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Simple one-pot process for the bioresolution of tertiary amino ester protic ionic liquids using subtilisin

Maude Brossat *, Thomas S. Moody *, Stephen J. C. Taylor, Jonathan W. Wiffen

Biocatalysis Group, Almac Sciences, David Keir Building, Stanmillis Road, Belfast BT9 5AG, Northern Ireland

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ABSTRACT

An efficient hydrolase-catalyzed bioresolution of tertiary amino ester protic ionic liquids has been demonstrated. Protic ionic liquids have been prepared in one step from the corresponding tertiary amino alcohols by treatment with butyric anhydride. After bioresolution, unreacted esters can be easily separated from the corresponding alcohols by extraction with hexane. Bioresolution of quinuclidin-3-yl butyrate has been performed with excellent selectivity.

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1. Introduction

Enzymatic kinetic resolution of racemic alcohols via acylation or hydrolysis has been extensively studied and established as one of the most effective methods for the preparation of optically active alcohols. Over the past three decades, novel media for biocatalytic processes such as organic solvents,[1] solvent-free systems,[2] gaseous media,[2] supercritical fluids[2] and ionic liquids[3] have been subjected to intense research. In particular, ionic liquids have received much attention due to their unique physiochemical properties such as their non-volatility and they have been generally recognized as green solvents for non-aqueous biocatalysis.[4]

The use of room temperature ionic liquids (RTILs) as new attractive reaction media for bioresolutions has been widely discussed.[5] The improvement of the enzyme stability and activity, the ease of product recovery combined with the enhancement of the enantioselectivity make them a powerful tool for biocatalysis.[6] In addition, the introduction of functional groups to ionic liquids by derivatisation of the cationic component or functionalisation of the anion resulted in the development of so-called 'task-specific' ionic liquids (TSILs).[7]

Afonso et al. reported the use of a TSIL as an acylating agent for the one-pot bioresolution-separation of secondary alcohols.[8] However in this case, the use of an additional ionic liquid as a co-solvent was required because of the viscosity of the medium.

Over the course of our studies on the bioresolution of tertiary amino alcohols, we investigated the feasibility of a cost effective and time efficient process for bioresolutions, which minimises processing time in the pilot manufacturing and uses inexpensive hydrolase enzymes.

Several examples have been reported in the literature for the kinetic bioresolution of quinuclidin-3-ol. The enantioselective hydrolysis of the corresponding butyrate ester of quinuclidin-3-ol has been demonstrated using horse serum butyrylcholine esterase,[9] although the main drawback of this method is the limited availability of the enzyme. The bioresolution of a tertiary 3-substituted acetylenic quinuclidinol butyrate ester has been reported using pig liver esterase in aqueous buffer.[10] Nomoto et al. have reported the bioresolution of the butyrate ester using *Aspergillus melleus* protease.[11] The butyrate ester hydrolysis has also been reported using commercially available subtilisin Carlsberg albeit under high dilution conditions.[12]

Herein we report a simple, efficient and inexpensive method for the bioresolution of tertiary amino ester protic ionic liquids without co-solvent as shown in Scheme 1, which offers processing benefits to the process chemist.

2. Results and discussion

2.1. Preparation of racemic amino ester substrates

The butyrate ester of quinuclidin-3-ol **2a** was prepared by mixing the amino alcohol **1** with 1.05 equiv of butyric anhydride (Scheme 2). The formation of the resulting mobile protic ionic liquid **2a** is quantitative and fast (10–30 min). The viscosity of **2a** was measured to be 30 mPa s at 25 °C and is comparable to the viscosity of ethylene glycol (16.1 mPa s at 25 °C), thereby is suitable for any pilot plant reactor and is easily stirred and handled at scale.[13]

2.2. Subtilisin-catalyzed kinetic bioresolution

The inexpensive, commercially available liquid form of the enzyme subtilisin (from *Bacillus licheniformis*) was used for bioresolution studies. The hydrolysis of quinuclidin-3-yl butyrate (*RS*)-**2a**

* Corresponding authors. Tel.: +44 (0) 28 3836 5906; fax: +44 (0) 28 9022 5544 (M.B.).
E-mail addresses: maude.brossat@almacgroup.com (M. Brossat), tom.moody@almacgroup.com (T.S. Moody).

