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Kinetic resolution of diastereomeric racemates of 7-bromo-3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one by immobilized CAL-B

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Abstract—Immobilized CAL-B catalyzed kinetic resolution of *syn*-7-bromo-3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one *syn*-(±)-**2** and its *anti*-diastereomer (±)-**3** were achieved with *E*-values over 200. Completely enantioselective acetylation of (1′*R*)-enantiomers in diastereomeric racemates with an opposite configuration at the second stereogenic center C(3) occured at substantially different rate ($t_{1/2syn}/t_{1/2anti}$ ca. 1/20). Conformational origins of enantioselection are also discussed.

1. Introduction

Biocatalytic methods for the stereoselective preparation of enantiomerically pure compounds with diverse properties, ranging from the biologically active, over catalytically active to those present in new materials with specific properties, e.g. liquid crystal ferroelectrics, are currently of great interest.[1–3] We have already used various enantioselective lipase catalyzed acylations either in the preparation of enantiomerically pure compounds with confirmed commercial value,[4] or in the studies of the conformational effects in the flexible racemic substrates on stereoselectivity of the lipase catalyzed reactions.[5]

In the framework of these studies we have recently reported on the lipase mediated kinetic resolution of racemic 3-hydroxymethyl-5-phenyl-1,4-benzodiazepines to obtain some natural compounds in their optically pure form, or compounds with specific biological activity.[4g] Continuing our studies towards biocatalytic approaches to chiral ligands and their application in organometallic catalysis,[4h] we herein report on the efficient resolution of diastereomeric racemates (±)-*syn*-**2** and (±)-*anti*-**3**, derivatives of 3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-1,4-benzodiazepin-2-one. The origin of the different reactivity at equally high enantioselectivity in acetylations catalyzed by commercially available immobilized lipase from *Candida antarctica* (CAL-B), is also discussed.

2. Results and discussion

Diastereomeric racemates *syn*-**2** and *anti*-**3**, are obtained from **1** by an aldol reaction (Scheme 1) according to a previously developed protocol.[6] The *syn*/*anti* ratio (23/77) was determined on a Eurospher C18 HPLC column and diastereomers were separated by chromatography on silica gel.

The relative *syn*-configuration is attributed to compound **2**, the faster running diastereomer on the HPLC column, on the basis of the coupling constant for H_a,H_b protons ($J_{a,b}$ 5.0 Hz), and *anti*-configuration to the slower running diastereomer **3** with $J_{a,b}$ 8.5 Hz. This assignment was already confirmed by X-ray structure analysis of structurally related diastereomers.[6b]

A number of commercial lipases were screened in the enantioselective acetylation of **2** and **3**, and proved non-effective. However both racemates were kinetically resolved by CAL-B with a very high efficiency. The kinetic resolution was followed by HPLC on the chiral column Chiralpak AS, and progress of acetylation on the Eurosphere C18 HPLC column. The results are

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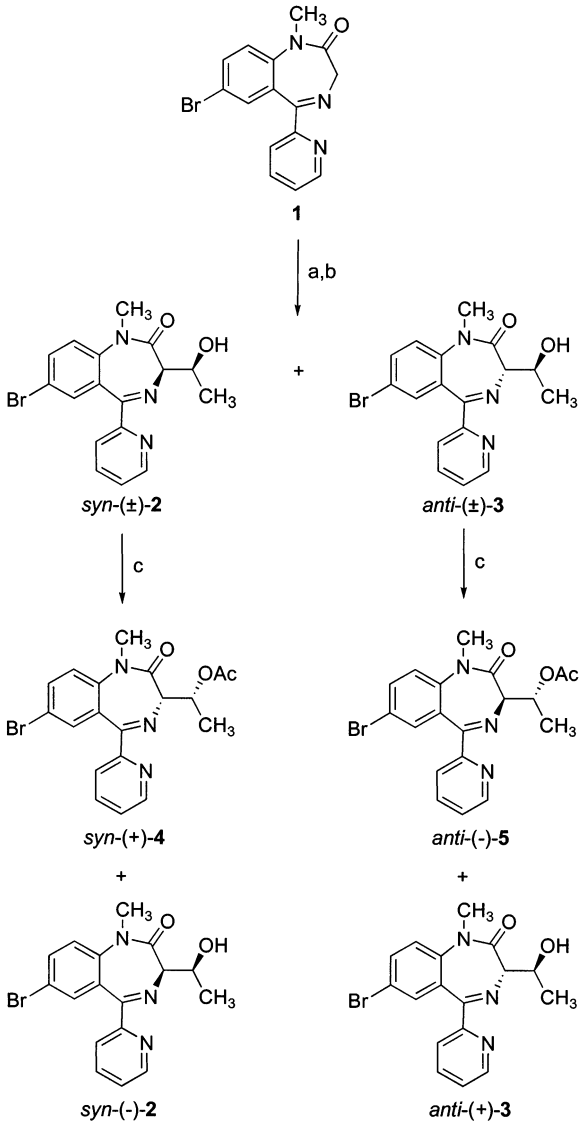


