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Convenient double-enzymatic synthesis of both enantiomers of 6-methyl-ε-caprolactone

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Abstract—Both enantiomers of 6-methyl-ε-caprolactone (6-MeCL) are obtained in high enantiomeric excess by the combination of an enzymatic ring opening of racemic 6-methyl-ε-caprolactone and subsequent enzymatic ring closure. Immobilized *Candida antarctica* lipase B (Novozym 435) was selected as the biocatalyst for both the ring-opening and the ring-closing reaction. This route provides ready access to enantiopure (*S*)-6-MeCL (ee = 99.6%) and (*R*)-6-MeCL (ee = 98.8%).

1. Introduction

Over the last decade, enantioselective (also referred to as stereoselective) polymerizations of racemic lactones have shown promise as a method for preparing optically active polymers. Lipases, for example, were found to be efficient catalysts for the enantioselective ring-opening polymerization of lactones.[1] The recent use of Novozym 435 (*Candida antarctica* lipase B immobilized on an acrylic resin) in the polymerization of 4-methyl-ε-caprolactone (4-MeCL) and 4-ethyl-ε-caprolactone (4-EtCL) led to the formation of enantioenriched poly-(*S*)-4-MeCL and poly-(*S*)-4-EtCL.[1e,2] The combination of the enantioselective polymerization of 4-MeCL with the controlled radical polymerization, such as an atom transfer radical polymerization or nitroxide-mediated radical polymerization, afforded chiral block copolymers through a one-pot approach.[2,3]

Recently, we have become interested in (*R*)- and (*S*)-6-ε-methylcaprolactone (6-MeCL) as building blocks for chiral poly-6-MeCL.[4] For a thorough study of this system, we considered it desirable to have both enantiomers of 6-MeCL at our disposal. Previous research has shown that (*R*)-6-MeCL can be easily obtained through kinetic resolution of 6-MeCL using lipases, since it is the slower reacting enantiomer.[5] However, to date the only method to obtain (*S*)-6-MeCL is through either an elaborate, multi-step procedure or by an enantioselective Bayer–Villiger oxidation using cyclohexanone monooxygenases.[6] Although the latter method affords (*S*)-6-MeCL in high enantiomeric excess (E >200), such biochemical oxidations require the use of whole cells of engineered *Escherichia coli* strains, which are not readily available in every chemical laboratory.

Recently, we observed that the enzymatic ring opening of 6-MeCL by an equimolar amount of a nucleophile is a reversible reaction and suffers from a rather unfavorable equilibrium. Therefore, we anticipated that it should be possible to isolate the ring-opening product and perform, quantitatively, an enzymatic ring closure by distilling off the regained cyclic structures. Herein, we report on a two-step enzymatic synthesis of both enantiomers of 6-MeCL using (1) the well-established selectivity of Novozym 435 to ring open the (*S*)-enantiomer and isolate the unreacted (*R*)-lactone and (2) enzymatic ring closure of the resulting ring-opening product yielding the (*S*)-lactone. This affords a straightforward and accessible approach that allows for the synthesis of both (*R*)- and (*S*)-6-MeCL (Scheme 1).

2. Results and discussion

Previous research has shown that the kinetic resolution of 6-MeCL by butanolysis using Novozym 435 shows (*S*)-selectivity, although with low enantioselectivity (E-values

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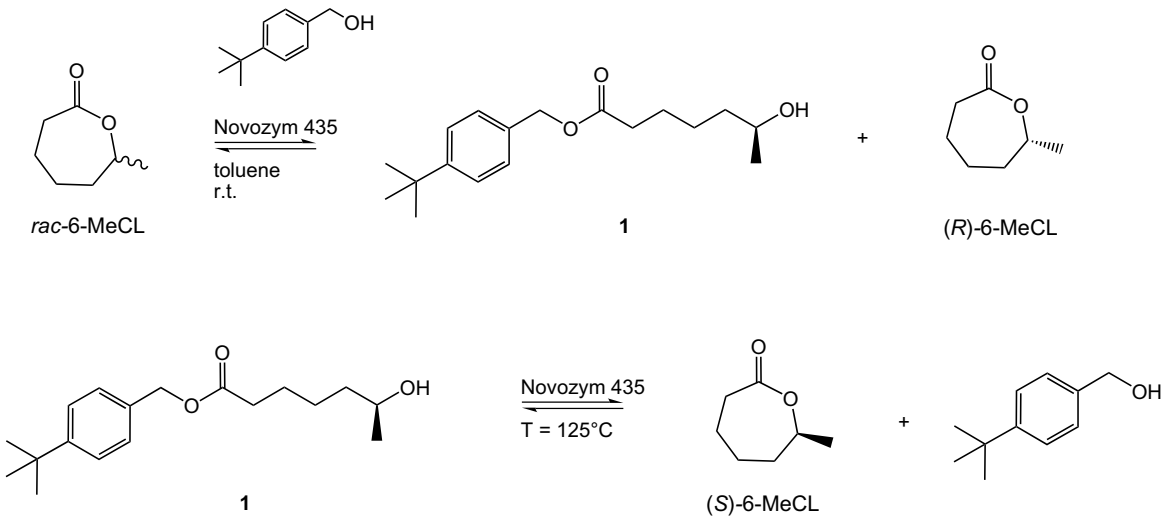


