

Notes

A Convenient Chemoenzymatic Synthesis of (4a*S*,5*S*)-(+)-4,4a,5,6,7,8-Hexahydro-5-hydroxy-4a-methylnaphthalen-2(3*H*)-one

Lee-Chiang Lo,* Jung-Jing Shie, and Tzyy-Chao Chou

Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

lclo@ccms.ntu.edu.tw

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Abstract: We have developed a convenient chemoenzymatic method for the preparation of (4a*S*,5*S*)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methylnaphthalen-2(3*H*)-one by taking advantage of the excellent enantioselectivity of alcalase. Four different esters were compared, and the butanoate ester was found to be the best substrate. The stereochemistry of the product is the same as the one predicted from the binding model of alcalase. A simple extraction/partition procedure was used to separate the hydroxyenone product from the remaining ester. This practical procedure would be very useful in a gram-scale operation for securing the title compound in high optical purity.

Optically pure Wieland–Miescher ketone (**1**)¹ (Figure 1) and its enantiomer are important starting materials for the syntheses of a variety of natural products, including terpenoids and steroids.² A number of recent publications have focused on how to secure large quantities of optically active **1**. Three different approaches, including asymmetric synthesis,³ enantioselective microbial reduction,⁴ and classical resolution through a hemiphthalate derivative,⁵ are currently utilized for this purpose. In the first approach, asymmetric cyclization of achiral 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione (**7**) in the presence of (*S*)-(-)-proline would offer optically enriched Wieland–Miescher ketone (**1**) (Scheme 1). However, the process is time consuming (3–5 days), and the optical purity of the product is frequently variable (40–

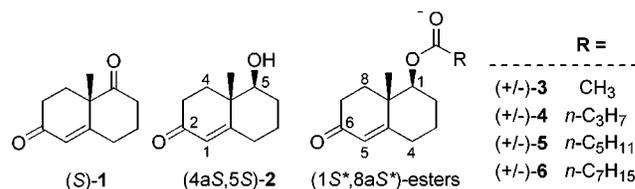
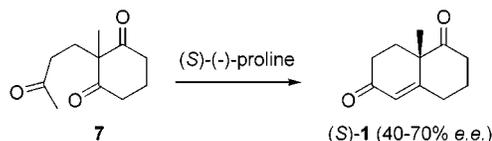


Figure 1. Structures of Wieland–Miescher ketone **1**, hydroxyenone **2**, and racemic ester derivatives **3–6**.

Scheme 1. Asymmetric Cyclization of Trione **7** in the Presence of (*S*)-proline To Form (*S*)-**1**



70%).^{3,6} A more recent example of this cyclization using catalytic antibodies,⁷ which gave Wieland–Miescher ketone in high optical purity, is yet to be applied to large-scale processes. The other two approaches also have room for improvement, as they would require either careful manipulations of the incubation medium and microbes or tedious crystallization procedures. The first step in many synthetic applications of (*S*)-**1** often involved its regio- and diastereoselective reduction with NaBH₄ to give (4a*S*,5*S*)-(+)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methylnaphthalen-2(3*H*)-one (**2**).⁸ Therefore, this hydroxyenone intermediate (4a*S*,5*S*)-**2** becomes an interesting target and could also serve as a starting point for future applications. Since compound **2** carries a secondary hydroxyl group, we envision that it would be well suited for enzyme-catalyzed kinetic resolution. We hereby report a convenient chemoenzymatic method using alcalase as an alternative approach for the preparation of (4a*S*,5*S*)-**2** (Scheme 2).