Scheme 1. *Reagents and conditions*: (a) LDA/CH_3CHO/THF/−60°C; (b) chromatography on silica gel; (c) hexane, vinylacetate, CAL-B, 45°C. Only one enantiomer of racemic **2** and **3** is presented.

presented in Table 1, and the progress curves in Figure 1.

The CAL-B enzyme was immobilized on a macroporous acrylic resin.[7] This enzyme has been reported by Uppenberg to prefer the (*R*)-enantiomers of *sec*-alcohols,[8] as predicted by Kazlauskas rule.[9] Recently it was shown that the immobilization method can greatly influence the enantioselectivity of CAL-B, varying for the same substrate from negligible to *E* over 400.[10]

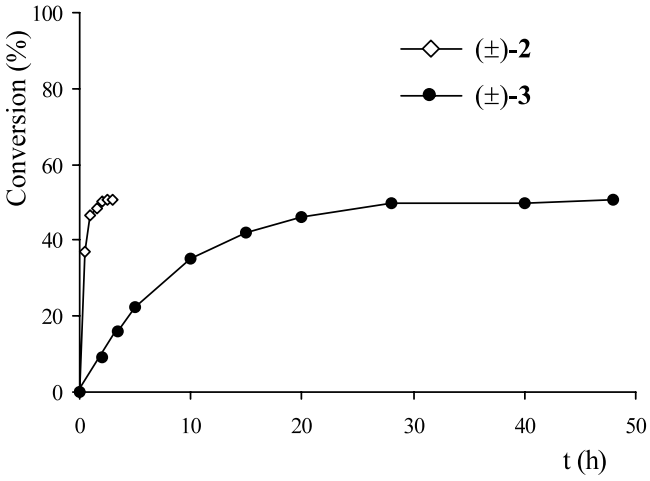


Figure 1. Progress curve of acetylation by CAL-B of (±)-**2** and (±)-**3**. Solvent: hexane; acylating agent: vinylacetate; temp.: 45°C.

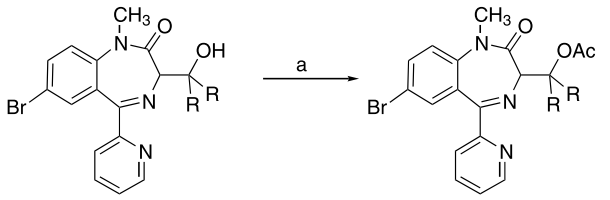
The results of kinetic resolution revealed a high enantiopreference of CAL-B towards (+)-**2** and (−)-**3**. The *E*-value for both substrates was found to be well over 200, as calculated according to Sih et al.[11] Surprisingly, however, this high enantioselectivity was retained irrespective of the rate of acetylation. 50% conversion of *syn*-**2** was achieved already after ca. 2 h, with complete enantioselective formation of *syn*-acetate (+)-**4** being observed. The same level of conversion of (−)-**3**, while maintaining equally high enantioselectivity, was reached with *anti*-**3** only after ca. 48 h. The half-time ratio $t_{1/2(+)\text{-}\mathbf{2}}/t_{1/2(-)\text{-}\mathbf{3}}$ was estimated at ca. 1:20. Such highly efficient kinetic resolutions of **2** and **3**, combined with the simple protocol for separation of acetates from alcohols followed as formerly reported,[4f,g] by hydrolysis of the 'eastern' part of the 1,4-benzodiazepine-2-one molecule to the α-amino-β-hydroxy acid, represents a novel approach to all four stereoisomers of threonine.

