Synthesis of Optically Active α-Methylene *β*-Lactams through Lipase-Catalyzed Kinetic Resolution

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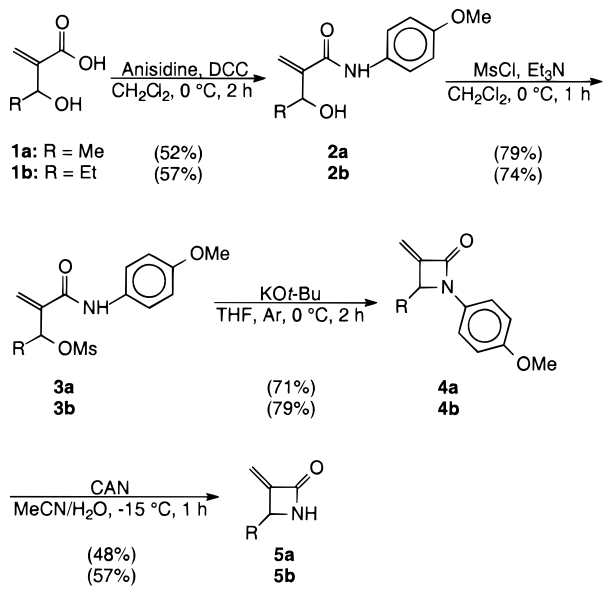
A convenient method for the preparation of the hitherto unknown chiral α-methylene *β*-lactam derivatives **5a**,**b** is reported. The optically active α-methylene *β*-lactams **5a**−**c**, and their corresponding amino acids **6a**−**c** have been readily made available through lipase-catalyzed kinetic resolution in high enantiomeric purity (up to 99% ee). The *N*-substituted *β*-lactam derivatives **4a**,**b** and **10** are not accepted by the lipases and were prepared in optically active form by chemical transformation.

Introduction

Enzymatic transformations of organic substrates have become a well-established method for the preparation of optically active compounds.[1] Enzymes efficiently catalyze a broad spectrum of chemical reactions under mild and environmentally acceptable conditions, often with very high selectivity. Among these biocatalysts, lipases play an outstanding role in enzymatic synthesis, since they are readily available, easy to handle, and require no cofactors nor any elaborate experimental setup.

Recently we have reported[2] the preparation of optically active α-methylene *β*-lactones, versatile building blocks in organic synthesis,[3] through lipase-catalyzed kinetic resolution. The nitrogen analogues of α-methylene *β*-lactones, in particular, the unprotected (free NH) α-methylene *β*-lactams, are so far not known in optically active form,[4] yet they may also serve as chiral synthons in organic synthesis. Therefore, there is a need for the preparation of optically active α-methylene *β*-lactams, e.g., through the possibility of enzymatic kinetic resolution.

Scheme 1. Synthesis of the Racemic α-Methylene *β*-Lactams 5a,b



Herein we report on the synthesis of two hitherto unknown racemic derivatives (**5a**,**b**) of α-methylene *β*-lactam (Scheme 1) and their kinetic resolution through lipase-catalyzed hydrolysis.

Furthermore, the preparation of the optically active *N*-protected derivatives **4a**,**b** and **10** is described by chemical transformations, because lipases do not serve the purpose.

Results and Discussion

The racemic α-methylene *β*-lactams **5a**,**b** were prepared in a multistep synthesis (Scheme 1) by amidation of the hydroxy acids **1** with *p*-anisidine as nitrogen source and dicyclohexylcarbodiimide (DCC) as coupling agent. The hydroxy amides **2** were converted to the mesyl

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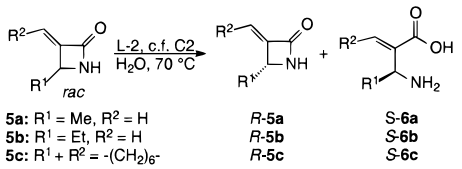
Table 1. Lipase-Catalyzed Resolution of β-Lactams 5



substrate	substrate: enzyme (μmol)/(mg)[c]	time (h)	convn[a] (%)	enantiomeric excess (% ee)[b] lactam **5**	enantiomeric excess (% ee)[b] amino acid **6**[d]	E[e]
5a	315:250	86	52	96	90	74
5b	315:250	24	50	99	98	>200
5c	315:500	68	49	94	97	>200