Alcalase,⁹ prepared from submerged fermentation of a selected strain of *Bacillus licheniformis*, is an inexpensive additive widely used in detergent formulations and the food industry. Its major enzyme component is a serine protease, subtilisin Carlsberg. Alcalase also possesses esterase activity with a high turnover rate and shows good stability in various organic solvents.¹⁰ In the meantime, the mechanism of subtilisin action in water and in various anhydrous solvents has been thoroughly studied

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(9) Alcalase, available from Novo Nordisk Biochem North America, Inc., Franklinton, NC, was a gift from Trump Chemical Corp., Taipei, Taiwan. The enzyme activity is expressed in Anson Units per gram (AU/g). <http://www.novozymes.com/eventure/demo/b1145a-gb.pdf>.

Scheme 2. Alcalase-Catalyzed Hydrolysis of Racemic Esters 3–6

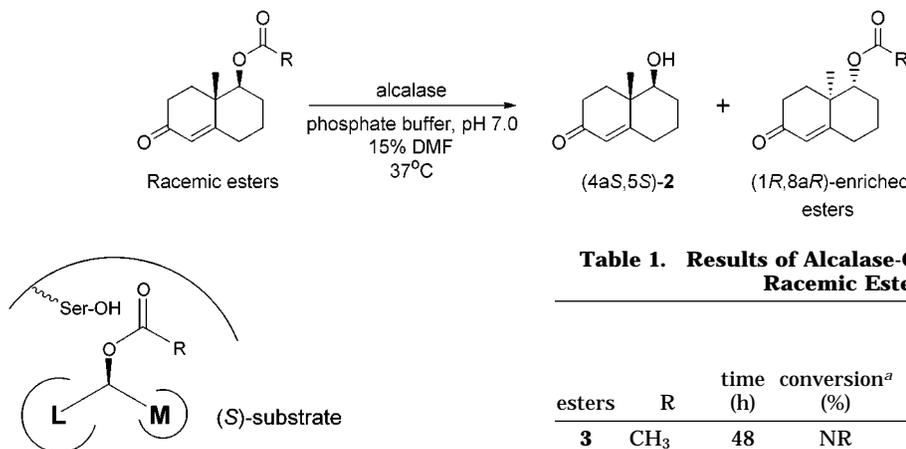


Figure 2. Preferred binding of (*S*)-substrate in the active site of subtilisin; L stands for the large substituent and M for the medium-sized substituent.

and found to be the same.¹¹ The enantioselectivity of subtilisin for the resolution of secondary alcohols was reported to be largely determined by the relative size of the two substituents.¹² The preferred binding of *S*-substrate in the active site is depicted in Figure 2, where L stands for the large substituent and M for the medium-sized substituent. The extensive information about the enantioselectivity of alcalase together with its ready availability have made it a useful reagent in organic synthesis.¹³ Based on this binding model, we predict that the secondary alcohol (4*aS*,5*S*)-**2** would meet the stereochemical requirement in the binding pocket of alcalase. We therefore prepared a series of four racemic esters **3–6** and carried out a preliminary screening using these substrates for alcalase-catalyzed hydrolytic reactions (Scheme 2). Esters **3–6**, including acetate **3**, butanoate **4**, hexanoate **5**, and octanoate **6**, are mainly different in the length of their carboxylate moiety (Figure 1). Their syntheses were achieved by acylation of the racemic alcohol **2** with suitable acylating agents in high yields.

The hydrolytic reactions were performed by incubating individual racemic ester substrate (50 mM) with Alcalase (2.4 AU/g)⁹ in 0.3 M phosphate buffer (pH 7.0) containing 15% DMF at 37 °C. Reactions were stopped at intervals to check the conversions as well as the enantiomeric excess of the remaining ester and the hydroxyenone product **2**. Under this screening condition, those four esters displayed a dramatic difference in their rates toward hydrolysis (Table 1). Only butanoate **4** gave a reasonable conversion after 12 h (~26%), whereas the other three substrates (**3**, **5**, and **6**) showed no appreciable