In order to obtain reference data for the interpretation of the results for (±)-**2** and (±)-**3**, the structurally related primary alcohol (±)-**6** and tertiary alcohol (±)-**7** were prepared (Scheme 2) and their kinetic resolution was studied. These substrates were selected because they have a single stereogenic center at the C(3) of 1,4-benzodiazepine ring, but very different steric perturbations around hydroxy group at the prochiral C(1′) atom. Very fast acetylation of (±)-**6** was observed; at 54% conversion after 15 min the e.e. of (+)-**6** was 17% (*E*-value 1.5). This result has revealed only slight enantiopreference for the (+)-enantiomer of **6**. The dimethyl derivative (±)-**7** proved completely inert in acetylation as has already been seen for most tertiary alcohols acting as substrates for CAL-B lipase.[12a,b]

Table 1. Results of acetylation by CAL-B of (±)-**2** and (±)-**3**

Substrate	*t* (h)	Conv. (%)	E.e. (%)[a]	Config.[a]	*E*
(±)-**2**	2	50	100	3*R*,1′*S*	>200
(±)-**3**	48	51	>99	3*S*,1′*S*	>200

[a] Unreacted alcohols.



(±)-**6**, R=H; (±)-**7**, R=CH_3

Scheme 2. *Reagents and conditions*: (a) hexane, vinylacetate, CAL-B, 30°C.

On the basis of the accumulated evidence concerning the sign of the Cotton effect at ca. 250 nm in the CD spectra, the absolute configuration at C(3) of the number of 3-substituted-5-aryl-1,4-benzodiazepin-2-ones can safely be assigned.[6a,13] This approach revealed a (3*S*)-configuration of the preferred (+)-**6** enantiomer in the acetylation of (±)-**6**. The problem remains, however, in determining the absolute configuration of the completely selectively acylated (+)-**2** and (−)-**3**, and to demonstrate which stereogenic center, in the ring or in the side chain, controls the enantioselection. In order to answer these questions, the absolute configuration at C(3) of the preferred enantiomers, *syn*-(+)-**2** and *anti*-(−)-**3** was deduced from the CD spectra of their unreacted enantiomers (−)-**2** and (+)-**3** (Fig. 2).

A negative CE for (−)-**2** revealed a (3*R*) configuration, and a positive CE for (+)-**3** revealed a (3*S*) configuration. Thus, the opposite configurations can be safely assigned to the reactive enantiomers; (3*S*) for (+)-**2** and (3*R*) for (−)-**3** (Fig. 2). From the relative *syn*-configuration of (+)-**2** and *anti*-configuration of (−)-**3**, as determined by ^{1}H NMR, their respective (3*S*,1′*R*) and (3*R*,1′*R*) configurations can be assigned with confidence. Consequently, in both racemic substrates, the **2** and **3** (1′*R*) enantiomers were acylated, with the stereogenic center at C(3) having no effect on the stereoselective bias. An overview of available data concerning lipase catalyzed enantioselective reactions has revealed only one example of acylations of the substrates schematic formulae **II**.[14a] It represented a 1-substituted ethanol derivative containing a second stereogenic center at the α-carbon atom, within a (hetero)cyclic unit (Fig. 3). Only a few examples of enantioselective acylation of 1-substituted ethanols with a prochiral α-carbon atom have been reported.[14b,c] These structures can be regarded as a specific case of 1-substituted ethanol derivatives **I**, for which Ema et al. have proposed an empirical enantioselection rule for acetylation by lipases.[15a,b]