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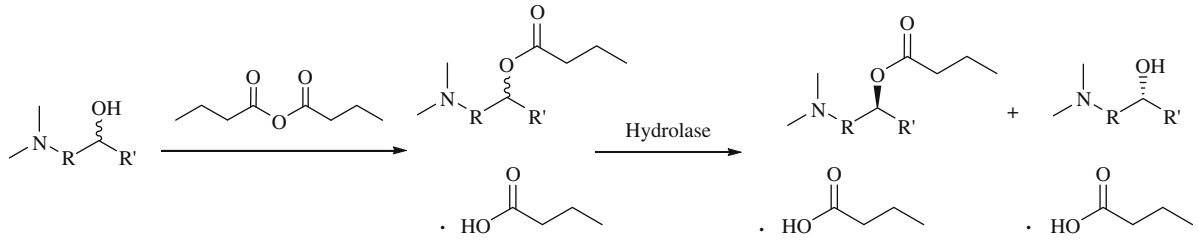
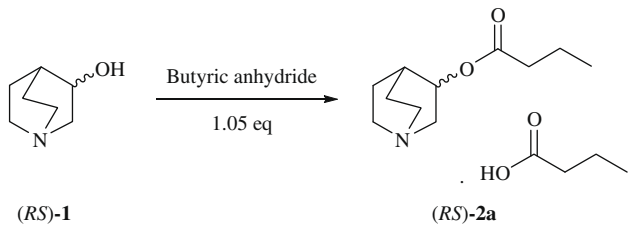


Scheme 1. Simple one pot process for the bioresolution of tertiary amino ester protic ionic liquids.



Scheme 2. Preparation of protic ionic liquid (*RS*)-**2a** from (*RS*)-**1**.

was performed by stirring at room temperature over 16 h with 100% v/v of liquid subtilisin as shown in Scheme 3, utilizing the water present in the enzyme for hydrolysis.

The bioresolution showed high conversion and (*S*)-selectivity with good ee for the (*R*)-butyrate **2a**[14] and the (*S*)-alcohol **3**[15] with a calculated *E*-value of 79 (Table 1, entry 1).

2.3. Application to other substrates

This methodology was expanded to other tertiary amino ester protic ionic liquids (*RS*)-**4a** to (*RS*)-**6a**, as shown in Figure 1.

The subtilisin-catalyzed bioresolution of butyrate ester protic ionic liquids (*RS*)-**2a**, (*RS*)-**4a** to (*RS*)-**6a** are shown in Table 1. The corresponding subtilisin catalyzed bioresolution of the free bases are shown in Table 2. The corresponding free bases are designated (*RS*)-**2b**, (*RS*)-**4b** to (*RS*)-**6b** and were prepared using a simple basic extraction from the parent protic ionic liquid.

Figure 2 shows the *E*-values obtained from the bioresolution of both the protic ionic liquids and corresponding free bases. For the bioresolution of quinuclidin-3-ol, the protic ionic liquid shows by far the best *E*-value for this series of compounds. An increase in *E*-value is obtained for compound (*RS*)-**4** when using the protic ionic liquid derivative. Resolution does occur for compounds (*RS*)-**5** and (*RS*)-**6** albeit with lower selectivity. Further work is currently underway to investigate alternative enzymes that show the desired selectivities for these substrates.

2.4. Scale-up

The bioresolution of substrate (*RS*)-**2a** was scaled to 10 g using 50% v/v of liquid subtilisin. An additional advantage of using the protic ionic liquid system is the self-buffering of the reaction, removing the necessity for pH control, which makes the reaction very easy to process, requiring no specialized equipment. The profile of the reaction is shown in Figure 3. After 16 h, 48% conversion was reached with 89.1% $ee_{alcohol}$ and 95.6% ee_{ester} corresponding to an *E*-value of 74.

The unreacted (*R*)-ester was separated from the (*S*)-alcohol by extraction with hexane and washing with saturated sodium carbonate as shown in Figure 4. The (*R*)-ester was recovered with 47% yield, 95.6% ee and 99% purity by NMR wt/wt assay.

