Lipase-Catalyzed Acetylation of 3-Substituted 2,3-Dihydro-1*H*-1,4-benzodiazepin-2-ones: Effect of Temperature and Conformation on Enantioselectivity and Configuration

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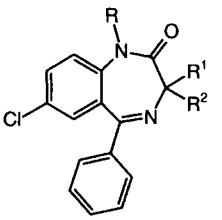
Enantioselectivity of acetylation by vinyl acetate/AcOEt catalyzed by immobilized *Candida antarctica* lipase (*Novozym 435*) is studied for *rac*-3-(hydroxymethyl)-1,4-benzodiazepin-2-ones **7**, **9**, **14** ($n = 1$; number of CH_2 groups in the chain at C(3)), **20** ($n = 2$), and for prochiral 3,3-bis(hydroxymethyl) derivative **16**. Enantiomeric excess (ee [%]) is correlated with conformational properties of substrates (relative conformation, energy difference betweeen two boat-like ground-state conformations, ring-inversion barrier) as determined by DNMR and MM2 calculations. (3*S*)-Enantiomers of acetates (+)-**8**, (+)-**10**, (+)-**15**, and (+)-**21** were preferentially formed. In the case of the acetate (–)-**17** (ee 90.2%), formation of the (3*R*)-enantiomer was favored. C(3)–OH Group with hemiaminal-like character in *rac*-**3** ($n = 0$) cannot be acetylated by any of 23 tested lipases and four esterases. For racemic alcohols **7**, **9**, **14**, and **20**, preferred acetylation of the (3*S*)-enantiomers, present in solution in absolute (*M*)-conformation, was established; only in prochiral diol **16** ($n = 1$) the CH_2OH group in the (*pro-R*)-position is prevalently acetylated, presumably due to the binding to the enzyme, in absolute (*P*)-conformation. Temperature dependence of enantioselectivity revealed inverse correlation of the *E* value of *rac*-**9**, and ee values for prochiral **16**, with T[K], indicating prevalent contribution of the enthalpy term to enantioselection. Absolute conformation (*M*/*P*) and absolute configuration at C(3) of all products was determined by combining CD and ^{1}H-NMR data.

1. Introduction. – Chiral 2,3-dihydro-1*H*-1,4-benzodiazepin-2-ones substitued at C(3) represent an important class of biologically active compounds. Unique variation of the profile of their biological activity with functional groups at C(3) is well-documented. Thus, 2,3-dihydro-3-hydroxy-1*H*-1,4-benzodiazepin-2-ones (oxazepam, temazepam *etc.*) [1], 3-(acyloxy) (camazepam) [2], 3-(alkyloxy), 3-carboxy [3], and 3-alkyl derivatives [4a,b] are active on central nervous system (CNS), whereas 3-amino derivatives are known as cholecystokinin (CCK) receptor antagonists [5a–e]. The latter have been proposed for treatment of pancreatic carcinoma [5f], biliary tract diseases, besides pain management and psychotic disorders [5b–d]. All these compounds possess a center of chirality, and different biological activities were usually observed for their different enantiomers [6]. The enantiomers of 3-hydroxy derivatives are shown to be configurationally unstable [7a,b]. However, although *in vitro* efficacy varies for single enantiomers [7c], no difference in biological activity *in vivo* can be expected for them as well as for their 3-(acyloxy) congeners. Conformational and chiroptical properties of 3-substituted 1,4-benzodiazepin-2-ones have already been studied [8a–d][9a,b], and it was shown that even achiral 1,4-benzodiazepin-2-ones are bound to biological molecules preferentially in one conformation, as, *e.g.*, on human serum albumine (HSA) [10a,b] or on CNS receptor [11].

3,3-Disubstituted 2,3-dihydro-1*H*-1,4-benzodiazepin-2-ones are nearly unknown in the literature. In one study, it was demonstrated that prochiral 3,3-dimethyl derivative

binds to HSA in its (*M*)-conformation [12]. When R ≠ R′, 3,3-disubstituted 1,4-benzodiazepin-2-ones become chiral; however, their conformational and chiroptical properties are also unknown. Continuing our project on the use of 3-substituted, conformationally unique 1,4-benzodiazepin-2-ones as starting materials in chemical [13] and enzymatic [14] approach to various α-amino-acid derivatives, we studied enzymatic enantioselective acetylation of a short series of chiral 3-(hydroxyalkyl) congeners **7**, **9**, **14**, and **20**, and prochiral (but not *meso*) 3,3-bis(hydroxymethyl) derivative **16**. These compounds are valuable intermediates for a wide range of enantiomerically pure α-alkyl-α-amino acids and their derivatives.



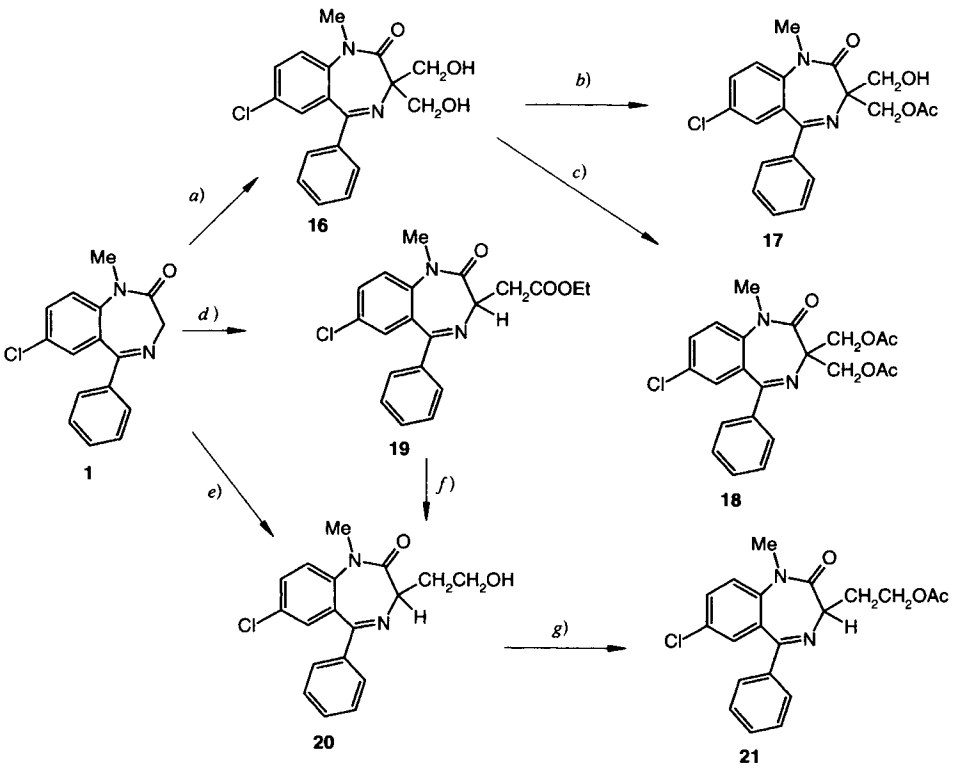
	R	R^1	R^2
1	Me	H	H
2	H	H	OH
3	Me	H	OH
4	Me	H	OAc
5	H	H	Me
6	Me	H	Me
7	H	H	CH_2OH
8	H	H	CH_2OAc
9	Me	H	CH_2OH
10	Me	H	CH_2OAc
11	H	COOEt	H
16	Me	CH_2OH	CH_2OH
22	Me	Me	CH_2OH
23	H	CH_2OH	CH_2OH

2. Results and Discussion. – 2.1. *Synthesis of Racemic and Prochiral 3-Substituted 1,4-Benzodiazepin-2-ones.* To introduce various functional groups at C(3), the C–H acidity of the substrates **1** and **11** has been exploited. Prochiral substrate **16** was prepared by double hydroxymethylation of **1** in the presence of $LiN(i\text{-}Pr)_2$ and excess of gaseous HCHO (*Scheme 1*). Racemic 2-hydroxyethyl derivative **20** was obtained by two different routes; the two-step approach afforded the final product in higher overall yield, however. Racemic **14** was prepared by methylation of **11** and subsequent regioselective reduction of **12** (*Scheme 2*).