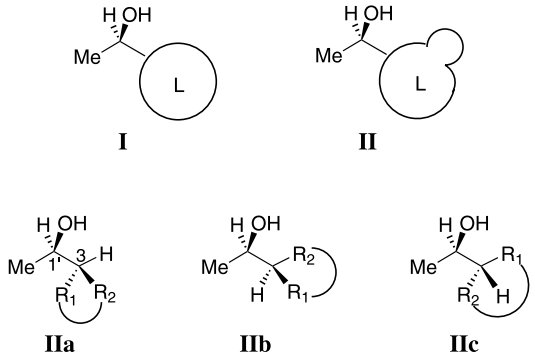


Figure 3. Extended empirical rule of Ema et al.[15c] for the lipase catalyzed kinetic resolution of 1-substituted ethanols.

These authors have also recently suggested the use of their model for lipase acetylation as a method for rapid

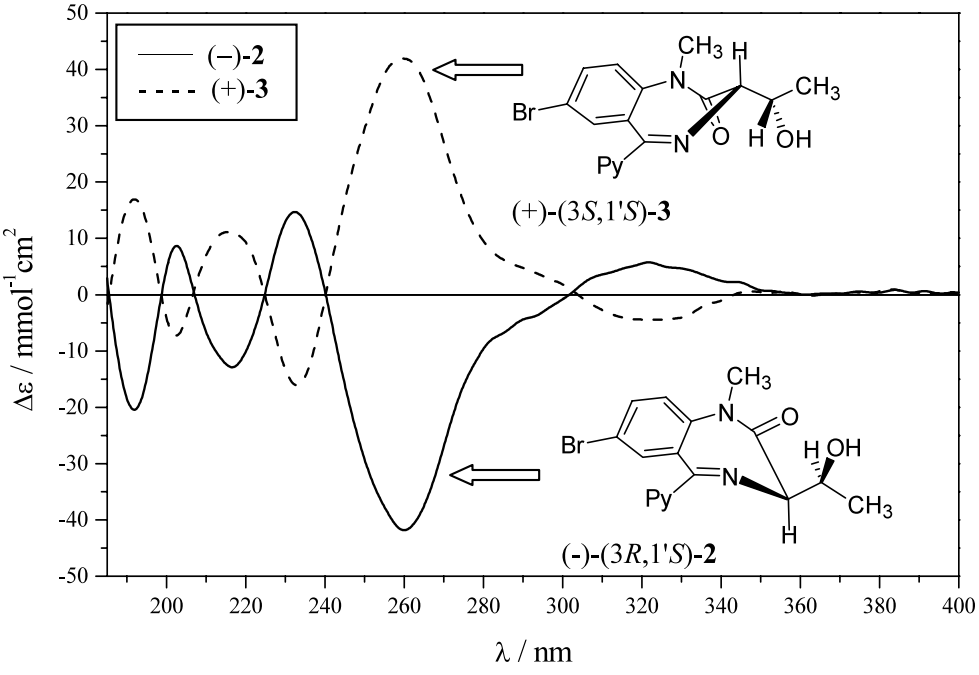


Figure 2. CD spectra of derivatives (−)-**2** and (+)-**3**, and their respective conformation and absolute configuration.

determination of the absolute configuration of 1-substituted ethanols.[15c] Importantly, according to this, the model conformation of 1-substituted ethanol unit is well defined by the requirements of the lipase active site.[15c] Considering these requirements, it can be assumed for (1′*R*) 1,4-benzodiazepine derivatives (+)-**2** and (−)-**3** that they on binding to the CAL-B active site adopt the side-chain conformation as presented in Figure 4.

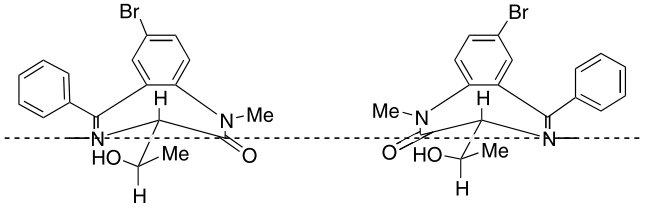


Figure 4. Schematic presentation of partial conformations for (3*S*,1′*R*)-**2** and (3*R*,1′*R*)-**3**.