Table 1. Results of Alcalase-Catalyzed Hydrolysis of Racemic Esters 3–6

| esters | R | time (h) | conversion ^a (%) | enantiomeric excess ^b (%) | | <i>E</i> value ^c |
|----------|--|----------|-----------------------------|--------------------------------------|--------------------------------------|-----------------------------|
| | | | | (1 <i>R</i> ,8 <i>aR</i>) ester | (4 <i>aS</i> ,5 <i>S</i>)- 2 | |
| 3 | CH ₃ | 48 | NR | | | |
| 4 | <i>n</i> -C ₃ H ₇ | 6 | 17 | 20 | >99 | |
| 4 | <i>n</i> -C ₃ H ₇ | 12 | 27 | 36 | >99 | |
| 4 | <i>n</i> -C ₃ H ₇ | 24 | 45 | 73 | 90 | 43 |
| 4 | <i>n</i> -C ₃ H ₇ | 48 | 52 | 90 | 84 | 41 |
| 5 | <i>n</i> -C ₅ H ₁₁ | 48 | NR | | | |
| 6 | <i>n</i> -C ₇ H ₁₅ | 48 | NR | | | |

^a NR indicates no reaction when checked with TLC. The conversions for ester **4** were calculated from the equation $c = ee_s / (ee_s + ee_p)$,¹⁴ where ee_s and ee_p represent the enantiomeric excess of ester starting material and alcohol product, respectively. ^b The enantiomeric excess was determined by HPLC with a Chiralcel OJ column. The retention times for the two pairs of enantiomers **4** and **2** are shown in parentheses (min); (1*S*,8*aS*)-**4** (7.36), (1*R*,8*aR*)-**4** (8.20), (4*aR*,5*R*)-**2** (18.47), and (4*aS*,5*S*)-**2** (23.76). ^c E (enantioselectivity) = $\ln[1 - c(1 + ee_p)] / \ln[1 - c(1 - ee_p)]$.

reactions even after 48 h when checked with TLC. This interesting result indicates that the carboxylate moiety also plays a critical role in the rate of hydrolysis in addition to the steric requirement for the substituents of the alcohol. This observation is not uncommon in enzyme-catalyzed reactions, as in some cases the rate and specificity could be manipulated by partially altering the structure of the substrates.¹⁵ Since the reaction only occurred with butanoate **4**, the results were further examined.

Butanoate **4** is a good substrate for alcalase-catalyzed hydrolysis, and it also exhibits excellent enantioselectivity¹⁴ as inferred from the optical purity of both remaining ester and hydroxyenone product **2** (Table 1). Determination of the enantiomeric excess was achieved by using HPLC with a Chiralcel OJ column. The absolute configuration of the major hydroxyenone product was established by comparing its optical rotation with that of authentic sample. The optical purity of (4*aS*,5*S*)-**2** remained high ($ee > 99\%$) at the conversion of 27%. It slightly dropped to 90% when the conversion reached 45% and further dropped to 84% when the conversion was over 52%. The calculated E values¹⁴ were larger than 40, favoring the formation of (4*aS*,5*S*)-(+)-**2**. The influence of DMF content on the rate and enantioselectivity was also studied, and the results showed that the enzyme still performed well in the presence of 15–35% DMF.

Since alcalase has great enantioselectivity on butanoate **4** ($E > 40$), we then combine this feature with

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the availability of (1*S*,8*aS*)-enriched ester **4** to develop a practical method for the large-scale preparation of enantiomerically pure (4*aS*,5*S*)-**2**. For a gram-scale operation, the condition for the resolution was the same as the one depicted in Scheme 2, except (1*S*,8*aS*)-enriched butanoate **4** (58% ee) instead of the racemate was used as the substrate. Optically enriched (*S*)-**1**^{3,6} (Scheme 1) was first reduced to hydroxyenone **2**, which was subjected to acylation with butanoyl chloride to offer (1*S*,8*aS*)-enriched butanoate **4** (58% ee). After enzymatic hydrolysis, both (4*aS*,5*S*)-**2** and the remaining ester were extracted from the mixture with EtOAc. These two compounds could easily be separated by partitioning in *n*-hexane/H₂O. The remaining ester goes to the hexane layer, while (4*aS*,5*S*)-**2** stays in the aqueous phase. This simple extraction/partition procedure is especially convenient and suitable for a large-scale operation. It avoids the need of extensive chromatography for the separation of (4*aS*,5*S*)-**2** from the remaining ester. Hydroxyenone (4*aS*,5*S*)-**2** of high optical purity (>97% ee) could thus be obtained in 57% yield.