Compound **6** was prepared by *N*-alkylation of the known compound **5** [9a]. Racemic monoacetylated derivatives **15**, **17**, and **21**, needed as standards for separation of enantiomers by HPLC on chiral columns, as well as diacetate **18**, were obtained in a usual manner [14]. Details of analytical separation of racemates on chiral HPLC columns and ee determination of enzymatically acetylated products are described in the *Exper. Part.*

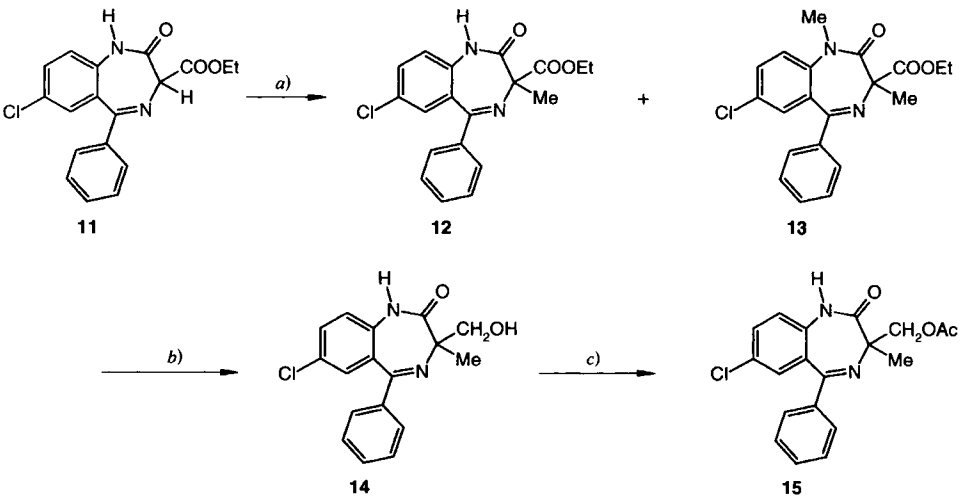
2.2. *Enzymatic Acetylation.* The fact that out of 23 tested lipases and four esterases none has acetylated *rac*-**3** seems rather to be a consequence of electronic surrounding (aminal-like character) of the 3-OH group than of sterical hindrance. Racemic, optically stable compounds **7**, **9**, **14**, and **20** are enantioselectively acetylated (kinetically resolved) by *Novozym 435*, obtained by recombinant DNA technology; the encoding gene has been transferred from a selected strain of *Candida antarctica* to the host organism, *Aspergillus oryzae*, and the isolated B-form of the enzyme immobilized on a macroporous acrylic resin. The immobilization contributes to enhanced thermal stability of the enzyme and, according to the results described below, presumably enlarges its substrate selectivity. The same enzyme has been used for acetylation of prochiral 3,3-bis(hydroxymethyl) derivative **16**.

Scheme 1



a) 1. BuLi/hexane; 2. $(i\text{-}Pr)_2NH$, −70°; 3. CH_2O, 25°. b) AcCl/**16** (1.2:1.0 mol-equiv.), pyridine, r.t. c) AcCl/16 (2.2: 1.0 mol-equiv.), pyridine, r.t. d) 1. BuLi/hexane; 2. $(i\text{-}Pr)_2NH$, 0°; 3. $BrCH_2COOEt$, 5°. e) 1. BuLi/hexane; 2. $(i\text{-}Pr)_2NH$, −50°; 3. oxirane, 25°. f) Et_2AlH_2Na, THF, 3°. g) AcCl, pyridine, r.t.

Scheme 2



a) 1. BuLi/hexane; 2. $(i\text{-}Pr)_2NH$, −50°; 3. MeI, 0°. *b*) Et_2AlH_2Na, THF, 0°. c) AcCl, pyridine, r.t.

The results of enzymatic acetylation for three racemic substrates **7**, **9**, and **14**, with one CH_2 group in the side chain ($n = 1$), and for 3-(2-hydroxyethyl) derivative **20** ($n = 2$) and for prochiral **16** are presented in *Table 1*.

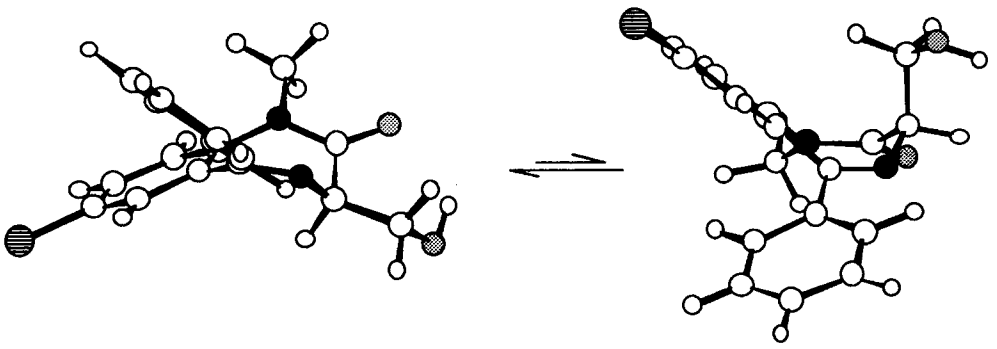
Table 1. *Data for* Novozym-435-*Catalyzed Acetylation of 3-(Hydroxyalkyl)-1,4-benzodiazepin-2-ones*

Compound	ee [%][a])		E[b])
	Alcohol	Acetate	
7	(−)-**7**, 83.5	(+)-**8**, 69.1	14.0
9	(−)-**9**, 81.8	(+)-**10**, 70.4	14.3
14	(−)-**14**, 33.8	(+)-**15**, 24.5	2.22
16	(−)-**17**, 90.2[c])		
20	(−)-**20**, 12.2	(+)-**21**, 10.2	1.37

[a]) In AcOEt at 30°. [b]) Enantioselectivity; calculated according [16]. [c]) Monoacetylation in the enantioselective step.

It is important to note that (*M*)- and (*P*)-conformers are diastereoisomeric for the chiral substrates, whereas, for the prochiral substrates, they are enantiomeric. Consequently, hydroxyalkyl groups in the racemic substrates are enantiotopic through external comparison, in the prochiral substrate **16** they are enantiotopic through internal comparison [15]; they became diastereotopic in two fixed conformations (*M*) and (*P*), however. This stereochemical relation is exemplified for (3*R*)-**9** (*Scheme 3*). In the more stable (*P*)-conformation, the larger CH_2OH group occupies a *pseudoequatorial* (ψe) position, whereas, in the less stable (*M*)-conformer, it occupies *pseudoaxial* (ψa) position, and falls into the anisotropy cone of the anellated aromatic ring.

Scheme 3



(3*R*,*P*)-**9** (3*R*,*M*)-**9**

In view of the conformational mobility of the prochiral compounds **16** and **23**, and high conformational stability of their chiral congeners **7**, **9**, **14**, and **22** (compounds **22** and **23** are not prepared), enantioselectivity of acetylation was investigated at various temperatures for two representatives, **9** and **16**. The results of temperature-dependent acetylation of *rac*-**9** are presented in *Table 2*, and, in *Table 3*, analogous data are collected for prochiral congener **16**.