Table 1
Subtilisin-catalyzed hydrolysis of (*RS*)-**2a**, (*RS*)-**4a** to (*RS*)-**6a**

Entry	Substrate	ee substrate[a] (%)	ee product[b] (%)	Conv.[c] (%)	*E*[c]
1	(*RS*)-**2a**	97.0	90.4	52	79
2	(*RS*)-**4a**	28.9	89.2	25	23
3	(*RS*)-**5a**	48.3	3.7	92	1.5
4	(*RS*)-**6a**	40.7	73.8	36	10

[a] Ee determined by extraction of the ester with hexane and basic hydrolysis in MeOH using 2 M aqueous NaOH followed by HPLC using CHIRALCEL AD-H column.
[b] Ee determined by HPLC using CHIRALCEL AD-H column.
[c] Calculated from $ee_{alcohol}$ and ee_{ester} after 16 h reaction.

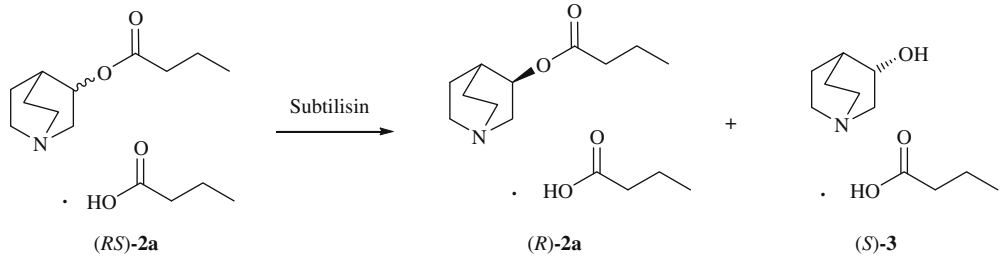
3. Conclusion

In conclusion, the bioresolution of tertiary amino alcohols has been demonstrated via their corresponding butyrate protic ionic liquids. This work has demonstrated that hydrolase enzymes, such as subtilisin, can tolerate high concentrations of ionic mixtures and still maintain high selectivity in the case of the quinuclidin-3-ol. Performing the bioresolution as the protic ionic liquid reduces processing time and removes the need to pH control the reaction and should be considered when performing other bioresolutions of this type to reduce costs for the plant chemist.

4. Experimental

4.1. General

Chemicals were purchased from Alfa Aesar. Protease enzyme subtilisin (from *B. licheniformis*) was obtained from Enzagen Ltd.



Scheme 3. Kinetic bioresolution of the butyrate ester (*RS*)-**2a**.

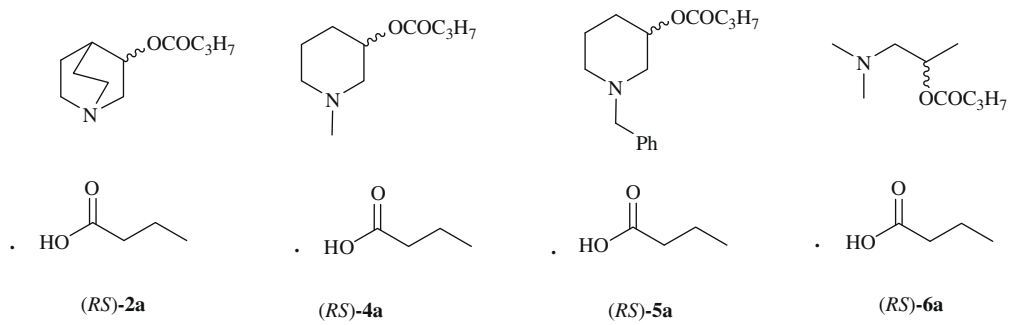


Figure 1. Tertiary amino ester protic ionic liquids (*RS*)-**2a**, (*RS*)-**4a** to (*RS*)-**6a**.

Table 2
Subtilisin-catalyzed hydrolysis of (*RS*)-**2b**, (*RS*)-**4b** to (*RS*)-**6b**

Entry	Substrate	ee substrate[a] (%)	ee product[b] (%)	Conv.[c] (%)	*E*[c]
1	(*RS*)-**2b**	55.8	13.2	81	2
2	(*RS*)-**4b**	92.8	61.4	60	13
3	(*RS*)-**5b**	97.3	62.7	61	17
4	(*RS*)-**6b**	98.2	58.9	62	16

[a] Ee determined by the extraction of the ester with hexane and basic hydrolysis in MeOH using 2 M aqueous NaOH followed by HPLC using CHIRALCEL AD-H column.[14]
[b] Ee determined by HPLC using CHIRALCEL AD-H column.[15]
[c] Calculated from $ee_{alcohol}$ and ee_{ester} after 16 h reaction.

