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A practical chemoenzymatic process to access (*R*)-quinuclidin-3-ol on scale

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Abstract—(±)-3-Butyryloxyquinuclidinium butyrate **6** (2 M, 571 g/L), prepared from (±)-quinuclidin-3-ol **1** and butyric anhydride, undergoes enantioselective hydrolysis by an *Aspergillus melleus* protease {1.0% (w/v)} in water in the presence of $Ca(OH)_2$ to keep the reaction at pH 7 and trap butyric acid that is introduced as part of (±)-**6** and generated by the enzymatic hydrolysis. After a 24 h period, extraction with *n*-heptane provides (*R*)-quinuclidin-3-yl butyrate **5a**, which, on methanolysis with Na_2CO_3, is converted into (*R*)-**1**, a common pharmacophore of neuromodulators acting on muscarinic receptors, in 96% ee and 42% overall yield from (±)-**1**. The unwanted antipode (*S*)-**1**, which is extracted into *n*-butanol and purified via its hydrochloride salt in 89% ee and 40% overall yield from (±)-**1**, can be racemized by the catalysis of Raney Co at 140°C under an atmosphere of H_2 (5 kg/cm^2) to regenerate (±)-**1** in 97% yield.

1. Introduction

The purpose of the present article is to disclose and elaborate on our process R & D program that has culminated in developing enzyme-catalyzed, enantioselective hydrolysis of (±)-quinuclidin-3-yl ester into a scalable, industrially viable process for (*R*)-quinuclidin-3-ol **1** (Fig. 1). Representing a common pharmacophore of neuromodulators acting on cholinergic muscarinic receptors, (*R*)-quinuclidin-3-ol **1** has been incorporated into therapeutic agents under clinical investigation, such as talsaclidine **2** (M_1 agonist; cognition enhancer),[1] and revatropate **3** (M_3 antagonist; anti-asthma agent),[2] while its racemic form was embedded in cevimeline **4** (M_1 and M_3 agonist; Evoxac®)[3] for the treatment of dry mouth (xerostomia) in patients suffering from Sjögren's syndrome (autoimmune, rheumatic disease).

To produce (*R*)-**1** of such pharmacological importance, five approaches have been developed to date, each, however, suffering from a practical drawback as indicated:[4] (1) resolution via diastereomeric salt formation

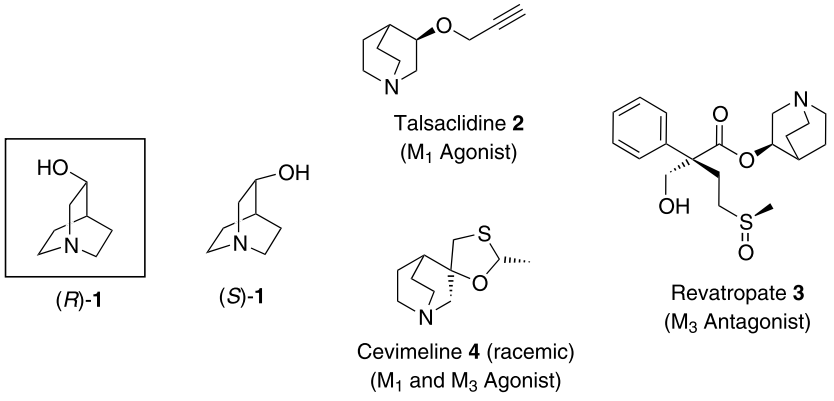

Figure 1. Structures of (*R*)-quinuclidin-3-ol **1**, talsaclidine **2**, revatropate **3**, and cevimeline **4**.

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of (±)-**1** with (1*S*)-(+)-camphorsulfonic acid {low overall yield and tedious repetition of fractional recrystallization};[5a] (2) kinetic resolution via enantioselective hydrolysis of (*R*)-quinuclidin-3-yl butyrate by horse-serum butyrylcholine esterase {limited availability of the enzyme};[6] (3) kinetic resolution via enantioselective hydrolysis of (*S*)-3-butyryloxyquinuclidinium butyrate by subtilisin Carlsberg {low volume efficiency as indicated by the substrate concentration of no more than 4.9% (w/v) in spite of high enantioselectivity leading to (*R*)-**1** of >95% ee};[7] (4) kinetic resolution via enantioselective acylation of (*S*)-**1** with vinyl butyrate by Chiro-CLEC™-BL, the cross-linked enzyme crystals of subtilisin, available from Altus biologics, Inc. {limited availability of the enzyme in a crystalline state suitable for immobilization into the cross-linked crystal form and a mediocre yield of 34% for (*R*)-**1** in spite of its high enantiomeric purity of 96.2% ee};[8] (5) asymmetric hydrogenation of 1-benzhydryl-3-oxoquinuclidinium bromide by a chiral rhodium complex catalyst prepared in situ from $[Rh(COD)Cl]_2$ and (*R*)-1-[(*S*)-2-(diphenylphosphino)-ferrocenyl]ethyl-di-*tert*-butylphosphine} in a 1:2 ratio {as low enantioselectivity as 58% ee}.[9]