Diastereomeric substrates (+)-**2** and (−)-**3** have opposite, low-energy conformations of the seven-membered ring.[5c,13] On binding they can either retain these opposite conformations or, less probably, one of them can be bound in the inverted less stable, conformation. If bound in their stable conformation, the side chain on C(3) in both substrates (+)-**2** and (−)-**3** is present in the *pseudoequatorial* (φ_e) position (Fig. 4). To adopt the required spatial orientation of the side-chain on the active site,[15b,c] these two substrates, conformationaly enantiomorphic when considering the seven-membered rings, presumably maintain the same absolute conformation of the side chain unit (C3)–(C1′)(H,OH)–Me, as indicated in Figure 4, but opposite, stable conformations of the seven-membered ring.

3. Conclusion

In conclusion, the substrates (+)-**2** and (−)-**3** with a (1′*R*) configuration are well accepted by CAL-B in spite of the opposite absolute conformations at the α-carbon, C(3) of the large bicyclic 1,4-benzodiazepine unit. According to the model of Ema et al.[15c] the orientation of the 1-substituted ethanol side-chain in both substrates should be maintained. Consequently, the substituents on N(1) and C(5) are oppositely oriented. The topology of the active site of the immobilized CAL-B presumably allows considerable difference in the steric requirements of the large, the stereogenic center containing heterocyclic unit. The higher acetylation rate of (+)-**2** indicated its better fit to the active site than (−)-**3**. The two examples presented here do not allow, however, any generalization of the steric requirements of CAL-B for the substrates general formulae **II**, and further studies of relative acylation rates and enantioselectivity bias of properly designed diastereomeric racemates is envisaged.

4. Experimental

Melting points were determined on Electrothermal 9100 apparatus, and are not corrected. IR spectra were recorded on a Perkin Elmer 297 spectrometer for KBr pallets. ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-GEM 300 spectrometer in $CDCl_3$ solution, δ is given in ppm relative to TMS as an internal reference, and J in Hz. Optical rotations were measured on an Optical Activity LTD automatic polarimeter AA-10. CD spectra were recorded on a Jasco J-810 spectropolarimeter. HPLC was performed on a Hawlett Packard instrument Series 1050 with UV detector at 254 nm. Reactions were monitored using a Eurosphere C18 column (250×4.6 mm). Enantiomeric excesses (e.e.s) were determined using a Chiralpak AS column (250×4.6 mm). Immobilized lipase B from *Candida antarctica* (Novozym 435) was a gift from Novozymes A/S (Bagsvaerd, Denmark).

4.1. *syn*/*anti*-Bromo-3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one, (±)-2/(±)-3

To a solution of $(i\text{-Pr})_2$NH (0.54 ml, 3.9 mmol) in dry THF (5 ml), 2.5 M solution of *n*-BuLi in hexane (1.6 ml, 3.9 mmol) was added under argon at 0°C. After 15 min of stirring, the reaction mixture was cooled to −78°C, a solution of **1** (1.0 g, 3.0 mmol) added at −60°C followed by MeCHO (0.5 g, 9.0 mmol) in THF (5 ml) after 30 min. Afterwards the reaction was quenched with 5% HCl and extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4, filtered and evaporated in vacuo. The diastereomeric ratio **2**/**3** (23:77, at 100% conversion) was determined by HPLC analysis using gradient 50% MeOH, 1% H_3PO_4 (0 min) to 100% MeOH (20 min). The crude product was then purified by chromatography on silica gel using CH_2Cl_2/MTBE/MeOH/Et_3N (50:50:2.5:0.5) as the eluant to obtain 0.15 g of a **2**/**3** mixture, 0.20 g of pure **2** and 0.74 g of pure **3**. Yield 97%.

