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Asymmetric desymmetrization of dialkyl bicyclo[2.2.1]hept-2,5-diene-2,3-dicarboxylates by a thermophilic esterase/lipase

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Abstract—A thermophilic enzyme efficiently monohydrolyzed a series of *meso* diesters, dialkyl bicyclo[2.2.1]hept-2,5-diene-2,3-dicarboxylates, in high chemical yields (72–98%) and high enantiomeric purities (>99% e.e.) in all the cases examined.

1. Introduction

Enzymatic asymmetric hydrolysis has been a powerful tool in the development of methodologies for the asymmetric synthesis of a variety of natural products. In particular, lipases and esterases are among those enzymes most widely applied in organic synthesis. Desymmetrization of symmetric diesters using these esterases/lipases is a very versatile concept because it potentially affords only one enantiomer in quantitative yield without wasting the other enantiomer, allowing regiospecific stereochemical conversions at later steps. Additionally, the starting diesters can be easily prepared from inexpensive sources.

Recently, enzymes from a diverse range of microorganisms in extreme environments of temperature, pH and osmotic and hydrostatic pressure as well as salt concentrations, have been showing new types of activities as well as substrate specificities.[1] These enzymes, representing a new class are referred to as 'extremozymes,' and thermophilic enzymes (thermophiles) are one example. However, the applications of these extremozymes in organic synthesis are still limited to only a few examples despite their expected utility, particularly in industrial processes, where their unique stability in extreme environments could lead to useful applications. Such enzymes are expected to be more thermally robust and more stable in organic solvents. Precedent examples, albeit few, include the resolution of epimers by ester hydrolysis and polymerization by lactone opening.[2]

Herein, we report the first example of desymmetrization by asymmetric monohydrolysis of symmetric diesters, applying a thermophilic esterase/lipase.

2. Results and discussion

Norbornane derivatives are known to be useful building blocks because of their uniquely strained skeletons. Half-esters obtained by desymmetrization of some norbornane derivatives have been shown to be useful in the development of elegant synthetic methodologies.[3] We therefore investigated the asymmetric monohydrolysis of a series of dialkyl bicyclo[2.2.1]hept-2-ene-2,5-dicarboxylates, **1a**–**c**, applying the thermophilic enzyme ESPESL 1864, which was developed as one of the enzymes in the thermophilic esterase/lipase library consisting of recombinant protein catalysts, many of which are derived from thermophilic organisms.[4] The diesters, **1a**–**c**, can be easily prepared quantitatively by a one-step Diels–Alder reaction of cyclopentadiene with the corresponding dialkyl acetylenedicarboxylates. Enantiomerically enriched half-esters of these derivatives could serve as very versatile building blocks for the synthesis of a variety of compounds. However, a more common enzyme, pig liver esterase (PLE), is known to monohydrolyze **1a** only in low enantiomeric purity.[5] In our experiments, PLE monohydrolyzed **1a**–**c**, with only 32% e.e. (**1a**), 41% e.e. (**1b**), and 71% e.e. (**1c**), although the chemical yields were reasonably high. Lipase AY from *Candida rugosa*[6] also showed modest reactivity, but the enantiomeric purities were also low. Lipases A, AK, D, F-AP15, G, M, PS, or S induced no reactions after incubation for 48 h (Scheme 1).[6]

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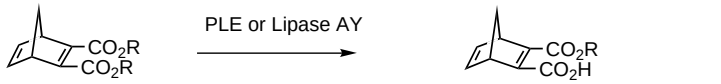


1a R=Me
1b R=Et
1c R=^{n}Pr

2a R=Me 99%y. 32% e.e. (by PLE)
61% y. 78% e.e. (by lipase AY)
2b R=Et 89%y. 38 % e.e. (by PLE)
44% y. 41% e.e. (by lipase AY)
2c R=^{n}Pr 99%y. 71% e.e. (by PLE)
48% y. 14% e.e. (by lipase AY)

Scheme 1.