Thus, weighing the pros and cons of such prior methodologies against industrial viability, we chose to explore the enzyme-catalyzed enantioselective hydrolysis of esters of (±)-quinuclidin-3-ol and develop the kinetic resolution processes for (*R*)-**1** amenable to scale-up.[10]

2. Results and discussion

2.1. Enzyme screen

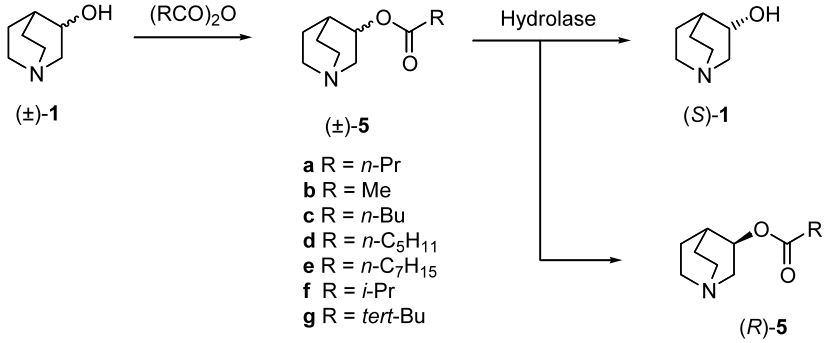
(±)-Quinuclidin-3-yl butyrate **5a** was prepared from (±)-**1** and butyric anhydride according to literature precedents,[5b,6] and seven commercially available hydrolases (three proteases and four lipases) were tested for the ability to hydrolyze (±)-**5a** in an enantioselective manner (Scheme 1). A mixture of (±)-**5a** (0.2 M), a hydrolase preparation {approximate 0.1% (w/v); a scoop of a microspatula} and a 0.2 M potassium phosphate buffer (pH 7.5) was agitated at 25°C for 16 h during which the reaction was analyzed by HPLC for the conversion {CAPCELL PAK C-18 (Shiseido)} and for the enantioselectivity {Chiralcel OD (Daicel)}. In the latter chiral analysis, authentic (*R*)-**5a** was prepared from (*R*)-**1** available from Acros Organics and used as reference to assign the absolute configuration of the left-over ester **5a** and hence that of the digested alcohol **1**.

As can be seen from Table 1 summarizing the enzyme screen, all the proteases tested showed (*S*)-selectivity, leaving (*R*)-butyrate **5a** unaffected (Scheme 1); and it was an *Aspergillus melleus* protease that showed the highest *E* value of 98 among the hydrolases evaluated. In contrast, no significant hydrolysis was observed with the lipase preparations tested except for the *Pseudomonas* sp. lipase (Sigma), the enantioselectivity of which, however, was so poor as to leave (*S*)-**5a** of 6% ee with $E^{11}=3$ at 12% conversion (16 h). Thus, the *A. melleus* protease was nominated for further investigation.

2.2. Structure–selectivity relationship

Six aliphatic esters of (±)-**1**, (±)-**5b** (acetyl), (±)-**5c** (valeryl), (±)-**5d** (caproyl), (±)-**5e** (capryl), (±)-**5f** (isobutyryl), and (±)-**5g** (pivaloyl), were prepared according to the usual procedures[5b] and subjected to the *A. melleus* protease-catalyzed hydrolysis to gain quick insight into the approximate structure-selectivity relationship and identify the fittest substrate for the enzyme: **5a**–**g**, 0.2 M; the *A. melleus* protease, 0.1% (w/v); a 0.2 M potassium phosphate buffer (pH 7.5); 25°C; 16 h (Table 2). While (±)-acetate **5b** (R = Ac), the shortest of the homologues tested, was reluctant to hydrolyse, the *A. melleus* protease could accommodate the straight higher homologues up to (±)-caprylate **5e** {R = *n*-C_7H_{15}; 40% conversion and 94% ee for (*R*)-**5e**} without significant loss of selectivity, showing substantial tolerance to the chain length of the acyl moiety. In contrast, however, branching in the acyl moiety caused a deleterious effect on the enzymatic catalysis as illustrated by (±)-isobutyrate **5f** {R = *i*-Pr; 28% conversion and 37% ee for (*R*)-**5f**} and (±)-pivalate **5g** (R = *tert*-Bu; 2% conversion).