4.1.1. *syn*-7-Bromo-3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one, (±)-2. Mp 87–89°C. IR (KBr) ν/cm^{-1}: 3440, 1670, 1320, 1100, 810. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, d, J=6.5 Hz), 3.38 (3H, s), 3.45 (1H, d, J=5.0 Hz), 4.61 (1H, dq, J_1=5.0 Hz, J_2=1.0 Hz), 7.25 (1H, d, J=9.0 Hz), 7.37 (1H, ddd, J_1=7.5 Hz, J_2=5.0 Hz, J_3=1.0 Hz), 7.53 (1H, d, J=2.5 Hz), 7.66 (1H, dd, J_1=9.0 Hz, J_2=2.5 Hz), 7.83 (1H, ddd, J_1=7.5 Hz, J_2=7.5 Hz, J_3=1.5 Hz), 8.21 (1H, d, J=7.5 Hz), 8.62 (1H, dd, J_1=5.0 Hz, J_2=1.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 19.0, 34.9, 66.5, 67.5, 116.8, 123.0, 123.8, 124.8, 129.5, 133.2, 134.2, 136.8, 142.1, 148.5, 154.9, 167.0, 169.75. Anal. calcd for $C_{17}H_{16}BrN_3O_2$ (*Mr* 374.23): C, 54.56; H, 4.31; N, 11.23. Found: C, 54.61; H, 4.34; N, 11.19.

4.1.2. *anti*-7-Bromo-3-(1′-hydroxyethyl)-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one, (±)-3. Mp 140–142°C. IR (KBr) ν/cm^{-1}: 3450, 1670, 1320, 1110, 810. ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, d, J=6.5 Hz), 3.39 (1H, d, J=8.5 Hz), 3.41 (3H, s), 4.76 (1H, dq,

$J_1=8.5$ Hz, $J_2=6.5$ Hz), 7.25 (1H, d, $J=9.0$ Hz), 7.40 (1H, ddd, $J_1=7.5$ Hz, $J_2=5.0$ Hz, $J_3=2.5$ Hz), 7.54 (1H, d, $J=2.0$ Hz), 7.72 (1H, dd, $J_1=9.0$ Hz, $J_2=2.0$ Hz), 7.84 (1H, ddd, $J_1=7.5$ Hz, $J_2=7.5$ Hz, $J_3=1.0$ Hz), 8.15 (1H, d, $J=7.5$ Hz), 8.63 (1H, dd, $J_1=5.0$ Hz $J_2=1.0$ Hz). ^{13}C NMR ($CDCl_3$) δ: 18.8, 35.0, 67.9, 69.3, 116.9, 123.0, 123.6, 124.8, 129.5, 133.2, 134.3, 136.8, 142.1, 148.5, 155.1, 165.9, 170.5. Anal. calcd for $C_{17}H_{16}BrN_3O_2$ (*Mr* 374.23): C, 54.56; H, 4.31; N, 11.23. Found: C, 54.60; H, 4.29; N, 11.27.

4.2. 7-Bromo-3-hydroxymethyl-1-methyl-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one, (±)-6

Starting from **1** (4.0 g, 12.1 mmol) and formaldehyde (0.54 g, 18.2 mmol), which was introduced at −40°C, the reaction was performed as previously described. The crude product was purified by chromatography on silica gel with $CH_2Cl_2/(i\text{-}Pr)_2O/MeOH/Et_3N$ (50:50:5:0.5) as the eluant, to obtain 2.66 g of pure **3**. Yield 61%. Mp 114–117°C. IR (KBr) ν/cm^{-1}: 3400, 1660, 1480, 1320, 1040, 830, 800, 750. ^{1}H NMR ($CDCl_3$) δ: 3.01 (1H, bs), 3.41 (3H, s), 3.83 (1H, dd, $J_1=7.0$ Hz, $J_2=7.0$ Hz), 4.21 (1H, m), 4.44 (1H, m), 7.24 (1H, d, $J=8.5$ Hz), 7.40 (1H, ddd, $J_1=7.5$ Hz, $J_2=4.5$ Hz, $J_3=1.0$ Hz), 7.52 (1H, d, $J=2.0$ Hz), 7.66 (1H, dd, $J_1=8.5$ Hz, $J_2=2.0$ Hz), 7.83 Hz (1H, ddd, $J_1=7.5$ Hz, $J_2=7.5$ Hz, $J_3=1.0$ Hz), 8.14 (1H, d, $J=7.5$ Hz), 8.64 (1H, dd, $J_1=4.5$ Hz, $J_2=1.0$ Hz). ^{13}C NMR ($CDCl_3$) δ: 34.9, 62.8, 64.1, 117.0, 123.0, 123.9, 124.9, 129.7, 133.4, 134.4, 136.9, 142.3, 148.8, 155.3, 166.8, 170.5. Anal. calcd for $C_{16}H_{14}BrN_3O_2$ (*Mr* 360.19): C, 53.35; H, 3.92; N, 11.67. Found: C, 53.20; H, 3.92; N, 11.53.