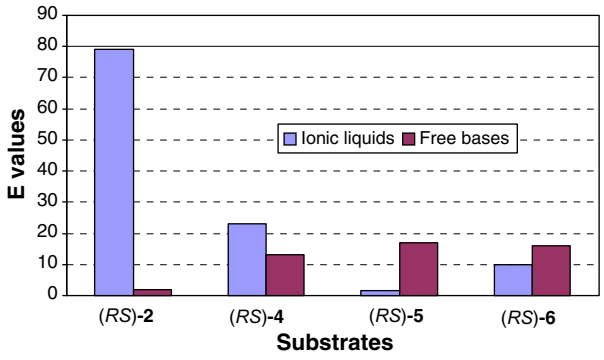


Figure 2. Comparison of the *E*-values for the bioresolution of protic ionic liquids (*RS*)-**2a**, (*RS*)-**4a** to (*RS*)-**6a** and free bases (*RS*)-**2b**, (*RS*)-**4b** to (*RS*)-**6b**.

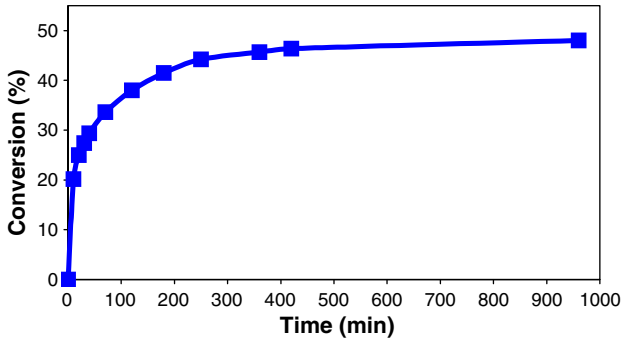


Figure 3. Reaction profile for the bioresolution of (*RS*)-**2a**.

^{1}H NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer, shifts are relative to internal TMS.

The enantiomeric excesses were measured by chiral stationary phase HPLC on Chiracel AD-H column (250 mm × 4.6 mm × 10 μm, Daicel Chemical Industries) with UV detection (λ = 254 nm).

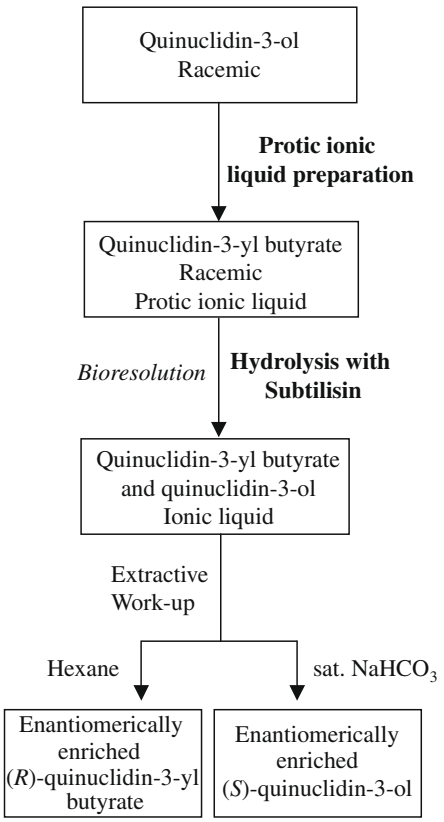


Figure 4. Facile processing for the bioresolution of racemic quinuclidin-3-ol.

Mass spectra (MS) were recorded on an ThermoQuest Finnigan LCQ Duo (electrospray) spectrometer.

4.2. General procedure for the synthesis of the butyrate ester protic ionic liquids, (*RS*)-2a, (*RS*)-4a to (*RS*)-6a

The protic ionic liquids were prepared by reacting the corresponding alcohols with butyric anhydride (1.05 equiv). The mixtures were stirred for 30 min at room temperature and used directly in the bioresolution studies.

4.2.1. Quinuclidin-3-yl butyrate. Butyric acid (*RS*)-2a

Transparent liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.91–4.95 (m, 1H), 3.37–3.31 (m, 1H), 3.04–2.84 (m, 5H), 2.20 (t, J = 8.0 Hz, 2H), 2.23–2.16 (m, 3H), 2.02–1.95 (m, 1H), 1.87–1.79 (m, 1H), 1.50–1.75 (m, 6H), 0.98–0.91 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.7, 173.1, 68.9, 53.4, 45.7, 44.9, 37.6, 36.2, 24.8, 22.6, 18.9, 18.4, 18.3, 13.9, 13.7. MS (ESI TOF) $[M+H]^+$ = 198. Mp −47 to −50 °C.