For the ester homologues of a medium-sized straight chain, the *A. melleus* protease exerted better enantiose-



Scheme 1. Enzyme-catalyzed enantioselective hydrolysis of (±)-quinuclidin-3-yl ester **5**.

Table 1. Enzyme screening for enantioselective hydrolysis of (±)-quinuclidin-3-yl butyrate **5a**[a]

Enzyme (Microbial source)	Conversion (%)[b]	Ee (%) for (*R*)-**5a**[c]	Ee (%) for (*S*)-**1**[d]	*E*[e]
Protease (*A. melleus*)[f]	44	75	95	98
Protease (*Bacillus subtilis*)[f]	17	18	93	33
Protease (*Aspergillus oryzae*)[f]	23	27	91	28
Lipase (*Pseudomonas* sp.)[g]	12	6 for (*S*)-**5a**	48 for (*R*)-**1**	3
Lipase (*Candida rugosa*)[h]	3	–[i]	–[i]	–[i]
Lipase (*Pseudomonas cepacia*)[j]	0	–[i]	–[i]	–[i]
Lipase (*Rhizopus japonicus*)[f]	0	–[i]	–[i]	–[i]

[a] [(±)-**5a**] = 0.2 M, [enzyme] = approximate 0.1% (w/v), a 0.2 M potassium phosphate buffer (pH 7.5), 25°C, 16 h.
[b] Determined by HPLC {CAPCELL PAK C-18 (Shiseido)} under the conditions detailed in Section 4.3.1.
[c] Determined by HPLC {Chiralcel OD (Daicel)} under the conditions detailed in Section 4.3.2.
[d] Calculated on the basis of conversion and ee values measured for (*R*)-**5a**.
[e] See Ref. 11.
[f] Available from Nagase ChemteX Corporation.
[g] Available from Sigma.
[h] Available from Meito Sangyo Co., Ltd.
[i] Not calculated.
[j] Available from Amano Enzyme, Ltd.

Table 2. Enantioselective hydrolysis of esters of (±)-quinuclidin-3-ol **5** by the *A. melleus* protease[a]

	R for (±)-**5**	Conversion (%)[b]	Ee (%) for (*R*)-**5**[c]	E[d]
a	*n*-Pr	44	75	98
b	Me	2	–[e]	–[e]
c	*n*-Bu	51	90	42
d	*n*-C_5H_{11}	53	99	95
e	*n*-C_7H_{15}	40	94	67
f	*i*-Pr	28	37	70
g	*tert*-Bu	2	–[e]	–[e]

[a] [(±)-**5**] = 0.2 M, [*A. mellelus* protease] = 0.1% (w/v), a 0.2 M potassium phosphate buffer (pH 7.5), 25°C, 16 h.
[b] Determined by HPLC {CAPCELL PAK C-18 (Shiseido)} under the conditions detailed in Section 4.3.1.
[c] Determined by HPLC {Chiralcel OD (Daicel)} under the conditions detailed in Section 4.3.2.
[d] See Ref. 11.
[e] Not calculated.

lection on (±)-butyrate **5a** (R = *n*-Pr; *E* = 98) than on (±)-valerate **5c** (R = *n*-Bu; *E* = 42) while it showed no substantial difference in the enantioselectivity between (±)-**5a** and (±)-caproate **5d** (R = *n*-C_5H_{11}; *E* = 95) (Table 2). However, (±)-caproate **5d** cost more to prepare than (±)-butyrate **5a** since caproic anhydride was much more expensive than butyric anhydride, caproic anhydride and butyric anhydride being available from Aldrich at the respective price of ¥11,900/250 g and ¥8,300/L, for instance. Hence, taking into account not only enzymological but also economical aspects, (±)-butyrate **5a** was nominated for further investigation into scale-up.