4.3. 7-Bromo-3-(1′-hydroxy-1-methylethyl)-5-(2′-pyridyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one, (±)-7

Using the same method and isolation protocol as described for **2**/**3**, starting from **1** (2.0 g, 6.0 mmol) and Me_2CO (1.5 ml), 0.42 g of (±)-**7** was obtained. Yield 18%. Mp 167–168°C. IR (KBr) ν/cm^{-1}: 3450, 1670, 1330, 1110, 820. ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, s), 1.46 (3H, s), 3.35 (3H, s), 3.40 (1H, s), 4.35 (1H, bs), 7.23 (1H, d, $J=8.5$ Hz), 7.35 (1H, m), 7.52 (1H, d, $J=1.5$ Hz), 7.63 (1H, dd, $J_1=8.5$ Hz, $J_2=1.5$ Hz), 7.80 (1H, t, $J=7.5$ Hz), 8.22 (1H, d, $J=7.5$ Hz), 8.57 (1H, d, $J=4.0$ Hz). ^{13}C NMR ($CDCl_3$) δ: 25.7, 27.6, 35.0, 69.4, 71.5, 117.1, 123.3, 123.9, 124.9, 129.6, 133.4, 134.4, 136.9, 142.3, 148.6, 155.4, 166.6, 170.2. Anal. calcd for $C_{18}H_{18}BrN_3O_2$ (*Mr* 388.26): C, 55.68; H, 4.67; N, 10.82. Found: C, 55.75; H, 4.61; N, 10.80.

4.4. General procedure for preparative enzymatic acetylation of *sec* alcohols (±)-2 and (±)-3

The reaction was conducted in a thermostated shaker at 45°C and 220 rpm. The substrate (100 mg) was dissolved in vinylacetate (10 ml), after which hexane (50 ml) was added, and reaction started by the addition of CAL-B lipase (1.0 g). Samples were taken at regular time intervals, evaporated, desolved in MeOH and analysed by HPLC with $MeOH/H_2O$ (80:20) as the eluant, at flow rates of 1 ml/min, $t_R=4.1$ min for **2**, $t_R=5.6$ min for **4**, $t_R=4.5$ min for **3**, $t_R=6.2$ min for **5**. After the indicated reaction periods, the reaction mixture was elaborated by standard work-up procedure.