In conclusion, we have developed a convenient chemo-enzymatic method for the preparation of (4*aS*,5*S*)-4-, 4*a*,5,6,7,8-hexahydro-5-hydroxy-4*a*-methyl-naphthalen-2(3*H*)-one (**2**) by taking advantage of the excellent enantioselectivity of alcalase. Butanoate **4** was found to be the best substrate, and it gave a product with the predicted absolute stereochemistry. The carboxylate moiety of butanoate **4** not only plays an important role in the rate of alcalase-catalyzed hydrolysis, it also offers the advantage of simplifying the purification procedure after enzymatic resolution. Although only alcalase was studied in this report, its low cost and readily availability would make this procedure very useful in a gram-scale operation for securing (4*aS*,5*S*)-(+)-**2** in high optical purity. We are currently surveying other hydrolytic enzymes, including esterases and lipases, for the hydrolysis of the butanoate **4**. It is interesting to note that lipases might display a reverse enantioselectivity for this substrate, based on the knowledge of the binding pocket of lipases.^{12b} A recent example of lipase-catalyzed transesterification on a structurally similar secondary alcohol also supports this prediction.¹⁶ We will report the results in due course.

Experimental Section:

General Methods. Melting points are uncorrected. ¹H and ¹³C NMR spectra were recorded at 400 and 100 MHz in CDCl₃, respectively. Analytical TLC (silica gel, 60F-54, Merck) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Flash column chromatography was performed with silica gel 60 (70–230 mesh, Merck). HPLC was performed on a Chiralcel OJ column (250 × 4.6 mm, *n*-hexane/*i*-PrOH = 94/6, 1 mL/min) monitored at 235 nm. Both racemic and optically enriched **2** were prepared according to literature procedures.^{3,8}

Acetic Acid (1*S*^{*},8*aS*^{*})-(±)-8*a*-Methyl-6-oxo-1,2,3,4,6,7,8,8*a*-octahydronaphthalen-1-yl Ester (3**).**¹⁷ To an ice-cooled solution of the racemic hydroxyenone **2** (760 mg, 4.22 mmol) in 6 mL of pyridine was slowly added 0.5 mL of Ac₂O (5.29 mmol). The reaction was kept at room temperature and stirred overnight. The progress of the reaction was monitored by TLC (hexane/EtOAc = 4/6). After the reaction was complete (~12 h), a few drops of H₂O were added to quench the reaction. The mixture was stirred for another 30 min, and pyridine was

removed under reduced pressure. The residual oil was dissolved in EtOAc and washed consecutively with NaHCO₃ (×3), 5% citric acid (×3), H₂O (×2), and brine (×1). After drying over anhydrous Na₂SO₄ and filtration, the desired ester **3** (890 mg, 95%) was purified by silica gel column chromatography eluted with hexane/EtOAc (80/20). *R*_f = 0.35 (hexane/EtOAc = 65/35). ¹H NMR (400 MHz, CDCl₃): δ 5.80 (d, *J* = 1.9 Hz, 1 H), 4.64 (dd, *J* = 11.1, 4.1 Hz, 1 H), 2.48–2.28 (m, 3 H), 2.22 (m, 1 H), 2.07 (s, 3 H), 1.98–1.62 (m, 5 H), 1.49 (m, 1 H), 1.26 (s, 3 H). ¹³C NMR (100 MHz, CDCl₃): δ 198.9, 170.3, 166.7, 125.8, 79.2, 40.4, 34.0, 33.5, 31.8, 26.9, 22.9, 21.1, 16.6. IR (neat): 2959, 2873, 1732, 1679, 1626, 1461, 1169, 1090, 1003, 864 cm⁻¹. HRMS: calcd for C₁₃H₁₉O₃ (M + 1)⁺ 223.1334, found 223.1332.