4.2.2. 1-Methyl-3-piperidinyl butyrate. Butyric acid (*RS*)-4a

Yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.96–4.93 (m, 1H), 2.81–2.70 (m, 1H), 2.57–2.45 (m, 3H), 2.36 (s, 3H), 2.30–2.25 (m, 4H), 1.83–1.72 (m, 2H), 1.70–1.54 (m, 5H), 1.52–1.45 (m, 1H), 1.00–0.92 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.1, 172.9, 67.8, 57.8, 54.4, 45.2, 36.7, 36.3, 28.2, 21.2, 18.5, 18.4, 13.8, 13.6. MS (ESI TOF) $[M+H]^+$ = 186.

4.2.3. 1-Benzyl-3-piperidinyl butyrate. Butyric acid (*RS*)-5a

Yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31–7.22 (m, 5H), 4.89–4.85 (m, 1H), 3.64–3.59 (m, 2H), 2.81–2.78 (m, 1H), 2.64–2.62 (m, 1H), 2.40 (t, J = 5.8 Hz, 2H), 2.38–2.21 (m, 4H), 1.86–1.73 (m, 2H), 1.69–1.55 (m, 5H), 1.45–1.38 (m, 1H), 0.97–0.85 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.0, 173.0, 129.7, 129.0, 128.3, 127.5, 68.7, 62.1, 55.9, 52.3, 37.1, 36.3, 29.2, 22.0, 18.5, 17.8, 13.7, 13.4. MS (ESI TOF) $[M+H]^+$ = 262. HPLC analysis: Chiracel AD-H column, eluent hexane/ethanol/diethylamine (85/15/0.1), flow rate 1 mL/min, λ = 254 nm, t_1 = 3.62, t_2 = 3.94.

4.2.4. 1-(Dimethylamino)-2-propanyl butyrate. Butyric acid (*RS*)-6a

Yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.13–5.11 (m, 1H), 2.80–2.75 (m, 1H), 2.50 (dd, J = 4.0 Hz, and 11.8 Hz, 1H), 2.37 (s, 6H), 2.27–2.19 (m, 4H), 1.63–1.57 (m, 4H), 1.19 (d, J = 5.0 Hz, 3H), 0.97–0.91 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.9, 173.1, 66.7, 62.4, 44.3, 37.0, 35.3, 18.8, 18.6, 18.3, 17.7, 13.9, 13.4. MS (ESI TOF) $[M+H]^+$ = 174.

4.3. General procedure for the synthesis of the free base butyrate esters, (*RS*)-2b, (*RS*)-4b to (*RS*)-6b

Isolation of the free base esters from the corresponding ionic liquids was achieved by washing with saturated sodium carbonate (2 vol) and extracting five times with hexane (1 vol). Evaporation of the solvent afforded the corresponding ester in 90–95% yield.

To calculate the ee of the ester, it was hydrolysed under basic conditions with 2 M NaOH in MeOH for 30 min followed by derivatisation with benzoic anhydride as described in Section 4.5.

4.3.1. Quinuclidin-3-yl butyrate (*RS*)-2b

Transparent liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.82–4.79 (m, 1H), 3.27–3.22 (m, 1H), 2.91–2.65 (m, 5H), 1.99 (t, J = 5.9 Hz, 2H), 2.00–1.98 (m, 1H), 1.84–1.83 (m, 1H), 1.71–1.63 (m, 3H), 1.57–1.55 (m, 1H), 1.40–1.37 (m, 1H), 0.97 (m, J = 5.9 Hz, 3H).

4.3.2. 1-Methyl-3-piperidinyl butyrate (*RS*)-4b

Transparent liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.90–4.86 (m, 1H), 2.64–2.61 (m, 1H), 2.42–2.39 (m, 1H), 2.30–2.23 (m, 7H), 1.78–1.75 (m, 2H), 1.67–1.57 (m, 3H), 1.45–1.43 (m, 1H), 0.97 (t, J = 5.9 Hz, 3H).