2.3. Enzymatic process on scale

To adapt the *A. melleus* protease-catalyzed enantioselective hydrolysis of (±)-**5a** for the scalable processes for (*R*)-quinuclidin-3-ol **1**, the following issues should be addressed (1) to merge the enzymatic hydrolysis with the preceding butyrate-forming step and establish a through process; and (2) to run the enzymatic hydrolysis at an industrially accepted substrate concentration (not less than 1 M) without compromising the enantioselectivity or conversion rate. To deal with these two problems, butyric anhydride was reacted with neat (±)-**1** according to Muchmore's procedures[7] and, without purification, the resulting (±)-3-butyryloxyquinuclidinium butyrate **6**, (±)-**5a**·*n*-$C_3H_7CO_2H$, was subjected to the *A. melleus* protease-catalyzed hydrolysis at 25°C where NaOH was used in place of a 0.2 M potassium phosphate buffer to adjust the pH of the reaction to 7.5. With (±)-**6** (1 M) and the *A. melleus* protease {0.5% (w/v)}, the enzymatic hydrolysis proceeded with high enantioselectivity at pH 7.5 (NaOH) and 25°C to afford the left-over (*R*)-butyrate **5a** of 94% ee at 50% conversion after 24 h (Table 3). However, when the concentration of (±)-**6** was increased beyond 2 M with a ratio of [(±)-**6** (M)] to [*A. melleus* protease {% (w/v)}] kept constant at 1:0.5, the enzymatic hydrolysis started to suffer not only a decrease in the enantiomeric purity of (*R*)-**5a**, but also a slight retardation in the progress: for

Table 3. Effect of inorganic bases on the *A. melleus* protease-catalyzed hydrolysis of (±)-**6** under condensed conditions

[(±)-**6**] (M)	Inorganic base	Conversion (%)[a]	Ee (%) for (*R*)-**5a**[b]
1.0[c]	NaOH	50	94
2.0[c]	NaOH	45	78
2.5[c]	NaOH	42	69
3.0[c]	NaOH	19	23
2.0[d]	$Ca(OH)_2$	51	96
2.0[c]	$Mg(OH)_2$	48	86

[a] Determined by HPLC {CAPCELL PAK C-18 (Shiseido)} under the conditions detailed in Section 4.3.1.
[b] Determined by HPLC {Chiralcel OD (Daicel)} under the conditions detailed in the Experimental section 4.3.2.
[c] [(±)-**6** (M)]:[*A. melleus* protease {% (w/v)}] = 1: 0.5, pH 7.5 (adjusted with each inorganic base), 25°C, 24 h.
[d] [(±)-**6** (M)] = 2 M, [*A. melleus* protease] = 1% (w/v), pH 7.5, 25°C, 24 h.

the enzymatic hydrolysis running at a 2 M concentration of (±)-**5a** for 24 h, the enantiomeric purity of (*R*)-**5a** went down to 78% ee with decrease in the conversion to 45% (Table 3).

Under the above-mentioned conditions using (±)-**6** at concentrations exceeding 2 M, surplus butyric acid was introduced as part of (±)-**6** to the reaction medium, where it built up further as the enzymatic hydrolysis proceeded. Thus, such deleterious phenomena should be ascribed to the unusually high concentration of butyric acid causing product inhibition against the *A. melleus* protease.[12] Hence, to keep the effective concentration of butyric acid below a harmless level, inorganic bases, such as $Ca(OH)_2$ and $Mg(OH)_2$, were tested for the ability to capture the butyric acid and squeeze it from the reaction milieu.