General Procedure for the Preparation of Racemic Esters 4–6. The procedure is similar to that for acetate **3**, except suitable acyl chlorides (butanoyl, hexanoyl, and octanoyl) instead of anhydrides were used as the acylating agents. The desired esters **4–6** were purified by silica gel column chromatography eluted with hexane/EtOAc (8/2 → 6/4).

Butanoic Acid (1*S*^{*},8*aS*^{*})-(±)-8*a*-Methyl-6-oxo-1,2,3,4,6,7,8,8*a*-octahydronaphthalen-1-yl Ester (4**).** Yield: 98%. Mp: 43–44 °C. *R*_f = 0.47 (hexane/EtOAc = 65/35). ¹H NMR (400 MHz, CDCl₃): δ 5.77 (d, *J* = 1.9 Hz, 1 H), 4.62 (dd, *J* = 12.0, 4.1 Hz, 1 H), 2.45–2.14 (m, 6 H), 1.98–1.58 (m, 7 H), 1.46 (m, 1 H), 1.24 (s, 3 H), 0.93 (t, *J* = 7.3 Hz, 3 H). ¹³C NMR (100 MHz, CDCl₃): δ 198.9, 172.9, 166.8, 125.8, 78.9, 40.4, 36.5, 34.0, 33.5, 31.8, 26.9, 22.9, 18.6, 16.7, 13.7. IR (neat): 2952, 1737, 1673, 1241, 1038 cm⁻¹. HRMS: calcd for C₁₅H₂₃O₃ (M + 1)⁺ 251.1647, found 223.1644. Optically enriched (1*S*,8*aS*)-**4** was similarly prepared from (4*S*,5*aS*)-enriched **2**.

Hexanoic Acid (1*S*^{*},8*aS*^{*})-(±)-8*a*-Methyl-6-oxo-1,2,3,4,6,7,8,8*a*-octahydronaphthalen-1-yl Ester (5**).** Yield: 90%. *R*_f = 0.52 (hexane/EtOAc = 65/35). ¹H NMR (400 MHz, CDCl₃): δ 5.76 (d, *J* = 1.9 Hz, 1 H), 4.61 (dd, *J* = 11.7, 4.1 Hz, 1 H), 2.43–2.18 (m, 6 H), 1.98–1.55 (m, 7 H), 1.46 (m, 1 H), 1.32–1.27 (m, 4 H), 1.23 (s, 3 H), 0.86 (t, *J* = 7 Hz, 3 H). ¹³C NMR (100 MHz, CDCl₃): δ 198.9, 173.1, 166.8, 125.8, 78.9, 40.4, 34.5, 34.0, 33.5, 31.8, 31.2, 26.9, 24.7, 22.9, 22.3, 16.7, 13.9. IR (neat): 2957, 2864, 1738, 1682, 1240, 1170, 1006, 774 cm⁻¹. HRMS: calcd for C₁₇H₂₇O₃ (M + 1)⁺ 279.1960, found 279.1964.