Table 2. *Temperature-Dependent Acetylation of* rac-**9**

Exper.	Lipase [mg]	(−)-(*R*)-**9** ee [%]	(+)-(*S*)-**10** ee [%]	Time [min]	Temp. [°C]	Conversion [%]	*E*
1	40	58.1	58.8	35	40	49.7	6.8
2	40	64.3	68.5	50	30	48.4	10.2
3	40	76.6	67.4	60	20	53.2	11.6
4	40	51.0	81.5	90	10	38.5	16.2
5	80	67.5	83.8	135	0	44.6	22.8
6	80	89.7	80.2	195	−10	52.8	27.4
7	80	83.0	85.7	195	−15	49.2	33.6

Table 3. *Temperature-Dependent Acetylation of Prochiral* **16**

Exper.	Lipase [mg]	Time [h]	Temp. [°C]	Conversion [%]	ee [%]
1	1800	8.0	0	70.2	90.2
2	1800	6.0	10	74.7	87.7
3	900	7.0	20	73.8	85.2
4	900	5.0	30	74.8	80.6
5	900	3.0	40	77.2	77.2
6	450	4.5	50	74.5	73.5
7	450	3.0	60	73.6	68.6

Decreasing the temperature from 40° to −15° enhanced the enantioselectivity for *rac*-**9**, expressed as *E* value according to *Sih* and co-workers [16], *ca.* 5 times. Since calculation of the *E* value for **16** would require exact data for the initial rates of four different steps that include mono- and diacetylation [17], we express the enhanced enantioselectivity by ee [%] at *ca.* 70–75% conversion. As seen from *Table 2*, the ee value drops from *ca.* 90% at 0° to 69% at 60°.

Further insight into the origin of enantioselection for **9** can be obtained from thermodynamic parameters, $\Delta\Delta H^{\neq}$ and $\Delta\Delta S^{\neq}$, as well as 'racemic temperature', T_r[1]), calculated as recently suggested by *Sakai et al.* [18a] (*Fig. 1*).

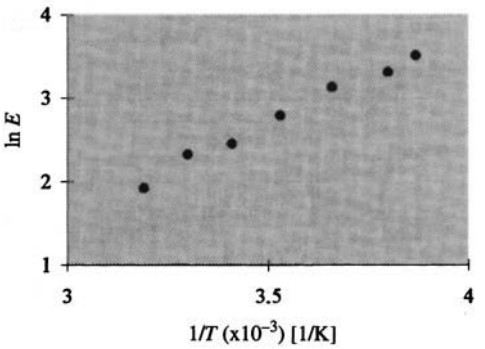


Fig. 1. *Temperature dependence of the ln* E *value for acetylation of* rac-**9** *by* Novozym 435

[1]) The term '*racemic temperature*' [18b] can be replaced by the more proper expression '*isoenantioselective temperature*'.

Since the E value is not easily available for the monoacylation step (desymmetrization) of prochiral substrates, we determined T_r value for **16** from the intercept of the linear plot (*Fig. 2*), based on the data in *Table 3*. For *rac*-**9** $T_r = 160°$, and for **16** $T_r = 252°$ were obtained. For the former substrate, $\Delta\Delta H^{\neq}$ (-4.57 kcal · mol^{-1}) and $\Delta\Delta S^{\neq}$ (-10.3 cal · K^{-1} · mol^{-1}) can be calculated. Comparing these data with those obtained for the conformationally rigid substrate **24** [18a], the structure of which can be regarded as 'truncated 1,4-benzodiazepin-2-one' **9**, one can observe a distinct lowering of T_r for both 1,4-benzodiazepin-2-one derivatives (*Figs. 1* and *2*).

Whereas the 2*H*-azirine derivative **24**, as model for the B-part of **9** and **16** (*cf. Fig. 3*), possesses no conformational mobility, **9** and **16** contain conformationally more flexible

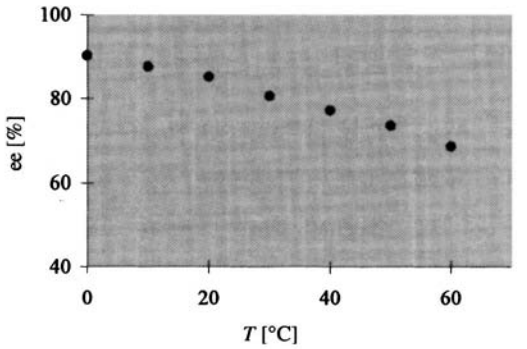


Fig. 2. *Temperature dependence of the* ee *value for acetylation of* **16** *by* Novozym 435

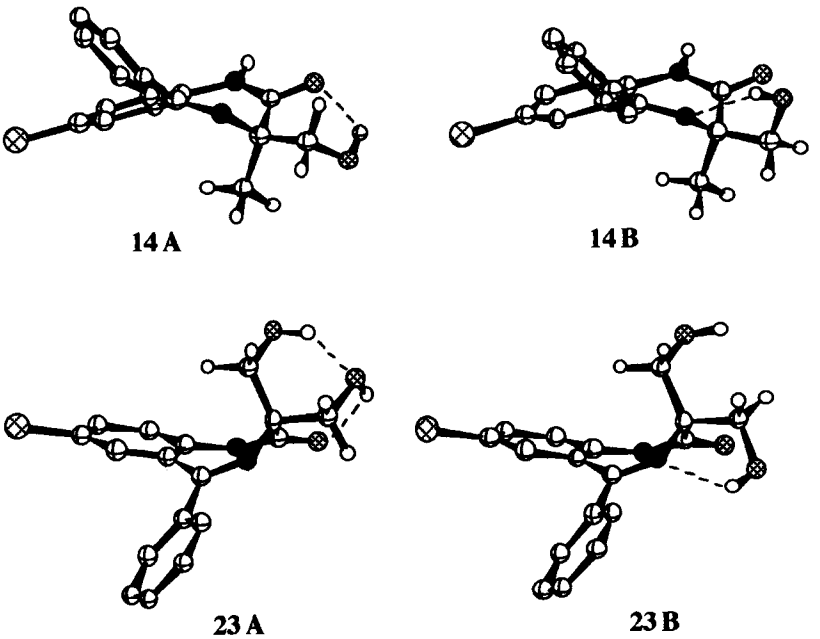
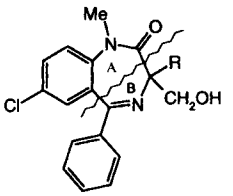


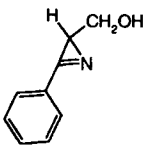
Fig. 3. *Stereoscopic view of energy-minimized conformers* **14A**/**14B** *and* **23A**/**23B**. Only H-atoms relevant for stereochemical considerations are displayed.

seven-membered ring. Since, for the present examples, even for the more flexible substrates **9** and **16**, the enthalpy term $\Delta\Delta H^{\ddagger}$ is more important for enantioselection than the entropy term $T \cdot \Delta\Delta S^{\ddagger}$, reverse relation between enantioselection and temperature was observed (*Figs. 1* and *2*). As presented in *Table 4* for **9** and **24**, there is, indeed, large enhancement of entropy factor for **9** that lowers T_r as compared to **24**.

Table 4. *Some Thermodynamic Parameters for the Enantioselective Acetylation of* **9**, **16**, *and* **24**



9 R = H
16 R = CH_2OH



24

	$\Delta\Delta H^{\ddagger}$ [kcal · mol^{-1}]	$\Delta\Delta S^{\ddagger}$ [cal · K^{-1} · mol^{-1}]	T_r [°C][a])
9	−4.5	−10.3	160
16	–	–	252
24	−3.0	−4.3	425

[a]) For definition, see the *Text*.

This means that, in spite of some polarity 'added' to **24** (represented, within the 1,4-benzodiazepin-2-one structure, as *B*) by *N*-acyl-4-chloroaniline unit (represented as *A*), which is expected to contribute to the enthalpy term of **9** and **16** by dipolar interactions, it is the conformational mobility of the seven-membered ring that mainly contributes to the entropy of activation of the latter two. From the higher T_r value calculated for **16** as compared to **9**, it could be concluded that the enthalpic effect of an additional CH_2OH group at C(3) largely outweighs conformational mobility of the ring (entropy term) (see *Sect. 2.3*). Conformational mobility of **16** is presumably restricted by H-bonding at the active site and/or by steric demand of the substituent.

2.3. *Conformational Studies by DNMR, MM2, and CD Method.* Characteristic data from ^{1}H- and ^{13}C-NMR spectra of some 3-substituted and 3,3-disubstituted 1,4-benzodiazepine-2-ones are presented in *Tables 5* and *6*.