4.4.1. Acetylation of alcohol (±)-2. The reaction was stopped after 2 h at 50% of conversion and the resulting reaction mixture filtered and evaporated. Alcohol and acetate were separated by chromatography on silica gel with $CH_2Cl_2/(i\text{-}Pr)_2O/MeOH/Et_3N$ (50:50:5:0.5) as the eluant. The enantiomerically pure alcohol (3*R*,1′*S*)-**2** (e.e. 100%; $[\alpha]_D=-214$ (*c* 1.0, CH_2Cl_2)) was isolated. Yield: 40 mg (80% of **2**). The optical purity of the alcohol was determined on a Chiralpak AS column with hexane/EtOH (88:12) as the eluent, flow rate 1 ml/min, $t_R=18$ min for (−)-**2**, $t_R=22$ min for (+)-**2**. Enantiomerically pure acetate (47 mg, 82% of **4**) (+)-**4** was also isolated. (+)-(3*S*,1′*R*)-**4**: $[\alpha]_D^{20}=+249$ (*c* 0.9, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, d, $J=6.5$ Hz), 2.11 (3H, s), 3.38 (3H, s), 3.67 (1H, d, $J=8.5$ Hz), 5.93 (1H, dq, $J_1=8.5$ Hz, $J_2=6.5$ Hz), 7.27 (1H, d, $J=9.0$ Hz), 7.37 (1H, ddd, $J_1=7.5$ Hz, $J_2=5.0$ Hz, $J_3=1.0$ Hz), 7.60 (1H, d, $J=2.5$ Hz), 7.67 (1H, dd, $J_1=9.0$ Hz, $J_2=2.5$ Hz), 7.82 (1H, ddd, $J_1=7.5$ Hz, $J_2=7.5$ Hz, $J_3=1.5$ Hz), 8.15 (1H, dd, $J_1=5.0$ Hz, $J_2=1.5$ Hz), 8.61 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 17.7, 21.3, 35.1, 67.1, 70.4, 116.9, 123.2, 123.6, 124.8, 129.4, 133.5, 134.3, 136.9, 142.5, 148.6, 155.2, 165.9, 167.7, 170.3. Anal. calcd for $C_{19}H_{18}BrN_3O_3$ (*Mr* 416.26): C, 54.82; H, 4.36; N, 10.09. Found: C, 54.90; H, 4.31; N, 10.00.

4.4.2. Acetylation of alcohol (±)-3. The reaction was stopped after 48 h at 51% of conversion with the work-up of the reaction mixture was performed as described for (±)-**2**. The enantiomerically pure alcohol (3*S*,1′*S*)-**3** (e.e. >99%; $[\alpha]_D=+243$ (*c* 1.0, CH_2Cl_2)) was isolated. Yield: 40 mg (80% of **3**). The optical purity of the alcohol was determined on a Chiralpak AS column with hexane/*i*-PrOH (70:30) as the eluent, flow rate 1 ml/min, $t_R=10$ min for (−)-**3**, $t_R=16$ min for (+)-**3**. Enantiomerically enriched acetate (48 mg, 86% of **5**) (−)-**5** was also isolated. (−)-(3*R*,1′*R*)-**5**: $[\alpha]_D^{20}=-156$ (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ: 1.53 (3H, d, $J=5.5$ Hz), 2.03 (3H, s), 3.70 (1H, d, $J=5.5$ Hz), 5.84 (1H, m), 7.28 (1H, d, $J=8.5$ Hz), 7.39 (1H, d, $J=5.0$ Hz), 7.58 (1H, s), 7.67(1H, d, $J=9.0$ Hz), 7.84 (1H, t, $J=7.5$ Hz), 8.21 (1H, d, $J=7.5$ Hz), 8.62 (1H, d, $J=4.5$ Hz). ^{13}C NMR ($CDCl_3$) δ: 16.6, 21.1, 35.0, 66.3, 70.0, 116.6, 123.1, 123.5, 124.7, 129.2, 133.3, 134.2, 136.7, 142.4, 148.4, 155.0, 166.0, 167.6, 169.8. Anal. calcd for $C_{19}H_{18}BrN_3O_3$ (*Mr* 416.26): C, 54.82; H, 4.36; N, 10.09. Found: C, 54.70; H, 4.39; N, 10.10.

4.5. Kinetic resolution of alcohol (±)-6

The substrate (10 mg) was dissolved in vinylacetate (1 ml) after which hexane (5 ml) was added and thermostated at 30°C. The reaction was initiated by the addition of CAL-B lipase (10 mg) and continued at 220 rpm. Samples were taken at regular time intervals, evaporated, desolved in the MeOH and analysed by HPLC. The enantiomeric excess of the alcohol was determined on a Chiralpak AS column with hexane/*i*-

PrOH (65:35) as the eluent, flow rate 1 ml/min, $t_R = 11$ min for (−)-**6**, $t_R = 14$ min for (+)-**6**.

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