Octanoic Acid (1*S*^{*},8*aS*^{*})-(±)-8*a*-Methyl-6-oxo-1,2,3,4,6,7,8,8*a*-octahydronaphthalen-1-yl Ester (6**).** Yield: 90%. *R*_f = 0.60 (hexane/EtOAc = 65/35). ¹H NMR (400 MHz, CDCl₃): δ 5.76 (d, *J* = 1.8 Hz, 1 H), 4.61 (dd, *J* = 12.7, 4.1 Hz, 1 H), 2.37–2.22 (m, 6 H), 1.98–1.58 (m, 7 H), 1.57–1.36 (m, 1 H), 1.32–1.27 (m, 11 H), 0.85–0.82 (m, 3 H). ¹³C NMR (100 MHz, CDCl₃): δ 198.8, 172.9, 166.7, 125.6, 78.7, 40.3, 34.4, 33.8, 33.3, 31.6, 31.5, 28.9, 28.7, 26.7, 24.9, 22.8, 22.4, 16.5, 13.9. IR (neat): 2934, 2863, 1738, 1684, 1469, 1452, 1166, 1006 cm⁻¹. HRMS: calcd for C₁₉H₃₁O₃ (M + 1)⁺ 307.2273, found 307.2271.

Alcalase-Catalyzed Hydrolysis of Racemic Esters 3–6 (Analytical Scale). Two stock solutions were first prepared; solution **A** contains 0.333 M of individual substrate in DMF and solution **B** contains 25% Alcalase 2.4L⁹ (v/v) in 0.3 M phosphate buffer (pH 7.0). For each reaction, 60 μL of solution **A** and 300 μL of phosphate buffer (0.3 M, pH 7.0) were first mixed. A 40 μL portion of solution **B** (total volume = 400 μL) was then added and the mixture placed in a shaker at 37 °C. The reactions were terminated at 6, 12, 24, and 48 h, respectively, by addition of EtOAc (20 mL) and H₂O (10 mL). The organic layer was collected and washed with H₂O (10 mL ×2) and brine (10 mL ×1). It was dried over anhydrous Na₂SO₄, filtered, and concentrated to dryness. The residue was dissolved in *n*-hexane/*i*-PrOH for HPLC analysis.

Alcalase-Catalyzed Hydrolysis of (1*S*,8*aS*)-Enriched Butanoic Acid 8*a*-Methyl-6-oxo-1,2,3,4,6,7,8,8*a*-octahydronaphthalen-1-yl Ester (4**) (Gram Scale).** To a solution of (1*S*,8*aS*)-enriched butanoate-**4** (1.00 g, 58% ee) in 12 mL of DMF was added 66 mL of phosphate buffer (0.3 M, pH 7.0) and 2 mL of alcalase. The mixture was placed in a shaker at 37 °C for 48 h. After enzymatic hydrolysis, both the hydroxyenone product **2** and the remaining ester **4** were extracted from the mixture with EtOAc (200 mL ×3). The EtOAc extracts were combined and concentrated. The residual oil was partitioned between hexane/H₂O. The remaining ester **4** goes to the hexane layer, while the hydroxyenone product **2** stays in aqueous phase. The aqueous phase was extracted with EtOAc (x3) and the desired product

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was purified by silica gel column chromatography eluted with hexane/EtOAc (6/4) to give (4a*S*,5*S*)-(+)-**2** (0.41 g, 57% yield). Its ee was found to be 98% by HPLC analysis, $[\alpha]_D^{25} = +174$ (*c* 1.0, benzene). ^1H NMR (400 MHz, CDCl_3): δ 5.76 (d, $J = 1.9$ Hz, 1 H), 3.40 (dd, $J = 11.7, 4.4$ Hz, 1 H), 2.50–2.22 (m, 3 H), 2.21–2.10 (m, 2 H), 1.90–1.58 (m, 5 H), 1.39 (m, 1 H), 1.17 (s, 3 H). ^{13}C NMR (100 MHz, CDCl_3): δ 199.8, 168.8, 125.4, 78.2, 41.6, 34.2, 33.6, 32.0, 30.2, 23.1, 15.2. HRMS: calcd for $\text{C}_{11}\text{H}_{17}\text{O}_2$

$(\text{M} + 1)^+$ 181.1228, found 181.1228. The spectroscopic data were identical to those reported in the literature.^{4,8}

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