[a] The % conversion was calculated from the enantiomeric excess of the starting material (ee_s) and the product (ee_p) according to % convn = $ee_s/(ee_s + ee_p)$ (ref 7). [b] The enantiomeric excess (ee) was determined by HPLC (Chiralcel OB-H and Chiralpak AS), error ca. 1% of the stated values. [c] Chirazyme L-2 from *Candida antartica*, carrier-fixed. [d] **6** was derivatized prior to HPLC analysis (see Supporting Information). [e] The enantioselectivity (E) was calculated (ref 7) from the enantiomeric excess of the starting material (ee_s) and the conversion according to

$$E = \ln[(1 - \text{convn})(1 - ee_s)]/\ln[(1 - \text{convn})(1 + ee_s)]$$

amides **3** in good yields with mesyl chloride and triethylamine. Treatment of the mesyl amides **3** with potassium *tert*-butoxide afforded the β-lactams **4** also in good yields, despite the considerable ring strain in the α-methylene β-lactam. The aryl-substituted lactams **4** were oxidatively[5] deprotected with ammonium cerium(IV) nitrate[6] at subambient temperatures. Due to the lability of these β lactams, especially toward acids, only moderate yields were achieved.

The racemic lactam **5b** was then submitted to enzyme screening to select the optimal biocatalyst for its enantioselective hydrolysis. From the numerous enzymes tested, only the lipase Chirazyme L-2 (from *Candida antartica*), which has been successfully employed in the resolution of α-methylene β-lactones,[2] accepted well the β-lactams **5** as substrates. Chirazyme L-1 (from *Burkholderia sp.*) and Chirazyme L-6 (from *Pseudomonas sp.*) showed no activity, even on prolonged reaction times. With Chirazyme P-1, a protease, and Penicillin G Amidase (PGA 450) also no conversion of the test substrate **5b** was observed.

Since the enzymatic hydrolysis of the lactam **5b** with the lipase L-2 proceeded highly selectively but very slowly at room temperature, the reaction temperature was raised to 70 °C to enhance the reaction rate. Fortunately, even at this relatively high temperature, the activity of the enzyme persisted sufficiently, but it rapidly lost activity at higher temperatures. To enhance enzyme stability and allow ease of separation of the enzyme from the amino acids **6** in the aqueous phase, the carrier-fixed L-2 enzyme was used. For the zwitterionic amino acids **6** there is no pH change of the solution as they are formed and, therefore, no buffer was necessary. In a control experiment without enzyme, the lactam **5b** was not hydrolyzed under these reaction conditions.

Scheme 2. Configurational Assignment of the β-Lactams 5a,b

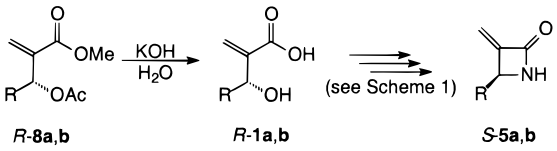
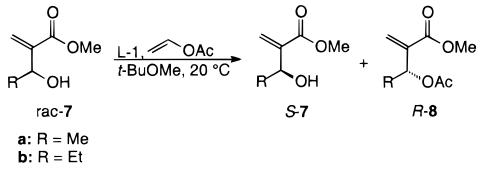


Table 2. Lipase-Catalyzed Resolution of Hydroxy Esters 7 on the Preparative Scale



substrate[d]	time (h)	convn[a] (%)	enantiomeric excess (% ee)[b] *S*-**7**	enantiomeric excess (% ee)[b] *R*-**8**	yield (%)[c] *S*-**7**	yield (%)[c] *R*-**8**	E[e]
7a	19	53	99	87	69	78	75
7b	91	50	98	98	83	77	>200

[a] See Table 1. [b] The enantiomeric excess (ee) was determined by HPLC (Chiralcel OD), error ca. 1% of the stated values. [c] Based on 50% conversion. [d] The substrate−enzyme ratio was 19.3 mmol/250 mg. [e] See Table 1.