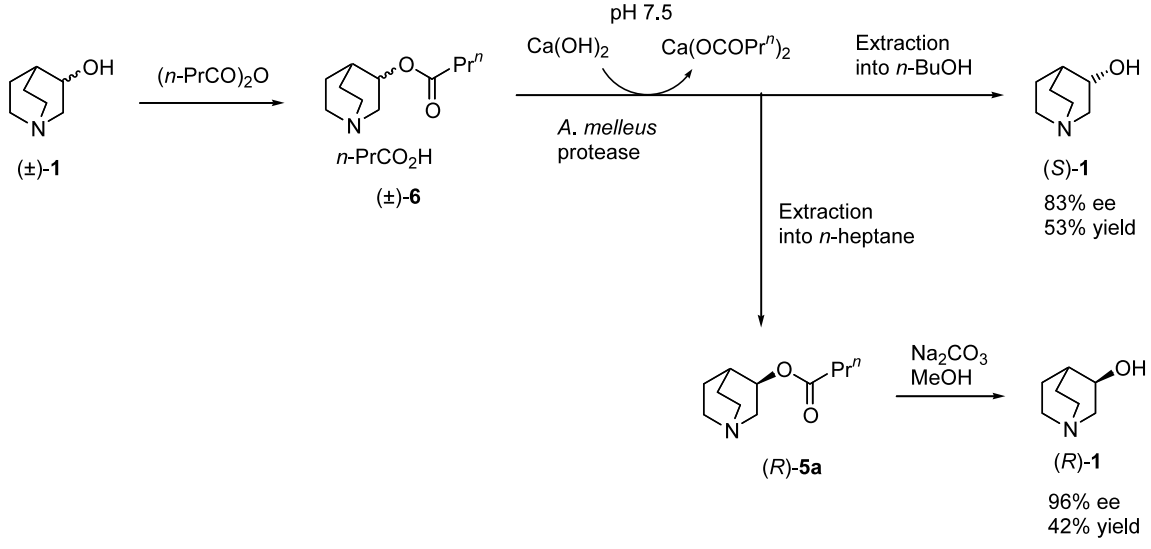
As can be deduced from Table 3, $Ca(OH)_2$ proved to be an effective scavenger for butyric acid, alleviating its inhibitory activity against the *A. melleus* protease, while no such beneficial effect was observed with $Mg(OH)_2$. In fact, when all the butyric acid present in the reaction medium was neutralized with $Ca(OH)_2$ and the pH of the reaction was kept at 7.5 throughout the enzymatic hydrolysis {(±)-**6**, 2.0 M; the *A. melleus* protease, 1.0% (w/v); 25°C, 24 h}, not only the enantioselectivity but also the conversion could be restored to the original level: 96% ee for (*R*)-**5a** at 51% conversion.

Having succeeded in telescoping preparation of (±)-**6** into its enzymatic hydrolysis at the concentration of 2 M (571 g/L), we directed our effort towards developing a practical method to separate the digested (*R*)-butyrate **5a** from the left-over (*S*)-alcohol **1** by partition. After much experimentation based on Muchmore's work,[7] the extractive separation method was devised which took advantage of the difference in hydrophobicity between (*R*)-**5a** and (*S*)-**1**: Extraction with *n*-heptane at pH 9.7 recovered the less polar (*R*)-**5a** selectively, while the more water-soluble (*S*)-**1** was kept confined in the aqueous phase. Once the butyric acid in the aqueous residue was extracted into *n*-heptane, (*S*)-**1** could be recovered successfully by extraction with *n*-butanol.

In the final analysis, the optimum conditions for the scalable kinetic resolution of (±)-quinuclidin-3-ol **1** via the enzyme-catalyzed enantioselective hydrolysis of (±)-**5a** were defined as follows (Scheme 2): On esterification of (±)-**1** with butyric anhydride, crude (±)-**6** (2 M) was treated with the *A. melleus* protease {1.0% (w/v)} in water at 25°C for 24 h. Throughout the enzymatic hydrolysis, solid Ca(OH) was added to keep the pH of the reaction at 7.5. When the hydrolysis reached 51% conversion, the reaction mixture was filtered to remove precipitated $Ca(OCOPr^n)_2$. The filtrate was basified to 9.7 with 48% aqueous NaOH, and extracted with *n*-heptane to recover the left-over (*R*)-butyrate **5a** of 96% ee, which, on Na_2CO_3-promoted methanolysis, provided (*R*)-**1** of 96% ee {chiral GLC (β-DEX 120, Sperco)} in an overall yield of 42% from (±)-**1**.[13] The aqueous residue was then made litmus-acidic with phosphoric acid, and extracted with *n*-heptane to remove butyric acid. Finally, extraction with *n*-butanol recovered crude (*S*)-alcohol **1**, which was purified via crystalline-salt formation with HCl to afford (*S*)-**1** of 89% ee {chiral GLC (α-DEX 120, Sperco)} in an overall yield of 40% from (±)-**1**.

2.4. Reuse of the unwanted antipode (*S*)-1 by racemization

The enzyme-catalyzed enantioselective hydrolysis being kinetic resolution by nature, its yield would not exceed 50% in theory. Thus, starting with (±)-quinuclidin-3-ol **1** that was costly due to its synthesis requiring multiple steps,[5a,14] the process would remain far from practical unless the unwanted enantiomer (*S*)-**1** could be reused. Thus, we undertook investigation into controlled racemization[15] of (*S*)-**1** to regenerate (±)-**1**.



Scheme 2. Chiral separation of (±)-quinuclidin-3-ol **1** by the catalysis of an *A. melleus* protease.

