33–2.06 (br. s, 2 H, OH, NH), 2.58–2.77 (m, 2 H, C*H*$_2$–CH$_2$–NH), 2.97–3.15 (m, 2 H, CH$_2$–C*H*$_2$–NH), 3.53–3.69 (m, 1 H, C*H*–CH$_2$–OH), 3.69–3.79 (dd, *J* = 10.7 Hz, *J* = 4.3 Hz, 1 H, CH–C*H*H–OH), 3.79–3.84 (s, 3 H, C*H*$_3$O–Ar), 3.84–3.90 (s, 3 H, C*H*$_3$O–Ar), 3.90–4.04 (dd, *J* = 9.2 Hz, *J* = 4.3 Hz, 1 H, CH–C*H*H–OH), 6.50–6.58 (s, 1 H, Ar), 6.58–6.67 (s, 1 H, Ar) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 29.8, 39.5, 56.5, 56.7, 64.8, 109.9, 112.7, 127.8, 128.4, 148.2, 148.5 ppm. $C_{12}H_{17}NO_3$ (223.27): calcd. C 64.55, H 7.67, N 6.27; found C 64.37, H 7.81, N 6.11.

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