In the enzyme-catalyzed enantioselective hydrolysis of the lactams **5a**−**c** (Table 1) with L-2 lipase under the above conditions, essentially perfect kinetic resolution was achieved for the ethyl derivative **5b**, for which enantiomeric excesses (ee) of 99% for the lactam **5b** and 98% for the corresponding amino acid **6b** were obtained. In the case of the sterically less-demanding methyl derivative **5a**, a slightly decreased enantioselectivity was found, with 96% ee for the residual lactam **5a** and 90% ee for the amino acid **6a**. Surprisingly, a significant longer (ca. 60 h) reaction time was necessary for this substrate in comparison to that of the ethyl derivative **5b** to reach a conversion of about 50%. For the bicyclic lactam **5c**, a conversion of 49% required a 2-fold excess of the L-2 enzyme and 68 h reaction time, which afforded ee values of 94% for the lactam **5c** and 97% for the amino acid **6c**.

The absolute configuration of the lactams **5a**,**b** was assessed by chemical correlation (Scheme 2). For this purpose, the hydroxy esters **7a**,**b** were prepared as optically active starting materials by lipase-catalyzed kinetic resolution (Table 2), which has been already reported,[8] but substantially improved through the use of the L-1 enzyme instead of Amano AK. In this way, the hydroxy esters **7a**,**b** were obtained with 99% and 98% ee, while the acetates **8a**,**b** possessed ee values of 87% and 98%. Hydrolysis of the esters **7** or the acetates **8** gave the optically active hydroxy acids **1a**,**b**, which were then employed in the lactam synthesis described for the racemic lactams **5a**,**b**. Due to the inversion at the stereogenic center during the ring closure, the *S*-configured lactams **5a**,**b** were obtained from the *R*-configured hydroxy acids **1a**,**b**.

The absolute configuration of lactam **5c** was established by the CD exciton chirality method.[9] For this

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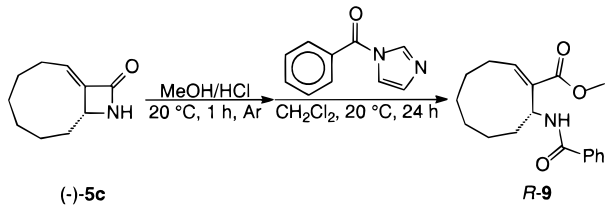
Scheme 3 Derivatization of the Lactam 5c for CD Spectroscopy

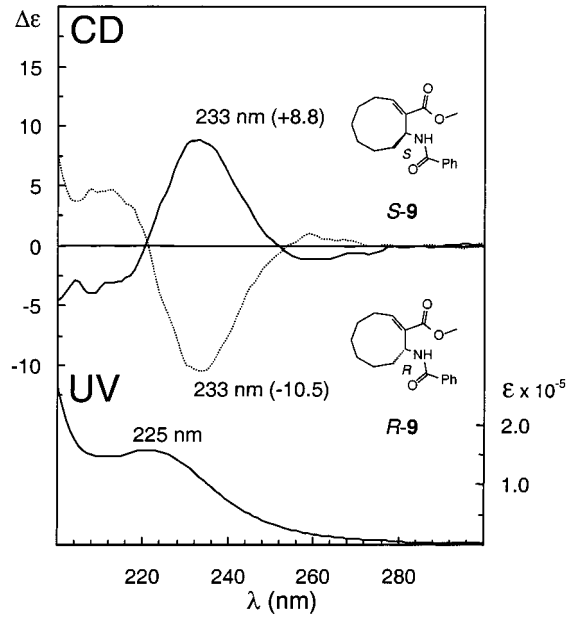


purpose, the benzoyl group, which has already been employed in the configurational assignment of allylic and homoallylic alcohols,[10] was introduced as second chromophore (Scheme 3). Therefore, the optically active lactam **5c** and amino acid **6c**, which were obtained by kinetic resolution of racemic **5c**, were converted first into the methyl ester with hydrogen chloride in methanol and then with benzoylimidazole into the benzoic amide **9**, the latter being directly used for the CD measurements.