Its stereogenic center residing in an isolated secondary carbinol carbon, (*S*)-**1** should be difficult to racemize by other method than simultaneous oxidation/reduction.[16] Thus, Raney alloys (Ni, Co, and Cu) were explored for the catalytic ability to oxidize (*S*)-**1** to quinuclidin-3-one **7** and reduce the latter to (±)-**1** in a concurrent fashion. As can be deduced from Table 4 summarizing the comparative study on catalyst and solvent species, Raney Co was identified as the catalyst of choice (Scheme 3).[17] After much experimentation, the optimum conditions were established as follows: An *o*-xylene solution of (*S*)-**1** was heated to 140°C with a Raney Co catalyst in the same weight as that of (*S*)-**1** under an atmosphere of H_2 (5 kg/cm^2). The racemization went to completion in half an hour to give (±)-**1** in 97% yield as monitored by chiral GLC {β-DEX 120 (Sperco)}.[18]

In the meantime, the redox mechanism was corroborated by the following control experiment: when a mixture of (*S*)-**1**, excess Raney Co catalyst, and *o*-xylene was heated at 142°C in the absence of H_2 for 10 h, (*S*)-**1** was converted to quinuclidin-3-one **7** in 97% conversion as assessed by GLC (Scheme 3).

3. Conclusion

To resolve (±)-quinuclidin-3-ol **1** into (*R*)-**1** of 96% ee on scale, the *A. melleus* protease-catalyzed enantioselective hydrolysis of (±)-quinuclidin-3-yl butyrate **5a** was developed successfully where the following tactical procedures were taken to increase the effective productivity for the overall processes to a practical level: (1) To allow the *A. melleus* protease to hydrolyze (±)-3-butyryloxyquinuclidinium butyrate **6** at its concentration of 2 M (571 g/L), $Ca(OH)_2$ was added to the reaction medium to squeeze the butyric acid that was introduced as part of (±)-**6** and generated by the enzymatic hydrolysis from there such that it would not cause inhibitory effect on the protease. (2) The unwanted antipode (*S*)-**1** was racemized by the catalysis of Raney Co under H_2 atmosphere (5 kg/cm^2) at 140°C to regenerate (±)-**1** in 97% yield.[19]

4. Experimental

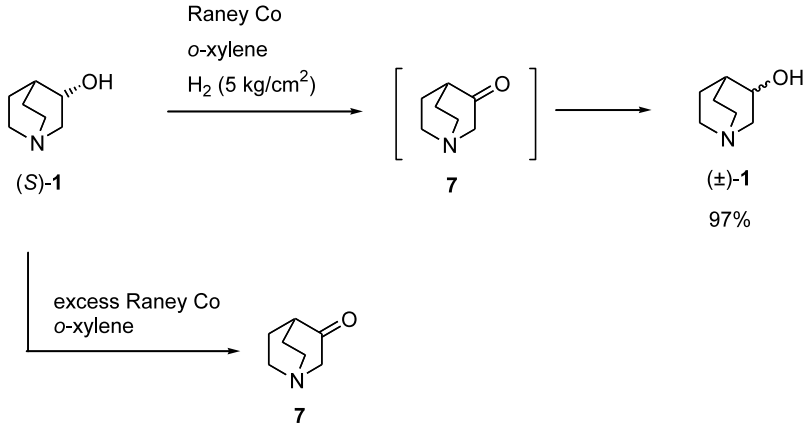
4.1. General

Melting points were measured on an Electrothermal 1A9100 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian UNITY-400 spectrometer in a $CDCl_3$ solution with tetramethylsilane as an internal standard. FT-IR spectra were recorded on a Nicolet AVATAR 360 spectrometer. Elemental analyses were performed on an Elementar vario EL analyzer. Optical rotations were measured on a Horiba SEPA-200 polarimeter.

Table 4. Raney alloy-catalyzed racemization of (*S*)-**1**[a]

Raney alloy	Solvent	Pressure of H_2 (kg/cm^2)	Time (h)	Yield (%)
Co[b]	Xylene	Atmospheric	0.5	68
Co[b]	Xylene	5	0.5	97
Co[b]	Cyclohexanol	Atmospheric	0.5	93
Co[b]	Cyclohexanol	5	0.5	97
Ni[c]	Cyclohexanol	5	1.0	98
Cu[c]	Cyclohexanol	5	1.0	97

[a] (*S*)-**1**, 6.4 g; solvent, 150 mL; 140°C.
[b] Catalyst weight, 6.4 g.
[c] Catalyst weight, 64 g.



Scheme 3. Racemization of (*S*)-**1** via quinuclidin-3-one **7** by Raney Co catalysis.