Relative configuration at C(3), *i.e.*, pseudoequatorial (ψe) *vs.* pseudoaxial (ψa) position of the substituent, can be deduced by comparison of the ^{1}H-NMR spectra of chiral compounds **9**, **14**, **15**, and **17** with their prochiral congeners **16** and **18**, as presented in *Table 5* for **16** and **18**; signals of two chemically equivalent groups at C(3) are separated by 0.4 and 0.8 ppm, respectively. The H-atoms at higher field fall into the anisotropy cone of the anellated aromatic ring, as first defined by *Breitmeier* and co-workers [19] for C(3)–CH_2 H-atoms in some 1,4-benzodiazepin-2-ones (*cf.* **1** in *Table 5*). This anisotropic effect gives rise to an upfield shift of ^{13}C(3) signals in **16** and **18** by 1.2 and 1.4 ppm, respectively (*Table 6*). It is straightforward that the signals of the CH_2 H-

Table 5. *^{1}H-NMR Data for Some Representative 1H-1,4-Benzodiazepin-2-ones* (chemical shifts δ in ppm rel. to Me_4Si)

Compound	R (δ, multiplicity)	R^1 (δ, multiplicity)	R^2 (δ, multiplicity)
1	Me (3.65, *s*)	H (4.07, *d*)	H (4.81, *d*)
4	Me (3.41, *s*)	H (5.94, *s*)	MeO (2.32, *s*)
5	H (9.95, *s*)	H (3.74, *q*)	Me (1.75, *d*)
9	Me (3.41, *s*)	H (3.67, *t*)	CH_2OH (4.28, *dd*)
14	H (9.20, *s*)	Me (1.14, *s*)	CH_2OH (4.06, *dd*)
15	H (9.82, *s*)	Me (1.21, *s*)	CH_2OAc (4.62, *dd*)
16	Me (3.47, *s*)	CH_2OH (3.93, *dd*)	CH_2OH (4.32, *dd*)
17	Me (3.44, *s*)	CH_2OH (3.94, *dd*)	CH_2OAc (4.23, *dd*)
18	Me (3.38, *s*)	CH_2OAc (3.80, *dd*)	CH_2OAc (4.65, *dd*)

Table 6. *^{13}C-NMR Data for Some Representative 1H-1,4-Benzodiazepin-2-ones* (chemical shifts δ in ppm rel. to Me_4Si)

Compound	R (δ)	δ(C(3))	R^1 (δ)	R^2 (δ)
1	Me (35.46)	58.59		
4	Me (35.15)	84.80		MeO (20.89)
5		58.55		Me (16.69)
9	Me (34.73)	62.94		CH_2OH (63.97)
14		65.43	Me (17.74)	CH_2OH (69.53)
15		64.92	Me (15.82)	CH_2OAc (69.12)
16	Me (36.73)	61.12	CH_2OH (69.42)	CH_2OH (70.63)
17	Me (36.75)	57.89	CH_2OH (68.16)	CH_2OAc (65.84)
18	Me (36.64)	57.54	CH_2OAc (66.31)	CH_2OAc (67.70)

and C-atoms appearing at higher field correspond to ψe-oriented groups in **16** and **18**. Consequently, in chiral compounds **9** and **14**, CH_2OH group is ψe- and in **15** and **17** $AcOCH_2$ group is ψe-oriented.

To gain more insight into the relative stability of the two diastereomorphic conformations of chiral **7**, **9**, **14**, and **22** (not prepared), MM2 calculations were performed (*Table 7*). They revealed ψe-O conformation, in which the ψe-CH_2OH group forms an intramolecular H-bond to the C=O group (OH·····O=C), as the most stable one for all examined conformers.

Two distinct energy minima (**A** and **B**) were found for ψe-oriented CH_2OH group as presented for **14A**/**14B** (*Fig. 3*).

According to the total energy content (E_{tot}), conformers **A** and **B** are by 2.6–4.6 and 1–2 kcal · mol^{-1}, respectively, more stable than those with the same group in ψa-position (*Table 7*). Stabilization of the ψe-O conformers results from the H-bonding to the amide O-atom, while the ψe-N conformers are stabilized by the H-bonding to the imine N-atom. Preferred H-bonding to the amide O-atom seems to be controlled by the ring size and higher negative charge on the acceptor atom.

Heats of formation (H_f in *Table 8*) offer an estimate of the relative ground-state stabilities of the ψe conformers of the compounds **7**, **9**, **14**, and **22**. There is a general trend of increasing H_f for N(1)–Me *vs.* N(1)–H derivatives; Me group at C(3) stabilizes compounds **14** and **22** by *ca.* 4.4 kcal · mol^{-1} and 2.9 kcal · mol^{-1} compared to **7** and **9**, respectively.

Table 7. *Total Energy (E_{tot}) of Conformers for Some Chiral 1H-1,4-Benzodiazepin-2-ones*[a])

Conformers	R	R^1	E_{tot}	δE [kcal · mol^{-1}]
7 (ψe-N)	H	H	26.3	
7 (ψe-O)	H	H	24.1	2.3
7 (ψa)	H	H	27.9	3.9
14 (ψe-N)	H	Me	29.4	
14 (ψe-O)	H	Me	27.8	1.6
14 (ψa)	H	Me	30.4	2.6
9 (ψe-N)	Me	H	36.1	
9 (ψe-O)	Me	H	33.5	2.6
9 (ψa)	Me	H	38.1	4.6
22 (ψe-N)	Me	Me	40.2	
22 (ψe-O)	Me	Me	38.4	1.8
22 (ψa)	Me	Me	41.2	2.8

[a]) ψe-N: pseudoequatorial (OH · · · · · N), ψe-O: pseudoequatorial (OH · · · · · O=C), ψa: pseudoaxial.

Table 8. *Heats of Formation* [kcal mol^{-1}] *for Some Chiral 1H-1,4-Benzodiazepin-2-ones*

Compound	R	R^1	H_f [kcal · mol^{-1}] (ψe conformer)	ΔH_f [kcal · mol^{-1}]
7	H	H	38.4	
14	H	Me	34.0	4.4
9	Me	H	46.8	
22	Me	Me	43.9	2.9

Two enantiomorphic conformations of prochiral 3,3-bis(hydroxymethyl)-benzodiazepines **16** and **23** (not prepared) are, of course, energetically equivalent; the type of H-bonding, presented in the formulae **23A** and **23B** (*Fig. 3*), determines relative stability. Conformer **23A** is more stable than conformer **23B** due to formation of two H-bonds in the former and only one in the latter. Stabilization energy amounts to 7.4 kcal · mol^{-1} and 7.1 kcal · mol^{-1} for **16** and **23**, respectively. A DNMR study was performed with compound **16**; from the line-broadening data, coalescence temperature (T_c), k_c, and $\Delta G^{\neq}$ were obtained [20]. T_c was found to be 65°, and k_c and $\Delta G^{\neq}$ were 660 s^{-1} and 15.9 kcal · mol^{-1}, respectively. For chiral substrates **7**, **9**, and **14**, no change in the NMR spectra was observed within the temperature range 0–150°, revealing high conformational stability of the seven-membered ring in these compounds, and equilibrium is completely shifted to the conformer with the sterically larger group in ψe orientation.