4.3.3. 1-Benzyl-3-piperidinyl butyrate (*RS*)-5b

Transparent liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36–7.28 (m, 5H), 4.91–4.94 (m, 1H), 3.66–3.63 (m, 2H), 2.85–2.83 (m, 1H), 2.67–2.66 (m, 1H), 2.46 (t, J = 5.8 Hz, 2H), 2.36–2.27 (m, 4H), 1.90–1.86 (m, 1H), 1.76–1.63 (m, 2H), 1.45–1.42 (m, 1H), 0.97 (t, J = 5.9 Hz, 3H). HPLC analysis: Chiracel AD-H column, eluent hexane/ethanol/diethylamine (85/15/0.1), flow rate 1 mL/min, λ = 254 nm, t_1 = 3.62, t_2 = 3.94.

4.3.4. 1-(Dimethylamino)-2-propanyl butyrate (*RS*)-6b

Yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.07–5.03 (m, 1H), 2.51–2.47 (m, 1H), 2.29–2.25 (m, 9H), 1.67–1.62 (m, 2H), 1.22 (d, J = 5.0 Hz, 3H), 0.97–0.91 (t, J = 5.9 Hz, 3H).

4.4. Large scale bioresolution of quinuclidin-3-ol (*RS*)-2a

(*RS*)-Quinuclidin-3-ol (10 g, 78.7 mmol) was added to butyric anhydride (13.3 mL, 82.7 mol) at room temperature. The resultant mixture was stirred at room temperature for 1 h. There is a moderate exotherm as the alcohol dissolves and the ester forms. The formation of the butyrate ester is quantitative and fast and the liquid product is easily stirred at room temperature (viscosity was measured to be 30 cP at 25 °C). The protic ionic liquid was used without work-up or further purification.

Liquid subtilisin (10 mL (50% v/v)) was then added directly in one portion. The reaction was stirred at 30 °C and ee of the residual ester monitored by chiral HPLC. After 16 h, the solution was diluted with 60 mL (6 vols) of saturated sodium carbonate and extracted five times with 30 mL (3 vols) of hexane to afford, after evaporation of the solvent, 7.32 g (47% yield) of the (*R*)-butyrate ester **2a** (95.6% ee) with 99% wt/wt purity by NMR assay.

4.5. Derivatisation method with benzoic anhydride

Aminoalcohol (~20 mg) and benzoic anhydride (~20 mg) were placed in a 1 ml HPLC vial and heated at 70 °C for 10 min. After cooling, 1 mL 2 M HCl was added followed by 1 mL MTBE. The vial was shaken to partition the amino ester into the acid, and the unreacted neutral organics and benzoic acid into the MTBE layer. The aqueous layer was pipetted into a fresh vial, pH adjusted to 13 with 2 M NaOH and 1 mL MTBE added. The vial was shaken to partition the derivatised amino ester into the organic phase. Hundred microlitres of the MTBE layer were added to 1.5 mL of mobile phase and the solution dried over $MgSO_4$ before HPLC injection.

For the benzoate ester of quinuclidin-3-ol, eluent hexane/ethanol/diethylamine (85/15/0.1); flow rate 1 mL/min; typical retention times were 9.6 min (*S*)-benzoate ester, 17.9 min (*R*)-benzoate ester. For substrate 1-methyl-3-piperidinol eluent hexane/ethanol/diethylamine (85/15/0.1); flow rate 1 mL/min; typical retention times were for benzoate esters 5.1 min and 5.4 min, 1-benzyl-3-piperidinol eluent hexane/ethanol/diethylamine (85/15/0.1); flow rate 1 mL/min; typical retention times for the alcohols were 5.3 min and 6.0 min, 1-(dimethylamino)-2-propanol eluent hexane/ethanol/diethylamine (95/5/0.1); flow rate 0.5 mL/min; typical retention times were for benzoate esters 9.1 min and 9.8 min.

The conversion for the hydrolysis of quinuclidin-3-yl butyrate was determined by GC under the following conditions: column Chiraldex B, 0.25 mm × 30 m, 17 psi He, injection temperature, 150 °C, then 10 °C /min to 210 °C, hold 4 min; retention times were 2.4 min for butyric acid, 4.3 min for quinuclidinol and 6.5 min for quinuclidinyl butyrate.

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14. The ee of the ester was determined after basic hydrolysis and derivatisation using benzoic anhydride and compared with a commercial reference.
15. The ee of the alcohol was determined by HPLC after derivatisation using benzoic anhydride and compared with a commercial reference.