Scheme 1.

of 3–7 were reported).[7] We adapted this procedure and performed the enzymatic ring opening of 6-MeCL in toluene at room temperature. 4-*tert*-Butylbenzyl alcohol (TBBA) was selected as the nucleophile, providing ring-opening product **1**, which has a sufficiently high boiling point for convenient removal by distillation of the remaining (*R*)-6-MeCL (Scheme 1). The addition of 1,2,3,4-tetramethylbenzene as an internal standard to the reaction mixture means that the reaction kinetics could be easily monitored by chiral GC. Fitting the enantiomeric excess (ee) of (*R*)-6-MeCL as a function of 6-MeCL conversion according to the method of Chen et al., resulted in an enantiomeric ratio (*E* ratio) of 11.[8]

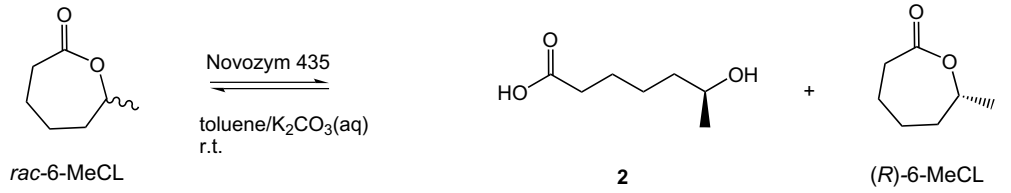
Using this approach, **1** was isolated with an ee of 62% (yield 47%).[9] Distillation and subsequent work-up of the remaining lactone afforded (*R*)-6-MeCL with an ee of 84.2% in a yield of 38% (see Table 1, entry 1). The enantiomeric excess of (*R*)-6-MeCL was further increased by enzymatic hydrolysis, which also occurs with preference for the (*S*)-enantiomer (Scheme 2).[5b] The reaction was carried out in a toluene/water biphasic system at room temperature. The addition of K_2CO_3 to the aqueous phase ensured that the hydroxyacid product **2** was extracted from the organic phase, shifting the equilibrium completely to the product side. The reaction was monitored by chiral GC, and, after complete hydrolysis of (*S*)-6-MeCL, (*R*)-6-MeCL, was isolated from the organic phase with an ee of 98.8% in an overall yield of 25% (see Table 1, entry 2).

Table 1. Results of the enzymatic ring opening and closing of 6-MeCL

Entry	Compound	$[\alpha]_D$[a]	ee (%)	Yield[b] (%)
1	(*R*)-6-MeCL	n/d[c]	84.2	38
2	(*R*)-6-MeCL	+24.8	98.8	25[d]
3	(*S*)-6-MeCL	n/d[c]	95.2	30
4	(*S*)-6-MeCL	−25.9	99.6	13[d]

[a] $c = 13$ g/100 mL in $CHCl_3$.
[b] Maximum attainable yield for each enantiomer = 50%.
[c] n/d = not determined.
[d] Overall yield.

Since enzymatic reactions are equilibria, **1** can be subjected to enzymatic ring closure, yielding enantiomerically enriched 6-MeCL and the starting alcohol TBBA (Scheme 1). By distilling off 6-MeCL and TBBA, the equilibrium is shifted completely to the lactone side. Since the sense of chirality for both the forward reaction as well as the reverse reaction is by definition the same, enzymatic ring closure will also occur with enantiopreference for the (*S*)-enantiomer of 6-MeCL.[8] Using this procedure, the enzymatic ring-closure distillation was performed under reduced pressure (0.05 mmHg) and at 125 °C. The absence of a non-enzymatic ring closure was confirmed in a blank reaction. After column chromatography to remove TBBA, (*S*)-6-MeCL was isolated with an ee of 95.2% and a yield of 30% (see Table 1, entry 3). This ring closure demonstrates that, as expected, the reverse reaction is (*S*)-selective, as the ee increased from 62.0% in **1** to 95.2% in (*S*)-6-MeCL. The remaining (*R*)-6-MeCL was recovered as oligomeric species in the residual fraction after distillation. After removal of



Scheme 2.

trace amounts of water by storage on molar sieves, (*S*)-6-MeCL crystallized as transparent needles (melting point 31 °C).