The UV and CD spectra of both enantiomers of **9** are shown in Figure 1. The CD spectrum of *S*-**9** revealed a positive Cotton effect at 233 nm ($\Delta\epsilon = +8.8$), and the *R* enantiomer showed a negative one also at 233 nm ($\Delta\epsilon = -10.5$). The CD of amide **9** results from the exciton coupling between the α,β-unsaturated ester (ca. 210 nm) and the ^1L_a transition band of the benzoate (ca. 230 nm). As observed for the optically active amide *S*-**9**, the first Cotton effect at 233 nm is positive if the axes of the two chromophores possess the sense of a right-handed screw. Since the exciton chirality depends on the conformation of the molecule, calculations with Macromodel 5.0[11] (modified Allinger MM2 force field) were performed to assess the preferred conformation. From these calculations it is seen that the two chromophores are oriented in a clockwise sense to each other in the *S* enantiomer and counterclockwise in the *R* enantiomer (Figure 1), which results in a positive first Cotton effect for *S*-**9** and a negative one for *R*-**9**, which is in very good agreement with our experimental data.

The results of the CD spectroscopy are also confirmed by the established enantioselectivity of the enzyme, since in all our cases the *S* enantiomer is accepted with high preference and hydrolyzed, while the optically active *R* enantiomer is left behind. Furthermore, the elution order for *S* and *R* enantiomers of the lactams **5a**−**c** on the chiral HPLC is also in agreement with the conformation assigned to the **5c** derivative.

In the enzymatic resolution of the α-methylene β-lactams, the ring-nitrogen atom of the lactam must not be substituted for lipase acceptance. This is evidenced by the fact that neither the *para*-methoxyphenyl-substituted lactams **4** nor the benzyl-substituted derivative **10** are converted under the reaction conditions used for the lactams **5a**−**c**. Nevertheless, the benzyl-protected lactam **10** may be synthesized from optically active amide **11** according to the route depicted in Scheme 1. The latter



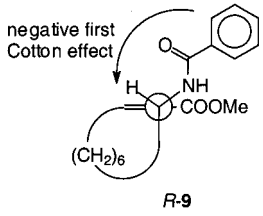
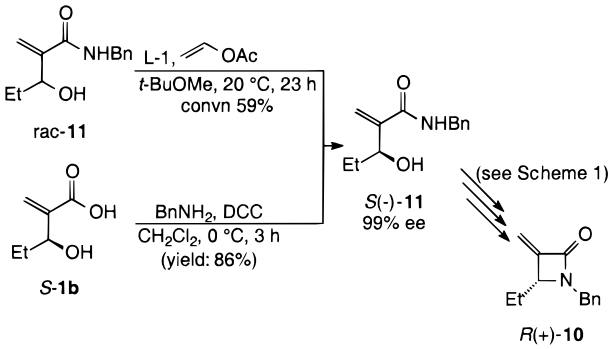


Figure 1. CD and UV spectra of the amino-ester derivatives *R*- and *S*-**9** in acetonitrile and the favored conformation assessed by computational studies with Macromodel 5.0.

Scheme 4. Preparation of the Optically Active *N*-Benzyl-Substituted β-Lactam 10



was made available by kinetic resolution with the L-1 enzyme to afford the amide *S*-**11** with an enantiomeric excess of 99% and the acetate *R*-**12** with 68% ee at a conversion of 59% (Scheme 4). Alternatively, the amide *S*-**11** is also accessible by coupling of benzylamine with the hydroxy acid *S*-**1b**.

Conclusions

Optically active α-methylene β-lactams **5**, which are attractive chiral building blocks for organic synthesis, have been prepared for the first time by lipase-catalyzed kinetic resolution in nearly enantiomerically pure form. Although the β-lactams **5** are good substrates for lipase L-2, the *N*-protected derivatives **4** are not converted by

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(11) All calculations were carried out with MM2 force field in $CHCl_3$; at least 1000 conformers were searched for each simulation.

this enzyme. The latter substrates are accessible in high optical purity from the corresponding optically active hydroxy acids **1**, which are again readily prepared by lipase-catalyzed kinetic resolution. Thus, a variety of optically active α-methylene *β*-lactams have been made available for synthetic applications.

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Supporting Information Available: Experimental details and the full characterization of the amides **2a**,**b**, the mesylates **3a**,**b**, the *N*-protected *β*-lactams **4a**,**b,** the α-methylene *β*-lactams **5a**,**b**, the amino acids **6a**,**b**, and the ester amide **12** are given. This material is available free of charge in the Internet under http://pubs.acs.org.

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