4.2. (±)-Quinuclidin-3-yl esters 5a–g

(±)-Quinuclidin-3-ol **1** was treated with acid anhydride $(RCO)_2O$ (R = *n*-Pr, Me, *n*-Bu, *n*-C_5H_{11}, *n*-C_7H_{15}, *i*-Pr, and *tert*-Bu) under the usual conditions[5b] to give the respective ester **5a**–**g** uneventfully.

4.3. Enzyme screen and structure-selectivity relationship for the enantioselective hydrolysis of (±)-quinuclidin-3-yl esters 5a–g

An Eppendorf® microtube (2.0 mL) was charged with (±)-quinuclidin-3-yl ester **5a**–**g**, an enzyme preparation, 0.2 M potassium phosphate buffer (pH 7.5) to set up reaction conditions designed for the specific objective (Tables 1–3). The reaction mixture was agitated at 25°C, and analyzed at appropriate intervals by HPLC for the conversion and enantioselectivity.

4.3.1. HPLC analysis to determine the conversion. An aliquot (40 μL) was taken from the reaction mixture and dissolved in MeCN (1.96 mL). A portion (10 μL) of the solution was injected to a chromatograph running under the following conditions: column, CAPCELL PAK C-18 (Shiseido), 0.46 cmϕ×25 cm; elution, MeOH/2.5% aqueous NH_3 (45:55), 1 mL/min; detection, UV at 210 nm; t_R 5.21 min for (±)-**1**. Quantity of (±)-**1** generated by the enzymatic hydrolysis was estimated by comparing the peak area with those of standard solutions of (±)-**1** (Aldrich) prepared at specific concentrations.

4.3.2. HPLC analysis to determine the enantiomeric composition of quinuclidin-3-yl ester 5. Authentic (*R*)-**5a**–**g** were all prepared from (*R*)-**1** (Acros Organics) under the standard conditions[5b,6] and used as reference in the following analysis. An aliquot (0.2 mL) was taken from the reaction mixture and extracted with AcOEt (1.0 mL). A portion (1.0 μL) of the AcOEt solution was injected to a chromatograph running under the following conditions: column, Chiralcel OD (Daicel), 0.46 cmϕ×25 cm; elution, *n*-hexane/*i*-PrOH/CF_3CO_2H (90:10:0.1), 1 mL/min; detection, UV at 210 nm; t_R 15.3 min for (*R*)-**5a**, 17.7 min for (*S*)-**5a**; 14.3 min for (*R*)-**5c**, 17.0 min for (*S*)-**5c**; 13.9 min for (*R*)-**5d**, 17.9 min for (*S*)-**5d**; 13.7 min for (*R*)-**5e**, 15.8 min for (*S*)-**5e**; 15.2 min for (*R*)-**5f**, 17.2 min for (*S*)-**5f**.