However, when these symmetric diesters were incubated with ESPESL 1864 at 70°C in sodium phosphate buffer containing 1% acetone, the corresponding optically active half-esters, **2a**–**c**, were obtained in quite high chemical yields (**2a**: 98%, **2b**: 93%, **2c**: 72%) after routine work-up and column chromatography. The enantiomeric purities for these half-esters were determined to be >99% in all cases, based on chiral gas chromatographic analysis. All reactions reached completion quickly under these conditions; the reaction times required for monohydrolysis of the dimethyl ester, **1a**, was 1.5 h; diethyl ester, **1b**, 1.5–2 h; and the di-*n*-propyl ester, 2 h. Interestingly, when the reaction was conducted at 37°C with all other conditions remaining the same, the reaction required a far longer time (2–3 days), and about 10% lower enantiomeric purities were observed for all the esters.

The absolute configurations of the half-esters, **2a**–**c**, were determined as depicted in Scheme 2 based on the sign of the specific rotation values for the same half-esters, **2a**–**c**, prepared from a D-mannitol derivative, which we reported earlier.[7]

It seems that this thermophilic enzyme has a higher affinity toward less hydrophobic ester groups than toward more hydrophobic groups. The decreased yield and somewhat longer reaction time required for the hydrolysis, especially for the hydrophobic diesters, **1c** and **1b**, may be related to this activity, while the yields and enantiomeric purities are still excellent.

Several other chiral building blocks that possess norbornane skeletons are known to be produced enzymatically in high enantiomeric purities.[8] While these derivatives are expected to serve as useful chiral building blocks, half-esters **2a**–**c** are easily obtained from diesters **1a**–**c**, which are easily and inexpensively prepared by a one-step Diels–Alder reaction. Therefore, these half-esters are also considered to be new versatile entities for which synthetic utility is expected.

3. Conclusions

In summary, we have found that the thermophilic enzyme ESPESL 1864 monohydrolyzes symmetric diesters, **1a**–**c**, in high chemical yields in enantiomerically pure form. Since these diesters can be prepared quite easily, synthetic utility of these half-esters is expected.

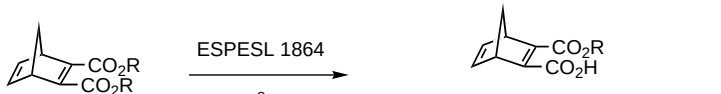
4. Experimental

4.1. General procedures

All melting points are uncorrected. ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz spectra were measured as solutions in $CDCl_3$ using TMS as an internal standard. The IR spectra were recorded on an FTIR instrument. The enantiomeric purity was measured by gas chromatography on a chiral column, CycloSil B, at 160°C for methyl and ethyl ester, **2a** and **2b**, and *n*-propyl ester, **2c**, at 170°C, under isothermal conditions. ESPESL 1864 was used as a sodium phosphate buffer solution (50 mM, pH 7), in which the enzyme concentration was 1 mg/50 μL buffer. The enzyme we applied here exhibited approximately 490 units/mL, which was determined using *para*-nitrophenyl butylate as a substrate.

4.2. Enzymatic monohydrolysis of dimethyl ester, 1a

Dimethyl ester **1a** (21 mg, 0.10 mmol) was dissolved in acetone (0.1 mL), and 0.1 M sodium phosphate buffer (pH 7.5, 10 mL) was added. To this mixture, ESPESL 1864 solution (0.4 mg, 20 μL of 1 mg enzyme/50 μL pH



1a R=Me
1b R=Et
1c R=^{n}Pr

2a R=Me 98%y. >99% e.e.
2b R=Et 93%y. >99 % e.e.
2c R=^{n}Pr 72%y. >99% e.e.

Scheme 2.

7 (50 mM) sodium phosphate buffer solution) was added, and the mixture was incubated at 70°C for 1.5 h with gentle stirring until the consumption of the starting diester was detected by thin layer chromatography. The reaction was cooled in a water bath (~5–10°C), adjusted to pH 10 with 1N NaOH solution, and then immediately washed with ethyl acetate (three times); this extract was discarded. The reaction mixture was immediately acidified with 1N HCl solution (pH 3), extracted with ethyl acetate (three times), washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to afford the half-ester, **2a**, as a white solid (19 mg, 98%). Mp 111–112°C (from hexane–ethyl acetate). >99% e.e. $[\alpha]_D^{22} = -25.7$ ($c = 1.9$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) $\delta = 2.12$ (1H, d, $J = 7.2$ Hz), 2.21 (1H, d, $J = 7.2$ Hz), 3.93 (3H, s), 4.1 (1H, br.s), 4.2 (1H, br.s), 6.8–6.9 (2H, m). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 53.37$, 53.75, 54.74, 72.69, 141.71, 142.71, 150.97, 162.45, 162.52, 168.16; IR (KBr, cm^{-1}) 2500–2800, 1720, 1631, 1601; anal. calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.86; H, 5.28%.