Finally, combined informations from the ^{1}H-NMR and CD spectra allowed the assignment of the absolute configurations of all enzymatically acetylated products. As demonstrated above, ^{1}H-NMR data allowed determination of the relative configuration (ψe *vs.* ψa) at C(3), whereas CD spectra allowed the assignment of the absolute conformation ((*M*) *vs.* (*P*)) of the seven-membered ring. CD Spectra of the alcohol/acetate pairs (–)-**7**/(+)-**8**, (–)-**9**/(+)-**10**, (–)-**14**/(+)-**15**, and (–)-**20**/(+)-**21** revealed negative *Cotton* effect (CE) at *ca.* 260 nm for alcohols and positive CE for acetates; only for monoacetate (–)-**17**, negative CE was observed. It is also worth mentioning that 3-monosubstituted chiral compounds, as, *e.g.*, (–)-**9**/(+)-**10** (*Fig. 4*) exhibit 2–4 times higher CD extrema

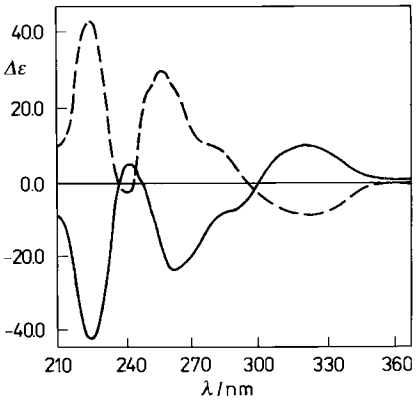


Fig. 4. *CD Spectra of the compounds (−)*-**9** (——) *and (+)*-**10** (– – – –)

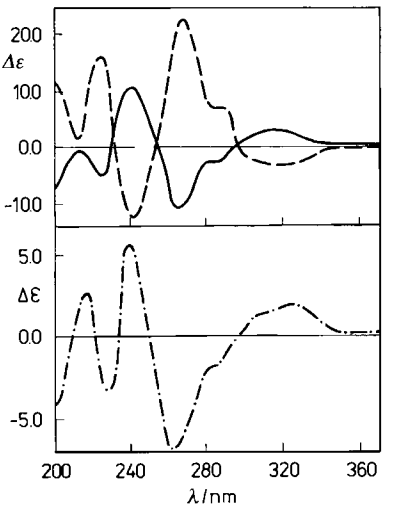


Fig. 5. *CD Spectra of the compounds (−)*-**14** (——) *and (+)*-**15** (– – – –), *and (−)*-**17** (– · – · – · –)

(calculated for 100% optical purity) than 3,3-disubstituted derivatives, as exemplified for (−)-**14**/(+)-**15** and (−)-**17** (*Fig. 5*). This effect might be tentatively explained by the enhanced torsional strain introduced by the second, ψa-oriented substituent, which, to a certain extent, planarizes the ring and reduces exciton interaction between two partial chromophores.

According to previous thorough analyses of the chromophoric system in 1,4-benzodiazepin-2-ones [9a][21], characteristic CEs at *ca.* 250–260 nm are linked to the moments of the B_{1u}-type transition of the two partial chromophores, *i.e.*, trisubstituted, anellated aromatic ring and the Ph ring at C(3). Positive exciton coupling (EC) within this band allowed the assignment of the absolute (*M*)-conformation ((*M*)-helicity) to (3*S*)-enantiomers of a number of 1,4-benzodiazepin-2-ones [9a]. This approach was corroborated by CNDO/S calculations of the same chromophoric system in 4,5-dihydro-1,4-benzodi-

azepin-2-ones; large positive value for B_{1u} transition was found for the geometry corresponding to the conformation with (*M*)-helicity [21]. Combining (*M*)-helicity of inherently chiral chromophore in acetates **8**, **10**, **15**, and **21** with ψe position of the $AcOCH_2$ group at C(3) allows to deduce the absolute (3*S*)-configuration; corresponding alcohols thus possessing the (3*R*)-configuration. The inversion of enantioselection, *i.e.*, prevalent acetylation of prochiral **16** to (3*R*)-**17** is firmly established but cannot be simply rationalized. We assume that the much less hindered, ψe-oriented group is regularly acetylated at the active site of the enzyme, and consequently **16** is prevalently bound and acetylated in absolute (*P*)-conformation, affording the acetylated (3*R*)-product.

In conclusion, using *Novozym 435* at a preparative scale, we accomplished efficient kinetic resolution of **7** and **9**, and desymmetrization of **16**. Kinetic resolution of **14** and **20** was less efficient, because of unfavorable steric interactions in the former, and because of the distance of the primary OH group from the perturbing groups at the chiral center in the latter. All alcohols and acetates obtained with high enantiomeric purity are valuable intermediates in preparation of α-substituted α-amino acids; representative examples will be published separately. Spectroscopic investigations (^{1}H-NMR, DNMR, CD) and force-field calculations reveal that larger and more polar substituents in **7**, **9**, **14**, **20**, and **22** preferentially adopt ψe orientation, whereby the (*M*)-conformation is presumably responsible for the acetylation at the enzyme active site. For prochiral **16**, which is acetylated to (3*R*)-monoacetate **17**, slight preference for binding of the (*P*)-conformer on the active site can be proposed.

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Experimental Part

General. HPLC: *HP 1050* Chromatograph with C_{18} *RP* (*Supelco*, 250 × 4.6 mm) reversed-phase column; separation was monitored by *HP 1050* UV detector (254 nm) connected to *HP 3396A* integrator. M.p.: *Electrothermal Apparatus*, not corrected. Optical rotations: *Optical Activity AA-10* automatic polarimeter in a 1-dm cell; *c* in g/100 ml. CD Spectra: *Jobin-Yvon*; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Perkin-Elmer 297* spectrometer for KBr pellets. ^{1}H- and ^{13}C-NMR Spectra: *Varian Gemini XL 300* spectrometer, measured in $CDCl_3$ solns. δ in ppm relative to TMS as internal reference, *J* in Hz.

Enzymes from the following microorganisms were screened for enzymatic acetylation: *Penicillium camemberti*, *Humicola lanuginosa*, *Penicillium roqueforti*, *Pseudomonas* species, *Mucor javanicus*, *Geotrichum candidum*, *Rhizopus niveus*, *Rhizopus delemar*, *Aspergillus niger*, *Candida cylindracea*, *Rhizopus oryzae* (all from *Amano*; lyophilized extracellular enzymes of unknown purity), *Candida cylindracea*, *Candida lipolytica*, lipase from porcine pancreas, pancreas-acetone powder, pancreatin and papain (from *Sigma*, unknown activity), *Mucor miehei*, *Novozym 435* (7300 PLU/g), *Lipozyme IM*, *Palatase M*, *Lipolase*, and *Palatase A* (all from *Novo Nordisk A/S*), proteinase from *Bacillus subtilis* var. *biotecus A*, *Pseudomonas fluorescens*, esterase from hog liver immobilized on *Eupergit C* (from *Fluka AG*), and α-chymotrypsin (type II, from bovine pancreas; *Aldrich Co.*).

2,3-Dihydro-1*H*-1,4-benzodiazepin-2-ones **1**–**3** and **11** were samples from *Compagnia di Ricerca Chimica*. All commercial reagents were used in their given purity. During usual workup, all org. extracts were dried over Na_2SO_4 or $MgSO_4$, and evaporated *in vacuo* on a *Büchi* rotavapor.

*7-Chloro-3,3-bis(hydroxymethyl)-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**16**). A soln. of BuLi in hexane (2.5M; 13.3 ml, 31.6 mmol) was added, at 0°, to the soln. of $(i\text{-}Pr)_2NH$ (4.42 ml, 31.6 mmol) in THF (15 ml) under Ar. The resulting soln. was stirred for 30 min, then cooled down to −78°. A soln. of *7-chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**1**; 3.0 g, 10.5 mmol, in 15 ml of THF) was added by syringe. After 30 min, the mixture was allowed to warm to −25° (CCl_4/dry ice), and dry HCHO gas (3.0 g,

0.1 mmol; obtained by thermic decomposition of paraformaldehyde) was introduced with the stream of N_2. After 2-h stirring, the reaction was quenched by addition of a 10% aq. NaOH soln. (10 ml) and then H_2O (20 ml). The mixture was extracted by CH_2Cl_2 (3 × 30 ml) and worked up as usual. The crude product was purified by chromatography (CH_2Cl_2/AcOEt 7:3), affording 2.37 g (65.3%) of pure **16**. M.p. 177–180°. IR: 1760, 1680, 1320, 1050, 700. ^{1}H-NMR: 3.36 (*d*, *J* = 10.7, 1 H); 3.47 (*s*, 3 H); 3.61 (*d*, *J* = 11.2, 1 H); 4.16 (*d*, *J* = 11.5, 1 H); 4.58 (*d*, *J* = 11.2, 1 H); 7.21–7.59 (*m*, 8 H). ^{13}C-NMR: 36.67; 61.21; 69.43; 70.63; 123.00; 128.34; 129.41; 129.48; 129.65; 130.78; 130.88; 131.92; 138.87, 141.16, 167.09, 172.41. Anal. calc. for $C_{18}H_{17}ClN_2O_3$ (344.78): C 62.70, H 4.97, N 8.12; found: C 62.81, H 4.82, N 8.23.