4.4. (*R*)-Quinuclidin-3-ol 1

(±)-Quinuclidin-3-ol **1** (12.7 g, 100 mmol; purchased from Aldrich) was added to butyric anhydride (17.4 g, 110 mmol) in portions at rt. The resultant mixture was stirred at 50°C for 2 h, and distilled water (22 mL) was added. To the mixture was added solid $Ca(OH)_2$ until pH of the mixture reached 7.5. XP-488 (0.5 g; a preparation of an *A. melleus* protease available from Nagase ChemteX Corporation) was added, and the mixture was stirred at 25°C for 24 h during which $Ca(OH)_2$ was added to keep the pH of the mixture at 7.5. The precipitated solids were filtered off. To the filtrate was added 10 M aqueous solution of NaOH to adjust the pH of the mixture to 9.7. The mixture was extracted with *n*-heptane (50 mL×1, 125 mL×1, 150 mL×1); a portion (10 μL) of the combined *n*-heptane extracts was analyzed by chiral HPLC for the enantiomeric purity of (*R*)-quinuclidin-3-yl butyrate **5a** under the conditions described above: (*R*)-**5a**, 98%; (*S*)-**5a**, 2%. The *n*-heptane solution was concentrated in vacuo until its weight diminished to 8.8 g. To the residue were added MeOH (25 mL) and Na_2CO_3 (5.0 g), and the mixture was stirred and heated at 65°C for 16 h. The mixture was allowed to cool to rt, and filtered to remove inorganic solids. The filtrate was concentrated in vacuo to dryness. To the residue was added $CHCl_3$ (30 mL), and the mixture was filtered to remove insoluble materials. The filtrate was concentrated in vacuo to give (*R*)-**1** (5.3 g, 42%), the enantiomeric purity of which was determined to be 96% ee by chiral GLC: A portion (approximate 10 mg) of it was dissolved in $CHCl_3$ (1 mL), and 5 μL of the $CHCl_3$ solution was injected to a chromatograph running under the following conditions: column, β-DEX 120 (Sperco), 0.25 mmϕ×30 m; carrier gas, He, 200 kPa; injection temperature, 220°C; column temperature, 140°C; detection, FID, 220°C; t_R 7.3 min for (*R*)-**1**, 6.9 min for (*S*)-**1**. IR ν_{max} (KBr) 3105, 2941, 2872, 1456, 1346, 1319, 1128, 1117, 1045, 991, 941, 818, 795, 775, 633 cm^{-1}; ^1H NMR δ 1.34 (m, 1H), 1.44 (m, 1H), 1.67 (m, 1H), 1.79 (m, 1H), 1.94 (m, 1H), 2.55–2.66 (m, 2H), 2.70–2.79 (m, 2H), 2.89 (m, 1H), 3.08 (m, 1H), 3.79 (m, 1H), 4.42 (br s, 1H). A portion of (*R*)-**1** thus obtained was further purified according to the literature procedures[7] to afford an analytical sample: mp 219°C {lit.:[6] 219–220°C}; $[\alpha]_D^{25}$ −44.9 (*c* 2.0, 1 M HCl) {lit.:[6] $[\alpha]_D^{25}$ −46±1 (*c* 2.0, 1 M HCl)}. Anal. found: C, 66.0%; H, 10.3%; N, 11.0%. calcd for $C_7H_{13}NO$: C, 66.09%; H, 10.32%; N 11.01%.

4.5. (*S*)-Quinuclidin-3-ol 1

On extraction with *n*-heptane at pH 9.7 as described in Section 4.4, H_3PO_4 (11 mL) was added to the aqueous residue. The mixture was extracted with *n*-heptane (30 mL × 3). The aqueous residue was concentrated in vacuo to remove remaining *n*-heptane. KOH (30 g) was added, and the aqueous mixture was extracted with *n*-butanol (45 mL × 3). To the combined *n*-butanol extracts was added 35% HCl (7 mL). The mixture was concentrated in vacuo to remove water azeotropically. The residual solution was allowed to cool to 5°C, and the precipitated solids were collected by filtration to give wet (*S*)-**1**·HCl (13.2 g), to which was added 5 M aqueous solution of NaOH (40 mL). The mixture was extracted with $CHCl_3$ (50 mL × 3). The combined $CHCl_3$ extracts were washed with 5 M aqueous solution of NaOH (20 mL × 1), dried (Na_2SO_4), and concentrated in vacuo to give (*S*)-**1** (5.1 g) in 89% ee and 40% overall yield from (±)-**1**; the enantiomeric purity of (*S*)-**1** was determined by chiral GLC under the conditions specified in Section 4.4.

4.6. Racemization of (*S*)-1

A bomb (300 mL) was charged with (*S*)-**1** (89% ee; 5.1 g, 40 mmol), *o*-xylene (120 mL), and Raney Co (5.1 g). The mixture was stirred and heated at 140°C under an

atmosphere of H_2 (5 kg/cm^2) for 1 h, during which the progress of the racemization was monitored by chiral GLC: From the supernatant of the reaction mixture was taken an aliquot (approximate 0.1 mL), to which was added $CHCl_3$ (0.2 mL). The mixture was filtered to remove solid materials and 5 μL of the filtrate was injected to a chromatograph running under the conditions recorded in Section 4.4. The mixture was allowed to cool to 90°C, and filtered to remove the catalyst. The filtrate was further cooled to 0°C. The precipitated solids were collected by filtration and air-dried to give (±)-**1** (4.9 g, 97%). Its spectral and physicochemical data were identical to those reported.[5a]

4.7. Oxidation of (*S*)-1 to quinuclidin-3-one 7

A mixture of (*S*)-**1** (0.64 g, 5.03 mmol), Raney Co (1.59 g), and *o*-xylene (15 mL) was stirred and heated at 142°C. After 10 h, the reaction mixture was analyzed by chiral GLC under the conditions recorded in Section 4.4: t_R 5.8 min for **7** (96.6%), 6.9 min for (*S*)-**1** (1.6%), 7.3 min for (*R*)-**1** (1.7%); authentic **7** was obtained by liberating it from the hydrochloride salt **7**·HCl (Aldrich) under the usual conditions.

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