To further increase the enantiomeric excess, (*S*)-6-MeCL with an ee of 95% was subjected to a second cycle of enzymatic ring opening and ring closure. This yielded nearly enantiomerically pure (*S*)-6-MeCL with an ee of 99.6% in an overall yield after two cycles of 13% (see Table 1, entry 4). Characterization of the enantiomers by specific rotation values showed that the $[\alpha]_D$ of (*R*)-6-MeCL was +24.8 (entry 2) and $[\alpha]_D$ of (*S*)-6-MeCL was −25.9 (entry 4). This is in good agreement with the previously reported values of +25.0 for (*R*)-6-MeCL an −25.1 for (*S*)-6-MeCL.[6b]

3. Conclusions

In conclusion, a double kinetic resolution involving enzymatic ring opening and ring closure was developed to obtain both enantiomers of 6-MeCL. Both (*R*)- and (*S*)-6-MeCL were obtained in excellent enantiomeric excess of 99% and in acceptable overall yields of 25% and 13%, respectively. This convenient route provides easy access to these enantiomers without the need for multi-step procedures or biochemical oxidations.

4. Experimental

4.1. Materials

6-Methyl-ε-caprolactone (6-MeCL) was synthesized by a Baeyer–Villiger oxidation of 2-methylcyclohexanone following a reported procedure.[2] 2-Methylcyclohexanone was purchased from Fluka and used as received. Novozym 435 was obtained from Novozymes A/S. All other chemicals were purchased from Aldrich and used as received unless otherwise noted.

4.2. Analytical methods

^{1}H and ^{13}C NMR spectra were measured using a Varian Mercury Vx 400 spectrometer (400 MHz) in $CDCl_3$. Chiral gas chromatography (GC) was performed on a Shimadzu 6C-17A GC equipped with an FID employing a Chrompack Chirasil-DEX CB (DF = 0.25) column. Injection and detection temperatures were set at 250 and 300 °C, respectively. Separations were carried out with the column temperature programmed to increase in temperature from 125 to 225 °C, which afforded in all cases baseline separation of the enantiomers of 6-MeCL. The internal standard method using 1,2,3,4-tetramethylbenzene as the internal standard was employed to determine the lactone conversions. The enantiomeric excess (ee) was calculated as follows: $ee = (R - S)/(R + S)$ where *R* and *S* represent the surfaces of the GC peaks of the (*R*)- and (*S*)-enantiomer, respectively. All samples were measured using a Shimadzu AOC-20i autosampler. The yields of the (*R*)- and (*S*)-enantiomers were calculated with the assumption that 50% is the maximum attainable yield in a kinetic resolution for each enantiomer. Optical rotations were measured at ambient temperature in $CHCl_3$ on a Jasco DIP-370 digital polarimeter. TLC staining was performed with 1% *p*-methoxybenzaldehyde in a mixture of ethanol, acetic acid, and H_2SO_4 90/5/5 v/v and subsequent heating with a heat gun.

4.3. Synthesis and isolation of (*R*)- and (*S*)-6-MeCL

4.3.1. Synthesis of compound 1 and isolation of (*R*)-6-MeCL. Novozym 435 (6.0 g) was added to a 10 mL sample vial. The vial was placed overnight in a vacuum oven (10 mmHg) at 50 °C in the presence of P_2O_5. The oven was backfilled with nitrogen and the vial removed from the oven. 4-*tert*-Butylbenzyl alcohol (TBBA, 14.7 g; 89.6 mmol), 6-MeCL (20.2 g; 157.6 mmol), 1,2,3,4-tetramethylbenzene (0.5 g; 4.2 mmol), and toluene (25 mL) were added to a 100 mL round-bottomed flask. The dried Novozym 435 was added to the flask, representing the start of reaction. The mixture was stirred at room temperature for 3 h. During the reaction, samples (~0.02 mL) were withdrawn from the reaction mixture using a glass Pasteur pipette. The sample was diluted with dichloromethane and the enzyme removed from the sample by filtration over cotton wool. The samples were analyzed by chiral GC for conversion of TBBA and both enantiomers of 6-MeCL. At 92% (*S*)-6-MeCL conversion, the enzymatic reaction was stopped by filtration using a class 3 glass filter. The residual enzyme was flushed three times with dichloromethane. The filtrate was concentrated and the remaining 6-MeCL and TBBA were removed by distillation using a Kugelrohr apparatus (115 °C, 0.05 mmHg), yielding **1** (24.4 g; 47%). ^{1}H NMR of **1**: δ (ppm) 7.3–7.4 (m, Ar–*H*), 5.1 (Ar–CH_2–O), 3.8 (m, C*H*(CH_3)OH), 2.4 (m, benzyl–CH_2–OCOCH_2), 1.70–1.35 (m, OCOCH_2(CH_2)$_3$), 1.3 (Ar–C(CH_3)$_3$), 1.15 (d, CH_3).