*(±)-7-Chloro-3-[(Ethoxycarbonyl)methyl]-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**19**). A soln. of BuLi in hexane (2.5M; 3.5 ml, 8.75 mmol) was added, at 0°, to a soln. of (i-Pr)$_2$NH (1.23 ml, 8.75 mmol) in THF (15 ml) under Ar. The resulting soln. was stirred for 30 min. Then, it was warmed up to 5°, and a soln. of **1** (1.78 g, 6.25 mmol) in THF (7 ml) was added by syringe. After 30 min, a soln. of $BrCH_2COOEt$ (1.38 ml, 12.5 mmol) in THF (5 ml) was added. TLC (CH_2Cl_2/AcOEt 7:3) revealed completion of the reaction after 1 h. After addition of 10% aq. HCl (5 ml), the soln. was extracted with CH_2Cl_2 (3 × 20 ml), the org. phase worked up, and the crude product recrystallized from cyclohexane: 1.46 g (62.9%) of pure **19**. M.p. 142–144°. IR: 1740, 1670, 1610, 1350, 1190, 710. ^{1}H-NMR: 1.29 (*t*, *J* = 7.3, 3 H); 3.21 (*dd*, *J* = 16.9, 6.5, 1 H); 3.41 (*s*, 3 H); 3.45 (*dd*, *J* = 16.9, 6.3, 1 H); 4.11–4.21 (*m*, 3 H); 7.26–7.58 (*m*, 8 H). ^{13}C-NMR: 13.99; 35.01; 36.76; 60.34; 60.38; 122.83; 128.37; 129.40; 129.52; 129.75; 130.32; 130.68; 131.58; 137.90; 142.13; 167.54; 169.75; 172.03. Anal. calc. for $C_{20}H_{19}ClN_2O_3$ (370.84): C 64.78, H 5.16, N 7.55; found: C 64.90, H 5.21, N 7.61.

*(±)-7-Chloro-3-(hydroxyethyl)-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**20**). *Method A*: A soln. of BuLi in hexane (2.5M; 3.5 ml, 8.75 mmol) was added, at 0°, to the soln. of (i-Pr)$_2$NH (1.23 ml, 8.75 mmol) in THF (15 ml) under Ar. The resulting soln. was stirred for 30 min, then cooled down to −50° (dry ice/acetone), and a soln. of **1** in THF (15 ml) was added *via* syringe. After 20 min stirring, the mixture was allowed to warm up to −20° (dry ice/CCl_4), and a 9.5% soln. of ethylene oxide in THF (13.8 ml, 30 mmol) was added by syringe within 30 min TLC (AcOEt/CH_2Cl_2 7:3) revealed completion of the reaction after 2 h. The reaction was quenched by addition of 10% aq. HCl (3 ml), and worked up as usual. The crude product was purified by flash chromatography (FC) (AcOEt/CH_2Cl_2 7:3): 0.94 g (31.2%) of pure **20**. Pale-yellow crystals.

Method B: To a soln. of **19** (1.0 g, 2.7 mmol) in THF (10 ml), a 2.0M soln. of $[(C_2H_5)_2AlH_2]Na$ (2 ml, 4.0 ml) was added. After 3 h, the reaction was completed, and 15% aq. NaOH (5 ml) was added, maintaining temp. below 3°. After 30 min stirring pH of the mixture was adjusted to 5.0 by aq. HCl (1:1), and the mixture stirred for 1 h. Extraction with AcOEt (3 × 25 ml) and usual workup afforded a crude product which was purified by chromatography (AcOEt/CH_2Cl_2 7:3): 640 mg (72.1%) of pale-yellow crystals. An anal. sample of **20** was obtained by crystallization from (i-Pr)$_2$O. M.p. 137–140°. IR: 3420, 1680, 1490, 1330, 1110, 700. ^{1}H-NMR: 3.41 (*s*, 3 H); 3.84 (*t*, *J* = 6.5, 1 H); 3.92–4.03 (*m*, 5 H); 7.14–7.69 (*m*, 8 H). ^{13}C-NMR: 33.60; 35.13; 60.17; 61.63; 122.74; 128.46; 129.46; 129.48; 129.81; 130.21; 130.80; 131.64; 137.86; 142.18; 167.81; 170.86. Anal. calc. for $C_{18}H_{17}ClN_2O_2$ (328.78): C 65.76, H 5.21, N 8.52; found: C 65.97, H 5.16, N 8.44.

*(±)-3-[2-(Acetoxy)ethyl]-7-chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**21**). To a soln. of **20** (0.2 g (0.6 mmol) in 5.0 ml of DMF), pyridine (0.2 ml) was added at r.t., and after 10 min, AcCl (50 µl, 0.68 mmol). Acetylation was followed by TLC using CH_2Cl_2/AcOEt 9:1. After 3 h stirring, the reaction was completed, and the solvent was evaporated. The yellow oily residue is taken into AcOEt (10 ml), washed with 10% aq. HCl (3 × 5 ml), then by sat. aq. $NaHCO_3$ soln. (3 × 5 ml) and H_2O (3 × 5 ml). Usual workup, followed by chromatography (CH_2Cl_2/AcOEt 9:1) afforded 186 mg (82.2%) of pure **21**. Pale-yellow crystals. M.p. 132–135°. IR: 1690, 1660, 1480, 1230, 1060, 700. ^{1}H-NMR: 1.98 (*s*, 3 H); 2.48–2.65 (*m*, 3 H); 3.41 (*s*, 3 H); 4.35 (*t*, *J* = 6.7, 2 H); 7.21–7.61 (*m*, 8 H). ^{13}C-NMR: 20.77; 30.80; 35.06; 60.11; 61.60; 122.73; 128.41; 129.28; 129.54; 129.74; 130.34; 130.74; 131.57; 137.98; 142.21; 167.64; 169.97; 171.21. Anal. calc. for $C_{20}H_{19}ClN_2O_3$ (370.83): C 64.78, H 5.16, N 7.55; found: C 64.92, H 6.25, N 7.72.

*(±)-3-(Acetoxymethyl)-7-chloro-3-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**15**). As described for **21**, crude **15** was prepared from 50 mg (0.16 mmol) of **14**. Chromatography with CH_2Cl_2/acetone 9:1 afforded 49.5 mg (87.4%) of crystalline **15**. M.p. 107–111°. IR: 1720, 1670, 1230, 1030, 700. ^{1}H-NMR: 1.21 (*s*, 3 H); 2.13 (*s*, 3 H); 4.58 (*d*, *J* = 11.0, 1 H); 4.66 (*d*, *J* = 11.0, 1 H); 7.10–7.51 (*m*, 8 H); 9.83 (*s*, 1 H). ^{13}C-NMR: 15.82; 20.91; 64.92; 69.12; 121.68; 128.26; 128.91; 129.66; 130.41; 130.51; 132.04; 133.18; 136.47; 139.95; 166.60; 171.21; 173.06. Anal. calc. for $C_{19}H_{17}ClN_2O_3$ (356.81): C 63.96, H 4.80, N 7.85; found: C 64.09, H 4.95, N 7.71.

*(±)-3-(Acetoxy)-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one* (**4**). As described for **21**, crude **4** was prepared from 200 mg (0.67 mmol) of **3**. Chromatography with CH_2Cl_2/acetone 8:2 afforded 194 mg (85.7%) of crystalline **4**. M.p. 157–161° ([22]: 156–160°; prepared *via Polonovski* rearrangement of *N*(4)-oxide).