4.3. Enzymatic monohydrolysis of diethyl ester, 1b

Diethyl ester **1b** (37 mg, 0.16 mmol) was dissolved in acetone (0.2 mL), and 0.1 M sodium phosphate buffer (pH 7.5; 20 mL) was added. To this mixture, ESPESL 1864 solution (0.4 mg, 20 μL of 1 mg enzyme/50 μL pH 7 (50 mM) sodium phosphate buffer solution) was added and the mixture was incubated at 70°C for 2 h with gentle stirring until the consumption of the starting diester was detected by thin layer chromatography. The reaction was cooled in a water-bath (~5–10°C), adjusted to pH 10 with 1N NaOH solution, and immediately washed with ethyl acetate (three times); this extract was discarded. The reaction mixture was immediately acidified with 1N HCl solution (pH 3), extracted with ethyl acetate (three times), washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to afford the half-ester, **2b**, as a white solid (30 mg, 93%). Mp 68–69°C (from ethyl acetate–hexane). >99% e.e. $[\alpha]_D^{22} = -13.4$ ($c = 2.4$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) $\delta = 1.39$ (3H, t, $J = 6.9$ Hz) 2.15 (1H, d, $J = 7.2$ Hz), 2.23 (1H, d, $J = 7.2$ Hz), 4.1 (1H, br. s), 4.2 (1H, br. s), 4.36 (2H, dq, $J = 6.9$, 12.0 Hz), 6.8–6.9 (2H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 13.95$, 53.37, 54.74, 63.33, 72.68, 141.72, 142.77, 151.36, 162.17, 162.64, 167.74; IR (KBr, cm^{-1}) 2500–2800, 1720, 1636, 1607; anal. calcd for $C_{11}H_{12}O_4$: C, 63.46; H, 5.80. Found: C, 63.21; H, 6.02%.

4.4. Enzymatic monohydrolysis of dipropyl ester, 1c

Dipropyl ester **1c** (36 mg, 0.14 mmol) was dissolved in acetone (0.2 mL), and 0.1 M sodium phosphate buffer (pH 7.5, 20 mL) was added. To this mixture, ESPESL 1864 solution (0.4 mg, 20 μL of 1 mg enzyme/50 μL pH 7 (50 mM) sodium phosphate buffer solution) was added and the mixture was incubated at 70°C for 2 h with gentle stirring until the consumption of the starting diester was detected by thin layer chromatography. The reaction was cooled in a water-bath (~5–10°C), adjusted to pH 10 with 1N NaOH solution, and immediately washed with ethyl acetate (three times); this extract was discarded. The reaction mixture was immediately acidified with 1N HCl solution (pH 3), extracted with ethyl acetate (three times), washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to afford the half-ester, **2c**, as a colorless oil (22 mg, 72%). >99% e.e. $[\alpha]_D^{23} = -9.7$ ($c = 2.6$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) $\delta = 0.99$ (3H, t, $J = 7.5$ Hz) 1.76 (2H, sextet, $J = 7.5$ Hz) 2.10 (1H, d, $J = 8.7$ Hz), 2.21 (1H, d, $J = 8.7$ Hz), 4.1 (1H, br. s), 4.2–4.3 (3H, m), 6.8–6.9 (2H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 10.21$, 21.61, 53.31, 54.63, 68.65, 72.57, 141.67, 142.71, 151.41, 162.03, 162.63, 167.74; IR (KBr, cm^{-1}) 2500–2800, 1729, 1643, 1609; anal. calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.74; H, 6.23%.

Acknowledgements

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