The mixture of TBBA, 1,2,3,4-tetramethylbenzene, and (*R*)-6-MeCL, was further purified by column chromatography over neutral aluminum oxide using dichloromethane/acetic acid 99/1 v/v as the eluent. Two fractions were obtained: fraction 1 ($R_f = 0.7$, (*R*)-6-MeCL, 7.75 g (38%), ee 84.2%, chemical purity 94%, impurities are 2-methyl-ε-caprolactone (5%) and 1,2,3,4-tetramethylbenzene (1%)) and fraction 2 ($R_f = 0.4$, TBBA, 1.3 g).

4.3.2. Enzymatic ring closure of 1. Novozym 435 (2.0 g) was added to a 5 mL sample vial. The vial was placed overnight in a vacuum oven (10 mmHg) at 50 °C in the presence of P_2O_5. The oven was backfilled with nitrogen and the vial removed from the oven. Compound **1** (24.4 g; 83.6 mmol) and the dried Novozym 435 were added to a Kugelrohr flask. The system was heated to 125 °C. The lactone and TBBA were distilled off under reduced pressure (0.05 mmHg). The mixture of TBBA and (*S*)-6-MeCL was separated by column chromatography over neutral aluminum oxide using dichloromethane/acetic acid 99/1 v/v as the eluent. Two fractions were obtained: fraction 1 ($R_f = 0.7$, (*S*)-6-MeCL) and fraction 2 ($R_f = 0.4$, TBBA, 8.9 g). After removal of trace water by storage on 3 Å molecular sieves, 5.7 g (*S*)-6-MeCL (30%) was obtained

as transparent needle crystals (ee 95.2%, chemical purity 99%, both determined by chiral GC). Mp = 31 °C.

4.3.3. Enzymatic hydrolysis of remaining (*S*)-6-MeCL in (*R*)-6-MeCL. (*R*)-6-MeCL (6.6 g; 51.5 mmol) with an ee of 84.2% was dissolved in 6 mL toluene and transferred to a 50 mL round-bottomed flask. Novozym 435 (0.4 g) and 3 mL of a 1 M K_2CO_3 solution in water were added to the mixture. The mixture was stirred vigorously at room temperature. During the reaction, samples (~0.02 mL) were withdrawn from the reaction mixture using a glass Pasteur pipette. The sample was diluted with dichloromethane and the enzyme was removed from the sample by filtration over $MgSO_4$ on cotton wool. The samples were analyzed by chiral GC for conversion of both enantiomers of 6-MeCL. After all (*S*)-6-MeCL had been consumed, the enzymatic reaction was stopped by filtration using a class 3 glass filter. The residual enzyme was flushed three times with dichloromethane. All solvents were removed in vacuo and the crude product was dissolved in dichloromethane. The dissolved product was washed twice with water and once with brine. The organic layer was concentrated, yielding 4.3 g of (*R*)-6-MeCL (65%, ee 98.8%, chemical purity 94%, impurities are 2-methyl-ε-caprolactone (5%) and 1,2,3,4-tetramethylbenzene (1%)). $[\alpha]_D = +24.8$ {lit.[6b] $[\alpha]_D = +25.0$ (*c* 1.8, $CHCl_3$)}.

4.3.4. Enzymatic ring opening and ring closure of (*S*)-6-MeCL. (*S*)-6-MeCL (4.46 g; 34.8 mmol, ee = 95.2%) was subjected to enzymatic ring opening by TBBA and subsequent enzymatic ring closure analogous to the procedure described above, using 10.7 g TBBA (65.2 mmol) and 3.0 g Novozym 435 for the ring-opening and 2.0 g Novozym 435 for the ring-closure procedure. The mixture of TBBA and (*S*)-6-MeCL was separated by column chromatography over neutral aluminum oxide using dichloromethane/acetic acid 99/1 v/v as the eluent. Then, 1.89 g (*S*)-6-MeCL (42%) was obtained with an optical purity of 99.6% and a chemical purity of 99% (both determined by chiral GC). $[\alpha]_D = -25.9$ {lit.[6b] $[\alpha]_D = -25.1$ (*c* 1.7, $CHCl_3$)}.

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9. The ee of the product was calculated based on conversions of both enantiomers of 6-MeCL (see Ref. 1e for details).