(±)-3-(Acetoxymethyl)-3-(hydroxymethyl)-7-chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one (**17**). As described for **21**, crude **17** was prepared from 100 mg (0.29 mmol) of **17** and 25 µl (0.34 mmol) of AcCl. Chromatography with CH_2Cl_2/acetone 9:1 afforded 75 g (67.3%) of crystalline **17**. M.p. 94–96°. IR (KBr): 3480, 1710, 1670, 1230, 1040, 700. ^{1}H-NMR: 1.97 (*s*, 3 H); 3.44 (*s*, 3 H); 3.88 (*d*, *J* = 11.3, 1 H); 3.98 (*d*, *J* = 11.3, 1 H); 4.23 (*s*, 2 H); 7.24–7.59 (*m*, 8 H). ^{13}C-NMR: 20.36; 22.62; 36.75; 57.89; 65.84; 122.96; 128.38; 128.63; 129.49; 129.83; 130.58; 130.95; 132.06; 138.83; 141.13; 168.21; 170.35; 171.00. Anal. calc. for $C_{20}H_{19}ClN_2O_4$ (386.80): C 62.10, H 4.95, N 7.24; found: C 62.26, H 5.07, N 7.16.

3,3-Bis(acetoxymethyl)-7-chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one (**18**). As described for **21**, crude **18** was prepared from 200 mg (0.29 mmol) of **16** and 50 µl (0.68 mmol) of AcCl. Chromatography with CH_2Cl_2/acetone 9:1 afforded 202 mg (81.3%) of crystalline **18**. M.p. 104–107°. IR: 1710, 1660, 1240, 1040, 700. ^{1}H-NMR ((D_6)DMSO): 1.93 (*s*, 3 H); 2.10 (*s*, 3 H); 3.45 (*s*, 3 H); 3.77 (*d*, *J* = 10.7, 1 H); 3.83 (*d*, *J* = 10.9, 1 H); 4.58 (*d*, *J* = 10.8, 1 H); 4.69 (*d*, *J* = 10.8, 1 H); 7.20–7.77 (*m*, 8 H). ^{13}C-NMR ((D_6)DMSO): 20.16; 20.74; 36.64; 57.54; 66.35; 67.70; 123.00; 128.17; 129.17; 129.34; 129.43; 130.49; 130.72; 131.86; 138.56; 141.04; 167.21; 169.00; 170.15; 170.78. Anal. calc. for $C_{22}H_{21}ClN_2O_5$ (428.86): C 61.61, H 4.93, N 6.53; found: C 61.76, H 4.97, N 6.70.

(−)-7-Chloro-1,3-dimethyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((−)-**6**). To a soln. of (−)-(3*R*)-**5** [9b] (1.35 g, 4.73 mmol) in MeOH (20 ml), a soln. of MeONa in MeOH (0.22 g (4.73 mmol) of Na in 5 ml of MeOH) was added portionwise. Then, Me_2SO_4 (0.90 ml, 9.45 mmol) was added, and the reaction was completed at r.t. in 2 h. Quenching was performed by 10% aq. NH_4OH (3 ml), and the mixture was evaporated to dryness. Residual solid was extracted with CH_2Cl_2 (3 × 15 ml), and the combined extracts were worked up as usual. Chromatography with CH_2Cl_2/acetone 9:1 afforded 1.15 g (80.6%) of crystalline (−)-**6**. M.p. 166–169°. $[\alpha]_D$ = − 295.0 (*c* = 1.0, CH_2Cl_2). IR: 1680, 1480, 1130, 840, 700. ^{1}H-NMR: 1.72 (*d*, *J* = 6.4, 3 H); 3.41 (*s*, 3 H); 3.71 (*q*, *J* = 6.4, 1 H); 7.26–7.61 (*m*, 8 H). ^{13}C-NMR: 17.25; 34.96; 58.75; 122.56; 128.37; 128.39; 129.09; 129.51; 129.63; 130.52; 131.37; 138.12; 142.33; 166.91; 171.27. Anal. calc. for $C_{17}H_{15}ClN_2O$ (298.76): C 68.34, H 5.06, N 9.37; found: C 68.45, H 5.14, N 9.27.

(±)-7-Chloro-3-(ethoxycarbonyl)-3-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one (**12**). A soln. of BuLi in hexane (2.0M; 13.3 ml, 26.25 mmol) was added, at 0°, to the soln. of $(i\text{-}Pr)_2NH$ (3.75 ml, 26.25 mmol) in THF (10 ml) under Ar. The resulting soln. was stirred for 30 min. Then, it was cooled down to − 50° and a soln. of **11** (3.0 g, 8.75 mmol) in THF (15 ml) was added by syringe. After 30 min, the soln. was slowly warmed up to −20°, and, within 45 min, MeI (1.64 ml, 26.25 mmol) was added. Over the next 1 h, reaction temp. was allowed to rise to 9°, and stirring was continued for 2 h (TLC control with CH_2Cl_2/acetone 9.5:0.5). Quenching was performed by addition of H_2O (30 ml), followed by extraction with CH_2Cl_2 (3 × 30 ml). Usual workup and chromatography of crude product with CH_2Cl_2/acetone 9.5:0.5 afforded **12** (2.17 g, 69.7%) and **13** (0.24 g, 7.4%).

Data of **12**: M.p. 181–184°. IR: 1760, 1690, 1330, 1240, 700. ^{1}H-NMR ((D_7)DMF): 1.02 (*t*, *J* = 7.0, 3 H); 2.07 (*s*, 3 H); 3.89 (*q*, *J* = 7.3, 2 H); 7.40–7.84 (*m*, 8 H); 11.01 (*s*, 1 H). ^{13}C-NMR ((D_7)DMF): 14.73; 26.56; 62.86; 72.30; 125.33; 129.08; 130.11; 131.12; 131.39; 131.46; 132.57; 133.77; 139.30; 140.86; 170.49; 171.08; 172.06. Anal. calc. for $C_{19}H_{17}ClN_2O_3$ (356.79): C 63.96, H 4.80, N 7.85; found: C 64.08, H 4.91, N 7.73.

Data of **13**: M.p. 189–192°. IR (KBr): 1760, 1680, 1350, 1240, 700. ^{1}H-NMR: 0.92 (*t*, *J* = 7.2, 3 H); 2.04 (*s*, 3 H); 3.45 (*s*, 3 H); 3.65 (*q*, *J* = 7.9, 2 H); 6.73–7.25 (*m*, 8 H). ^{13}C-NMR: 13.56; 24.48; 58.72; 61.32; 64.80; 123.69; 128.32; 128.43; 128.51; 128.72; 131.91; 133.43; 136.87; 139.03; 140.29; 169.00; 171.13; 173.56. Anal. calc. for $C_{20}H_{19}ClN_2O_3$ (370.82): C 64.78, H 5.16, N 7.55; found: C 64.91, H 5.22, N 7.14.

(±)-7-Chloro-3-(hydroxymethyl)-3-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine-2-one (**14**). To the soln. of **12** (1.2 g, 3.35 mmol) in THF (10 ml) at 0°, a 2.0M soln. of $[(C_2H_5)_2AlH_2]Na$ (3.0 ml, 6.0 mmol) was added. After 3 h, the reaction is completed, and 15% aq. NaOH (5 ml) was added maintaining the temp. below 3°. The pH of the mixture was adjusted to 5.0 by aq. HCl (1:1), and the mixture was stirred for 1 h. Extraction with AcOEt (3 × 20 ml) and usual workup afforded a crude product which was purified by chromatography with CH_2Cl_2/acetone 9:1: 750 mg (71.4%) of **14**. Colorless crystals. M.p. 180–183°. IR: 3480, 1680, 1420, 1050, 700. ^{1}H-NMR: 1.14 (*s*, 3 H); 3.30 (*dd*, *J* = 5.3, 5.0, 1 H); 4.02 (*dd*, *J* = 11.0, 5.3, 1 H); 4.13 (*dd*, *J* = 11.2, 5.0, 1 H); 7.06–7.51 (*m*, 8 H); 9.20 (*s*, 1 H). ^{13}C-NMR: 14.74; 65.43; 69.53; 121.63; 128.20; 128.32; 128.94; 129.49; 130.40; 130.54; 131.97; 136.32; 139.86; 166.98; 175.36. Anal. calc. for $C_{17}H_{15}ClN_2O_2$ (314.76): C 64.87, H 4.80, N 8.90; found: C 64.99, H 4.97, N 8.77.

Enzyme-Catalyzed Acetylation of Racemic Substrates **7**, **9**, **14**, **20**, *and Prochiral* **16**. The reactions were performed in a thermostated shaker (*Tehtnica Co.*) at 30 ± 0.5° and 225 rpm. Enzymatic acetylations at 0°, 10°, 15°, and 60° were performed in a double-wall, magnetically stirred glass reactor connected to kryostat (*Haake, F3*). Substrate/enzyme ratio was 1:5 (g/g), if not stated otherwise.

(+)-3-(Acetoxymethyl)-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((+)-**8**). *rac*-**7** (0.35 g, 1.00 mmol) and *Novozym 435* (0.35 g) were slurried in AcOEt (20 ml) and shaken at 225 rpm and 30°. The reaction was initiated by addition of vinyl acetate (3.0 ml). Samples (20 µl) were taken at 15-min intervals over 45 min, filtered through a *Teflon* filter, and analyzed by HPLC. The reaction was stopped after 45 min at 54.7% conversion. The mixture was filtered and the filtrate evaporated to dryness. Crude product was purified by FC (CH_2Cl_2/AcOEt 7:3). First, (+)-**8** (184 mg, 46.3%, 69.1% ee) was eluted. M.p. 155–157°. $[\alpha]_D$ = + 157.9 (*c* = 0.90, THF). CD (MeCN): 225 (+ 44.17), 257 (+ 30.25), 283 (+ 8.12), 318 (– 9.07). Then, (–)-**7** (140 mg, 40.1%, 83.5% ee) followed. M.p. 197–199°. $[\alpha]_D$ = – 272.7 (*c* = 0.90, THF). CD (MeCN): 225 (–45.21), 261 (–24.71), 283 (–9.01), 319 (+ 9.66).

(+)-3-(Acetoxymethyl)-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((+)-**10**). *rac*-**9** (0.5 g, 1.59 mmol) and *Novozym 435* (0.5 g) were slurried in AcOEt (25 ml) and shaken at 225 rpm and 30°. The reaction was initiated by addition of vinyl acetate (3.0 ml). Samples (20 µl) were taken at 10-min intervals over 40 min, filtered through a *Teflon* filter, and analyzed by HPLC. The reaction was stopped after 40 min at 52.7% conversion. The mixture was filtered and the filtrate evaporated to dryness. Crude product was purified by FC (CH_2Cl_2/AcOEt 7:3). First, (+)-**10** (238 mg, 43.5%, 70.4% ee) was eluted. M.p. 203–206°. $[\alpha]_D$ = + 163.8 (*c* = 0.90, THF). CD (MeCN): 225 (+ 44.23), 265 (+ 25.25), 281 (+ 10.13), 328 (– 10.15). Then, (–)-**9** (200 mg, 39.8%, 81.8% ee) followed. M.p. 183–186°, $[\alpha]_D$ = – 265.8 (*c* = 0.90, THF). CD (MeCN): 225 (–43.91), 264 (–24.12), 282 (–90.2), 325 (+ 10.37).

(+)-3-[2-(Acetoxy)ethyl]-7-chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((+)-**21**). *rac*-**20** (0.21 g, 0.64 mmol) and *Novozym 435* (25 mg) were slurried in AcOEt (20 ml), and shaken at 225 rpm and 30°. The reaction was initiated by addition of vinyl acetate (2 ml). Samples (20 µl) were taken at regular time intervals, filtered through a *Teflon* filter, and analyzed by HPLC. The reaction was stopped at 53.4% conversion. The mixture was filtered and filtrate evaporated to dryness. Crude product was purified by FC (CH_2Cl_2/AcOEt 1:1). First, (+)-**21** (104 mg, 45.3%, 10.2% ee) was eluted. M.p. 133–136°. $[\alpha]_D$ = + 23.6 (*c* = 1.1, THF). CD (MeCN): 223 (+ 29.70), 262 (+ 52.81), 287 (+ 13.80), 318 (– 9.11). Then, (–)-**20** (76 mg, 36.1%, 12.2% ee) followed. M.p. 138–141°. $[\alpha]_D$ = – 25.4 (*c* = 1.1, THF). CD (MeCN): 223 (–29.33), 262 (–52.64), 287 (–12.34), 320 (+ 9.72).

(+)-3-(Acetoxymethyl)-7-chloro-3-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((+)-**15**). *rac*-**14** (0.35 g, 1.10 mmol) and *Novozym 435* (1.75 g) were slurried in AcOEt (50 ml), and stirred in glass reactor at 10°. The reaction was initiated by addition of vinyl acetate (5 ml). Samples (20 µl) were taken at regular time intervals, filtered through a *Teflon* filter, and analyzed by HPLC. Reaction was stopped at 54.7% conversion. The mixture was filtered and filtrate evaporated to dryness. Crude product was purified by FC (CH_2Cl_2/AcOEt 9:1). First, (+)-**15** (190 mg, 47.9%, 24.5% ee) was eluted. M.p. 108–111°. $[\alpha]_D$ = + 49.5 (*c* = 1.92, THF). CD (MeCN): 221 (+ 16.97), 238 (–12.12), 264 (+ 23.30), 293 (+ 6.32), 328 (–2.00). Then, (–)-**14** (125 mg, 35.7%, 33.8% ee) followed. M.p. 180–182°. $[\alpha]_D$ = – 62.2 (*c* = 2.33, THF). CD (MeCN): 219 (–5.51), 238 (+ 9.15), 264 (–10.89), 292 (–2.10), 325 (+ 1.66).

Table 9. *HPLC Resolution of the Racemic Compounds* **7**, **8**, **14**, **15**, **17**, **20**, *and* **21**

Compound	Column	Mobile phase	k_1'	α	R_s
7	*OD-R*	70% $NaClO_4$/$HClO_4$ pH 2, 30% MeCN	2.13	1.41	1.79
8	*OD-R*	50% $NaClO_4$/$HClO_4$ pH 2, 50% MeCN	1.71	2.15	3.58
14	*OD-R*	70% $NaClO_4$/$HClO_4$ pH 2, 30% MeCN	3.98	1.08	0.83
15	*OD-R*	30% $NaClO_4$/$HClO_4$ pH 2, 70% MeCN	1.02	1.23	1.98
17	*Chiralpak AS*	80% hexane 20% abs. EtOH	1.17	1.26	1.18
20	*Chiralpak AS*	80% hexane 20% abs. EtOH	1.99	1.43	1.94
21	*Chiralpak AS*	80% hexane 20% abs. EtOH	1.40	1.43	1.94

(–)-3-(Acetoxymethyl)-7-chloro-3-(hydroxymethyl)-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-2-one ((–)-**17**). Compound **16** (0.42 g, 1.22 mmol) and *Novozym 435* (2.1 g) are slurried in AcOEt (60 ml) at 0°, and vinyl acetate (7 ml) was added under conditions described for (+)-**15**. After 187 h, the reaction was stopped at 91.1% conversion and worked up as described before. Pure product was obtained by FC (CH_2Cl_2/acetone 9:1): **17** (408 mg; 86.5%, 90.2% ee). M.p. 95–98°. $[\alpha]_D = -141.4$ ($c = 0.99$, THF). CD (MeCN): 211 (+1.99), 224 (–3.54), 237 (+5.51), 265 (–6.08), 290 (–1.69), 325 (+1.28).

Enantiomeric purity was determined by HPLC on a series of chiral columns selected according to their resolution efficacy, as determined for racemic mixtures of the compounds listed in *Table 9*.

The chromatographic parameters were calculated according to the standard method described in [23].

Thermodynamic parameters of the temp.-dependent kinetic resolution were calculated according to [18a,b], using equation $\ln E = \Delta\Delta S^{\neq}/R - \Delta\Delta H^{\neq}/RT$; r^2 was over 0.98.

MM2 Calculations were performed by *ChemOffice Ultra 4.5*, *CambridgeSoft Corp.*, Massachusetts, USA.

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